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CONTENTS

DIFFERENTIAL SUSCEPTIBILITY AND DIFFEREN-
TIAL INHIBITION IN THE DEVELOPMENT OF
POLYCHETE ANNELIDS

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FIFTY-SEVEN FIGURES

The existence of gradients in susceptibility to various agents has been demonstrated for the eggs and developmental stages of starfish and sea-urchin (Child, '15 c, '16 a), and the control and modification of development through differential susceptibility in the sea urchin (Child, '16 c) has shown the fundamental significance for the development of this form of the axial gradients in metabolic activity and protoplasmic condition of which the susceptibility gradients are indicators. In the light of the results obtained with echinoderms, the desirability of extending investigations along these lines to other forms is evident. Since the polychete differs so widely from the echinoderm in its highly determinate cleavage, its very early larval differentiation and its apparently limited regulatory capacity in early developmental stages, the investigation of its development by means of the susceptibility methods is of particular interest, not only for the purpose of comparison of the two sharply contrasting forms, but as a further contribution to the general theory of organization. Such an investigation must determine as far as possible, first, whether axial gradients are present or not, second, what modifications they undergo during development and third, their rôle in development. The data presented below, the result of work done during the last four summers at the Marine Biological Laboratory, Woods Hole, Mass., throw some light on these problems.

The differential susceptibility along an axis may appear in sufficiently high concentrations of the agent employed as a

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difference in survival time, a death gradient (Child, '15 c, '16 a), and, in many cases, certain changes preceding death, such as change in state of aggregation of protoplasm, swelling and separation of cells or change in the color of a vital stain, for example, neutral red, used as an indicator, are readily observed and show the same gradient as the final death-change. With somewhat lower concentrations the differential susceptibility may be made evident as a differential inhibition, or, in cases of acclimation or recovery, as a differential acceleration of development following a primary inhibition (Child, '16 c). In this work on the polychetes, disintegration and death and inhibition of development have been used as a means of demonstrating the differences in susceptibility. Little can be accomplished with differential acclimation because larval development is far advanced before any considerable degree of acclimation occurs.

For purely practical reasons, namely, because eggs and sperm could be obtained at any time and in almost any quantity desired from males and females kept in the laboratory, Chaetopterus pergamentaceus was used as material to a greater extent than other species, but Nereis limbata and Arenicola cristata were also used so far as material was available, and some work was done with Hydroides dianthus. Since a considerable number of eggs must be used in each experiment and a considerable range of concentrations of each agent used must be tested repeatedly with different stages of development, an abundance of material obtainable as desired is essential for any thorough study, particularly of the modification of development. The material available has not been sufficient for all purposes, but certain definite and characteristic results which permit certain conclusions have been obtained.

DISINTEGRATION AND DEATH GRADIENTS

The gradients in disintegration and other protoplasmic changes and in decoloration after staining with neutral red have been demonstrated by means of various agents: KNC $m/1000-m/100$, alcohol 2.5 to 5 per cent, $HgCl_2$ $m/500000-m/50000$, $CuSO_4$ $m/100000$ $m/20000$, NaOH $m/10$ approximately. These agents

were used, either after staining with neutral red as an indicator
for death (Child, '16 b, '16 d) or without neutral red, or neutral
red was used after exposure to the agent for various periods,
and the results as regards the existence of axial differences in
susceptibility were essentially the same with all procedures,
though differences in the degree and character of protoplasmic
change appear with different agents.

Chaetopterus

Since it is evident from well known facts that in Chaetopterus
as well as the other species under consideration, the polar axis
of the egg after fertilization has not been determined during
fertilization, but must have been present in earlier stages, and,
since the susceptibility of the fertilized egg to the agents used
is higher and the axial gradient more distinct, little time has
been spent in demonstrating the polar gradient in the unfertilized
egg.

In the unfertilized egg of Chaetopterus, however, a very dis-
tinct apico-basal susceptibility gradient has been observed, by
means of KNC $m/100$ after neutral red. The change of the
neutral red to yellow, as the protoplasm dies and the alkali of
the KNC solution penetrates the cytoplasm, and the following
decoloration begin at the apical or animal pole and progress
basipetally through the egg. After five to six hours in the solu-
tion most eggs show a beautiful color gradation from light yellow
or complete absence of the stain in the apical region to deep red
in the basal region, and after eight or nine hours the change is
usually complete. The color change is accompanied by an in-
creasing cytoplasmic translucency, and sometimes the membrane
is ruptured at some point, and the egg substance bursts out,
evidently under high pressure in consequence of a tendency to
swell in the solution. In such cases the cytoplasm disintegrates
and dies rapidly in the solution and the egg is of no further value
for demonstration of a gradient.

In the fertilized egg of Chaetopterus before cleavage, swelling
and bursting occur very frequently in the higher concentrations
of KNC and alcohol, but where bursting does not occur, the

apico-basal gradient can be demonstrated with any of the agents
mentioned above, especially after staining with neutral red, and
the same gradient appears in eggs placed in a thin layer of sea
water exposed to evaporation and so becoming increasingly
hypertonic. The most marked differences along the egg axis
in decoloration, swelling and disintegration which serve as indi-
cators of susceptibility, appear in $HgCl_2$ and $CuSO_4$.

Figures 1 to 5 show some of the protoplasmic indications of
the gradient as they appear in $HgCl_2$ $m/500000$. These eggs
remained in sea water for thirty minutes after fertilization, when
the first polar body was forming or had formed. Then they were
stained to a uniform red color in neutral red—five or ten minutes
being usually sufficient—and were then placed in the $HgCl_2$
solution. Swelling and increasing translucency soon began at
the apical or animal pole and were followed by decoloration of the
swollen region. Other regions of the eggs usually showed little
or no swelling, but the decoloration gradually progressed basip-
etally through the protoplasm. After two hours in $HgCl_2$ the
eggs showed the forms indicated in figures 1 to 4. In many
the apical region, the region about the polar spindle has under-
gone marked swelling, is almost hyaline in appearance and pro-
trudes as a more or less sharply defined area from the egg surface
(figs. 1 and 2). In such cases this region usually contains a
mass so deeply stained with neutral red as to appear black or
blackish. This mass is the nucleus or chromatin, and perhaps
includes more or less of the spindle region. Occasionally two
masses appear, unquestionably the two groups of chromosomes
of the second polar spindle in anaphase. These black masses
decolorize much later than the cytoplasm of this region, which
at this time is usually completely decolorized. In the basipetal
direction a decoloration gradient appears, from complete de-
coloration apically through all gradations of color to deep red
basally.

In other eggs (figs. 3 and 4) the apical swelling is less sharply
defined and the black mass is less distinct or not visible. These
differences are doubtless associated with different stages of
maturation. In eggs like figures 3 and 4, the apical region is

almost hyaline and there is a gradation in granulation basipetally from it, as well as a color gradient ranging from complete decoloration apically to deep red basally.

After three and one half hours in $HgCl_2$ the swollen apical region has undergone complete disintegration in many cases, in most of the eggs decoloration is also complete, and the protoplasm has undergone a change in appearance, being more distinctly granular except where separation of granules and hyaline substance has occurred in the apical region.

The apico-basal gradient appears in these cases, both in the degree of physical alteration in the protoplasm, which is greatest apically and decreases basipetally, and in the progress of decoloration from the apical to the basal pole, which can be followed continuously.

With $CuSO_4$ $m/100000$ the results are essentially the same as with $HgCl_2$ except that there is less apical swelling. In KNC $m/200$ or $m/100$ after staining with neutral red, the decoloration gradient is the same as in that described above, and in many cases some apical swelling and disintegration occurs.

In the living egg of Chaetopterus at the time of maturation an area about the polar spindle is distinctly less opaque than other parts of the egg and it is this area which is chiefly involved in the swelling and protrusion and separation of hyaline protoplasm. The gradient, however, does not consist merely in the difference between this area and other parts of the egg, but exists in the more basal regions as well. The less opaque apical area is associated with the occurrence of mitosis and the position of the spindle, and the clear area about it is of course itself an expression of the apico-basal gradient.

During the earlier cleavage stages the apico-basal gradient in susceptibility to the various agents appears both in the physical changes of the protoplasm and in decoloration. In the eight cell stage and later, the micromeres swell, become more hyaline and undergo decoloration much earlier than the macromeres. In many cases the egg membrane is ruptured by the internal pressure and the micromeres become spherical and separate completely from each other, while the macromeres are still quite

unchanged. The swelling and separation of the micromeres occur before their decoloration, but decoloration occurs in general before decoloration of the macromeres. Since the cell divisions do not occur simultaneously in all blastomeres and since periods of increased susceptibility are associated with division periods, irregularities are not infrequent, one or two macromeres sometimes dying before some of the micromeres, etc., but in general the difference in susceptibility between micromeres and macromeres is perfectly distinct.

In the later cleavage stages a susceptibility gradient exists in the ectoderm. The swelling and separation of blastomeres begins in the small cells of the apical region, progresses over the ectoderm and occurs in the entodermal cells last of all. Decoloration follows in general the same course. The first somatoblast and mesoblast (Wilson, '92, Mead, '97 b, Child, '00), which arise dorsally and which form respectively the ectoderm and the mesoderm of the trunk region of the worm, are certainly less susceptible than the cells of the apical region, and many cases were observed in which the mesoblast was less susceptible than the somatoblast, but further work is necessary for certainty concerning this point, although other lines of evidence indicate that such a difference does exist.

Four to five hours after fertilization some of the embryos begin to move, and after six to seven hours they are swimming actively. At this stage (seven hours) the apical or anterior region is still the most susceptible region, but a second region of high susceptibility is arising in the basal or posterior region. The swelling and separation of cells begins, first, in the apical region, but soon after or at about the same time in the posterior or dorso-posterior region slightly in front of the extreme posterior end, for instance in the region of the somatic plate, while the intervening regions of the ectoderm remain intact for a considerably longer time. In one series, for example, in KNC $m/200$, the swelling and extension of cells began after two hours in KNC, usually chiefly the anterior region, but often in both anterior and posterior, and sometimes in the posterior earlier than in the anterior region. After three hours in KNC the larvae were still moving slightly,

but the ectoderm of anterior and posterior regions was largely disintegrated into separate cells while the middle regions were still largely intact, though giving off single cells here and there. After four hours the ectoderm had completely disintegrated in more than 50 per cent of the embryos, but the remainder showed various stages of anterior and posterior disintegration the process being usually more advanced in the anterior region. A small percentage still showed slight movement at this time. After five hours the ectoderm was completely or almost completely disintegrated in all, but the entoderm was still largely intact. Decoloration in KNC or other agents, after staining with neutral red, follows the same order as the swelling and cell separation.

As will appear below, the basal or posterior region of high susceptibility, which is appearing at this stage, is the first physiological indication of the growing region of the somatic plate which gives rise to the ectoderm of the trunk segments. My observations show that during the early development all parts of the body undergo an increase in susceptibility as they become physiologically younger (Child, '15 a, Chapter XV), but that after the somatic plate is formed and begins to grow, its susceptibility increases more rapidly and to a greater degree than that of other parts. The first indications of this change appear at the stage just described in which the susceptibility of the somatic plate region is almost as high as that of the anterior region.

During the following stages of development the relative increase in the susceptibility of the posterior growing region continues. Twenty to twenty-four hours after fertilization this region is the most susceptible of the whole ectoderm. The swelling and separation of cells occur first of all there (figs. 6 and 7). Very frequently, in stages of about twenty hours, the first cells to swell and protrude from the ectodermal epithelium are two cells on each side of the body in the posterior region (fig. 6), evidently cells of the somatic plate. Whether they represent the early stages of the seta-sacs could not be determined with certainty, but at this time they are the most susceptible cells of the ectoderm.

The disintegration of the ectoderm in the posterior growing region proceeds as indicated in the dorsal view (fig. 7) and the more advanced stage of disintegration (fig. 8), in which cells are also being given off from the apical region. In side view (fig. 9) the posterior area of disintegration extends around the body and frequently an area of disintegration appears where the stomodeum is forming, suggesting increased activity in that region.

It is of interest to note that the susceptibility of the prototroch region in this stage is relatively low, a zone including the prototroch being usually the last portion of the ectoderm to disintegrate. Apparently the cells of the prototroch have undergone a rapid decrease in susceptibility during their differentiation.

Mesoderm and entoderm are much less susceptible than ectoderm in these stages, and the entoderm is apparently less susceptible than the mesoderm, though after the body is covered with the swollen ectoderm cells it is often difficult to determine just when the mesoderm begins to disintegrate.

Decoloration in some killing agent after neutral red follows the same course as the swelling and disintegration, both as regards the different regions of the ectoderm, and the differences between ectoderm, mesoderm and entoderm, the entoderm undergoing decoloration last of all. The apparent low susceptibility of entoderm is certainly not wholly due to protection by the ectoderm from the action of the killing agent, for the difference is distinct while the entoblasts are still exposed. The entoderm remains intact for hours after the ectoderm has completely disintegrated and the cells are widely scattered.

In the fully developed larva of forty-eight hours or more, the region of highest susceptibility is still the posterior growing region and the posterior segment (fig. 10), although in some cases the susceptibility of the apical pretrochal region is almost as great. At this stage cell separation and disintegration begin in the growing region, just in front of the extreme posterior tip, and progress anteriorly over the segments, but much more rapidly ventrally than dorsally (fig. 11), that is, the ventro-latero-dorsal gradient characteristic of the bilateral invertebrates

is now evident. After posterior elongation begins, the susceptibility of the apical region is usually slightly lower than that of the posterior growing region (fig. 11) and the trochal region and the dorsal part of the first body segment are the least susceptible ectodermal regions. As in earlier stages, mesoderm and entoderm are less susceptible than ectoderm, entoderm least susceptible of all. The stomodeum, however, shows its ectodermal origin in a susceptibility considerably higher than that of the entoderm. Essentially the same relations persist as long as the larvae live.

Nereis

The differences in susceptibility as indicated by disintegration and death in different regions of the embryonic and larval body of Nereis limbata are similar to those observed in Chaetopterus. In the early stages of development the apical region is most susceptible, the basal least. As development proceeds, the susceptibility of all parts increases up to the fully developed larval stage (Child, '15 a, p. 414), but in the later larval period there is apparently a slight decrease in susceptibility which may be merely incidental to the gradual starvation of the larvae which, in these forms, does not bring about extensive reduction and increase in metabolic rate, as in many of the lower animals (Child, '15 a, Chapter VII), but soon ends in death, as in the higher forms (Child, '15 a, pp. 297–301). As soon as posterior elongation begins, however, the somatic plate, and particularly the posterior growing region of the somatic plate, shows a more rapid increase in susceptibility than other body-regions and soon becomes and remains the most susceptible region of the body. The extreme posterior terminal region is less susceptible than the growing region immediately anterior to it. In the fully developed larva with three segments death begins in the ectoderm of the posterior growing region and progresses anteriorly over the larval segments, more rapidly on the ventral than on the dorsal side. Death begins in the apical region somewhat later than in the posterior growing region and spreads over the pretrochal region, the two death gradients meeting in the region

of the prototroch which is the last portion of the ectoderm to die.
As the larval parapodia develop, they become regions of high
susceptibility and the developing tentacles and cirri also show a
relatively high susceptibility.

As regards the different body layers the mes-entodermal
region is certainly less susceptible than the ectoderm before
gastrulation is completed, for instance, while the cells of this
region are directly exposed to the action of the agent. In some
experiments on temporary inhibition in the earlier stages of
development, followed by return to water, some degree of differ-
ential recovery occurs if the concentration is not too high and
time of exposure too long, and in many such cases the entoblast,
mesoblast and sometimes the first somatoblast fail to recover
and die and disintegrate some time, perhaps even a day or two
after return to water, while the apical and antero-ventral ecto-
derm recover and continue development. As I have shown
(Child, '16 c), differential recovery resembles differential acclima-
tion in that the regions of low susceptibility to the direct action
of the agent show less capacity for recovery than those of high
susceptibility when the inhibiting action has not been too severe.
These cases of partial recovery in Nereis are, I believe, cases of
this sort and constitute further evidence for the lower metabolic
activity of the mesodermal and entodermal regions as compared
with the ectoderm and particularly the apical and antero-ventral
ectoderm. These experiments indicate, further, that the somato-
blast which gives rise to the ectoderm of the trunk region has a
lower metabolic rate in the earlier stages than the ectoderm of
the more strictly larval regions.

Although the general susceptibility gradients are the same in
Nereis as in Chaetopterus, the behavior of the two protoplasms
in the killing agents differs in certain respects. In Nereis there
is little or none of the swelling and separation of cells so char-
acteristic of all stages of Chaetopterus, death occurs with com-
paratively little change in appearance, and the death point is
most easily determined by the decoloration after staining with
neutral red.

In KNC and various other agents, however, the Nereis pro-
toplasm, like that of Chaetopterus, undoubtedly does become

more fluid, for a stratification of the cell contents with reference to gravity appears, particularly in the micromeres of the earlier cleavage stages. Such a stratification occurs in Chaetopterus, but is more distinct in Nereis stained with neutral red, because of the greater transparency of the cells. When the four-cell stage is killed in the proper concentrations, the higher susceptibility of the apical region of each cell, which would later give rise to a micromere, is indicated by the appearance of stratification there, while other regions are unchanged. This protoplasmic stratification precedes death, but shows the same regional differences as regards susceptibility. As the cells become smaller with advancing development, these changes become less conspicuous.

The relation between physiological condition of the cells and their ability to hold neutral red can be demonstrated very clearly in Nereis. In the earlier cleavage stages, where both micromeres and macromeres are directly exposed to the neutral red, both stain very readily and deeply and undergo decoloration only as they die. In later stages, for example, in young, nearly spherical, swimming trochophores, in which the ectoderm has completely overgrown the entoderm, the ectoderm stains rapidly and deeply, but little or none of the neutral red reaches the enclosed macromeres until the ectoderm cells approach death. Then they are unable to hold the neutral red and it passes through them to the macromeres which stain rapidly. The decreasing ability of the ectoderm cells to hold neutral red as they approach death can be shown even more clearly in another way. Young trochophores are placed in KNC, $HgCl_2$ or some other agent used and after various periods of exposure to the killing agents are removed to a dilute solution of neutral red in sea water. After short periods of exposure to the killing agent the ectoderm stains rapidly and the macromeres very slowly or not at all, as in normal forms. Later as the ectoderm cells approach death they stain much less rapidly and less deeply, but the enclosed macromeres now stain more rapidly. After still longer periods of exposure the ectoderm is dead and does not stain at all but the entoderm still stains very rapidly and deeply. Since, in order to reach the entoderm at this stage, the neutral red must pass through the ectoderm, and since the macromeres

stain at once when the ectoderm is torn open or partly removed, as well as when the ectoderm is approaching death, it is evident that their slow staining or failure to stain in normal animals after complete overgrowth by ectoderm is due simply to the storing up in some way of the neutral red in the ectoderm cells as rapidly or almost as rapidly as it enters. As the action of the toxic agent progresses, the ectoderm cells become less and less able to hold neutral red, and the neutral red which enters them passes through and enters the macromeres, which are as yet but little or not at all affected by the killing agent.

These same relations may be shown by still another modification of procedure. The young larvae are first stained, for instance, the ectoderm is stained, but little or no neutral red reaches the inclosed macromeres, and then, after washing, the larvae are placed in the killing agent without any admixture of neutral red. As the ectoderm cells approach death they begin to give up their neutral red, and at least a part of it passes to the macromeres which stain more and more deeply until, finally, the ectoderm is dead and decolorized, and the entoderm deeply stained. The change in relative physiological condition of ectoderm and entoderm has evidently determined the redistribution of the neutral red. Later, of course, the entoderm also undergoes decoloration, but, whether it is directly exposed to the action of the toxic agent or inclosed in ectoderm, its susceptibility is very much lower than that of the ectoderm.

The behavior of the trochoblasts and the prototroch is of interest. So far as I have been able to determine, the trochoblasts, when first formed, do not show any marked differences in susceptibility from adjoining cells. This suceptibility, like that of other cells of the more apical portions of the egg is high. At the time ciliation develops, and perhaps for some time after, the cells of the prototroch are apparently the most susceptible cells of the apical hemisphere, and when eggs are placed soon after fertilization in concentrations of KNC low enough to permit development to progress slowly as far as the early trochophore stage, but not low enough to permit acclimation (for example $m/25000$ and in some cases $m/50000$), the cells of the prototroch

usually die before any other part of the ectoderm, but only after they have formed the prototroch. In later stages, and particularly after the deposition of pigment begins, the susceptibility of the trochal region decreases rapidly until, in the more advanced larvae, it is the least susceptible region of the ectoderm. Apparently the trochal cells attain their maximum physiological activity or physiologically youngest stage very early and after that undergo rapid senescence.

After it has attained its definitive condition, the prototroch region stains with neutral red more rapidly than other parts of the ectoderm, but like other ectodermal regions, gradually loses its capacity to hold the dye when subjected to the action of the various agents used.

Arenicola

The earlier stages of development have not been available for susceptibility determinations, for none of the egg strings collected showed anything earlier than the first motile stages, and I have never yet succeeded in making fertilizations with eggs and sperm from the body cavities of the animals.

In the stages available the susceptibility relations are similar to those in Chaetopterus and Nereis. In the earliest motile stages posterior elongation is already beginning and the somatic plate region is usually the most susceptible part of the ectoderm, the apical region slightly less so and the trochal and intermediate region least susceptible. In the three-segmented trochophore the same relations persist. Death usually appears, first in the ectoderm of the posterior growing region and slightly later in the pretrochal region, though in some cases it begins at the same time in both. From the posterior growing region death progresses anteriorly, in the order third, second, first body segments, and the trochal region is the last portion of the ectoderm to die. Mesoderm dies later and entoderm much later than ectoderm.

The Arenicola larva undergoes complete metamorphosis in the laboratory without any external source of nutrition. Young worms with five or six body segments can be obtained in large

numbers, and, by feeding and proper care, development can be carried still farther.

The susceptibility relations in a stage of six segments differ in certain respects from those in the three-segmented trochophore. The posterior growing region and the head are still the most susceptible regions, the latter being usually slightly less susceptible than the former, and in the body region the susceptibility decreases anteriorly from the sixth to the fourth or third segment. In the first three or four segments, however, the relations have undergone change. Of these the first segment is most, the second less and the third and sometimes the fourth still less susceptible, just the reverse of the relations in the early three-segmented trochophore, but even in the first segment the susceptibility is lower than in the head.

In the six-segmented worm then two opposed susceptibility gradients appear more or less clearly in the ectoderm, and I think also in the mesoderm, for instance, death progresses posteriorly from the head' region through the first three or four segments and anteriorly from the posterior growing region through the segments developed later. The possible significance of the changes in these segments and the two opposed gradients in the body will be considered below in the general section (p. 35).

As in Nereis and other forms, the susceptibility in general increases during the earlier stages of development as the developing animal becomes physiologically younger, but in Arenicola it continues to increase up to or slightly beyond metamorphosis. How much of this latter increase is due to actual intrinsic physiological rejuvenescence and how much to increased sensory, muscular and other special functional activity, the susceptibility method does not tell us, but I am inclined to believe that the special functional factors are largely concerned in the increase in susceptibility during and after metamorphosis, and that in this way the earliest stages of senescence are masked and, with this method, become visible only later. For the determination of its intrinsic physiological condition the cell should be isolated from sources of stimulation and this, of course, is less the case in later than in earlier stages of development.

Hydroides

A few observations on the susceptibility of the cleavage stages and twenty-four hour trochophores of Hydroides dyanthus showed the same susceptibility relations as in the other forms, namely, in the earlier stages an apico-basal gradient, with apical region showing the highest susceptibility and in the twenty-four hour trochophore two regions of high susceptibility, the apical or anterior (pretrochal) region and the posterior growing region.

DIFFERENTIAL INHIBITION OF DEVELOPMENT

In the sea urchin the differences in susceptibility along the axes afford a basis for modifying and controlling development to a very high degree (Child, '16 c) by means of differential inhibition, acclimation and recovery. In the polychetes the possibilities of modification are, as might be expected, much more narrowly limited. The work of Wilson ('04 a, '04 b) and others has shown that the course of larval development is very definitely and fixedly determined and that apparently but little regulatory capacity exists in molluscs and polychetes. Moreover, the earlier larval stages develop within a few hours and with very little growth of any part, and even in the fully developed trochophore, growth is very largely limited to the posterior region. Physiologically speaking the polychete egg has apparently attained an advanced stage of specialization of some sort, at least as regards the larval structures, before cleavage begins, and both the number of cell divisions and the amount of protoplasmic growth are very much less than in the sea urchin.

In consequence of these conditions, not only is the time within which the action of an inhibiting agent may be effective in altering development very short, except as regards the posterior growing region, but the amount of growth under the experimental inhibiting conditions is small, again except in the posterior growing region. In view of these facts it might be expected that the modifications produced in larval development by differential inhibition would be relatively slight, and that there would be

still less prospect of producing modifications by differential acclimation, since this requires a considerably longer time than differential inhibition.

In spite of this situation, however, I have found it possible to bring about some degree of modification in these forms. Two chief lines of modification appear. If exposure to the inhibiting agent begin very soon after fertilization, when a simple apical-basal susceptibility gradient is present, with the highest susceptibility in the apical region, the larvae are more or less microcephalic and the posterior growing region is of course also more or less inhibited. If, on the other hand, exposure to the inhibiting agent begin at a later stage of development, say sixteen to twenty-four hours (for instance after the larval head region has undergone a large part of its development and the posterior growing region has attained a high susceptibility, then the larvae will be more or less megacephalic and the inhibition will be chiefly posterior. Of course special organs, such as tentacles, cirri, paropodia, may be partially or completely inhibited according to the experimental conditions. The characteristic forms produced in my experiments are described below. In these experiments only KNC and HCl have been used thus far as inhibiting agents. Work with various other agents is desirable and certain unpublished experiments on the sea urchin suggest the possibility that some other agents may have a greater differential effect and so determine a greater degree of modification than the two used, but it has not yet been possible to test them.

The results presented below of this first attempt to use differential susceptibility as a means of modifying polychete development are by no means exhaustive. Attention has been directed chiefly to the general change in form and proportion resulting from the chief regional differences in susceptibility, and many details remain to be investigated. The figures are semi-diagrammatic and are intended, primarily, to show as simply as possible the general modifications in size and proportions of body-regions.

Chaetopterus

The usual course of larval development in sea water at ordinary temperature is indicated in figures 12 to 14, *A* being the lateral, *B*, the ventral aspect of the larva. Figure 12 is a 12 hour stage, figure 13, *A*, *B*, twenty-four hours and figure 14 *A*, *B*, forty-eight hours.

Figures 15 to 17 show three-day larvae, the most advanced stages attained by eggs placed in KNC $m/100000$ thirty minutes after fertilization and undergoing their whole development in this solution. As compared with normal larvae of forty-eight hours (fig. 14), the three-day larvae of figures 15 and 16 are smaller, shorter and very evidently less advanced in development. The head-region is distinctly inhibited, the region of the first two larval segments is almost as well developed as in the normal animal and the posterior region again shows marked inhibition.

The larva of figure 17 is a form characteristic of both KNC and HCl cultures, sometimes constituting 25 to 50 per cent of the living individuals. As regards the stage of development attained, the larva is evidently inhibited, like those of figures 15 and 16, but the body-region is much enlarged or distended. All degrees of such enlargement, ranging from forms like figures 15 and 16 to those like figure 17 and even still more enlarged forms, occur. As regards general activity and behavior these larvae do not differ from the others and do not die earlier than the others. Since they are not merely an occasional but a characteristic feature of the inhibition cultures, I am inclined at present to regard them as a result of differential inhibition and to account for them as follows: they appear to be due to an excess of fluid in the body cavity and perhaps also in the enteric cavity. The coelomic fluid at least is, to some extent, a metabolic product, for instance directly or indirectly the result of cellular activity. If the cells chiefly concerned in its production possess a relatively low susceptibility, their activity in these differentially inhibited larvae will be relatively greater and may occur to a greater extent at the expense of other parts than in normal

animals, consequently the amount of coelomic fluid formed may be relatively greater and the larvae therefore more distended than the normal. In the differential inhibition of the sea urchin one result may be over-development of the skeleton, because the mesenchyme cells are the least susceptible and therefore least inhibited cells of the larva, and, in differentially inhibited individuals, are able to obtain more than their normal share of nutritive material and to increase in numbers and to produce skeletal substance greatly in excess of the normal (Child '16 c, p. 91). It seems probable that these distended polychete larvae, like the plutei with skeletal over-development, result from a differential alteration by the inhibiting agent of the normal metabolic relations between different parts of the body.

Figures 18 and 19 show three-day larval forms resulting from exposure to KNC $m/10000$ for eleven hours, beginning forty-five minutes after fertilization. In these cases there is of course more or less recovery, but here as in the sea-urchin, the differential inhibiting action of KNC is remarkably persistent. In these cases the head is markedly inhibited, the first larval segment is overdeveloped, at least as regards size and, to a lesser degree, the second also, while the third segment and the posterior growing region are present merely as a more slender posterior prolongation (fig. 18) or in cases with a slightly greater degree of recovery as a distinct region (fig. 19). The posterior terminal appendage is almost completely (fig. 18) or completely (fig. 19) absent. In these cases the metabolic activity of the head-region and, secondarily, of the posterior growing region has undergone a relatively greater decrease than that of the first and second larval segments, and these therefore show a relative increase in size. Incidentally it may be noted that the peculiar proportions of these larvae, appear relatively late. At two days they are much like those of figures 15 and 16 though somewhat larger, but on the third day the regional differences become more marked. The distended forms resembling figure 17, but somewhat more elongated, also occur frequently and in all degrees in these series.

The differential results obtained with HCl do not differ in any essential way from those with KNC. Much higher con-

centrations of HCl than of KNC must, of course, be used and,
even in concentrations high enough to retard the earlier stages
of development and give differential inhibitory effects, some
degree of differential acclimation may occur in the later stages.
The differences in action between KNC and HCl are, in general,
the same for the polychete as for the sea urchin (Child '16 c).
The larval forms produced by development in HCl $m/2500$
from thirty minutes after fertilization (figs. 20, 21) are not dis-
tinguishable in any characteristic way from the KNC forms
(figs. 15, 16), and the distended forms (fig. 17) occur as frequently
in HCl as in KNC. In some lots development goes no farther
in HCl m 2500, but in others some degree of differential acclima-
tion occurs, giving forms like figure 22 in which the development
of head region and posterior end is progressing slowly and the
body is becoming more elongated as in the normal animal.

When development is allowed to proceed for twenty-four
hours in water and the younger trochophores are then placed in
KNC or HCl, the head and anterior body region are relatively
large, and the inhibition is limited almost entirely to the posterior
body-region (fig. 23). At twenty-four hours the posterior region
has become the most active and so the most susceptible region
of the young larva. Exposure to KNC and HCl after eight to
twelve hours development in water gives results intermediate
between those of exposure from the beginning of development
and exposure after twenty-four hours development. The head
region is relatively smaller than in figure 23, but not so small as
in figures 15 to 21.

As regards the limits of concentration of agents used, KNC
$m/100000$ for the whole period of development usually gives a
high percentage of differential inhibitions and a considerable
mortality within three days. Some lots of eggs will develop
in KNC $m/50000$, others die after two days with greatly retarded
development. Higher concentrations can be used only for tem-
porary exposure. KNC $m/10000$ can be used for periods up to
twelve to fourteen hours at the beginning of development, but
this length of time will kill larvae twenty-four hours or more old.
HCl $m/5000$ for the whole developmental period does not pro-

duce much differential effect, and HCl $m/2000$ usually kills in a day or two. HCl $m/2500$ has been chiefly used for differential inhibition. Higher concentrations of HCl with temporary exposure have not as yet been used.

Nereis

Because of the impossibility of obtaining abundant material as desired, the data concerning modification of development in Nereis are rather fragmentary. Since the chief object of the present paper is the demonstration of the general regional differences and changes in susceptibility and their relation to the form and proportions of the larvae, the figures are drawn to illustrate these points, most of the details of larval structure being omitted. In all cases the figures show merely the dorsal aspect of the body, because the side-views show in general the same modifications of form and proportion as the dorsal. As regards the inhibition or modification of particular organs and parts a few facts are mentioned. There is no doubt that, with sufficient material, much may be accomplished in the way of selective or differential inhibition of particular organs by varying concentrations of agents used and periods of exposure over a wide range, and beginning exposure at different stages of development. By means of such a procedure a particular organ is most inhibited when the period of action of the agent in high concentration coincides with its period of highest susceptibility or highest metabolic activity, and since this period occurs in different organs at different stages of development, it is possible to inhibit a particular organ relatively more at one stage, relatively less at another, the selective action at a given stage being primarily not a matter of specific chemical constitution but of metabolic activity and susceptibility. My experiments also indicate that something may be done in the way of differential acclimation (Child '16 c) with low concentrations, at least with some agents, although effects of this kind will appear only in the later stages, so far as they appear at all. All these variations of the susceptibility method are however limited in their applicability by the rapid development and by the fact that the inhibiting agent

requires a certain amount of time to penetrate and produce its effect, during which time development is progressing and conditions are changing more or less rapidly.

The general form-modifications observed are indicated in figures 24 to 47. Figures 24 to 27 show the normal development at one, one and one-half, two and one-half and four and one-half days respectively. Figures 28 to 37 show inhibited larval forms obtained when eggs develop in KNC from a stage just preceding the first cleavage. Figure 28 shows the characteristic condition after three days in KNC $m/50000$. The pretrochal region is small and the post-trochal region large, as compared with corresponding normal stages (fig. 25), setae are absent, and there is no definite posterior elongation. In this concentration death usually occurs about the fourth day without any marked further advance in development. Figures 29 to 37 show larvae after four to six days in KNC $m/100000$, beginning at the first cleavage. In all cases the development is of course retarded, but the pretrochal region is relatively smaller and the post-trochal region relatively larger than in normal forms. In more advanced stages this differential effect of the KNC becomes still more marked, but at the same time the later development of the posterior growing region and the development of the third body-segment are more or less completely inhibited (figs. 34 to 36, five days). The development of the setae is also markedly inhibited in many cases, some individuals having only a few setae (figs. 31, 32, 34, 35) while others are entirely without setae and remain so (figs. 33, 36). Figure 36 shows a six-day old KNC larva from the same series. Here the head is still relatively smaller and the body region relatively larger than normal (fig. 27), though evidently retarded in development.

These larval forms, like those of Chaetopterus inhibited from the beginning of development, show some degree of microcephaly and in later stages an inhibition of the posterior growing region. It is possible that forms like figure 37, which are found after six days or more in KNC, represent some slight degree of differential acclimation in the posterior growing region, for this region apparently shows an increase in rapidity of development

at about this time and a large third body-segment differentiates.
Since this growing region is the most active region of the larva
in the later stages acclimation is to be expected there if any-
where (Child '16 c).

Since different eggs, even those from the same female, differ
more or less in susceptibility, there is a considerable range of
variation in the effect of any concentration of any agent. In
KNC $m/100000$ from the first cleavage the range is from almost
normal, somewhat retarded larvae, through the forms of figures
29 to 36 which represent the most advanced stages of the in-
dividuals figured, to cases where development is completely
inhibited at an earlier stage and death occurs in a few days. In
general the greater the degree of inhibition the earlier death
occurs. In KNC $m/200000$ from the first cleavage the inhibiting
effect is slight and the larvae are nearly all normal. In KNC
$m/50000$ development rarely proceeds beyond the stage of figure
23 and death occurs in two or three days.

Figures 38 to 42 show the effect of KNC where exposure began
twelve hours after fertilization. In figure 38, a two-day stage
in KNC $m/50000$ from twelve hours, the pretrochal region is of
normal size and the larva is in general not very different from a
one-day normal larva, the posterior growing region being evi-
dently completely inhibited. In this concentration the larvae
usually die without much further advance in development.

Figures 39 to 41 show larval forms after four days in KNC
$m/100000$ from twelve hours after fertilization. The most
conspicuous features are the relatively large head and relatively
small body-region, a modification in the opposite direction from
that of figures 29 to 33. In figure 39 the first two body segments
are distinguishable and bear a few setae, while in figure 40 the
differentiation of segments is almost, and in figure 41 entirely
inhibited, and no setae appear. These forms are characteristic
for this concentration and time. After six days in KNC most
of the individuals which are still alive show no further advance,
but some, like figure 42, show what seems to be some degree
of acclimation of the posterior growing region. Here the first
body segment is small, but the second and third segments are

larger, indicating that the growing region has recovered its activity to some extent in the later stages. The head remains relatively large and is more advanced than in the six-day stage in KNC from the first cleavage (fig. 37) but setae are still entirely absent. Some individuals of the same type show a few setae.

A few experiments on temporary exposure to much higher concentrations of KNC were performed, but the material was not sufficient for any large number of these. In these experiments young moving larvae twelve hours after fertilization were placed in KNC $m/1000$ and returned to sea water after four, five, six and one-half and eight hours. The four-hour lot produced forms ranging from practically normal to slightly megacephalic, with slightly inhibited posterior body-region and few or no setae. The five-hour lot produced forms like figures 43 to 47, megacephalic, with extreme posterior inhibition and entirely without setae. In many of these, as in figures 45 to 47, the posterior growing region develops only as a small single or bilobed outgrowth, the body segments never differentiate visibly, and in many cases bilaterality does not appear in the segmental region. Of the six-and-a-half hour lot 20 to 25 per cent died wholly or in part within two days and 60 to 75 per cent within four days, but a few individuals produced forms like figures 43 to 47. Of the eight hour lot 80 to 90 per cent died wholly or in part within two days. A few individuals lived to four days, producing forms like figures 44 to 47.

With a few lots of eggs HCl was used as inhibiting agent. In HCl $m/10000$ or below, development is practically normal. In HCl $m/5000$ the results are in general very similar to those obtained with KNC $m/100000$, but with a higher percentage of deaths within the first three days. In HCl $m/2500$ most of the larvae died within two or three days without developing beyond the early trochophore stage.

As regards particular regions and organs, it is evident from the figures that the development of the setae, segmental cirri and tentacles may be completely inhibited, even when the general developmental modifications are not extreme. Evidently these organs in their early stages are regions of high metabolic activity

and therefore of high susceptibility and so are very readily
inhibited. There is no evidence of any specificity of action of the
agents in these inhibitions, for they show all gradations, they
occur in essentially the same way with different agents, and the
killing experiments show that these are regions of high suscepti-
bility when the organs begin to grow. Evidently the inhibiting
agents either prevent the establishment of the high metabolic
activity necessary for the formation of these organs or, because
of their high metabolic rate, inhibit their development after
it has begun.

The trochal cells, as noted above, show a high susceptibility
in their earlier stages and the development of trochal cilia may
be completely inhibited. The formation of the trochal pigment
is also more or less completely inhibited by the agents used, the
prototroch being entirely without visible pigment in many cases
where the inhibiting agent acts on the earlier stages. In some
cases, also, the later divisions of the trochoblasts are inhibited
and if the young larvae are returned to sea-water and recover,
the prototroch may consist of four separate groups of cells with
distinct gaps between them.

To what extent the inhibiting agents may induce variations in
cleavage or changes in cell lineage has not been determined, but
the indications of regulation which I have observed incidentally,
suggest that interesting facts remain for future investigation in
this field.

Arenicola

The earliest stages of Arenicola available for this work were
stages in which the prototroch was already formed and elongation
of the embryo just beginning. Development in KNC and HCl
from these stages on gives megacephalic forms with more or less
inhibited posterior segmental region. Figures 48 to 50 show
three stages in the later normal development and figures 51 to
55 show forms which have developed in KNC from the beginning
of elongation. Arenicola is less susceptible than Chaetopterus
and Nereis, development in KNC $m/100000$ being practically
normal and only slightly retarded in $m/50000$. In $m/25000$

differential inhibition occurs (fig. 53), but the percentage of the more extreme forms is small and most animals complete metamorphosis, while in $m/10000$ nearly all individuals show more or less differential inhibition, and the majority die before completing metamorphosis. After four days in KNC $m/10000$ from the beginning of elongation the alteration in the larval form is present but slight, the posterior region being relatively small (fig. 51) as compared with the corresponding normal stage (fig. 48). After six days (fig. 52) the head-region is distinctly larger and the posterior region smaller than the normal (fig. 49) and setae are usually entirely absent. Very similar forms occur, though less frequently, in $m/25000$ and usually show setae on one or more segments (fig. 53). Figures 54 and 55 show similar forms after eight days in KNC $m/10000$, the latter being a case in which the development of the paratroch is completely inhibited and bilaterality of form is almost entirely absent from the segmental region.

Forms like the figures died in my experiments without completing metamorphosis, but individuals which are less inhibited may develop into worms like figure 56, which shows relative inhibition of the posterior segmental and growing region as compared with the normal worm (fig. 50). The large transverse diameter of the body in such forms is apparently due to distension of the coelom by fluid, a condition somewhat like that in Chaetopterus (p. 17), but less extreme.

A few experiments with HCl, beginning at the same stage, gave similar but less extreme differential results, and more or less differential acclimation usually occurred in later stages in concentrations which permitted the completion of metamorphosis. As regards concentrations, HCl $m/10000$ has no appreciable effect, $m/5000$ retards development slightly, but gives no marked differential effect, $m/2500$ gives differential inhibitions of the same sort, but less extreme than figures 52 to 55, and the posterior growing region shows acclimation and increase in growth rate in the later stages; and, finally, $m/1000$ kills within three or four days with but little advance in development.

DISCUSSION

Metabolic relations during early development

The results of the two modifications of the susceptibility method, differential killing and differential inhibition of development, agree in that in the early stages of development in the polychetes susceptibility is highest in the apical region and decreases basally, while, as development proceeds, the somatic plate, or more particularly the posterior growing region, shows a more rapid increase in susceptibility, until, after elongation begins, it is the most susceptible region of the body. In a recent study of the oligochetes Hyman ('16) has found that the posterior growing region is, in most forms, the region of highest susceptibility, from an early stage on and in the adult.

The method also shows, as far as observations go, that the primary gradient becomes more complicated as development goes on, by the appearance of particular organs or parts as regions of relatively high susceptibility as they begin rapid growth. In the prototroch cells, on the other hand, we have apparently a case of rapid decrease in susceptibility, a senescence following the completion of growth and differentiation. The general relation between susceptibility, metabolic rate, physiological condition, vitality, protoplasmic activity or whatever we prefer to call it, has been discussed elsewhere (Child '13; '15 a, Chapters III, IX; '15 b, Chapter III; '16 b; '16 c) and need not be further considered here.

Developmental modifications

The general modifications of development resulting from differential inhibition are of two kinds: differential inhibition beginning with the beginning of development at first inhibits the apical region more than other parts, but later, as the activity of the posterior growing region increases, it also is inhibited, and the result is a more or less microcephalic form with a secondary inhibition of the posterior growing region. If, however, the inhibiting action begin later (with the early trochophore

stage), the result is a megacephalic form with more or less inhibited posterior growing region.

The results of differential inhibition of sea urchin development (Child '16 c, as well as various other data as yet unpublished) indicate that two factors are involved in the modifications of development by this means. The first of these factors is the direct effect of the agent upon each part. In general a decrease in the metabolic activity of a developing, growing part is accompanied by a decrease in the rate of growth and development. The second factor is the alteration of the metabolic relations existing between the various parts. The general proportions and the relative sizes of the various organs in any organism must be, to a greater or less extent, an expression of the relative metabolic activities of the different regions and parts. This is particularly true in the earlier stages of development, where metabolism is largely concerned with growth. If nutritive material be present in excess, the region with the more intense metabolic activity may simply grow more rapidly than a less active region, until its growth is retarded by senescence or other conditions. If nutritive material be limited and insufficient, a region of higher metabolic rate may not merely grow more rapidly than, but may live and grow at the expense of, a region of lower rate and so may not only retard or prevent its growth, but may bring about its reduction or atrophy. If we inhibit certain regions of the annelid larva to a greater degree than others, we not only retard this growth, but we make it possible for other less inhibited regions to grow relatively more rapidly because nutritive material which, under normal conditions, would go to supply the demands of the more active regions, is thus made available to a greater degree for other regions. The effect of this factor will, of course, be in the same direction as the direct effect of the differential inhibition of different regions and will merely tend to increase the changes in form and proportion produced directly by the differential inhibition.

To what extent each of these two factors is concerned in the modifications of form in the annelid larva it is impossible to determine, but that they both play a part here as well as in

normal development cannot be doubted. In the metamorphosis of the polychete a decrease in size of the head-region is a very characteristic feature. It appears in Chaetopterus (figs. 12 to 14), Nereis (figs. 24 to 27) and Arenicola (figs. 48 to 50), and in some annelids, for example Polygordius, it is much more marked than in these three. In the light of what the susceptibility method has shown us concerning the changes in metabolic relations in the different regions of the body during development, there is every reason to believe that the reduction of the head-region in metamorphosis is an indirect result of increase in activity of the posterior growing region, until it is the most active region of the body. Under these conditions, particularly in the later stages when the nutritive supply of the egg is approaching exhaustion, the posterior growing region and the more posterior segments apparently grow to a greater or less extent at the expense of the head-region and the more anterior segments. Probably the cephalic ganglia, which undoubtedly represent a region of higher metabolic rate than the rest of the head ectoderm, also grow to some extent at the expense of other parts of the head.

If we alter the normal relations by inhibiting both head and posterior growing region more than the anterior segmental region, as when the inhibiting action begins at the beginning of development, the latter may become not merely relatively, but in time absolutely larger than in the normal animal, as in the case in many of the figures above. If, on the other hand, we inhibit the posterior growing region to a greater extent than the head and anterior region, as in the cases where inhibition begins with the early trochophore stage, these regions may become absolutely larger than in the normal larva and their reduction may be delayed or may be less than normal as long as the differential inhibition persists. This condition also appears in the figures. If this interpretation be correct, it is evident that, at least from the metabolic point of view, the larval development of the polychete is not a mosaic of independent parts as Wilson and others have maintained.

The fact remains, however, that the modifications produced by differential inhibition are much less extreme than those produced in the sea urchin (Child '16 c). As noted above, one reason for this is the short time available for the action of experimental conditions in consequence of the very small amount of growth and the extremely early complete differentiation of the strictly larval structures. As regards the trunk and the posterior growing region, where growth and development continue, it is evident that extensive experimental modification is possible. Visible differentiation of segments may be completely inhibited, the size relations of successive segments may be altered, the growing region itself may be obliterated as a growing region, the setae, the cirri and the paratroch may fail to develop, without the actual killing or removal of any portion of the larva. But even in the head-region some differential modifications have been produced, for instance in Nereis the development of the prototroch as four isolated groups of cells and the absence of ciliation of the prototroch. Moreover, I have some evidence that the cephalic ganglia may be differentially delayed in development and therefore smaller than in corresponding normal stages, though further investigation concerning this point is necessary. The possibility that the distension of the coelom by fluid in Chaetopterus is also a result of differential inhibition has already been mentioned (p. 17). In short, in those parts where any considerable degree of growth occurs, extensive modification is possible by altering the metabolic relations through differential inhibition and, even in the more strictly larval parts, size and form relations may be altered to a very appreciable degree.

Regional specialization

The facts indicate that the more apical, for instance, more strictly larval regions of the egg are in some way more highly specialized and determined than in the sea urchin egg before embryonic development begins. I believe, however, that this difference is of the same sort as that between earlier and later stages of development in any species. The egg of the polychete has passed beyond the stage of the simple axial gradient in me-

tabolic rate, at least as regards the more apical regions, before embryonic development begins. If the axial gradient has been present during the growth period of the oocyte, we should expect to find the specialization more marked apically than basally, and this is apparently the case. Differential inhibition of the oocyte, perhaps during the growth period, would probably produce more extensive differential modification of even the more apical regions.

If this point of view be correct, there is no good reason for maintaining, as I did in an earlier paper (Child, '00), that the cytoplasm of the annelid egg is fundamentally no more highly specialized than that of the sea urchin, nor is there any reason for asserting, as some others have done, that the fate of cytoplasmic regions is as definitely determined normally in the sea urchin as in the annelid egg. In the annelid, cytoplasmic specialization of some sort apparently occurs to a larger extent, particularly in the more apical portions, before embryonic development begins, than in the sea urchin. The facts seem to indicate, however, that this specialization of the annelid cytoplasm is associated with an axial gradient or gradients and is progressive during the history of the egg. During the process of rejuvenescence in early embryonic development (Child, '15 a, Chapter XV) the annelid cytoplasm still retains its specialization to a considerable degree, while the sea urchin approaches more nearly equipotentiality of parts.

Concerning the nature of this specialization we know little. The various investigations on the effect of centrifugal force have shown what I maintained earlier (Child, '06), namely that it is not dependent upon the visible so-called formative substances of the cytoplasm, but is a feature of the cytoplasmic substratum, the 'ground substance.' If it originate in differences in metabolic rate, as suggested above, we should expect to find it primarily in the 'ground substance' not in the visible depositions and enclosures of the cytoplasm, which are secondary features. The apicobasal metabolic gradient is present at the beginning of embryonic development in the annelid, as in the sea urchin, but in the annelid modification of the cytoplasm

along its course is greater in degree and less readily altered or obliterated than in the latter. Nevertheless, the differences in metabolic rate in different regions still play a rôle in further development, even in the annelid, though a less fundamental rôle, except in the posterior region, than in the sea urchin.

The origin of the segmental region in relation to the axial gradients

It is perhaps a significant fact that the cells which constitute the posterior growing region arise from the dorso-posterior region of the egg, the ectoblast being the most anterior, the mesoblast further posterior and the entoblasts most posterior of all. In the flatworms, and probably also in other bilaterally symmetrical invertebrates, the region of highest susceptibility and metabolic rate in the axes of bilaterality is primarily the ventral region, and susceptibility and metabolic rate decrease laterally and dorsally (Child, '13; '15 b, pp. 60, 67 to 69). This is true, at least in the earlier stages of development, though changes may occur later. In such forms, then, the dorso-posterior regions are the regions of lowest metabolic rate in the body. In the annelid egg the cells which take almost no part in the formation of the rapidly differentiating, specialized strictly larval structures, arise from the posterior region of the egg. As regards the relative susceptibility of this region, it is certainly true that the somatoblast from which the ectoderm of the growing region arises is, in many cases, less susceptible when first formed than other parts of the ectoderm; the mesoblast is still less susceptible and the entoblasts are least susceptible of all. If the symmetry gradient as well as the polar gradient is present in the egg from the beginning of development, then these cells represent, in general, the region of lowest metabolic activity in the egg cytoplasm, and the ectodermal somatoblast arises from the most active part of this region, the entoblasts from the least active and the mesoblast from a region intermediate between the two. This region is least involved in the larval specialization and is therefore capable of undergoing a greater degree of rejuvenescence and so becomes more embryonic. It possesses in all cases a wide range of developmental potentialities and is

often 'totipotent,' as is evident from those cases in which the segmental region remains capable, even in the adult, of giving rise to a new head, as well as to all other parts, when physiologically or physically isolated from the head-region.

The formation of the posterior growing region and of the segments

From the work of Hyman ('16) on the oligochetes and the present paper on the polychetes, it appears that the metabolic activity of the entoderm of that portion of the embryo or larva which becomes the growing region increases more rapidly than that of other regions during early development, so that, finally, it becomes the most active region and is embryonic in character and capable of long continued or indefinite growth. In the first place the more rapid and more extreme rejuvenescence of this region is doubtless associated with its less extreme specialization.

In forms such as the oligochetes and particularly the leeches, where teloblasts persist in the growing region, the physiologically youngest region with the highest metabolic rate in both ectoderm and mesoderm is probably somewhat anterior to the teloblasts, where the cells have begun to divide and grow. In any case there is in all annelids at some stage a posterior, more or less embryonic growing region of high metabolic activity.

From this region segments arise successively, and the problem of the physiological conditions and processes concerned in their origin must be briefly considered. The facts suggest two possibilities: one that the segment is essentially a new individual or zooid, and the formation of segments a process of reproduction which is inhibited by the physiological integration of the segments into a composite individual. The other possibility is that segmentation is primarily a mesodermal process a reproduction or reduplication of coelom sacs, other duplications being secondary to these. The morphological theories of segmentation fall into two groups corresponding more or less closely to these two conceptions.

If the segment is a new zooid, the process of segment-formation is undoubtedly the result of physiological isolation in the growing region. As this region becomes more active, it becomes more

independent of regions anterior to it and, as in various flatworms (Child, '11), the development of a new individual begins, but is later inhibited by a new physiological integration through the nervous system. If segmentation be a process of asexual reproduction of this kind, it must begin in the ectoderm, since this is the most active body-layer and precedes the others in development, and the fundamental feature of the segment is a new dominant region, which becomes a new nervous center, a ganglion. Other processes, for example those in the mesoderm, must be secondary to the ectodermal processes.

If, on the other hand, segmentation be primarily a mesodermal or mesoblastic process, it may be conceived as a sort of reproductive process in the mesoblast. As the mesoblast bands grow, groups of cells become successively separated, either by a process of physiological isolation within the mesoblast bands or, perhaps, by a physical separation of the cells into groups which form sacs by the secretion of fluid from their internal surfaces. If the process of segmentation is of this character the ectodermal reduplications must be secondary features.

Experimental evidence is not yet available for a final choice between these two alternatives, and the morphological evidence points toward one alternative in some cases and toward the other in others and cannot be regarded as conclusive. In embryonic development the ectoderm arises from the most active regions of the egg and morphogenesis begins in it, and we know that physiological differences and specialization may exist before visible morphological differences arise. In the growing region of the annelid body the ectoderm is certainly the most active body-layer, and there is no reason to doubt that there, as in the embryo, it is physiologically in advance of the other layers. Moreover, if segmentation be fundamentally a mesoblastic process, it is difficult to account for the reduplication in the nervous system. Reduplication of intestinal branches and testes occurs in the flatworms, without any corresponding reduplication of nerve centers, and it is not clear why or how the reduplication of coelom sacs in the annelids can bring about reduplication in the nervous system. Moreover, the synchron-

ism in the behavior of the two separate mesoblast bands on the two sides of the body indicates the existence of some physiological coordinating factor external to the mesoblast bands themselves and we can expect to find such a factor only in the ectoderm.

In Planaria the posterior zooids are distinguishable physiologically in various ways, for example, by the independent motor reactions of the zooid under certain conditions, by the susceptibility gradient in the ectoderm as well as in other parts, and by the capacity for head-formation in isolated pieces (Child, '11), but no certain morphological indications of the existence of these zooids have ever been discovered. The susceptibility method in its present form is not sufficiently delicate to distinguish the earliest stages in segment-formation, but the data presented above on developmental modification in annelids show that segment-formation may be more or less completely inhibited, and we know that the ectoderm is much more susceptible than the mesoderm to the concentrations of agents use for this purpose. This fact is of course only suggestive, not conclusive.

These various lines of evidence seem to me to favor the conclusion that the formation of a segment is physiologically a new individuation resulting from physiological isolation and fundamentally similar to embryonic development in its earlier stages. According to this view, the head region of the annelid represents the primary individual, the primary gradient and the addition of segments is the result of successive physiological isolations resulting from lower specialization and greater growth capacity of the dorso-posterior region of this individual and bringing about a series of reproductions producing individuals, which are prevented from developing a head by the process of integration into a 'composite' individual through the development of the nervous system and the extension of nervous control which proceeds from the apical or anterior end.

The process of segmentation is then fundamentally similar to the process of zooid-formation in Planaria and other forms (Child, '11; '15 b, Chapter V), but the segments of the annelid

do not finally become capable of independent motor reaction and so separate and develop into complete individuals, as do the zooids of Planaria, because of the greater effectiveness and more rapid extension, with advancing development in the annelid of nervous control from the apical region. Because of this the segmental ganglia remain to a greater or less degree subordinate constituent members of a more or less integrated whole.

It is evident from the preceding discussion that the theory of the axial gradients serves as a basis for the interpretation and synthesis of a series of facts which otherwise remain merely isolated data of observation and experiment. From this point of view, the axial relations in the bilateral invertebrates, the regional metabolic relations in the annelid egg, embryo and larva, the origin of the segmental region from a certain region of the egg, the formation of a posterior growing region with a wide range of developmental potentialities and capacity for long continued growth, and, lastly, the successive formation of segments, all appear to be connected and to have a common basis in the axial gradients. Moreover, viewed in this light, annelid development appears as the physiological activity of an organism rather than as a mosaic of independent parts, a mysterious predetermined harmony. The apparent mosaic condition is secondary, not primary, concerns only certain regions of the egg and moreover, is not a mosaic in the strict sense. In short, the interpretation and synthesis of facts on this basis makes it possible to discern a fundamental similarity between the physiology of annelid development and that of other forms.

Changes in the antero-posterior gradient after metamorphosis

In the three-segmented larva the third or most posterior segment is the most susceptible, the second less and the first least susceptible. In Arenicola it has been found that, after metamorphosis, in the worm of six segments the susceptibility of the whole segmental region is higher than in the three-segmented stage, but the susceptibility decreases from the head through the first body-segment to the third or fourth, while

posterior to the third or fourth segment the susceptibility increases again posteriorly to the growing region (p. 14). Here, then, two opposed gradients are present in the body. Hyman ('16) has shown that a similar condition exists in most oligochetes from an early stage, and there is little doubt that the same is true for other polychetes besides Arenicola.

As Hyman has suggested, these two gradients are probably somewhat different in character, but the susceptibility method fails to show the difference. The gradient in the three-segmented larva and in the region posterior to the third or fourth segment in the young worm is undoubtedly a secondary developmental or age gradient. Each segment arises earlier than the segment next posterior to it and is therefore, at least for a time, more advanced in development, more differentiated, physiologically older and possesses a lower metabolic rate than the latter. Consequently the metabolic rate decreases from the posterior growing region to the first body segment. Essentially the same relations appear in the posterior zooids of Planaria (Child, '11, '13). In the annelid, however, the development of the nervous system and the extension of functional dominance from the head posteriorly bring about integration of the segments into a composite individual. With the differentiation of the cephalic ganglia and the development of the sense organs the head-region receives stimuli from its environment which increase its metabolic activity, and, as nervous connection with the segments is established, transmission to these becomes more and more effective, and this, together with the differentiation of the segmental ganglia, brings about increased metabolic activity in the segments, but at the same time determines their subordination to the head or to more anterior segments. Under these conditions the most anterior segment being subject to the earliest, most frequent and most intense excitation from the head-region acquires a higher metabolic rate than the second, this a higher rate than the third, and so on. As development progresses, the length of body thus brought under nervous control undoubtedly increases, but a longer or shorter posterior region may still retain the developmental gradient in at least some tissues of

the body, and apparently in some cases in the nervous system. Even in such a case, however, some degree of nervous control by more anterior over more posterior regions is possible because nervous differentiation with its peculiar possibilities of rapid and intense excitation and transmission progresses posteriorly. In other words, the increasing excitability and conductivity, with increasing differentiation of the nervous system, makes it possible for a segment or a group of segments to control to some extent more posterior, physiologically younger segments with a higher intrinsic metabolic rate, especially when the excitation is intense, for a sufficiently intense excitation may proceed for a considerable distance up the developmental gradient in the posterior region. Observation of the behavior of many of the elongated oligochetes suggests that the posterior body-region, in which the gradient rises toward the posterior end is not completely subordinated to more anterior regions, except in relatively intense excitation. As regards the polychetes, the behavior and the degree of segmental coördination indicates that the degree of segmental integration is greater in at least many forms than in the oligochetes referred to. Probably a study of the susceptibility gradients in the adult polychetes will show a shorter or in forms with a definite number of segments no posterior region in which the secondary developmental gradient persists.

According to this interpretation, the gradient which first appears in the segmental region, a gradient decreasing in rate from the growing region anteriorly is an intrinsic developmental gradient of secondary origin, for instance, it is an indicator of the physiological age and stage of differentiation of the successive segments. The later gradient, decreasing in rate from the first body-segment posteriorly, is a gradient of extrinsic stimulation, resulting from the physiological integration of the segments through the development of functional nervous control. It may be called the integrative gradient. Since the head region is, to a greater or less extent, the dominant region in such functional nervous control, this later and persistent gradient is in the same direction as the primary apico-basal gradient and rep-

resents the final developmental result of the existence of the primary gradient.

If this interpretation be correct, the segmental region of the annelid body passes through three successive physiological stages: in its origin and earliest development it represents the region of lowest metabolic activity in the egg; second, it becomes the most active region of the larva and gives rise to segments in which a developmental gradient decreasing in rate anteriorly appears; third, development of the nervous system brings about a physiological integration of these segments, and a gradient of nervous stimulation decreasing in rate posteriorly from the first segment appears. This final gradient may, sooner or later, extend to the extreme posterior end of the body, or the developmental gradient may persist in a longer or shorter posterior region, as in the six-segmented Arenicola and in most oligochetes (Hyman, '16).

Certain differences between the different species

In the killing experiments the egg, the blastomeres, and the cells of later stages of Chaetopterus undergo marked swelling and separate from each other very completely before death, in the agents used, while in Nereis and Arenicola almost no swelling and cell separation occurs, and death takes place with little change in appearance. This difference in behavior is evidentl due to some differences in protoplasmic constitution, but the experiments throw no light on the question of its nature.

Another interesting difference appears in the size relations between egg and larva. In Chaetopterus the egg is much smaller than the eggs of Nereis and Arenicola, but the young trochophore is distinctly larger, the fully developed elongated larva is much larger than the egg, and the decrease in size of the head region is slight. In Nereis, where the egg is large, the young trochophore differs but little in size from the egg, and after posterior elongation begins, the head region undergoes a very marked reduction and the three-segmented Nereis larva is but little larger than the egg. Arenicola is intermediate between Nereis and Chaetopterus in these respects. The egg is larger than that of Chaetopterus and smaller than that of Nereis, and the young trochophore

is little larger than the egg, but the elongating trunk region is relatively larger than in Nereis, and the development of the first three trunk segments is accompanied by little reduction of the head, so that the three-segmented larva is considerably larger in relation to the egg than in Nereis. Later, however, as additional segments arise, the head region undergoes marked reduction.

The figures of the different stages of each species are all drawn as nearly as possible on the same scale, but the figures of the different species are drawn with different magnifications, those of Chaetopterus being on a considerably larger scale than the others, and those of Arenicola being on a somewhat larger scale than those of Nereis. Consequently the figures show the size relations for different stages of each species, but not those between the different species.

The size relations between egg and larva of each species depend, in part, on the size of the coelomic cavity which is larger in Chaetopterus than in the other forms and may be enlarged still further by altering the metabolic relations between different regions (fig. 17, A, B,). The proportions of the larvae also are evidently determined by the metabolic relations between the different regions for they too are altered by altering these relations. In Nereis, for example, differential inhibition from the beginning of development, that is, a greater inhibition of the head than of the posterior region, produces larval forms like figures 29 to 37 which, as regards general proportions of anterior and posterior regions, approach the Arenicola type (figs. 48, 49). On the other hand, differential inhibition, beginning at the young trochophore stage in Arenicola, for instance, greater inhibition of the posterior region than of the head, gives larval forms (figs. 51 to 56) whose proportions resemble somewhat those of certain stages of Nereis (fig. 26). Evidently the proportions of the three-segmented larva depend, to a considerable degree, on the metabolic relations between the head, the body segments and the posterior growing region. The Chaetopterus larva is somewhat different in form, but its proportions can be altered in the same ways.

The amount of nutritive material in the egg and its physical condition, water content, etcetera and very probably its chemical constitution must also play a part in determining the size relations between egg and larva. In Arenicola the posterior region grows to a considerable size before reduction of the head occurs, while in Nereis posterior growth apparently occurs, largely at the expense of the head region after the earlier stages. Apparently the supply of nutrition in Arenicola suffices for the demands of all parts up to a later stage than in Nereis. Moreover, the Arenicola larva, even in the three-segment stage of figure 49, is still almost completely opaque because of the small 'yolk' granules distributed through the protoplasm of all cells, and is able to continue its development to a stage of five or six segments without external nutrition, but, during this development, the granules gradually disappear and the protoplasm of the young worm is highly translucent. In Nereis, however, the nutritive supply of the egg is used up much earlier, practically no visible traces of it remaining in the fully developed three-segmented larva, and, in the absence of food from without, development ceases. Summing up, the facts of observation and experiment indicate that the general size relations between egg and larva, and the larval proportions are matters of amount, availability, and perhaps efficiency of food supply in the egg for the metabolism of each species and of the relations of metabolic rate between different parts.

The course of development in other segmented animals

While the data are still too fragmentary to permit the formulation of a general theory of segmental development, various facts are at hand which suggest that the course of events in other forms is more or less like that in the annelids. In segmented forms, generally, the order of segment-formation is the same as in the annelid, and in the teleosts and amphibia, the only vertebrate groups studied in this way as yet, a secondary posterior growing region of high susceptibility appears, at least in the development of the tail. Somites may arise anterior to this growing region, doubtless through physiological isolation, and

in this region of the body the primary gradient, decreasing in rate posteriorly, may always be present and the developmental gradient characteristic of the earlier stages of annelid segmentation may never appear, although it does appear in the tail bud. Such differences are, however, incidental rather than fundamental.

These facts are mentioned merely by way of calling attention to the possibility of a general physiological conception of the process of segmentation in animals and it remains for the future to determine whether the course of events in the annelids has any general significance.

SUMMARY

1. The results of the susceptibility method, both in the killing experiments and in modification of development, indicate that at the beginning of development the apical region is the most active region metabolically and that metabolic rate decreases toward the basal pole.

2. As development proceeds the posterior portion of the somatic plate shows a relatively rapid increase in susceptibility and, as elongation begins, or soon after, the posterior growing region becomes the most active region of the larva.

3. The general modifications of larval form and proportions by differential inhibition are of two sorts. Inhibiting agents, acting from the beginning of development, produce forms more or less microcephalic, with relatively large anterior segments and more or less completely inhibited posterior region. Inhibition beginning at the stage of the young trochophore, for instance, after the increased activity of the posterior region has occurred, produces forms more or less megacephalic with more or less completely inhibited posterior growing region.

4. Particular organs such as the prototroch, the seta-sacs, the tentacles and cirri can be differentially inhibited in the early stages of their development, an indication that they are the seat of high metabolic activity at that time.

5. The facts indicate that, in general, the more apical or anterior and probably the antero-ventral regions of the egg and embryo

are more highly specialized than the basal or posterior and dorso-posterior, and it is suggested that the origin of the body-region with its high developmental potentialities and capacity for long-continued growth from the dorso-posterior region of the egg is connected with these differences. This region undergoes a greater degree of rejuvenescence during early development than the other egg-regions and gives rise to the posterior growing region. This interpretation brings the peculiar course of anne-lid development into relation with the axial gradients and with the development of other forms.

6. The developmental gradient decreasing in rate anteriorly from the posterior growing region, which is characteristic of the whole segmental region in the three-segment stage, is replaced, after metamorphosis, in the more anterior segments of Areni-cola and undoubtedly also of other forms by a gradient in the opposite direction, which is an indicator of the progressive nervous integration of the segments and the dominance of the head-region and may be called the integrative gradient. This integrative gradient coincides in direction with the primary, apico-basal gradient and is, physiologically speaking, the final developmental consequence of the existence of the primary gradient.

7. Some differences in size relations between eggs and larvae and larval proportions in the different species are shown to be associated with the metabolic relations of different regions and the nutritive supply of the egg.

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Chaetopterus. Susceptibility gradients in egg and larva

1 to 5   Susceptibility gradient in eggs thirty minutes after fertilization in $HgCl_2$ $m/500000$.

6   The beginning of swelling and cell separation in twenty-four hour stage.

7 to 9   Later stages of cell separation in twenty-four hour stage.

10, 11   Early and later stage of disintegration in fully developed larvae.
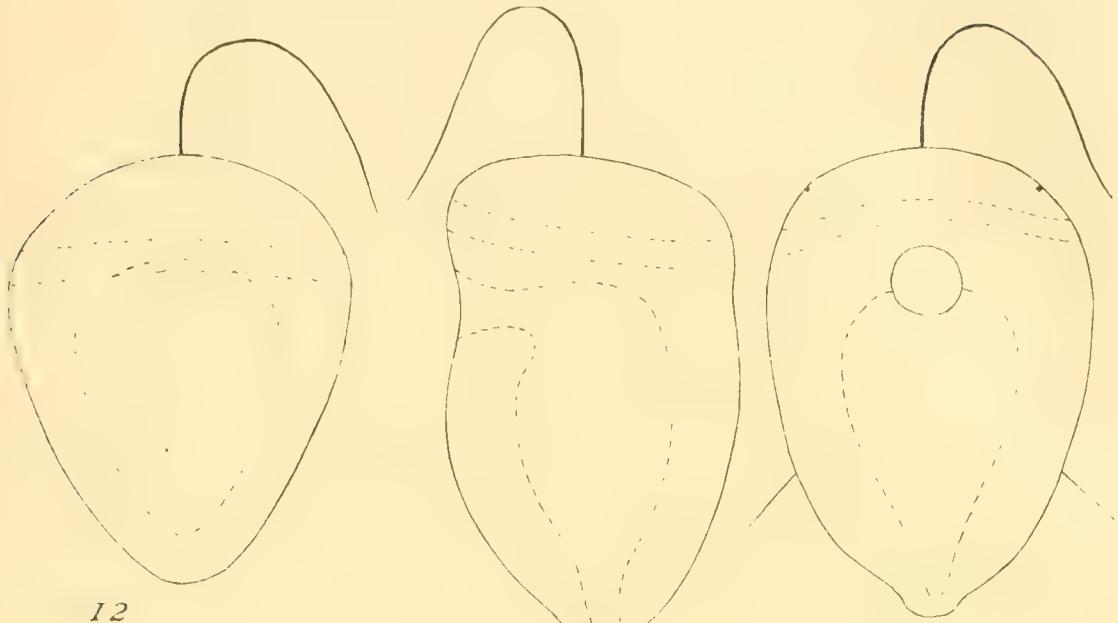
12

13A

13B

14A

14B
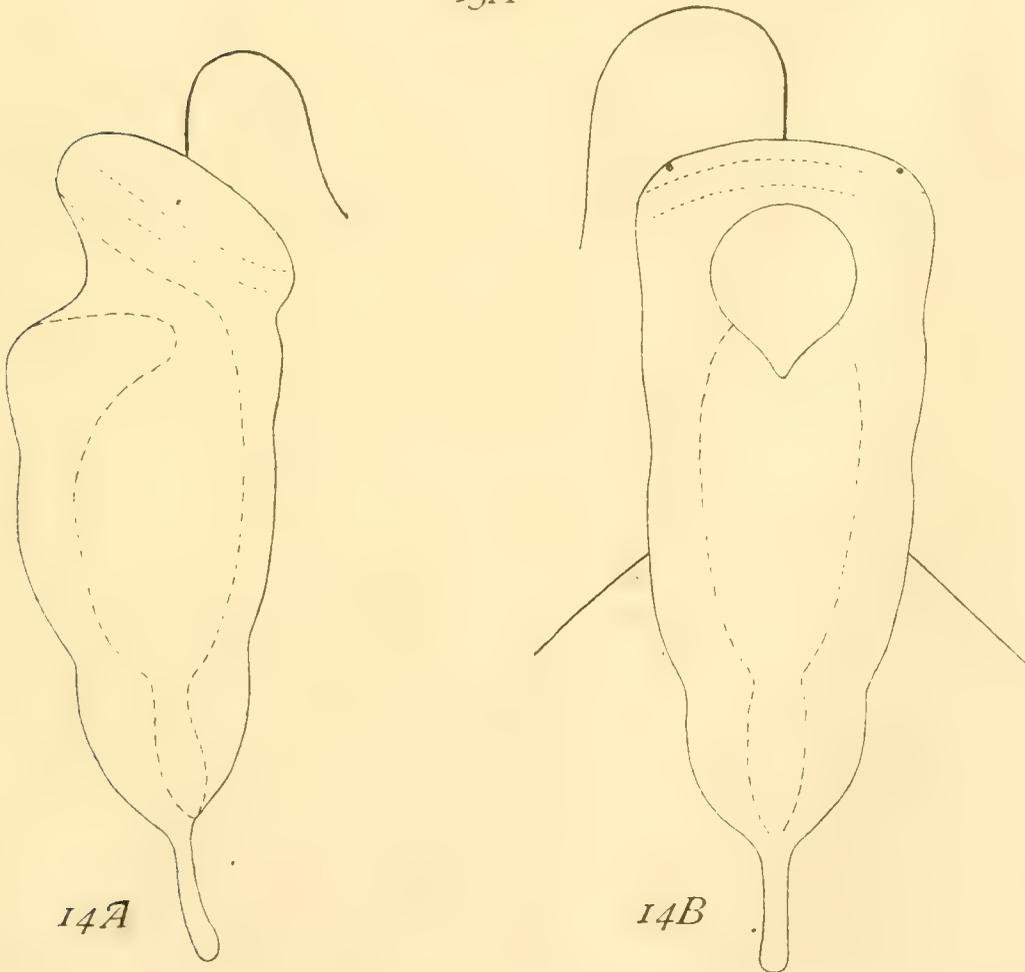
PLATE 3

Chaetopterus.   Development in KNC $m/100000$, from thirty minutes after fertilization

15 to 16   Larvae with relatively small heads and inhibited posterior growing region.   *A* lateral, *B* ventral view.

15A

15B

16A

16B

49
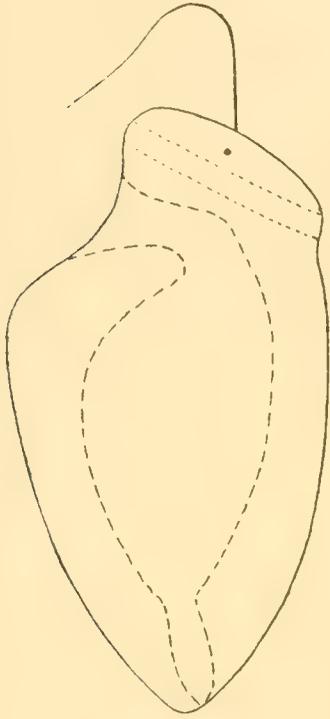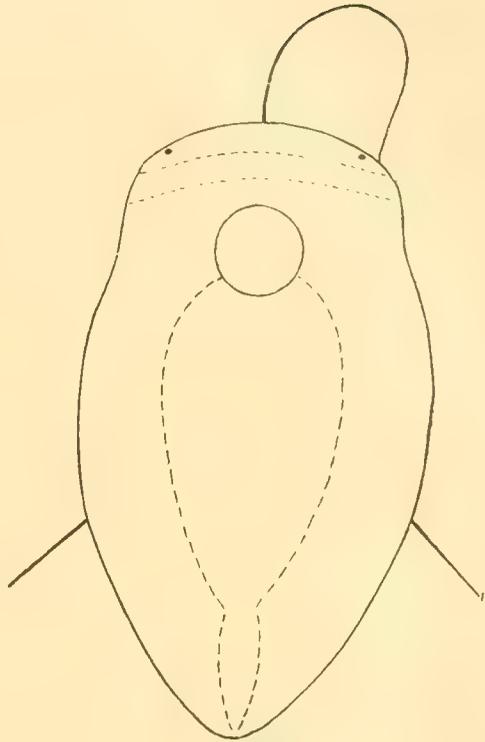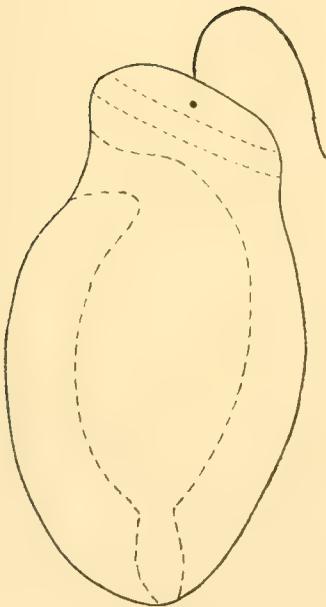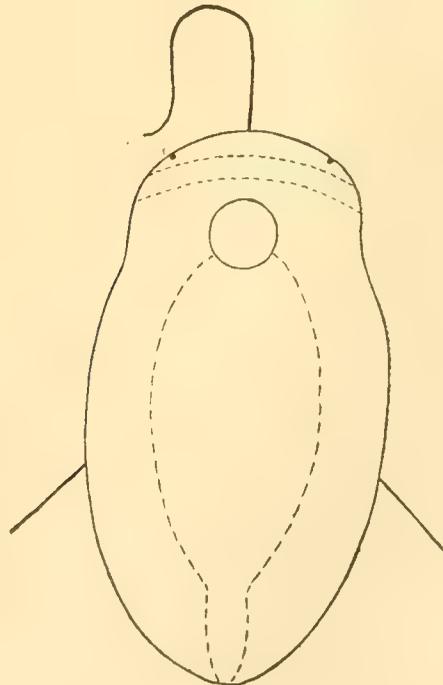
PLATE 4

Chaetopterus.   Development in KNC $m/100000$

17 *A B*   Distended type in KNC and HCl from thirty minutes after fertilization.

18 *A B*   Larval form showing differential inhibition and partial recovery. KNC eleven hours, beginning forty-five minutes after fertilization; then returned to sea water.

A lateral, *B* ventral view.

17A

18A

17B

18B

51
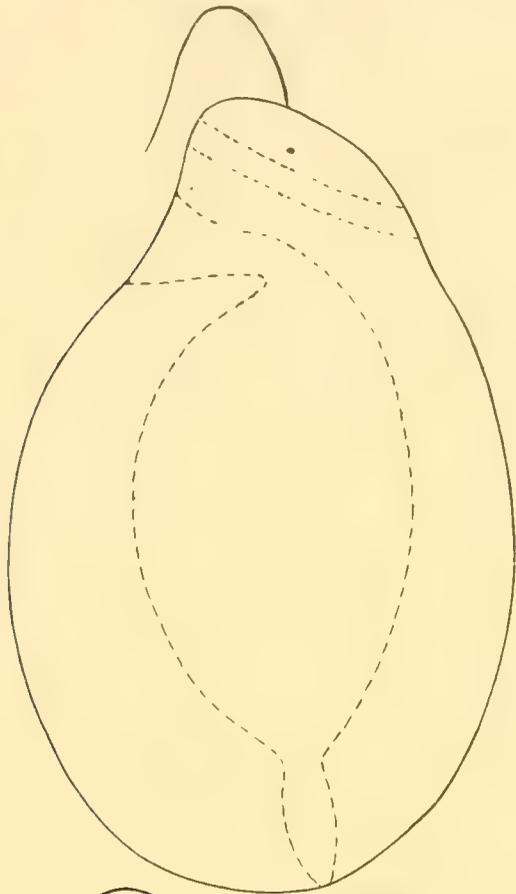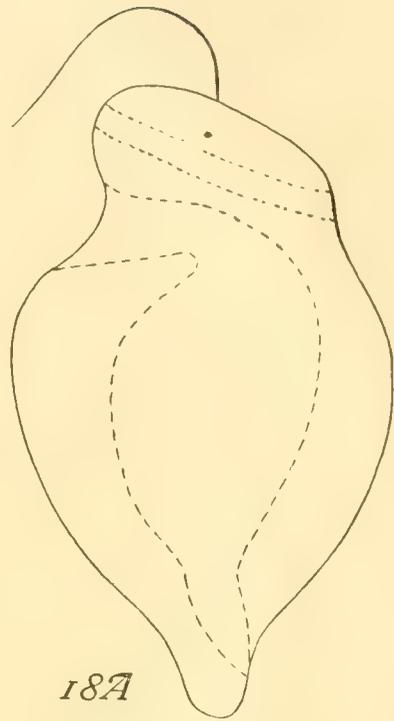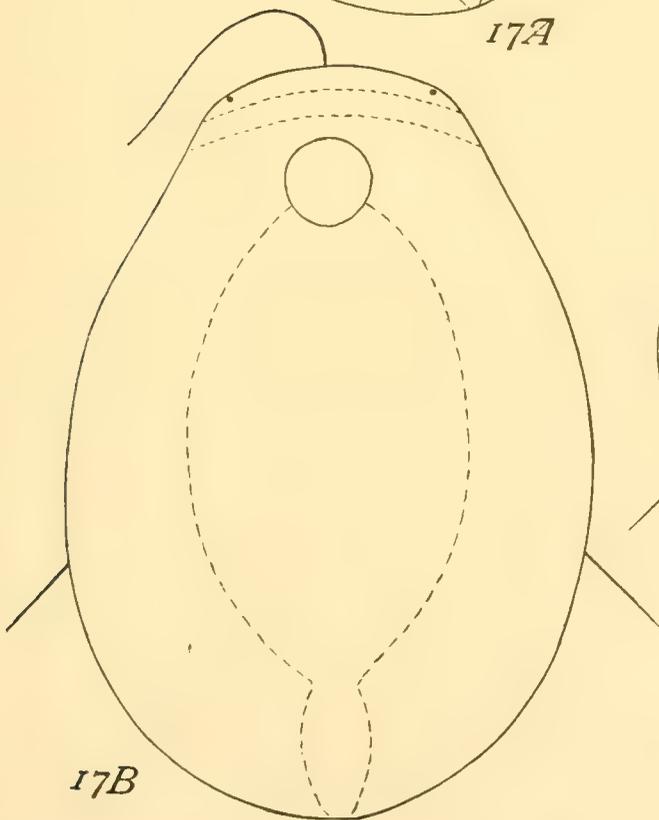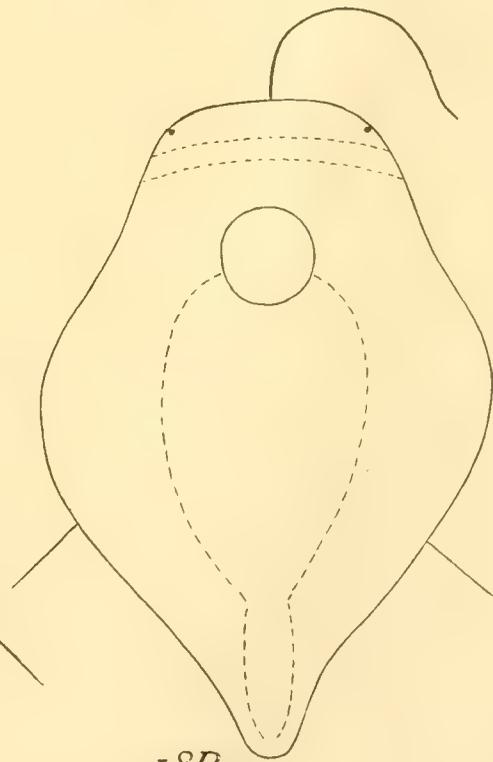
PLATE 5

Chaetopterus.   Development in KNC and HCl

19 *A B*   Larval form showing differential inhibition and partial recovery. KNC eleven hours, beginning forty-five minutes after fertilization; then returned to sea water.

20 *A B*   Development in HCl $m/2500$ from thirty minutes after fertilization. *A* lateral, *B* ventral view.
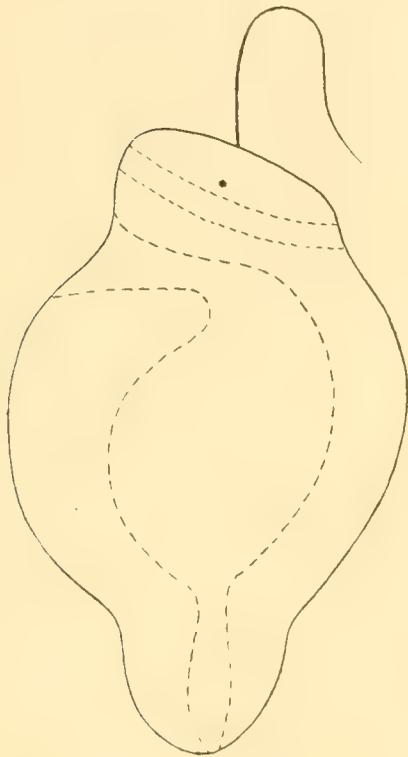
19A

19B

20A

20B
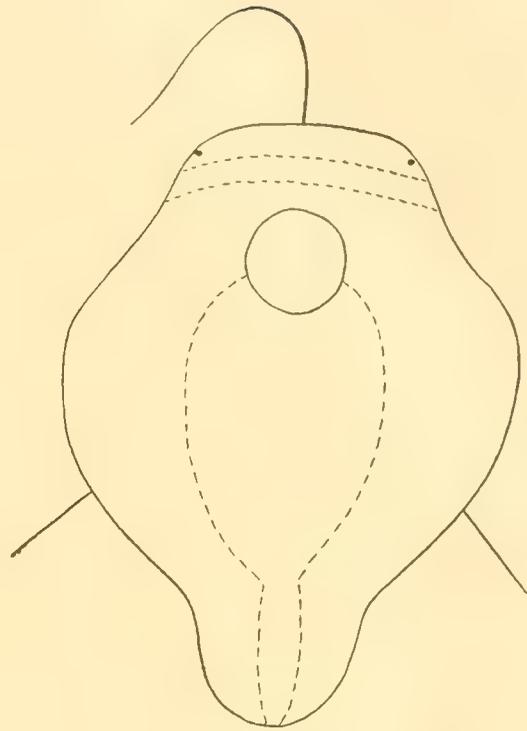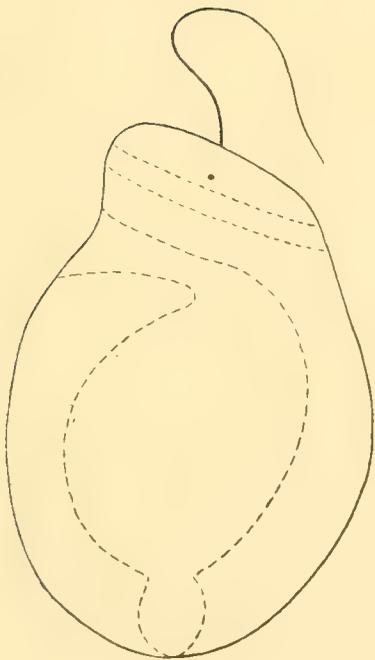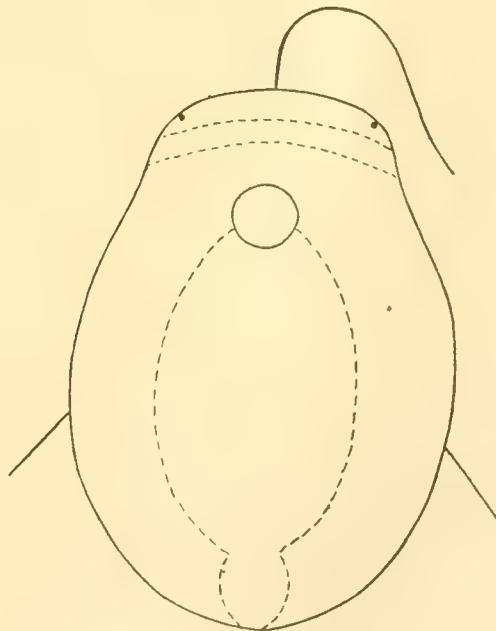
PLATE 6

Chaetopterus.   Development in HCl and KNC

21 *A B*   Development in HCl *m*/2500 from thirty minutes after fertilization.

22   Differential acclimation in HCl *m*/2500.

23 *A B*   Development in KNC *m*/100000 from twenty-four hour stage.

A lateral, *B* ventral view.

21A

21B

22
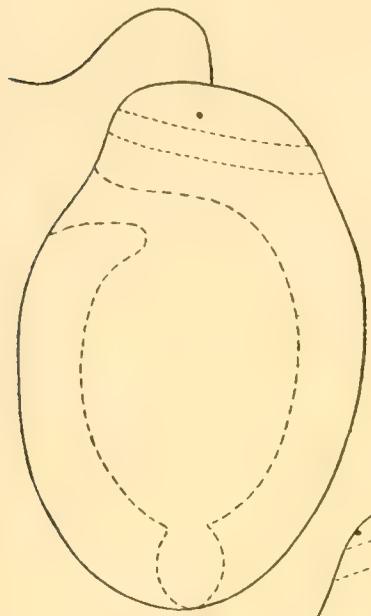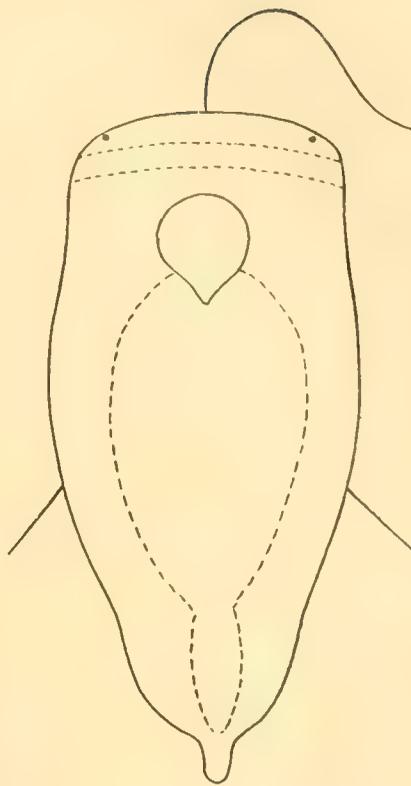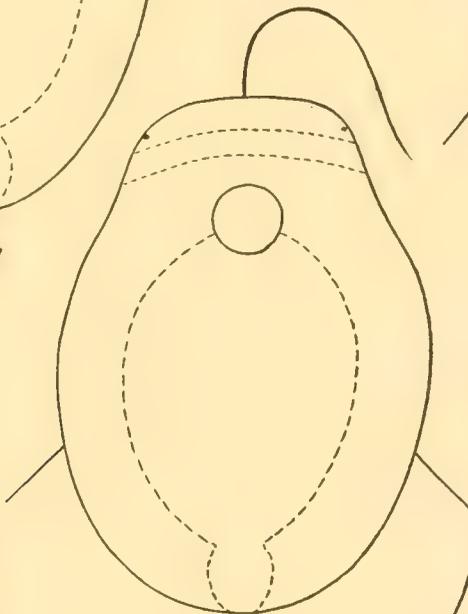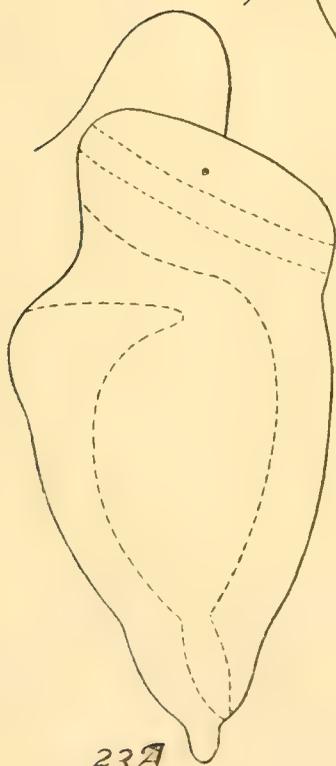
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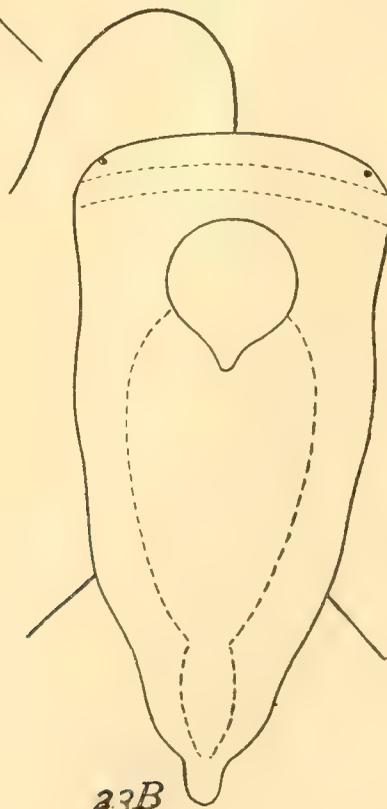
23B

PLATE 7

EXPLANATION OF FIGURES

Nereis.   Normal and inhibited development

24 to 27   Normal development; one, one and one-half, two and one-half and four and one-half days.

28   Stage attained after three days in KNC $m/50000$, beginning just before first cleavage.

29   Four days in KNC $m/100000$ from stage just before cleavage.

24

25

26

27

28

29

Nereis.   Development in KNC

30 to 33   Four days in KNC $m/100000$ from stage just before first cleavage.

34 to 36   Five days in KNC $m/100000$ from stage just before first cleavage.

37   Six days in KNC $m/100000$ from stage just before first cleavage.

38   Two days in KNC $m/50000$ beginning twelve hours after fertilization.
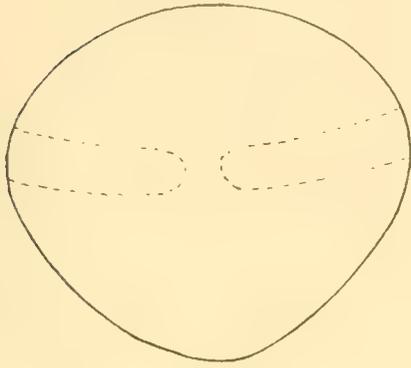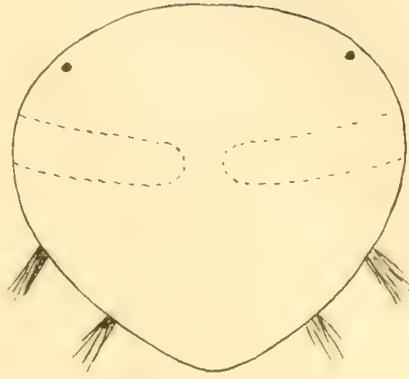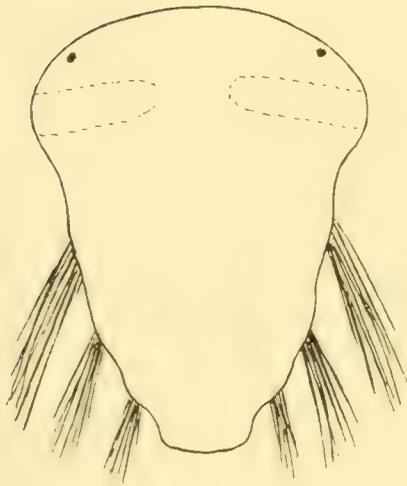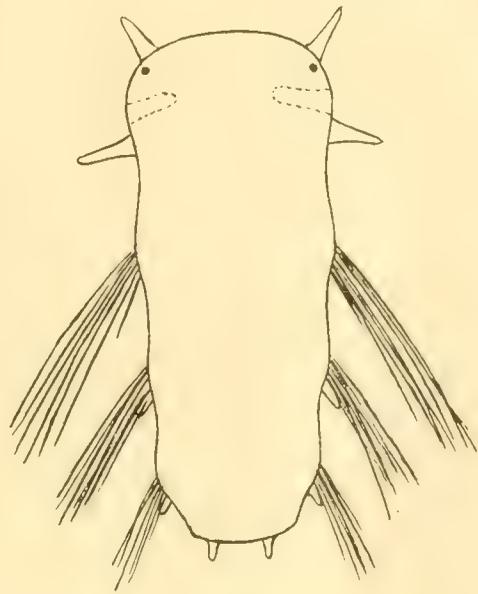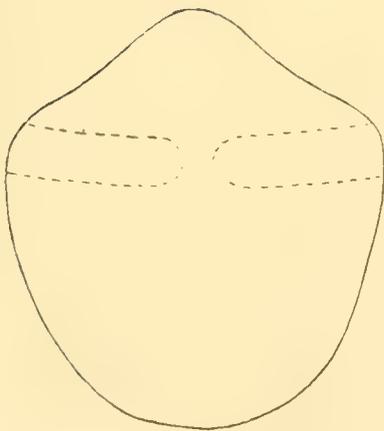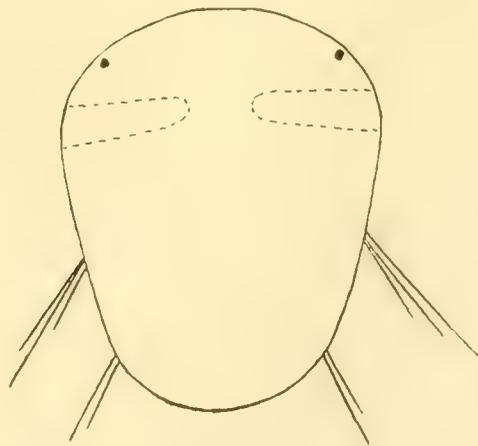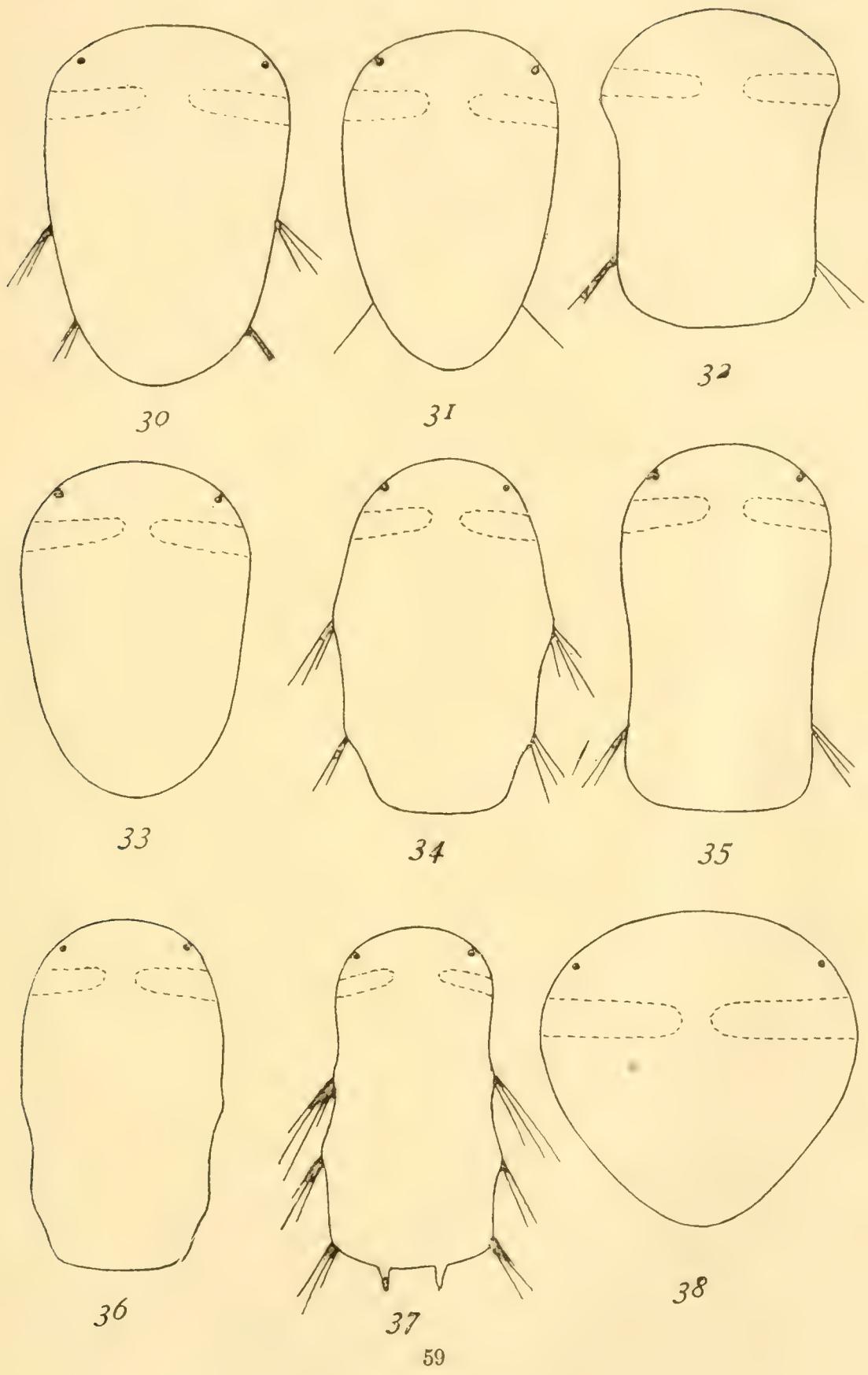
EXPLANATION OF FIGURES

Nereis. Development in KNC

39 to 41   Four days in KNC $m/100000$, beginning twelve hours after fertilization.

42   Six days in KNC $m/100000$, beginning twelve hours after fertilization. Possible acclimation in posterior region.

43 to 47   Five hours in KNC $m/1000$, beginning twelve hours after fertilization.
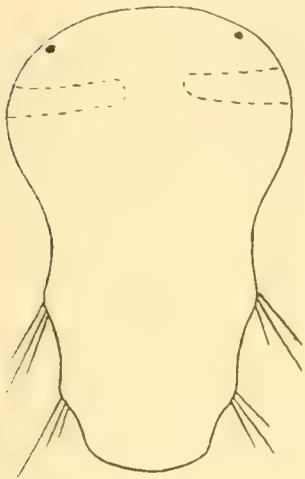
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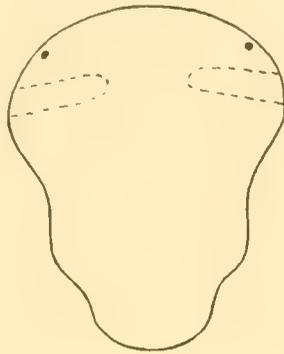
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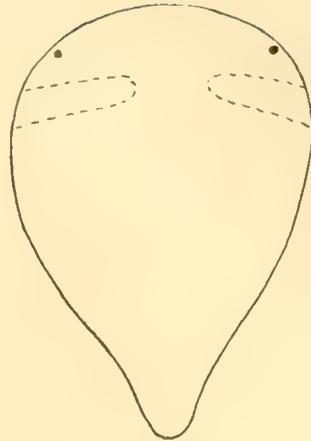
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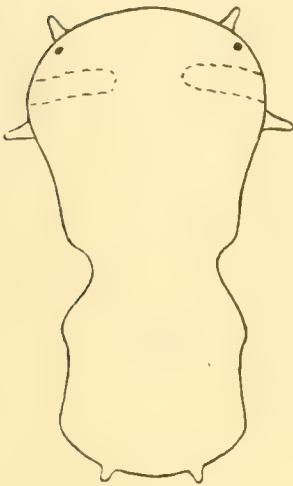
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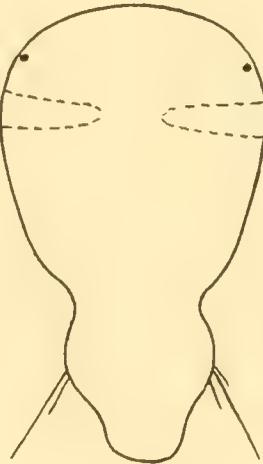
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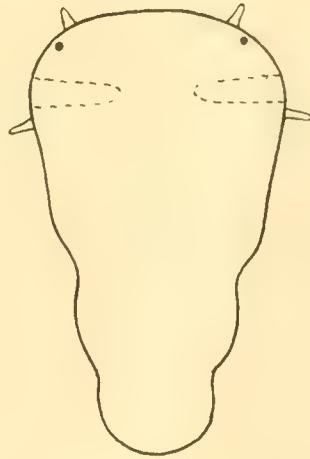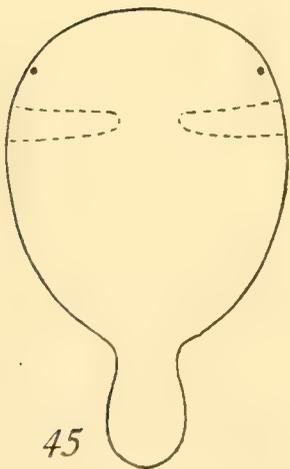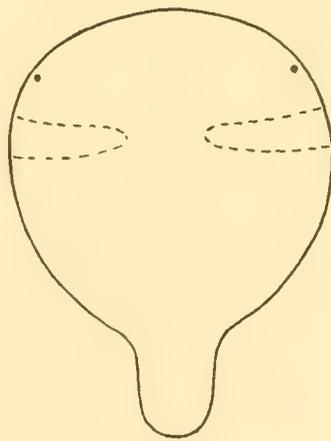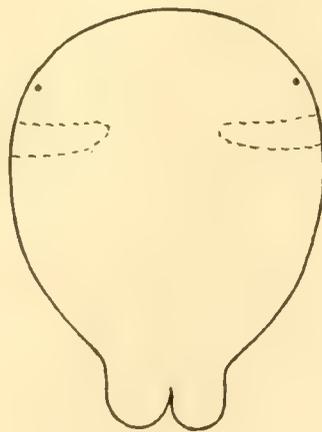
PLATE 10

Arenicola. Normal and inhibited development

48 to 50   Normal development.

51   Four days in KNC $m/100000$ from the beginning of elongation.

52   Six days in KNC $m/10000$ from the beginning of elongation.

53   Six days in KNC $m/25000$ from the beginning of elongation.

54 to 55   Eight days in KNC $m/10000$ from beginning of elongation.

56   A worm which has completed metamorphosis in KNC $m/25000$.
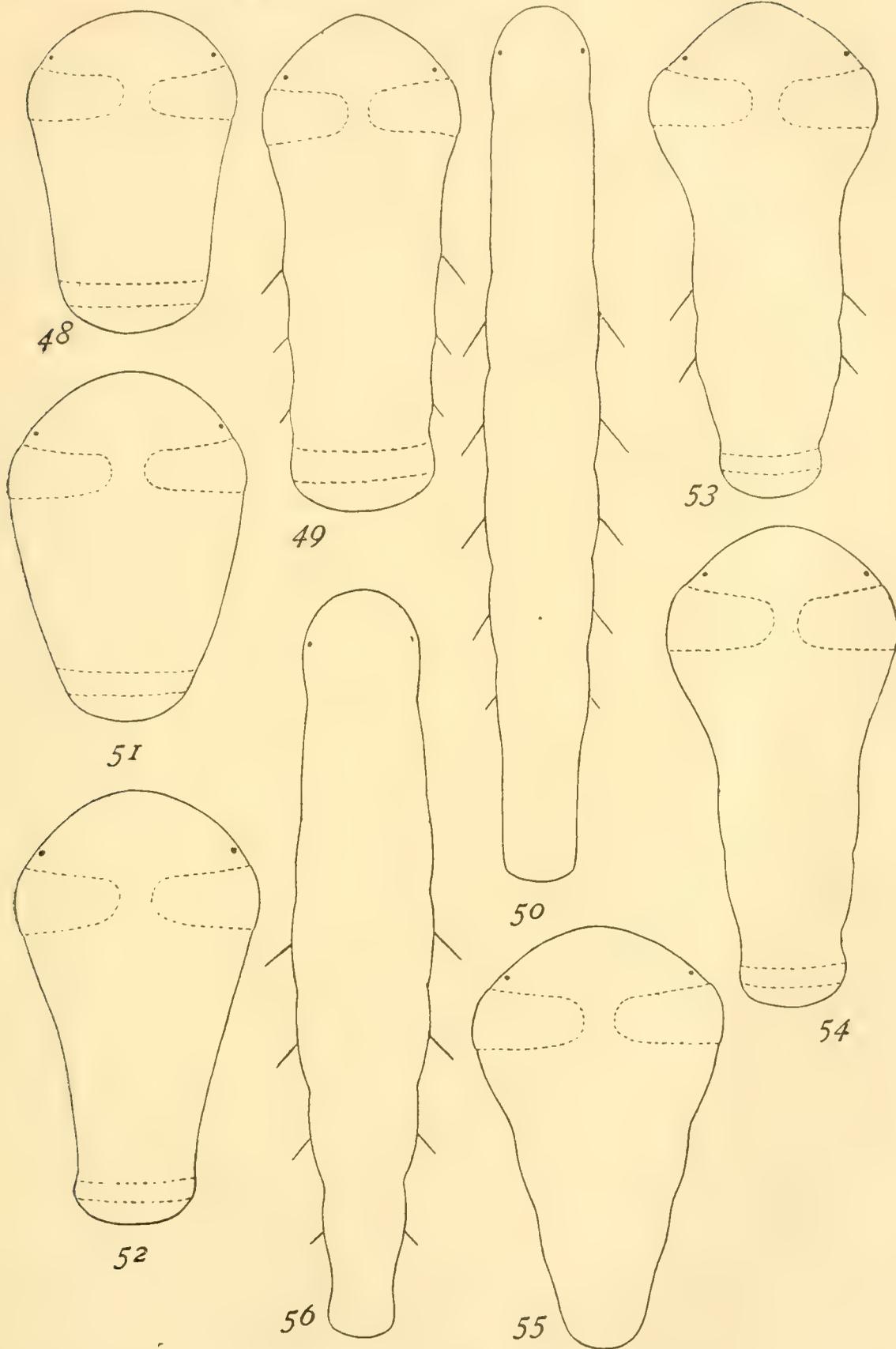
A CASE OF A SYNDACTYLOUS CAT

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SIXTEEN FIGURES

An interesting case of syndactyly in a cat was brought to my attention by Prof. H. B. Torrey. The kitten was two or three months old when given to him. It lived for six weeks afterward. During this time it ate very little and this was obviously followed by much discomfort. Its death seemed to be due to starvation. At Professor Torrey's suggestion and under his direction I undertook to investigate the case. Very little could be learned of the ancestry. The mother was apparently normal, four years old. She had had but one kitten previous to this one and that was said to have been polydactylous with some deformity of the leg bones. The first kitten had been killed and nothing further could be learned about it. The father in the two cases is unknown.

The only other recorded case of a syndactylous cat which I have been able to find is that one noted by Freeland Howe, Jr. ('02). This cat had syndactylous hind paws each bearing two toes, and polydactylous fore paws, each with six toes. There was no description of the condition of the syndactylous feet and no account of the heredity. Very many cases of polydactyly occurring in cats have been recorded and a description of a case of syndactyly should be of value as affording a basis for comparison. In order to establish a working theory of the causes of such abnormalities in the extremities it is neccessary to have a record of the modifications of the abnormality which occur.

The significant features of this particular case of syndactyly are the very obvious tendency to a pronounced distal fusion, brought about by a partial obliteration of the central digits of

the hands and feet, and the correspondence between the skeletal irregularities and the irregularities of the muscles, pads, and claws.

Externally each of the two fore paws (fig. 1) shows two toes, and the skeleton reveals a pronounced symmetry. The condition of the skeleton of the hind feet is less regular. Externally the left hind paw has two toes and the right hind paw, three (fig. 2). These skeletal abnormalities may be seen by comparing the skiographs of the feet of this cat (figs. 3 and 5) with the skiographs of the feet of a normal cat (figs. 4 and 6).

Fig. 1   Dorsal view of the fore feet of the abnormal cat.

In describing the bones reference will be made to the diagrams rather than to the skiographs. It must be remembered that the skiographs are shadow pictures and the prints therefore represent a mirror image of the actual condition. The foot on the right of the skiograph, then, must be compared with the diagram of the left foot. Certain discrepancies may be noticed between the diagrams and the skiographs. It was impossible to flatten the foot absolutely under the X-ray and, as a result, the pictures are a little distorted. The diagrams are drawn with the aid of dividers from the dorsal side of the actual bones.

Left fore foot. The bones of the first digit are normal throughout (fig. 7). The other digits are modified as follows. The diaphyses of the 2d and 3rd metacarpals are distinct, and there is a bone which seems to have formed from the fused distal epiphyses of these bones. The distal boundary of this fused bone is partially obliterated in the skiograph. One bone represents the proximal phalanges of digits 2 and 3, and another, partially fused with this, the medial phalanges. The skiograph indicates a complete separation which is not found in the actual bones. There is no movable joint between the metacarpals

Fig. 2   Dorsal view of the hind feet of the abnormal cat.

and the proximal phalanx. Digits 4 and 5 indicate a similar pairing. The diaphyses of metacarpals 4 and 5 are separate. Metacarpal 5 has a distinct epiphysis. The epiphysis of metacarpal 4 has apparently fused with the bone which represents the 4th and 5th proximal phalanges. The proximal epiphysis of proximal phalanx 5 is distinct from the fused bone, although this does not show clearly in the skiograph. The medial phalanges of digits 4 and 5 are represented by one bone. Here, again, there is no movable joint between the metacarpals and the proximal phalanx, nor between the proximal and medial phalanges. There is but one distal phalanx, bearing one claw,
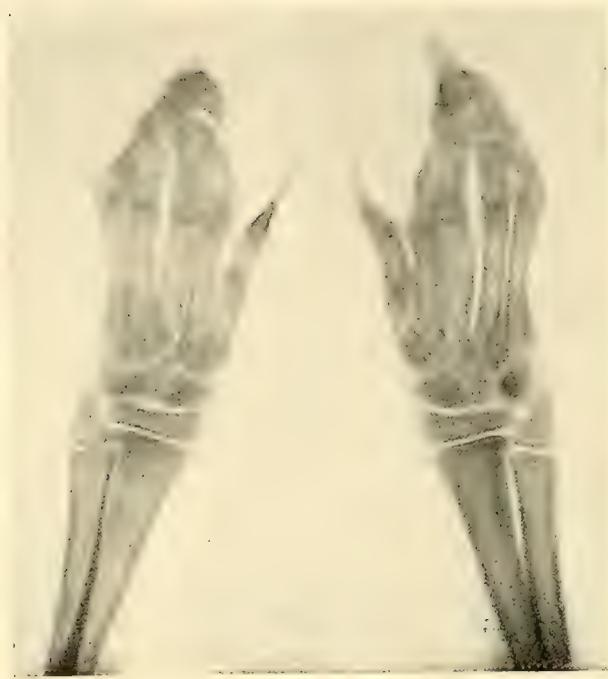
Fig. 3   Skiograph of the fore feet of the abnormal cat.   The right foot is on the left.   (The skiographs are shadow pictures and the prints therefore represent a mirror image of the actual condition.)
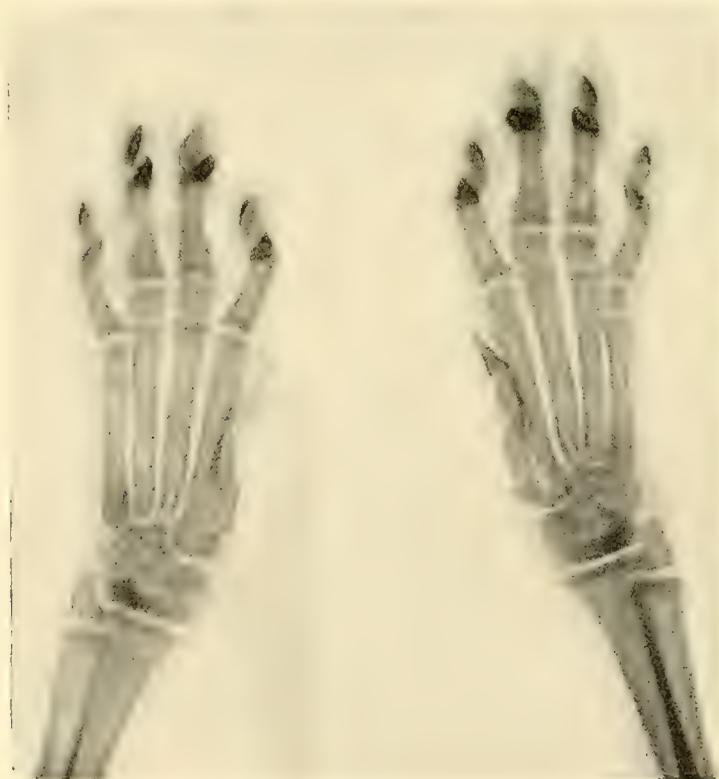
Fig. 4   Skiograph of the fore feet of a normal cat.   The right foot is on the left.

Fig. 5   Skiograph of the hind feet of the abnormal cat.   The right foot is on the left.

Fig. 6   Skiograph of the hind feet of a normal cat.   The right foot is on the left.

to represent the four normally found. This distal phalanx articulates with medial phalanges 2–3 and 4–5.

Right fore foot. In the bones of this foot (fig. 8) the condition is so closely symmetrical to that described for the left that only the variations need be noted. The epiphysis of metacarpal 2 is distinct instead of being fused with the epiphysis of metacarpal 3. The epiphysis of metacarpal 3 is fused with the bone representing proximal phalanges 2 and 3. The proximal epiphysis of metacarpal 4 is distinct. There are apparent dis-
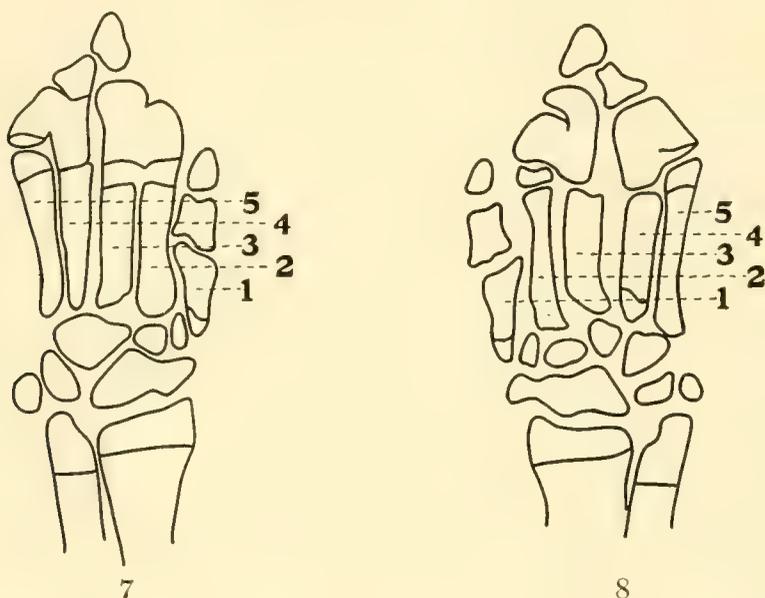
Fig. 7   Diagram of the bones of the left fore foot of the abnormal cat.
Fig. 8   Diagram of the bones of the right fore foot of the abnormal cat.

crepancies between the skiograph and the diagram in the relative dimensions of some of the bones of this foot. This is due to the fact that the foot was placed under the X-ray in a plane not parallel to that of the photographic plate.

Left hind foot. The bones of digit 2 and the rudimentary bone representing the degenerate first digit are normal (fig. 9). The other digits are modified as follows. The diaphyses of metatarsals 3 and 4 have partially fused at the distal end. Their two epiphyses have fused. A single proximal and a single medial phalanx represent the proximal and medial phalanges of digits 3 and 4. Metatarsal 5 shows a distinct diaphysis and distal

epiphysis, a proximal and a medial phalanx. Each of the proximal and medial phalanges shows a distinct proximal epiphysis. The distal phalanx representing digit 3-4 and the distal phalanx of digit 5 have fused in a curious way. The bone thus formed articulates with the distal end of medial phalanx 3-4 and with the side of medial phalanx 5. The claw borne by this distal phalanx shows the parts of two claws quite distinctly (fig. 2).
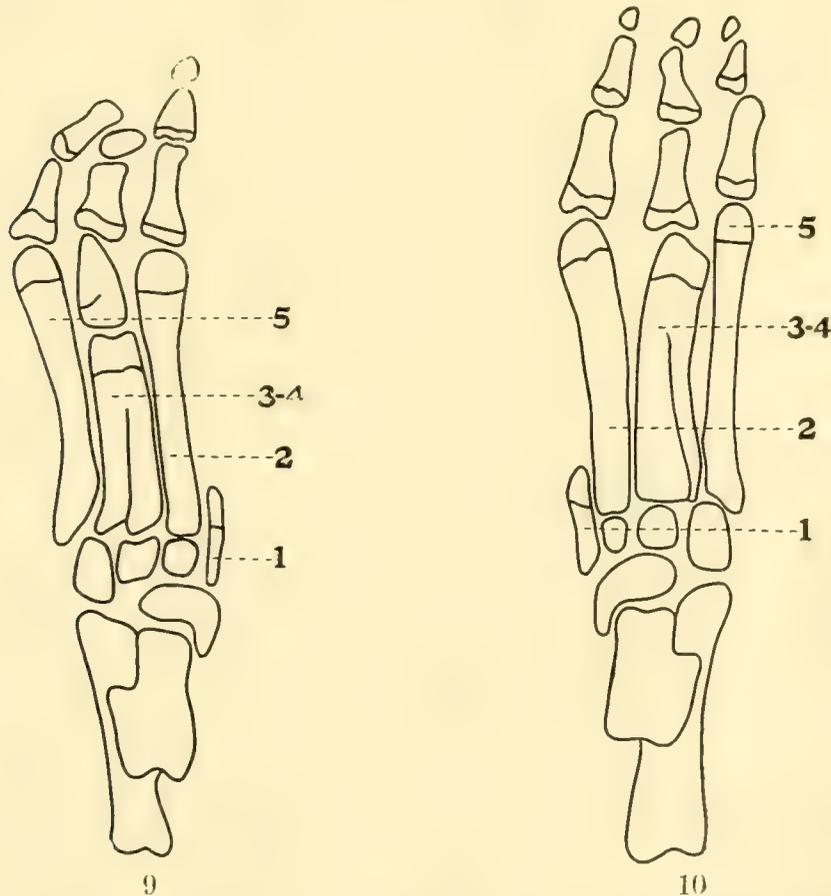
Fig 9 Diagram of the bones of the left hind foot of the abnormal cat.
Fig. 10 Diagram of the bones of the right hind foot of the abnormal cat.

The metatarsal bones of digit 3–4 are relatively short. As a result the distal end of the medial phalanx is approximately even with the distal end of the proximal phalanx of digit 5. This discrepancy in length accounts for the peculiar articulation of the distal phalanx. The fusion of these distal phalanges has resulted in a bending inward of the phalanges of digit 5 (fig. 5).

Right hind foot. Digits 2 and 5 and the rudimentary bone representing digit 1 are normal (fig. 10). The diaphyses of the 3rd and 4th metatarsals have partially fused at the distal end; their epiphyses are completely fused, and there is a single representative of each of the phalanges. The diaphysis of digit 3–4 is short, but not so pronouncedly as in the left pes. This shortness is compensated for in the length of the phalanges, so that the middle digit is not shorter than the lateral ones.

The wrist bones present few irregularities. In the left carpus the magnum and unciform have fused; otherwise the bones are normal. In the right carpus the magnum is small and does not articulate with the fourth metacarpal. The ankle bones are normal.

The tendency to obliteration in the central digits and to convergence to a point distally is such as one would expect to find resulting from a pressure directed proximad and effective along the distal margin and to some extent along the lateral margins. This tendency shows itself in two ways. In the fore paws the 3rd and 4th digits are shorter instead of longer than the others and the epiphyses are less frequently distinct from their diaphyses In the hind paws there is a conspicuous shortening of the partially fused 3rd and 4th metatarsals, and the phalanges of these have completely merged their identity. As a result of this convergence and tendency to a dropping out or shortening of the central elements, the hands do not spread out in a distal direction as is normal, but have the shape, in general, of an inverted *V*. This is not so conspicuous in the feet, but in both cases there may be said to be a greater tendency to fusion distally than proximally.

There is in the fore feet also, a tendency to a synarthrosis, rather than a diarthrosis at the finger joints, that is, some of the joints which are normally articulate are non-articulate in this cat. Apparently at these joints the joint cavities failed to form, possibly because of some pressure at the distal margin, and the intervening tissue developed into a cartilaginous mass.

The non-articulate structure of the feet was partially responsible for the awkward gait of the kitten which at once

attracted attention. In addition to inability to flex the bones normally, there was an abnormal muscular condition. The emaciated condition of the cat made dissection of the muscles of the feet difficult, but certain facts were discernable. There were no flexor muscles for the claws so that they could not be retracted. The plan of the musculature followed the skeletal plan, in general. Where the bones of the two digits were represented by single bones, the muscles were correspondingly represented by single muscles in the main. Certain muscles seemed to be lacking entirely and all were degenerate.

Fig. 11   Ventral view of the fore feet of the abnormal cat showing the abnormal condition of the pads.

Even the pads show an irregular condition (figs. 11 and 12) and those of the fore feet are again the more abnormal. A comparison of the diagrams of the normal (figs. 14 and 16) and abnormal (figs. 13 and 15) pads makes the discrepancies at once obvious. The pad usually found on the pollex of the first digit of the fore foot is found on the abnormal paws (figs. 13 and 14, a). The four pads normally found on the other digits (fig. 14, b, c, d, e) are represented on the paws of the abnormal cat by one large pad (fig. 13, b). The three lobed metacarpal pads normally found (fig. 14, f) are represented in the abnormal condition by

two separate pads on the right paw (fig. 13, f, f^1) and by three
separate pads on the left paw (fig. 13, f, f^1, f^2). The proximal
pads (figs. 13 and 14, g) seem to be similar in the two cases al-
though the shorter hand in the case of the abnormal cat brings
them in that case nearer the digital pads.

On the right hind paw there are three digital pads (fig. 15,
b, c, e) corresponding to the normal four (fig. 16, b, c, d, e). On

Fig. 12   Ventral view of the hind feet of the abnormal cat showing the pads.

13   14
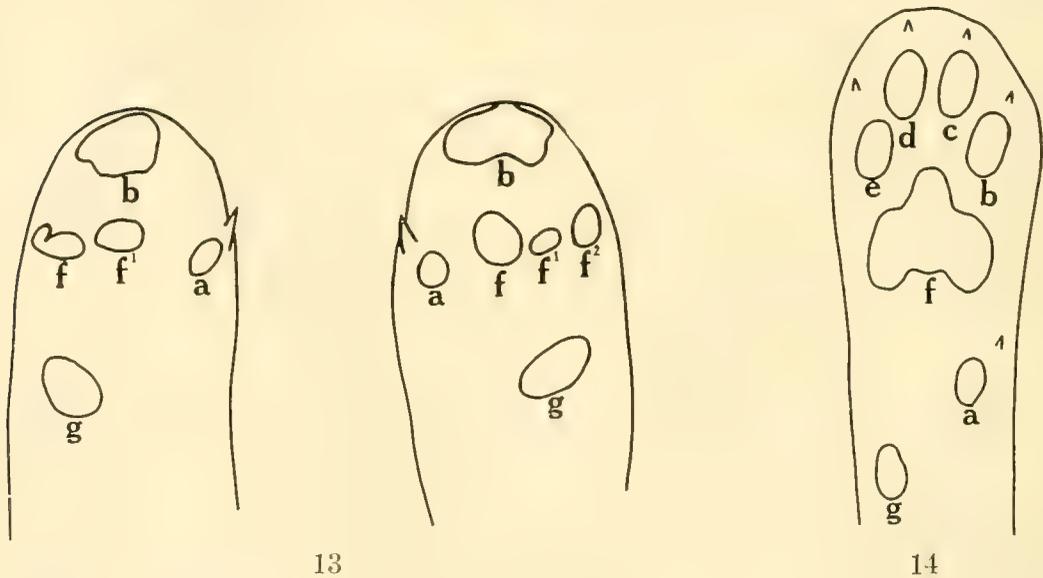
Fig. 13   Diagram of the pads on the fore feet of the abnormal cat. a, b,
digital pads, f, f^1, f^2, metacarpal pads, g, proximal pads.

Fig. 14   Diagram of the pads on the right fore foot of a normal cat. a, b, c, d,
e, digital pads, f, metacarpal pad, g, proximal pad.

the left foot there are but two digital pads, (fig. 15, *b*, *c*,). The metatarsal pads in each case are three lobed (fig. 15, *f*) as is normal (fig. 16, *f*).

There exists then, a definite correlation between the variations of the bones, muscles, and pads.

In this cat a condition of malnutrition existed in the post-foetal life. The difficulty was not so much one of non-assimilation as of actual starvation. The cat ate very little after it was brought into the laboratory and this was obviously followed by

15   16
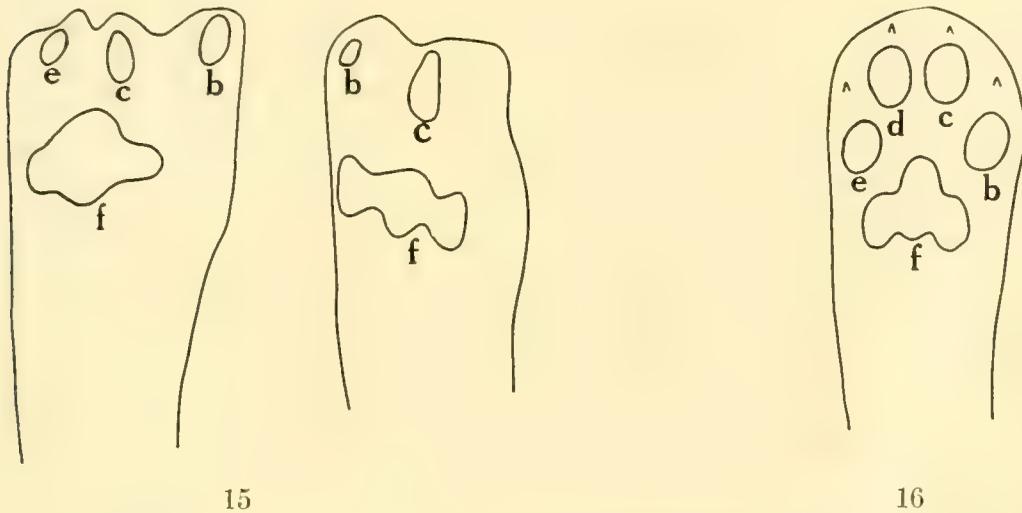
Fig. 15   Diagram of the pads on the hind feet of the abnormal cat.   *b*, *c*, *e*, digital pads, *f*, metatarsal pads.

Fig. 16   Diagram of the pads on the right hind foot of a normal cat.   *b*, *c*, *d*, *e*, digital pads, *f*, metatarsal pad.

much discomfort. At death the body was exceedingly emaciated. It was thought that there might be some clogging of the intestines but a dissection of the complete length of the alimentary canal revealed no mechanical obstruction. The stomach was quite empty and the intestines contained very little, a few wads of hair, three small thread worms, and several small quantities of waste material. Both the small and large intestine were greatly shrunken, although the lumen remained open. Owing to this, the diameter of the large intestine was less in proportion than is normal. Malnutrition undoubtedly caused the death of the kitten, but it is difficult to see how it could have factored

in causing the defects which originated during foetal development. Both the physical deformities and the failure to eat, however, may have been the result of the same physiological disturbance.

Other than the variations noted the cat seemed to be quite normal. The thymus and pituitary body appeared normal. The thyreoid was a little small when compared to the thyreoid of a normal kitten of the same size. It was at first suspected that the upper leg bones were relatively short but a comparison of the radio-humeral and tibio-femoral indices with those of normal cats of the same age indicated no such unusual condition. The abnormalities under consideration were confined to the feet.

This case raises several questions. Is the deformity heritable? If so what is the heritable character? What are the relations of the various forms of abnormality occurring in the extremities? What are the causes? Are such deformities commonly associated with any other abnormal conditions?

Poulton ('83, '86), Bateson ('94) and Torrey ('02) have indicated that polydactyly is strongly hereditary in cats. Lewis ('09) and Broman ('04) establish the heritability of both polydactyly and syndactyly among human beings. Broman ('04, p. 62) says,

Viele, ja vielleicht die meisten angeborenen Missbildungen und Anomalien sind ausgesprochen erblich, und zwar sowohl Hemmungs-Missbildungen wie andere. Sehr erblich sind z. B. Hypospadie, Poly- und Syndactylie und die angeborene Hüftgelenksluxation (Hoffa, '04).

Both these authorities note the frequent association of polydactyly and syndactyly in the same individual. Broman ('04, p. 233) says,

"Die Syndactylie ist eine relativ gewöhnliche Missbildung, Sie kommt nicht selten entweder mit Perodactylie oder mit Polydactylie kombiniert vor."

In order to establish a working theory concerning the origin of polydactyly, where there is a greater than normal number of elements, and of syndactyly, where the number of elements is less than the normal number, it will be necessary to indicate the

various types of malformation which occur in the extremities. Bateson ('94, p. 399) finds the cases of polydactyly more regular than those of syndactyly. Polydactyly, in its simplest form, may consist in the reduplication of a single phalanx. The intermediate step between it and the normal condition is the development of a third phalanx on the first digit or thumb. The reduplication may proceed so far as to include the metacarpal and metatarsal bones of several digits of hands and feet. Syndactyly seems to be more irregular in its manifestations. Cutaneous and fibrous forms occur in which only the fleshy parts of the extremities are affected. It may result from the omission of certain skeletal elements or from fusion of some of the bones. In such cases there is a corresponding syndactyly of the fleshy parts. The omission may occur in either marginal or central digits. The latter is less common and is called split hand or split foot (Broman, '04, pp. 651, 230–231). According to Lewis ('09, p. 7), split foot may be accompanied by polydactylous hands and split foot is never found unaccompanied by split foot. Defects in the wrist bones may occur. A single carpal or tarsal bone may completely fail or may fuse with a neighboring bone. In the case of an omission of one of the carpal or tarsal bones, there is usually a coincident defect of the bones either distal or proximal. (Broman, '04, p. 651).

In either polydactyly or syndactyly the abnormal condition is apt to be symmetrical (Broman, '04, p. 231) and to be more pronounced distally than proximally. This distal syndactyly was particularly conspicuous in the cat under discussion. Bateson finds this difficult to understand. He says ('94, p. 357).

The rule that in the lowest condition of syndactylism of the bones it is commonly at the periphery that the union is most complete is also difficult to understand in connexion with the fact that the division of digits in the lowest forms of polydactylism appears also in the *peripheral* phalanges.

Syndactyly and polydactyly, then, are both heritable and are frequently found in association. Each consists in an abnormal number of skeletal parts, the one in less and the other in greater number. The abnormality is usually more pronounced at the

distal extremity. In analysing the possible causes of such conditions one must recognize that, although a given case may not be hereditary, it may be the result of conditions identical with those effective in hereditray cases. The difference would be that in the former case the effective factors must have arisen locally, while in the hereditary case such factors were carried in the germ cells.

The question of when the unknown factors causing polydactyly or syndactyly may be effective is important in determining what these factors may be and this can be answered only by a consideration of the course of normal development. Since no material on the cat is available, a review of the essential points of the development of the human arm will suffice. The lower extremities develop in a way essentially similar to the upper, and the facts regarding the cat may be considered different only in unessential details.

The skeleton of the hand first appears outlined in a bedplate of scleroblastema. In this, differentiation proceeds from base to tip, the humerus, radius and ulna being laid down in cartilage before the carpus and fingers. In the hand the metacarpal cartilage forms, then the carpal, and finally the cartilages of the phalanges; the cartilages of the distal phalanges are the last to appear. The first ossification occurs in the long bones. In the hand the distal phalanges ossify first, the metacarpals and proximal phalanges next, then the medial phalanges and finally the carpal bones (Bardeen '10, pp. 366–398).

As the various parts of the skeletal anlage become differentiated, the hand region assumes the form of a rounded pad. It is probably at this or an earlier stage that later poly- or syndactyly is determined. It is difficult to conceive how, after the cartilages had been laid down, certain elements could be eliminated in the case of syndactyly or how new cartilaginous elements could form in the case of polydactyly. It is quite conceivable, however, that, if this pad were prevented from rounding out to the normal width, differentiation might progress, with a crowding together of the elements such that some would fuse or, in extreme cases, even drop out. On the other hand if the pad were to widen to a

greater degree than ordinarily, superfluous elements might be formed and a condition of polydactyly result. If the syndactyly or polydactyly occurred slightly later, after the anlagen of the digits were differentiated, it would have to result either from a fusion or from added separation. Syndactyly might be a result of some early fusion of distinct or partially distinct elements and polydactyly a result of a division of one or more of the digit anlagen. Broman ('04) supports both the idea that syndactyly arises early and the idea that it may be in some way a pressure effect. He says (p. 651).

In anderen Fällen ist die anscheinend kleinere Zahl der Finger (Zehen) auf mangelhafte Trennung (Syndactylie), der in normaler Zahl vorhanden Fingeranlagen zurückzuführen (fig. 199–202 s. 232). Die Skeletteile zweier mangelhaft getrennten Finger können entweder getrennt bleiben oder verschmelzen (Syndactylie ossea).

He does not explain what causes this defective separation nor does he give any evidence for assuming that syndactyly is brought about at this time. In regard to pressure he says ('04, p. 233),

"In vielen Fällen von Syndactylie scheint man berechtigt zu sein, die Missbildung auf frühzeitige Druckwirkung und Raumbeengung durch das Amnion zurückzuführen (Klaussner 1904)."

Neither of these views, which are as yet unsupported by adequate evidence, involves a consideration of the relation of syndactyly to polydactyly. There is no definite evidence pointing to distinct origins of these two conditions. On the contrary they are frequently found in close relation. This suggests that both may be due to the operation of a single fundamental factor. It would be interesting to determine whether or not the heritable character of each of these abnormalities might be a tendency to instability in the formation of the extremity. If such were the case, the resulting polydactyly or syndactyly would depend upon individual conditions; if it were not, the two might be fundamentally different in origin. In the recorded strains of polydactylous cats there was no constant number of digits characteristic of a given family, but there seemed to be a tendency to increase the number of superfluous digits in the

later generations. This probably points to some unstable factor in the underlying situation.

At present it seems to be simplest to think of either poly-dactyly or syndactyly as due to the introduction of some factor not normally present. Local conditions are never identically the same, and the variations in the abnormality may be the result of variations in the local conditions rather than the result of variations in the unusual factor. This idea can be expressed in abstract terms. The individual variable conditions govern-ing the formation of a given extremity may be represented by x. A constant factor, K, may or may not be introduced. If introduced an abnormal condition is produced. $x + K$, then, may be said to represent the situation when syndactylous extremities are formed. If, however, the local conditions are sufficiently changed, and if to these changed local conditions the constant factor, K, is added, $X + K$ may produce polydactylous extremi-ties. In a given individual the physiological condition of the upper extremities might be represented by X and that of the lower extremities by x. If, then, the disturbing factor, K, were introduced such an individual would have polydactylous hands and syndactylous feet.

Such a factor might be either physiological or mechanical. If physiological, it must be a factor which occasionally affects only the upper or lower extremities. Three possible physio-logical factors at once occur to one, infection, malnutrition, and deficient blood supply. In practical effect the last two are the same, for the physiological condition would be the same whether the lack of material was occasioned by an actual lack of the blood supply, or by a lack of the essential constituents in the blood. The evidence taken from cases of rickets makes it clear that there is a distinct connection between nutrition and bone formation (Still, '14, p. 776). If the factor be mechanical it must tend to exert its effect symmetrically. The conception of pressure as the determining factor is not new. The amnion has been thought to be a possible source of pressure, causing both poly- and syndactyly. It has been suggested that polydactyly may result from pressure of amniotic threads on the distal

margin of the hand plate (Broman, '04). To effect syndactyly the pressure would have to be exerted laterally. There are two chief difficulties in the way of such an explanation. If the amnion is the determining factor some evidence pointing to this should have come to light (Broman, '04). The chief difficulty, perhaps, is presented in the general symmetry of the abnormalities. It is difficult to see why the pressure, unless itself the result of some physiological condition, should be effective symmetrically. It is conceivable that lateral pressure might be effective in causing a change in the normal shape of the early limb pads, as was suggested above, but it is difficult to explain the source of the pressure.

Various other abnormalities are occasionally associated with polydactyly or syndactyly. No causal connection between these defects has been established and they are of no significance for the general condition under discussion. None of these defects were present in the kitten. Bateson ('94, p. 399) finds occasionally phocomely, cyclopia, double uterus, hare lip, defective dentition, defect of tibia, associated with polydactyly. Lewis ('09, p. 10) finds polydactyly occasionally in conjunction with hare lip and abnormalities of the limb bones. Bateson ('94, p. 399) finds syndactyly associated very often with general deformity and with many forms of arrested development.

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ORIGIN OF THE CASTES OF THE COMMON TERMITE, LEUCOTERMES FLAVIPES KOL

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FORTY-TWO FIGURES

CONTENTS

INTRODUCTION

The origin of the different castes of termites has been discussed for many years and from many points of view, and is a question of far reaching biological significance, in that its solution may

throw light upon the greater question whether the heritable
bodily structure is determined by extrinsic factors, such as food
and environment, or by intrinsic factors within the germ plasm.
The related problems of polymorphism and the meaning of the
castes of social insects may also be better understood when the
question of caste origin is settled.

Two views of the origin of termite castes, both based upon
observation and experiment, are held today. According to the
first view, the young are all alike and undifferentiated at the
time of hatching, but differentiate into the various adult castes
through the influence of external factors, such as food, the pres-
ence of parasites, the care received from the older workers, etc.

According to the second view, the young are not all alike at
the time of hatching; some, if not all, of the different castes are
distinguishable from the beginning, and the castes are therefore
predetermined in the egg or embryo by intrinsic factors.

The first view has the authority of age and of the number of
its supporters; its weakness lies in the fact that it is based upon
assumptions which either have not been proved or which can
be disproved. For example, there is no experimental proof that
food is the cause of differentiation in the young nymphs, nor
even exact knowledge of what their food is, and the present writer
has evidence to disprove the statement that the newly hatched
nymphs are all alike.

The second view was regarded as possible by a few of the older
naturalists; the evidence for it is to be found in the recent writings
of Bugnion ('12, '13), and in the work of the present writer, to
be presented in this paper. The observations of Knower ('94)
and Snyder ('13, '15) on the origin of the soldier caste furnish
additional evidence, and important arguments in favor of this
view may be found in certain studies upon other social insects,
notably, von Ihering ('03), upon the development of the stingless
South American bees, and Wheeler ('07), upon polymorphism
in ants.

HISTORICAL REVIEW

I

In this section the work of those writers will be reviewed who consider extrinsic factors—food, care, parasitic influence—as the determining cause, either directly or indirectly, of caste; or who, as in the case of some of the older writers, do not especially consider the question of caste origin.

The work of König (1779) may be regarded as one of the first exact accounts of termite life. A résumé of his observations is given in Hagen's monograph ('55–'60). König lived for many years in India and made numerous observations upon the termites in his vicinity. He described the nests, the eggs and young, and both the young and the enlarged queens. The workers he erroneously regarded as males.

Another early account of the biology of termites is found in a letter from Smeathman (1781) to the Royal Society of London. In his travels in Africa Smeathman became well acquainted with termites, and records his observations in a most interesting although somewhat inaccurate manner. He observed three castes of termites: "(1) the labourers, (2) the soldiers, (3) the perfect insects." He described the different kinds of nests, the royal chamber, the enlarged queen, and the habits of the castes. A plate, which is now classic, shows the royal cell and its many occupants. Alluding to the small cavities containing the young, he says:

I call them the nurseries because they are invariably occupied by the eggs, and young ones, which appear first in the shape of labourers, but white as snow.

Between the years 1840 and 1850, a termite, since identified as Leucotermes lucifugus, caused great damage in France, in the villages of the Charente-Inférieure. The anatomy and biology of these termites and the means of preventing their ravages began to be studied by a group of French naturalists, among whom were Du Four ('41), Bobe-Moreau ('43), Joly ('49), Boffinet ('53), Quatrefages ('53), Lespès ('56). The names of

Bobe-Moreau and Boffinet should be recorded among the fathers of economic entomology.

Lespès is prominent among the men of this group who interested themselves especially in the biology and anatomy of termites. His monograph ('56) upon the structure and habits of Termes (Leucotermes) lucifugus, based upon careful observations and minute dissections, was an important contribution to science.

Referring to the youngest nymphs[1], Lespès says:

les larves les plus petites que j'aie pu voir avaient environ 1 millimetre de long; elles venaient de sortir de l'oeuf. Chez elles, il est impossible de distinguer plusieurs formes . . . . ce sont, pour moi, les larves du premier âge. . . . . Ainsi, tous les termites se ressemblent pendant le premier âge. La différence des neutres et des sexués commence au second.

Lespès also gives an account of the metamorphosis of the soldier from a worker-like nymph, which will be quoted in a later section, p. 124.

Darwin ('59) in the eighth chapter of the 'Origin of Species', under the heading of "Objections to the Theory of Natural Selection as applied to Instincts: Neuter and Sterile Insects" states:

The subject well deserves to be discussed at great length, but I will here take only a single case, that of working or sterile ants. How the workers have been rendered sterile is a difficulty; but not much greater than that of any other modification of structure. . . . . But I must pass over the preliminary difficulty. The great difficulty lies in the working ants differing widely from both the males and the fertile females in structure, as in the shape of the thorax, and in being destitute of wings and sometimes of eyes, and in instinct. . . . . According to M. Verlot, some varieties of the double annual stock from having been long and carefully selected to the right degree, always produce a large proportion of seedlings bearing double and quite sterile flowers; but they likewise yield some single and fertile plants. These latter, by which alone the variety can be propagated, may be compared with the fertile male and female ants, and the double sterile

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[1] The term nymph is used in this paper to denote any developmental stage of an insect with incomplete metamorphosis, whether the form possesses wing pads or not. The older authors used the term 'larva' to denote the younger nymphs in which wing pads were not visible to the naked eye.

plants with the neuters of the same community. As with the varieties of the stock, so with social insects, selection has been applied to the family, and not to the individual, for the sake of gaining a serviceable end.

Fritz Müller ('73–'75), who devoted many years to the study of termites, states that he, in accordance with Lespès, found the young 'larvae' of the different castes all alike, but that, before they have attained half the size of a worker, the future sexual forms may be recognized by their wing buds, the future workers and soldiers by their thicker heads and the absence of wing buds.

The causes, phylogenetic as well as ontogenetic, of the castes of the social Hymenoptera, are discussed by Weismann in several of his works ('92, '93, '94, '02). In his famous controversy with Herbert Spencer in the Contemporary Review during the years '93–'94, Weismann held that food is merely a stimulus acting upon predetermined units in the egg. In agreement with Darwin, Weismann held that the castes of the social Hymenoptera arose phylogenetically by the action of natural selection upon the fertile females, those which produced sterile offspring as well as fertile having a greater selection value. For the ontogenetic development of the castes Weismann assumed that the eggs of the fertile female contain a set of 'determinants' for every caste. In the development of the egg one set of determinants comes into activity, the others remaining inactive; the stimulus which causes the development of one set of determinants rather than another being the quality of the larval food.

In Weismann's own words (The Germ Plasm):

The individual is determined at the time of fertilization. The germ plasm must contain double-determinants for certain parts of the body of the queen and workers respectively. In the case of bees, the factor that determines which of the two halves of the 'female' determinants is to become active, seems to be the quality of the food applied to the larva.

It is well known of course that Weismann's theory is an argument for predetermination, but the complexity of his architecture of the germ plasm forced him to postulate a selective stimulus in the larval food.

Herbert Spencer ('93–'94) in his controversy with Weismann, defended, as is well known, the inheritance of acquired characters, and, as a logical consequence, believed in the direct action of food and environment upon the body. In regard to the origin of the castes of social insects Spencer held that:

the different structures of queen and workers are determinable by differences of feeding. Therefore the production of their different castes does not result from the natural selection of varying germ plasm.

A work which has been very widely read and quoted, and which has exerted an influence, probably greater than any other, upon biologists' views of the origin of termite castes, is a monograph by Grassi ('93–'94) in collaboration with A. Sandias. This monograph was translated by Blandford and published in '96–'97; the quotations here given are from the translation.

In his introduction Grassi states that the elucidation of the origin of castes in termites is of the highest interest, since it involves the theory of evolution, the question of the inheritance of acquired characters, and the constitution of the soma and germ plasm. His interest has been aroused by the study of bees, where nutrition apparently has a great influence upon the sex organs, which

would indicate that the environment has a powerful and direct influence upon the genitalia, and would therefore tend indirectly to show that the much disputed inheritance of acquired characteristics is a possibility.

The work deals with the habits, structure, and development of two termites, Calotermes flavicollis Fabr., and Termes (Leucotermes) lucifugus Rossi. The following is a brief statement of Grassi's observations and conclusions upon the origin of termite castes.

The newly hatched 'larvae,' 1 mm. long, are all alike, or undifferentiated; by the time they have attained a length of 2 mm. two types may be distinguished, with large and small heads respectively. From the 'large headed larvae' the sterile workers and soldiers develop; the 'small headed larvae' give rise to the reproductive forms and also to some sterile forms. Some 'undifferentiated larvae' are always kept in reserve by the colony,

and, at need, may be made into the 'substitute' or 'complemental' royal forms. The chief factor in differentiation is thought to be nutrition, the amount and quality of the food determining the large and small headed nymphs and from them the adult castes[2]. The presence or absence of parasitic protozoa in the alimentary tract and their inhibiting effect upon the developing sex organs of the workers and soldiers is regarded as a secondary factor, which itself may be affected by the quality of the food. In other words, the food, 'saliva,' administered to those 'larvae' which are to become sexual forms, has the property of destroying the protozoa which may enter the intestine; the food of the 'larvae' to become workers and soldiers permits the intestinal protozoa to live, and in some way the protozoa check the growth of the developing sex organs.

The following quotations will serve as a further exposition of Grassi's view.

The gonads are sexually differentiated, and the vasa deferentia and oviducts are present at the time of birth, but there is no trace of the external genitalia, which do not become evident until the larvae are divisible by the greater or less size of the head. . . . . Newly born larvae receive nothing but saliva, but little or none is administered later to those in progress of becoming workers or soldiers. . . . . A large amount of saliva is administered to examples destined to become neoteinic; it causes the disappearance of the parasitic Protozoa. The importance of this disappearance is not clearly understood, but it is certainly insufficient by itself to produce neoteinia. . . . . Although details cannot be given it may be concluded that the development of the soldiers and workers is consequent on the less quantity of saliva which they receive; and with this is associated the earlier appearance of Protozoa, and their constant presence in great numbers. The method by which the soldier is further differentiated from the worker is a more obscure matter to determine. Is the larger amount of nutriment which the latter receives the cause of such a phenomenon? In any case it is certain that the essential factor is one of nutrition.

Emery, in various writings upon the polymorphism of social insects ('93, '96, '04, '10) advances a peculiar view which seems like a special modification of Weismann's theory. Emery

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[2] Grassi, distinguishes three kinds of fertile adults; 'perfect insects,' 'substitute' or 'neoteinic' forms, 'complemental' forms.

believes that the distinctive characters of the castes of social insects are present in all eggs, not as determinants, but as special sensitive conditions of the germ plasm, which manifest themselves only under specific external conditions or with specific stimuli. The quality and the quantity of the food are important stimuli and indirectly produce differentiation. "Senza dubbio si regola l'evoluzione individuale delle Termiti con mezzi che sono, almeno in massina parte, dietetici, ma non sappiamo quali."

Silvestri ('02, '03) holds the view that food is the determining factor in caste differentiation, although an indirect factor, since the germ plasm is also involved. He writes:

Abbiamo visto che le uova delle Termiti sono tutte uguali fra di loro e che da esse, a volontà degli operai, per mezzo di una speciale nutrizione si possono svillupare o individui alati o operai o soldati, quindi l'idioplasma di ciascun uovo sotto lo stimolo di un cibo diverso è capace di reagire diversamente: di far svilluppare alcuni caratteri somatici ed altri arrestarne. Ciò è ormai un fatto accertato per tutti gli insetti sociali e non si può intorno ad esso sollevare dubbio di sorta.

La questione sta nel come l'idioplasma di un uova di Termitide possiede anche i germi dei caratteri di operai e soldati una volta che esso è generato da individui sessuati con alcuni caratteri affatto diversi da quelli degli operai e soldati, che sono sterili.

Heath ('03) studied the habits of three California termites, Termopsis angusticollis, Calotermes castaneus, and Termes (Leucotermes) lucifugus, and makes the following general statement regarding the origin of the castes:

It is now thoroughly well established that the soldier and worker in the termite colony are not the result of the arrested development of the reproductive organs. It has also been shown that they are not restricted to either sex. And it is almost equally certain that their differentiation is not to be traced back to the newly hatched young. The latter, when they first appear. are exactly alike in form and color, though they may exhibit slight differences in size, and the characteristics of the different castes develop at varying times after the first molt. If then neither arrested development, nor sex nor heredity are directly responsible for the production of soldiers and workers, what is the agency immediately concerned? Grassi is of the opinion that it is the food. Owing to its character or amount or both, the royal pairs of the colony are able to transform the larvae into soldiers, workers or perfect insects. . . . . For months I have fed a large number of termite

colonies of all ages, with or without royal pairs, on various kinds and amounts of food—proctodeal food dissected from workers or in other cases from royal forms, stomodeal food from the same sources, sawdust to which various nutritious ingredients had been added—but in spite of all I cannot feel perfectly sure that I have influenced in any unusual way the growth of a single individual.

It is interesting to note that these careful experiments produce only negative evidence for the food hypothesis.

Desneux has written a number of papers on the biology and the classification of termites. In the Genera Insectorum, Isoptera ('04), he states his view that the different castes arise from eggs that are identical, that the newly hatched 'larvae' are all alike, and that the probable cause of the differentiation of the young 'larvae' is a particular diet.

Escherich ('09) reaffirms Grassi's view of the origin of termite castes:

Gehen wir auf das erste, jüngste Stadium zurück, so gibt es überhaupt noch keine Unterschiede, sondern sind sich alle Jugendformen vollkommen gleich. Nicht nur äusserlich, sondern auch ihren Werte, ihren Entwicklungspotenzen nach, d. h. die eben ausgeschlüpften Larven sind noch völlig indifferent und können sich sowohl zu Arbeitern, als Soldaten, als auch geflügelten entwickeln.

Escherich believes that food plays the chief rôle in the differentiation of the castes, but he assumes, with Weismann, that food is not the direct cause, but merely the stimulus which brings into being the potentialities of one or another of the different castes, these potentialities being present in every egg.

Holmgren ('09), who has written exhaustively upon the systematic position and the anatomy of termites, explains the origin of castes by his exudation theory (Exudat-theorie). According to this view the newly hatched nymphs are 'indifferent' or all alike in appearance, but from the beginning some nymphs may receive a little more food and may produce a little more exudation than the others, and are consequently more frequently licked and better cared for by the workers, and finally develop into the 'small headed' or sexual forms. The nymphs, which at first get less food and produce less exudation, receive less care

and food from the workers and become the 'large headed' or sterile forms.

Holmgren's own words are:

Wenn nun der oben gegebene Zusammenhang zwischen Fütterung und Exsudatabsonderung gilt, indem die Menge der Exsudatabsonderung die Art der Fütterung bestimmt, so scheint es selbstverständlich, dass die Exsudatabsonderung mit der Kastenbildung in nahem Zusammenhang steht. Denn Grassi und Sandias haben gezeigt, dass die Fütterung wahrscheinlich als Faktor der Kastenbildung anzusehen ist. Diese Auffassung ist auch allgemein angenommen worden.

Holmgren also states that food may be the determining cause of sex, more food producing females, less, males.

Feytaud ('12) has made a careful and very valuable study of the common European termite, Leucotermes lucifugus Rossi, supplemented by some comparative work with certain other species. In regard to the origin of the castes he states that the newly hatched nymphs are all alike, and that the differences between the future neuters and sexual forms appear only after the first molt, which takes place when the nymphs have attained a length of 2 mm. and have twelve antennary segments. In agreement with Grassi, Feytaud recognizes, after the first molt, the large headed nymphs destined to become workers and soldiers, and the small headed nymphs which will develop into the sexual forms. The cause of the differentiation of these two forms, and of the differentiation from them of the future castes, is, according to Feytaud, the treatment and food given by the parents or the adult workers to selected groups of the developing nymphs.

But, although Feytaud accepts the prevailing view that food and care determine the castes, in his careful and exact study of the internal anatomy of the youngest forms, there is evidence which gives strong support to our second view that the castes are determined before hatching. This evidence is to be found in Feytaud's statement that, possibly, two types of nymphs— with differences in the degree of development of the sex organs— exist from the time of hatching.

Les larves au stade le plus jeune ont des organes génitaux extrême-
ment réduits. . . . . Il est possible que, dès cette période de l'exist-
ence des larves, il y ait déjà lieu de distinguer deux catégories d'individus
au point de vue du développement des organes génitaux, ceux qui
doivent devenir sexués les ayant plus développés.

Again, in his study of the sex organs of the older stages there
is further evidence in favor of caste predetermination, but once
more Feytaud attributes these conditions to differences in régime.
He finds that in the second stage (2.25 to 3.75 mm.) the sex
organs are larger in the small headed, sexual, nymphs than in
the worker-soldier nymphs. The sex organs of both types are
now larger than in the first stage, which indicates that no arrest
of development has yet taken place in the worker-soldier gonads.
In stages III and IV (3.75 to 6 mm.) the sex organs of the sexual
nymphs increase greatly in size, and the cells are in the phases
of ovocytes and spermatocytes; on the other hand, the sex organs
of the worker-soldier nymphs have remained stationary, and
continue so into the adult stage, with the size found in stage II,
and the cells in the phases of ovogonia and spermatogonia.
Feytaud believes that the arrest of development is not final in
the worker-soldier type, but that development may continue
when an individual has the same treatment as a sexual nymph:

Il est probable que l'arrêt de développement des organes génitaux
n'est pas forcément définitif, et que ces organes conservent la faculté
de poursuivre leur évolution, si, à une période quelconque, l'individu
est soumis au même régime que les larves de sexués.

Feytaud notes that the nymphs of the second form have larger
and better developed sex organs than the nymphs of the first
form of similar age, and concludes that there is acceleration of
development in one case, retardation in the other, and that both
are due to differences in food and care.

Les nymphes de 2me formé ont donc des organes génitaux beaucoup
mieux développés que les nymphes de 1re forme du même âge.
"Les deux catégories de nymphes proviennent de larves qui, au
1re âge, étaient identiques. Il s'est donc produit une accélération de
développement dans un cas, un rétard dans l'autre. Cette différence
d'évolution est certainement sous la dépendence d'une différence de
régime.

v. Rosen ('13) in a study of the eyes of termites, beginning with the newly hatched nymphs, writes:

Wie wir gesehen haben, besitzen die jüngsten Termitenlarven alle gleichgestaltete Augenanlagen. Dieses Verhältnis bleibt auch noch kurze Zeit bestehen, nachdem die Differenzierung in Larven mit grossen und solche mit kleinen Kopf eingetreten ist. Man kann nur annehmen dass durch verschiedene Ernahrung aus der indifferenten Larve beide Formen gezüchtet werden.

In L. flavipes the 'anlagen' of the eyes are not all alike in the youngest nymphs, but two distinct types of eyes may be distinguished at the time of hatching. The present writer believes that a reexamination of the youngest nymphs of L. lucifugus would also reveal two types of eyes.

II

This section deals, in the first place, with the works of those writers who believe that some, if not all, of the castes of termites are present at the time of hatching, and that the castes are therefore predetermined in the egg by intrinsic factors which are yet to be discovered. In the second place, those writers will be considered whose work is in harmony with, and indeed furnishes evidence for the above stated view.

In the monograph of Bobe-Moreau ('43) we find what is probably the first observation of any caste differentiation among newly hatched nymphs. He states:

Un très-petit soldat, né au printemps 1842, mêlé à des larves que j'avais rassemblées, me fut une preuve qu'il était sorti de l'oeuf avec sa forme. Les soldats ne subissent donc point de métamorphose.

This observation of Bobe-Moreau's is attributed by Fritz Müller ('73–'75) to Boffinet. On p. 463 Müller remarks:

Eine bis jetzt vereinzelte Ausnahme bildet der von Bofinet beobachtete Soldat, der so klein war, dass er als solcher das Ei verlassen zu haben schien.

I can find no description of any such soldier nymph in the writings of Boffinet, so that I am inclined to believe the sub-

stitution of one name for the other was merely an error on the part of Müller.

Hagen ('55–'60) begins a section entitled "The different kinds of larvae and nymphs" with the remark, "Der dunkelste Punkt in der Naturgeschichte der Termiten ist die Erklärung der verschiedenen Arten von Larven." Hagen believed that the worker and soldier are arrested 'larval' stages, while other 'larvae' continue to develop and become the nymphs and imago; he states that he bases this view partly upon his own observations, partly upon two letters from H. W. Bates, then in Santarem.

Under the date of April 19, 1854, Bates writes[3]:

I believe that the larval forms from which the nymph and imago arise are a special caste, and that the workers and soldiers, the feeding caste, perhaps immature males and females, undergo no further development.

In a letter of April 29, 1855, Bates writes:

I think you will agree with me that the different castes in termites are composed of (1) soldiers and workers, which begin and end their lives as such, (2) individuals which develop into nymphs and imago, and from their earliest age form a separate caste. The point which still needs investigation is, whether these latter forms hatch from the egg as a special caste, or whether they are produced by artificial means, namely by special food in special chambers, as the queen bee is made from a worker. . . . . I have observed the queen in her cell and the heaps of eggs which swarms of the workers were carrying to all parts of the nest, but I found no differences in the eggs, nor any definite method of distributing them in different parts of the nest. In the second place, I saw small pale individuals which had evidently just hatched, but I could not distinguish among them those which would develop into nymphs. In the third place, in a larger stage of the young pale individuals, I could instantly distinguish the future nymphs. . . . . I have come to the conclusion that the individuals from which the nymphs and imago arise are not produced by a special food, because at the time when nymphs, young workers, and soldiers can be distinguished (before the wing buds appear) they are found in the same chambers with other individuals of different castes, of all ages and sizes, all massed together and feeding. Whenever I open a mound I notice this. It is not impossible that a still earlier age may be the time when the individual receives a special care, but it seems improbable. So that it must be assumed that all individuals from which the males and females develop form a special caste from the time of hatching.

[3] The above quotation is translated from Hagen's text, which is a translation of Bates' original letters.

Dewitz ('78) writing of the postembryonic development in insects, thinks it highly improbable that the workers can affect the eggs and the young by treatment, such as warmth, food, licking. He considers that the great difference between queen and worker ant could not have been caused by the care of the adult workers, but that the eggs must be predetermined within the body of the mother.

In the case of termites Dewitz finds it hard to believe that the workers can exert any influence upon the young 'larvae,' since the latter can get their own food. He concludes that, in spite of the similarity of the young 'larvae,' the germ of the future form, either winged or wingless, lies ready in the egg. Referring to the work of Dzierzon, Dewitz ends with the remark: "Möchten recht bald zwei ähnliche Männer auftreten, welche gleiches Licht über die Entwicklung der Ameisen und Termiten verbreiteten."

Herbst ('01) in a work entitled "Formative Reize in der thierisschen Ontogenese" devotes one section to the consideration of "external formative (morphogenic) stimuli." Beginning with the developmental causes of the castes of social insects, he reviews the work of Grassi and Sandias on termites, and remarks that it must be recognized at once that there are many gaps in our knowledge of caste differentiation. He points out that while Grassi states that 'saliva' is the cause of differentiation in termites, nothing is known about its nature except that it "è un liquido incoloro spiccatamente alcalino e non continente alcun elemento rilevabile al microscopio." After discussing Grassi's hypothesis of the effect of saliva upon the parasitic protozoa and their possible influence upon the sex organs, the question of the origin of worker and soldier, and the production of the 'substitute' royal forms, Herbst concludes with the words,

Es dürfte aus vorstehenden Erörterungen hervorgehen, dass unsere Kenntnisse von den Bildungsursachen den verschiedenen Termiten- kasten noch nicht gerade sehr tief gehen.

Having analyzed the question of caste differentiation in the social Hymenoptera, Herbst suggests that an internal secretion

from the sex organs may be the cause of the differentiation of the worker caste, and adds that this view has already been advanced by O. Hertwig ('98).

Forel ('04) in a delightfully broad analysis of the subject of polymorphism and variation in ants, remarks upon the authority that may cling to a formerly widely accepted dogma. He cites the recent work of Janet and Wheeler as evidence against the hypothesis of qualitative feeding as a factor in caste production.

Aber auch mit der künstlichen Ernährung zu verschiedenen polymorphen Formen, an welcher Prof. Emery fest hielt, habe ich mich aus verschiedenen Grunden nie befreunden können. Wie könnte die verschiedene Quantität der Nahrung 3 so verschiedene Formen wie Weibchen, Soldat und Arbeiter hervorrufen?

Von Ihering ('03) in a study of the stingless bees of Brazil shows that in the genus Melipona the brood cells are all of equal size. The same kind of food, honey and pollen, is placed in each cell, an egg is laid in each, and the cells are then sealed up. From these similar cells, containing similar food, the three well defined castes emerge. The queen of Melipona hatches with small immature eggs in her ovaries.

In the genus Trigona, the queens hatch from large brood cells which project beyond the edge of the comb and contain a large store of honey and pollen; the drones and workers emerge from smaller cells. The queen of Trigona hatches with large ripe eggs ready to lay. The conclusion is that the larger queen cells and the greater amount of food are only the means of hastening the development of the sex organs, and not the factor that produces a queen. Wheeler ('07) suggests, in commenting upon von Ihering's observations, that the 'royal jelly' fed to the queen larva of the honey bee is "merely an adaptation for accelerating the development of the ovaries," since the queen honey bee hatches in about sixteen days and with nearly ripe eggs in the ovaries, while the worker requires four or five days longer for her development. "If this interpretation is correct the qualitative feeding of the queen larva is not primarily a morphogenic but a growth stimulus." It is disappointing that Nelson ('15) does not discuss this question in his "Embryology of the Honey Bee."

Wheeler ('07), writing on the polymorphism of ants, makes a careful examination of the two opposed theories of the influence of food upon organisms and of hereditary predetermination. He draws attention to the tendency of the physiologist to study an animal from the point of view of its reactions to external stimuli, while an embryologist would look rather for evidences of predetermined differentiations, and he remarks, further, that the embryologist has been at a serious disadvantage in his study of the social Hymenoptera, in which, so far, no determining caste characters have been detected in either the eggs or young larvae.

On the question of the influence of larval food Wheeler states:

Closer examination of the subject, however, cannot fail to show that larval alimentation among such highly specialized animals as the social insects . . . . is a subject of considerable complexity. In the first place, it is evident that it is not the food administered that acts as a stimulus, but the portion that is assimilated by the living tissues of the larva. . . . . In the second place, while experiments on many organisms have shown that the quality of assimilated food may produce great changes in size or stature, there is practically nothing to show that even very great differences in the quality of the food can bring about morphological differences of such magnitude as those which separate the queens and workers of many ants.

Wheeler next enumerates a number of concrete instances tending to disprove the assumption that qualitative feeding can produce morphological differences in the worker, but he goes on to state that quantitative feeding may produce diminution of stature and some of the atypical phases found in ants.

Such variations are of the fluctuating type and are therefore attributable to the direct effects of the environment. The soldier and worker, however, differ from the queen in the absence of certain characters, like the wings, wing-muscles, spermatheca, some of the ovarian tubules, etc., and the presence of other characters, like the peculiar shape of the head and mandibles. In these respects the sterile castes may be regarded as mutants, and Weismann's contention that such characters cannot be produced by external conditions, such as feeding, is in full accord with de Vries's hypothesis. . . . . It must be admitted that a direct causal connection between underfeeding on the one hand and the ontogenetic loss or development of characters on the other, has not been satisfactorily established. The conditions in the termites, which are often cited as furnishing proof of this connection, are even more complicated and obscure than those of the social Hymenoptera.

Bugnion, who has made extensive studies upon the termites of Ceylon, is the first modern biologist to present evidence for the view that the castes of termites are determined in the egg, before hatching. He states, ('12, '13), that the generally accepted view that the castes are produced by food, the presence of parasites etc. is not in accord with the facts that he has personally observed.

Among a number of newly hatched nymphs of Eutermes lacustris, examined under the microscope, Bugnion found one individual, 1.3 mm. long, with the distinct frontal horn and well formed frontal gland characteristic of the soldier caste. After further investigation, he states that two or three such nymphs are to be found in every hundred of the newly hatched forms. He concludes from these observations that, in general, the termite soldier type is differentiated in the egg, and that its origin goes back to a deep seated cause, probably analogous to the cause of sex.

The following facts are brought forward by Bugnion as additional evidence for this view:

1. In the mushroom-feeding termites no one has observed that the future sexual forms are given a special food (saliva) at first.

2. There are no actual observations that the action of saliva affects the egg; the eggs are not sorted into lots, but lie in masses in the niches of the nests, and no one has recorded an observation that some are licked more than others.

3. The supposed action of the protozoan parasites is not to exert an injurious effect upon the development of the termites; on the contrary, the protozoa are of use, by partly digesting the wood in the intestine before being in their turn digested and absorbed.

Mon impression, après avoir examiné la question sous tous ses faces, est que les Trichonymphides ne sont pas des parasites, mais que leur existence est liée à celle des Calotermes par une vraie symbiose.

4. These protozoa are not present in all termites; they are absent from the higher Indian forms, where caste differentiation

is greatest, but are present in the lower genus Calotermes, where the castes are few.

Bugnion states his general conclusion in the following words: "Ma conclusion est que la différentiation s'effectue dans la phase embryonnaire pour les trois castes."

Knower ('94) reports the origin of the soldier of Eutermes (rippertii?) from a form which resembles a worker.

The adult soldier of this Eutermes has a frontal horn or process, a large frontal gland, small jaws, and thirteen antennary segments. The adult worker lacks the frontal horn and frontal gland, but possesses very large jaws and fourteen antennary segments. Among his first collections Knower could distinguish only worker 'larvae.' He then found a 'larva' with thirteen antennary segments, which was worker-like in its head and jaws but possessed a small frontal gland. Later a young soldier was discovered in the act of molting. The cast skin was worker-like, as shown by the head and jaws, but the newly molted soldier had the characteristic small jaws, longer head with the frontal horn, and a large frontal gland.

This discovery of Knower's seems to indicate that, although in its early stages the soldier caste of this species of Eutermes can not be distinguished from the worker by external characters, it does possess, probably from the moment of hatching, one internal character, namely the frontal gland, and probably more, which differentiate it from the worker caste.

Snyder ('15), in a study of the biology of the termites of the eastern United States, has investigated the later stages of development of the nymphs of Leucotermes flavipes, L. virginicus, and Termospis angusticollis. In regard to the earlier stages of development Snyder merely restates Grassi's view that the recently hatched 'larvae' are all alike and undifferentiated; the following quotations, however, show that he may doubt the accuracy of Grassi's classic theory:

P. 41, the writer has observed quiescent stages of undifferentiated (?) larvae . . . . p. 43, the larvae having belonged *to all external appearances*[4] to the undifferentiated group."

[4] Italics of the present writer.

Snyder records numerous observations on the quiescent stage of termites preceding a molt, which was first observed in L. flavipes by Wheeler, and described by Strickland ('11). He also describes and figures ('15, Pl. IX), the molting of soldier nymphs from worker-like forms with mandibles of the worker type. This phenomenon was observed in the three termites: L. flavipes, L. virginicus, and Termopsis angusticollis.

Mr. Snyder has kindly allowed the present writer to examine the mounted heads of soldiers of L. flavipes and L. virginicus which had just molted from a worker-like skin, on which the workerlike mandibles are clearly seen. It is interesting to note that in both species the newly molted soldiers have a large well defined frontal gland, which, in the worker, is merely vestigial (Thompson ('16) pp. 582–583). This fact proves for Leucotermes, as Knower's work did for Eutermes (rippertii?), that the worker-like nymph from which the soldier develops resembles the worker in external characters only, but internally possesses the distinctive organs of the soldier caste.

THE ORIGIN OF THE CASTES OF L. FLAVIPES AND L. VIRGINICUS

A. INTRODUCTORY REMARKS

The present writer approached the problem of the origin of termite castes with the belief that the food hypothesis, founded as it is upon unproved assumptions, is inadequate to explain the phenomenon of differentiation, and with the hope that careful staining methods might reveal structural differences in the 'undifferentiated' or newly hatched nymphs.

My lack of belief in the food hypothesis is based upon the following reasons:

1. It has been stated that certain groups of the young nymphs are selected by the colony for special care, but, in opening a nest of L. flavipes, the youngest forms are found in masses, packed close together and filling a small cavity. There does not seem space enough for any effective selection within a group, and it is not known that the younger stages of the different castes are kept apart or in separate places in the nest; indeed, in col-

lecting the somewhat older nymphs that are distinguishable one from another, the different castes are always found mixed together.

2. It is stated that the diet of the newly hatched nymphs may vary in quantity and in quality. Weismann's experiments with the blow fly ('94) show that quantitative feeding produces changes in stature that are not inherited; on the other hand, von Ihering ('03) has shown that qualitative feeding is merely a stimulus for rapid development.

3. Even without the evidence of Weismann, von Ihering, and others, it is inconceivable that there could be a food powerful enough to act selectively upon certain organs of the body, for example, the brain and the sex organs, and produce types as diverse as the reproductive forms and the workers and soldiers. It was this difficulty that led certain supporters of the food hypothesis to assume the presence in a termite egg of the potential structural 'tendencies' of all the different castes, or of special sensitive conditions of germ plasm, which were manifested only by food, food therefore playing the rôle of the indirect rather than the direct cause of differentiation.

Now, instead of assuming the presence of the characters of all the different castes in a single egg, and in all eggs, how much simpler is the assumption that there are different kinds of eggs, and that each egg contains the predetermined characters of a single caste. With this assumption, the structural differentiations of the various castes, or their prototypes, may be looked for at the time of hatching.

My hope of finding internal structural differences in the newly hatched nymphs of L. flavipes and L. virginicus has been very satisfactorily realized, and these differentiations will form the subject matter of the remainder of this paper.

B. MATERIAL AND METHODS

My material was collected in the summer and autumn of 1916, partly by the writer, partly by Mr. T. E. Snyder, of the Bureau of Entomology of the U. S. Department of Agriculture, to whom

I am indebted for specimens of the young of both L. virginicus and L. flavipes. The photo-micrographs shown in figures 38 to 42 were taken, from slides made by the writer, by Mr. John H. Paine, of the Bureau of Entomology, United States Department of Agriculture, and I wish to thank the Bureau of Entomology for the many courtesies extended to me.

The nests were found in the interior of partly decayed logs, which were chopped open, and the young nymphs were then picked up with fine forceps and dropped at once into the fixative. Bouin's fluid, which has proved most successful for the older stages of Leucotermes, was the chief fixative used; and it is very satisfactory for all except the youngest nymphs. For these, a fixative with a more rapid penetration would be better; Bouin's fluid penetrates slowly, and if the digestive tract contains much food, the posterior part of the intestine and sometimes even the oesophagus is protruded, injuring the end of the abdomen and the sex organs, or the brain. Unfortunately I did not discover this until the collecting season was past, but out of the large numbers of very young nymphs collected there were many in good condition. One lot of material fixed in alcoholic corrosive-acetic gave excellent results.

Before staining it is necessary to puncture the body cuticula with very fine needles sharpened on a stone. For preliminary study whole mounts were made of the entire insect by staining for two days in Conklin's picro-haematoxylin and destaining in acid alcohol from thirty-six to forty-eight hours, until the desired differentiation was obtained. The specimens were then rapidly dehydrated, otherwise they become very brittle, cleared in cedar oil by the sinking method, and mounted in damar. Meyer's carmalum was also tried for staining whole mounts, but although it is more permanent than the picro-haematoxylin, it gives a much less sharp differentiation and has not been used to any extent.

The specimens to be embedded and sectioned were also stained by the Conklin method, and each individual was examined in cedar oil under the dissecting lens in order to determine the type, sex, etc.; the different types were then kept separate. The

specimens were next transferred from cedar oil to xylol and embedded in paraffin of 54° melting point for one hour or longer, according to the size. The previous staining was found indisspensable for orienting the insect in paraffin. Most sections were cut in the horizontal, frontal, plane, 5 micra thick, and were stained on the slide with iron haematoxylin and orange G. The chitin is so thin in the youngest nymphs that complete series of sections can be obtained.

C. SCOPE OF THE WORK

My work deals chiefly with the development of the nymphs of L. flavipes, from the time of hatching, when they are about one millimeter long, up to the first molt, when they have attained a length of 2.25 mm. The newly hatched nymphs and a few of the older phases of the southern species, L. virginicus, have been studied, but not with the sequence and detail given to L. flavipes; enough has been done, however, to state that the development of the two species is very similar and probably identical. To determine the origin of the soldier caste of L. flavipes, older nymphs, from 2 to 4 mm. long have been studied.

D. THE DEVELOPMENT OF L. FLAVIPES, FROM THE TIME OF HATCHING (1.1 MM.) TO THE FIRST MOLT (2.25 MM.)

The period between hatching and the first molt was called stage I by Grassi, who described it for Leucotermes lucifugus as follows:

"I. Very young larvae, the head of which is alike in those of equal length."

These Grassi divides into: Forms scarcely 1 mm. long, antennae of eleven segments, the third bare; Forms 1 to 2 mm. long, antennae with eleven hairy segments, or twelve, the third and fourth bare; Forms 2 mm. long, antennae with twelve segments, the third bare.

Stage II of Grassi consists of forms from 2.25 mm. to 3.75 mm. long, with twelve or thirteen antennary segments, and contains: 'Larvae' with large heads, and 'Larvae' with small heads.

In the present study of L. flavipes I have divided the period between hatching and the first molt, which is, of course, a continuous series of phases, into the early, middle, and late phases of growth.

In selecting specimens for preliminary study, body length was at first used as a criterion of age, but, except with living material, body length is such a variable quantity, depending largely upon the presence or absence of food in the alimentary tract, that it was soon abandoned, and the exact age of a specimen was determined by the number of antennary segments and the condition of the third segment, whether entire or grooved, bare or hairy.

In the phases selected for description, see table 1, the number of antennary segments is as follows: early phases, (1) nine, third segment grooved; (2) ten, third segment entire and bare; middle phases, (3) ten, third segment grooved; (4) eleven; end phases, (5) twelve, third segment entire and bare; (6) twelve, third segment hairy. The approximate body lengths for these phases are: early phases, 1.1 to 1.2 mm.; middle phases, 1.3 to 1.6 mm.; end phases, 1.7 to 2 mm.

It will be noted that the number of antennary segments in the newly hatched nymphs of both L. flavipes and L. virginicus is nine, which differs from the number, eleven, stated by Grassi for L. lucifugus. It will further be noted, table 1 and figures 1, 3, 6, 7, 39, that two distinct types, which I have termed the reproductive type and the worker-soldier type, are distinguishable at the time of hatching, and thereafter may be traced throughout the series of phases from hatching to the first molt, figures 6 to 17. In other words, the 'small-headed' and 'large-headed' forms of Grassi do not appear for the first time after the first molt, but may be traced back to their prototypes in the newly hatched nymphs, namely, to the reproductive type and to the worker-soldier type respectively.

The general characters by which the reproductive type and the worker-soldier type may be distinguished are: (1) the size and character of the brain and its relation to the size of the head;

TABLE 1

Leucotermes flavipes Kol. Phases in the first stage of development, from time of hatching to first molt

PHASE	TYPE	CASTE	NUMBER OF ANTENNAL SEGMENTS	LENGTH ON BODY (mm.)	MEASUREMENTS OF HEAD — Width of head (spaces)	MEASUREMENTS OF HEAD — Width of head (spaces)	TYPE OF BRAIN — Bulk	TYPE OF BRAIN — Size of optic lobes	EYES	SEX ORGANS
Early — 1	Reproductive		9, 3d segment grooved	1.1	22*	20	Large	Well developed	Small	Large
	Worker-Soldier		9, 3d segment grooved	1.1	20	13	Small	Small	Small	Small
Early — 2	Reproductive		10, 3d segment entire	1.2	24	22	Large	Well developed	Small	Large
	Worker-Soldier		10, 3d segment entire	1.2	23	17	Small	Small	Small	Small
3	Reproductive	1st form	10, 3d segment grooved	1.3	23	20	Large	Well developed	Large	Large
		2d form			23	19	Slightly smaller	Well developed	Large	Medium sized
	Worker-Soldier		10, 3d segment grooved	1.4	25	18	Small	Small	Small	Small
Middle — 4	Reproductive	1st form	11, 3d segment entire	1.4	26	24	Large	Well developed	Large	Large
		2d form			26	23	Slightly smaller	Well developed	Large	Medium sized
	Worker-Soldier		11, 3d segment grooved	1.6	30	20	Small	Small	Small	Small
5	Reproductive	1st form	12, 3d segment not hairy	1.7	29	27	Large	Well developed	Large	Large
		2d form			29	26	Slightly smaller	Well developed	Large	Medium sized
	Worker-Soldier		12, 3d segment not hairy	1.8	32	25	Smaller	Small	Small	Small
End — 6	Reproductive	1st form	12, 3d segment hairy	2	30	27	Large	Well developed	Large	Large
		2d form			30	25	Slightly smaller	Well developed	Large	Medium sized
	Worker-Soldier		12, 3d segment hairy	2	40	26	Smaller	Small	Small	Small

* 1 Micrometer space = 0.016 mm.

(2) the size and structure of the compound eyes; (3) the size of the sex organs.

In the whole mounts from which figures 6 to 17 are drawn, the eyes of the reproductive and worker-soldier types appear equal in size, but figures 18 to 23, drawn from sections with high magnification, show that from the beginning there are marked, although slight, differences in the two types of eyes.

I wish to emphasize the value of well stained and cleared whole mounts, for without them this work could hardly have been accomplished. It is possible, however, to distinguish the two types of nymphs in unstained alcoholic specimens, the size of the brain making a good criterion in a well preserved individual. These specimens also show that the abdomen of the worker-soldier type is frequently more translucent than that of the reproductive type, owing to the difference in the amount of fatty tissue.

Early phases

Phase 1. The newly hatched nymphs of L. flavipes. (figs. 1, 6, 7, 39.) A newly hatched living nymph of L. flavipes is pure white and about one millimeter long; in well preserved specimens the length, after fixation, is 1.1 mm.

The antennae consist of nine segments, eight of which are hairy; the third segment is without hairs, and its surface is slightly grooved, so that the later number ten is indicated, but the interior of the third segment is wholly undivided.

The heads of all the newly hatched nymphs are about equal in size (figs. 6, 7), the slight differences which may be noted in some specimens being due to individual contraction or expansion; the thorax and abdomen are also similar in specimens that are not contracted. The newly hatched nymphs of L. flavipes are therefore externally all alike.

This, however, is by no means true of the internal structure, as seen in the stained whole mounts and in sections, since very striking differences are to be observed within the head and in the posterior part of the abdomen, namely, in the brain and in the sex organs. These differences are so great and so constant

that they constitute the two sharply defined types of individuals which I have termed the reproductive type and the worker-soldier type, which, as stated above, are the prototypes of the 'small-headed' and 'large-headed' forms of Grassi, and also of the future reproductive castes and the future workers and soldiers (table 1 and figs. 6 to 17). The most striking differences between these two types are the absolute size and degree of differentiation of the brain, and the relative size of brain and head.
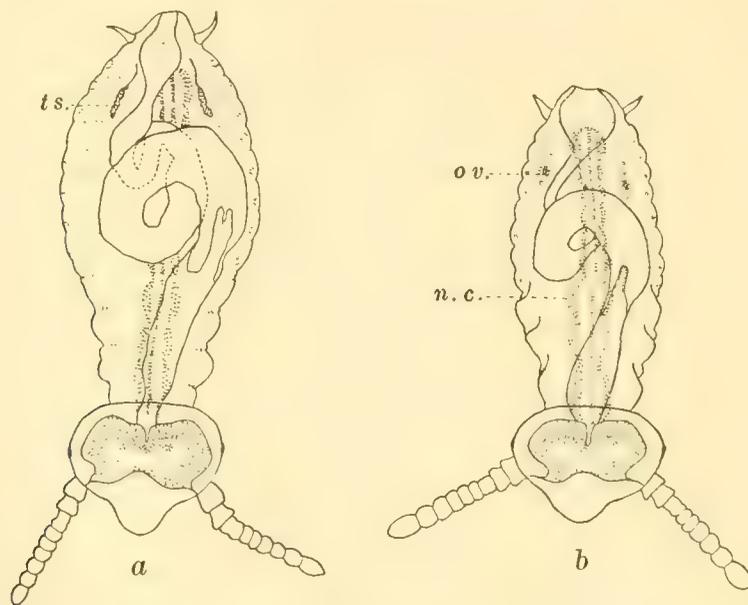
Fig. 1   Newly hatched nymphs of L. flavipes.   A, reproductive type, male; b, worker-soldier type, female; ts, testis; ov, ovary; n.c., nerve cord.   Oc. 6, obj. 16, reduced one half.

In the reproductive type (figs. 1a, 6,), the brain fills nearly the entire head, and is separated from the hypodermis by a very narrow area; the mushroom bodies, the optic lobes and the antennary lobes are all large, prominent and well developed.

In the worker-soldier type (figs. 1b, 7, 39), the brain fills only a portion of the head and is widely separated from the hypodermis; the bulk of the brain is much less than that of the reproductive type, and the mushroom bodies, the optic lobes, and the antennary lobes are all smaller and less differentiated.

Figure 2 a, in which the outlines of the two types of brains are superimposed one upon the other, shows the relative size

and differentiation of the two brains and their parts in the newly hatched nymphs.

The mushroom bodies of the reproductive type project prominently from the future dorsal[5] surface of the brain, and their surfaces are grooved, so that the outer and inner lobes are already indicated, and the pointed outer lobes are especially prominent. The mushroom bodies of the worker-soldier type are smaller and much less differentiated at this age. The outer
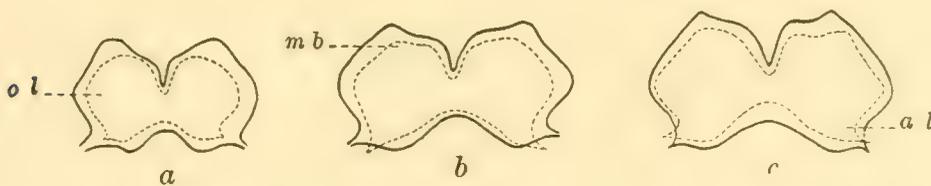
Fig. 2 Superimposed outlines of the brains of the two types of L. flavipes, the reproductive type in heavy outline, the worker-soldier type in broken line. A, brains of the newly hatched nymphs, body length 1.1 mm.; b, brains of nymphs with eleven antennary segments, body length 1.4 mm.; c, brains of nymphs with twelve antennary segments, body length 2 mm.; m.b. mushroom bodies; o.l. optic lobes; a.l. antennary lobes. Oc. 6, obj. 16, table level, reduced one half.

surface is smooth, since the inner and outer lobes are not yet indicated. The optic lobes of the reproductive type are very large and project laterally and downward toward the antennae. The outline of the downward curve of the optic lobes of this type is the best means I know for distinguishing the two kinds of brains, (figs. 41, 42), In the worker-soldier type the optic lobes are small and do not point downward. The antennary lobes are far greater in bulk in the reproductive than in the worker-soldier type. The relative size of these three parts of the brain is even more apparent in sections than in whole mounts.

[5] In life the heads of these termite nymphs are held in a slightly slanting position, making a large obtuse angle with the long axis of the body, so that the morphological dorsal surface becomes frontal or anterior. In the whole mounts the heads are flattened out and the frontal surface becomes dorsal again. The same terms of direction, however, will be used as for the adult termites. In describing the heads the terms anterior and posterior imply toward and away from the frontal surface, in like manner dorsal and ventral imply toward or away from the vertex of the head.

Sections show also that the brains are differentiated into the central fibrous core and the enveloping nerve cell layer, all the cells of which are alike and undifferentiated at this age, and in which mitotic figures are frequent.

All the parts of the brain which are present in the adult are found in the brains of the newly hatched nymphs, and with little difference from the adult except in size, with the one exception of the optic lobes, which, in both types, have but one fiber mass, instead of the three of the adult.

The head of the newly hatched nymph, as seen in frontal section, (figs. 33, 34, 41) is covered by a very thin cuticula, scarcely thicker than the layer of hypodermal cells which has secreted it. Within the head, and attached to the hypodermis on each side of the median line, are two broad bands of very slender muscle fibers which are the beginnings of the great mandibular muscles, the m. adductor magnus mandibulae of Holmgren.

The frontal gland—a gland found near the postero-dorsal surface of the brain, and which in a former paper (Thompson, '16), I have described as a degenerate median ocellus—is very slightly differentiated at the time of hatching and is barely distinguishable. It consists, in individuals of the reproductive type, of a very slight invagination of the hypodermal cells in the median line of the frontal surface of the head. In frontal sections, (fig. 33, *f.g.*) the invagination lies above the more frontal portion of the brain. Beneath the hypodermal invagination mesenchym cells form a delicate membranous sheath, the future basement membrane of the frontal gland, which extends backward to the posterior limits of the brain; the hypodermal invagination, however, has a much smaller extent, and is present only in three sections. In figures 34 *f.g.* the empty cup-shaped basement membrane is seen, at some distance posterior to the hypodermal invagination. Attached to the sides of the basement membrane are two slender muscles, the m. retractor fontanellae of Holmgren, which run down behind the brain to their distal attachment to the tentorium. In individuals of the worker-soldier type there is as yet no hypodermal invagination of the frontal gland, and the only traces of this organ are the

two slender mm. retractor fontanellae, shown in figure, 35 *f.g.*, attached to the mesenchymatous sheath, just beneath the hypodermis in the frontal region. The empty basement membrane may be observed above the brain in subsequent sections.

It will be recalled that the tentorium, or the chitinous framework within the head, originates as a cuticular excretion from the surface of a number of hypodermal invaginations that later meet and fuse. The tentorial invaginations still retain their connections with the hypodermis in this and in several succeeding phases, but very little of the chitinous cuticula is yet formed. The great delicacy of all parts of the head except the brain, is one of the causes of the frequent shrinkage observed in the heads of fixed specimens of the early phases. So far I have been unable to distinguish the lateral ocelli in the newly hatched nymphs.

The compound eyes are small and apparently equal in size in the two types of the newly hatched nymphs as seen in the whole mounts from which figures 6 and 7 are drawn. But in sections of this phase, (figs. 18, 19, 41, 42,) it may be seen that, from the beginning, there are differences between the two kinds of eyes. The compound eyes of the worker-soldier type of newly hatched nymph (figs. 19, 42) are very simple in structure, consisting merely of two ill defined layers of scattered undifferentiated cells. In one newly hatched worker-soldier individual, not figured, the eyes were composed of only a single row of cells. In the compound eye of the reproductive type (figs. 18, 41) the cells are in two layers, and a few are differentiated into large and small cells which already show a tendency toward the future grouping. This eye is almost twice as thick as that of the worker-soldier and contains a few more cells, but the two sections have about the same length, indicating that the diameter is nearly similar in both types of eyes. The fibers of the optic nerve may be observed issuing from the inner surface of the eye of the reproductive type, figure 18. These were not observed in the worker-soldier eye of this age, although present in older phases.

v. Rosen ('13), in a study of the termite eye, states that in L. lucifugus the compound eye 'Anlagen' are similar in all 'indifferent larvae,' and in the youngest 'small headed' and 'large

headed larvae.' He describes the eye of an 'indifferent larva' as a mass of undifferentiated cells, which later will differentiate into two layers of small outer cells and larger inner cells. He then describes and figures (figs. 11, 12) two sections from the eye of a small headed nymph with twelve antennary segments, which he evidently considers typical for all nymphs with this number of segments. On the contrary, the eye shown in v. Rosen's figures 11 and 12, is undoubtedly that of a reproductive type, and quite different from the eye of the worker-soldier type of the same age, which he has not recognized. v. Rosen's figure 13, a section of the eye of a 'small headed larva' with fourteen to fifteen antennary segments, is, of course, that of a reproductive type; his figure 14, from a 'large headed larva' with fourteen to fifteen antennary segments, is a typical worker-soldier type of eye.

I must repeat once more that, in L. flavipes, both types of eye are found from the beginning, side by side in the reproductive and worker-soldier types of the recently hatched nymphs, and it is probable that the same is true of L. lucifugus.

The reproductive organs of both sexes are distinguishable at the time of hatching and differ in size in the two types of newly hatched nymphs. This size difference is especially noticeable in the females, but it is also true of the males (figs. 24 to 27).

Grassi states that in L. lucifugus "there is no trace of the external genitalia, which do not become evident until the larvae are divisible by the greater or less size of the heads;" by 'external genitalia' evidently meaning the pair of cerci on the median line of the posterior edge of the ninth abdominal sternite. In L. flavipes and L. virginicus these cerci, as well as the more laterally placed pair on the tenth segment, are present in all nymphs at the time of hatching.

In the newly hatched reproductive type the ovaries are about one-third larger in general bulk than in the worker-soldier type. The oviduct is large and conspicuous, the ovarian tubules are long and numerous, and the egg cells are large (fig. 24). All of these characters are diminished in the worker-soldier ovaries. The oviduct is smaller, the ovarian tubules are fewer and shorter,

and the majority of the egg cells are smaller (fig. 25). In both types the ovarian tubules open into the sides of the oviduct, a characteristic of termites, and unlike the higher insects.

The testes are small in both types at the time of hatching, but the slight size difference is in favor of the reproductive type (figs. 26, 27). At this stage the testis has the general appearance of an irregularly swollen but slender tube, which at its posterior end is continued into the still more slender sperm duct. I am unable to distinguish any difference in the size of the male germ cells in the two types of nymphs at the time of hatching (figs. 26, 27).

The newly hatched nymphs of L. virginicus. The newly hatched nymphs of L. virginicus are about 0.8 mm. long in preserved specimens—I have not seen them alive—and externally are all alike, but internally they are differentiated, as in L. flavipes, into the reproductive and the worker-soldier types.

The same distinctive characteristics prevail in the two types of the two species (fig. 3 *a, b*). The reproductive type has the greater brain, with large mushroom bodies, optic lobes and antennary lobes, and larger sex organs; the worker-soldier type has the smaller brain, with small mushroom bodies, optic lobes and antennary lobes, and smaller sex organs. The antennae of both types have nine segments, the third segment grooved and bare. *Phase 2. Nymphs with ten antennary segments, the third segment entire and bare; body length, 1.2 mm.* (figs. 8, 9).[6]

The differences between the brains of the two types are as marked as in the first phase, although the mushroom bodies of the worker-soldier type are increasing in size and are becoming more differentiated, in that the outer, pointed, lobes can now be recognized. The individual of the reproductive type from which figure 8 is drawn, has evidently been slightly compressed either in fixation or after mounting. The position of the antennae and the distance between the antennary lobes indicates that there has been a slight horizontal compression of the head, which makes the brain appear a little larger than that of

[6] The nymphs described in phases 2 to 6 belong to the species flavipes, unless otherwise stated.

the reproductive type in figure 6 (cf. figs. 6, 8, 10). The compound eyes of both types have increased in size, but are still of about equal diameter, as seen in the who'e mounts. The sex organs are also growing larger.

Middle phases

Phase 3. Nymphs with ten antennary segments, the third segment gro ved; body length 1.3 to 1.4 mm., (figs. 10, 11, 38). The greater bulk and relatively higher degree of differentiation of the brain of the reproductive type is still to be observed in this phase, but the mushroom bodies of the worker-soldier brain are continuing to increase in size and complexity (figs. 10, 11).
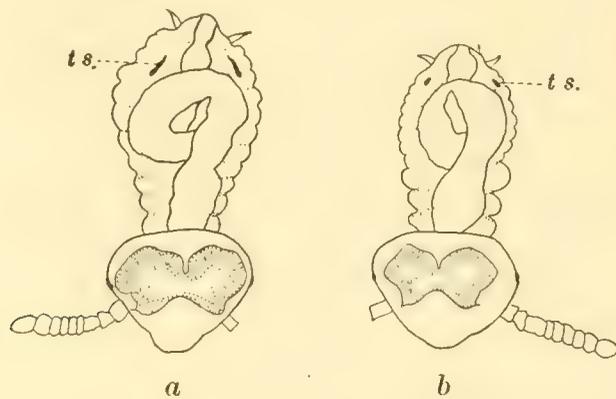
Fig. 3   Newly hatched nymphs of L. virginicus.   *A*, reproductive type, male; *b*, worker-soldier type, male; *ts*, testis.   Oc. 6, obj. 16, reduced one half.

By actual measurement under the compound microscope the head of the worker-soldier type is 2 micra wider than that of the reproductive type, (figs. 10, 11), table 1. It is therefore at this point in the first stage of development—one-fifth of the way between hatching and the first molt—that the first size differences in the heads of the famous 'large headed' and 'smal. headed' nymphs are to be noted.

Within the head the mandibular muscles have increased in bulk, spreading out as great fan shaped masses on each side of the median line. The tentorial invaginations of the hypodermis are still very conspicuous in this phase, and a thin cuticula, the future tentorium, is forming on their inner, opposed, surfaces;

the cuticula on the outside of the head is slightly thicker, and especially on the edges of the mandibles.

The frontal gland of both types consists of a small anterior hypodermal invagination and a posterior cup shaped basement membrane. The hypodermal invagination at this age extends only through three or four sections in the anterior or frontal part of the brain. The cup shaped basement membrane, however, extends backward through about ten more sections to the point where the fontanelle nerve will enter the posterior part of the brain, namely: between the posterior roots of the mushroom bodies. At this age, therefore, the frontal gland of both types of individuals consists of two different regions, the anterior formed by the hypodermal invagination resting on the basement membrane; the posterior, into which the hypodermal invagination has not yet grown, consisting only of the cup shaped basement membrane, partly filled by loose mesenchyme cells, and to the sides of which a pair of muscles, the m. retractor fontenellae are attached, The hypodermal invagination is slightly larger in individuals of the reproductive than in the worker-soldier type.

The compound eyes of the worker-soldier type have not increased in size to any extent; indeed, in surface views of the head, (figs. 10, 11), they appear of the same diameter as in the last phase. The eyes of the reproductive type, however, are considerably larger.

Another and a still more important differentiation has now manifested itself. The individuals of the reproductive type with a body length of 1.3 to 1.4 mm. are divisible into two groups: (1) forms with a more highly differentiated brain, as shown by the mushroom bodies, and large sex organs (fig. 4 *a*); (2) forms with a less highly differentiated brain, as shown by the mushroom bodies, and sex organs that are smaller, (fig. 4 *b*), although not so small as those of the worker-soldier individual drawn in figure 4 *c*.

Figure 5 *a* is a male of the reproductive type, 1.3 mm. long, with large brain and large sex organs. The male of the reproductive type with smaller brain and sex organs has not been figured,

since the brain is similar to that of the female in figure 4 *b*, and the sex organs are only slightly smaller than those of the male shown in figure 5 *a*.

The sex organs of the two varieties of the reproductive type and those of the worker-soldier type, drawn from whole mounts with an immersion lens, are shown in figures 28 to 32. Figure 30 represents a large ovary of the reproductive type. The oviduct is a tube with a wide lumen into which the ovarian tubules
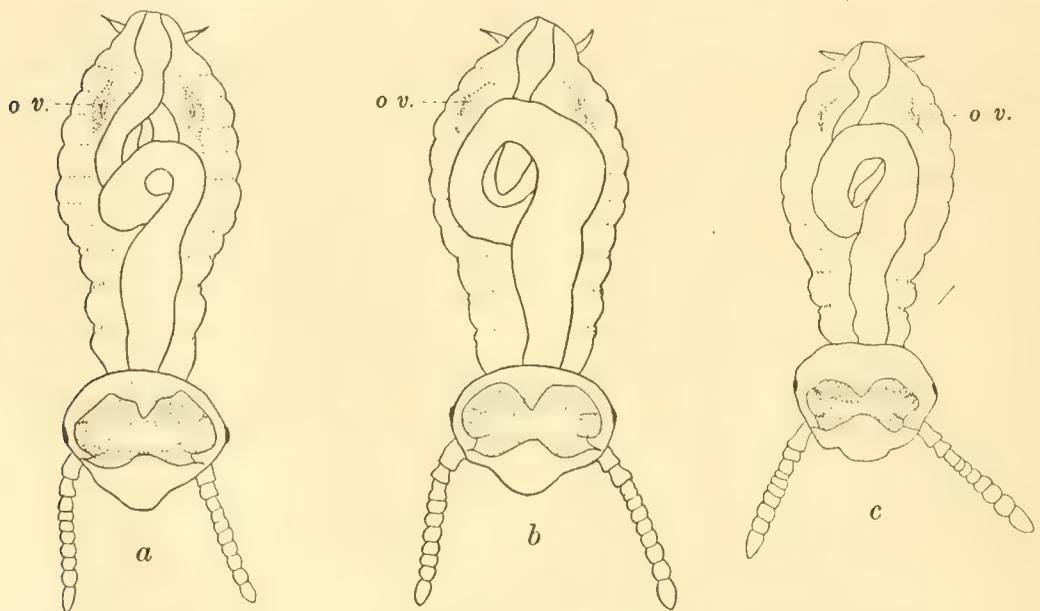
Fig. 4 Female nymphs of L. flavipes, with ten antennary segments, body length 1.3 mm. *A*, reproductive type of the first form, with large brain and large ovaries; *b*, reproductive type of the second form, with slightly smaller brain and smaller ovaries; *c*, worker-soldier type, with very small ovaries; *ov.*, ovary, Oc. 6, obj. 16, reduced one half.

open. The ovarian tubules are long and numerous, about seven to eight on the outer side, and eleven to thirteen on the inner, the proximal egg cells are large, the most distal ones very minute. Mitotic figures occur in a number of the larger egg cells. I am unable to make any statement in regard to the cytology of the egg cells. Figure 31 represents a small ovary of the reproductive type. The oviduct is small; the ovarian tubules are shorter and more slender and, possibly, less numerous, although I think that the number is about the same as in the larger ovary; the largest egg cells are smaller than those in the large ovary. In the very

small ovary of the worker-soldier type, the oviduct is smaller, the ovarian tubules less numerous and more slender, and the egg cells are smaller than in either of the reproductive types.

Figure 28 represents a testis from a male of the reproductive type with large brain and large testes. The testis has the appearance of an irregularly swollen tube, with about eight swellings, or lobules. At its posterior end the testis is continued backward into the very slender sperm duct. The male germ
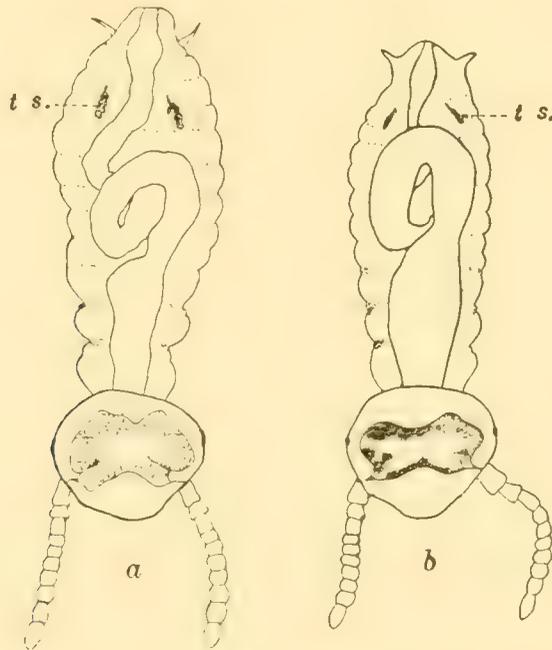
Fig. 5 Male nymphs of L. flavipes, with ten antennary segments, body length 1.3 to 1.4 mm. A, reproductive type of the first form, with large brain and large testis; b, worker-soldier type, with small brain and very small testis, ts, testis. Oc. 6, obj. 16, reduced one half.

cells at this age are all small and of similar size; I can make no statement in regard to their cytology. Males of the reproductive type of this age with smaller brains have also smaller testes, but these have not been figured. Figure 29, represents the testis of a worker-soldier individual, and is smaller in bulk than that of either of the reproductive types; the sex cells however appear equal in size to those of the reproductive types.

Since the two groups of individuals of the reproductive type are constant and do not intergrade, it is evident that they must be the prototypes of the two well known kinds of older reproduc-

tive nymphs, namely: the 'nymphs of the first form,' with long wing pads, and the 'nymphs of the second form,' with short wing pads, which, it will be remembered, develop respectively into two different adult castes, the 'true' royal pair, or adults with long wings, and the neoteinic adults with short scale-like wing pads But here, at first, a difficulty presented itself. Which of these two varieties of the reproductive type is the prototype of the nymph of the first form? and which of the nymphs of the second form? It will be recalled (Thompson, '16, figs. 5, 6 a, 8, 10), that the nymphs of the first form have a large brain, a large frontal gland, and pigmented compound eyes; the nymphs of the second form have a smaller brain, in which the mushroom bodies and the optic lobes are of diminished size, a smaller frontal gland, and compound eyes almost devoid of pigment. But, and here lay the difficulty, the sex organs of the nearly mature nymphs of the second forms are larger than those of the first form of similar age. Both Grassi and Feytaud make this statement and the writer corroborates it. We are therefore confronted with the difficulty of two prototypes, one with large brain and large sex organs (figs. 4 a, 5 a), the other with smaller brain and smaller sex organs (fig. 4 b), while the older nymphs have, in one case, a larger brain and smaller sex organs (nymph of the first form), and in the second case a smaller brain and larger sex organs (nymph of the second form).

To solve the difficulty we must pass over the nymphs of the first and second form to their respective adult stages; the 'true' adults with long wings, and the 'neoteinic' forms with short wing pads, and especially to the older queens of these two castes, after they have undergone the characteristic post-adult growth, and have become the enlarged queens which may attain a body length of 14 mm. and 9 mm. respectively, Thompson ('16) and Snyder ('16).

It is true that the sex organs of the winged adults which are ready to swarm are not so large in bulk as those of the 'neoteinic' adults with short wing pads of similar age. It is also well known that the sex organs in the enlarged egg laying queens with the stubs of the former long wings are far larger than those of the

'neoteinic' enlarged queens with short wing pads Since, there-
fore, the adults with wing stubs possess larger sex organs in the
final, post-adult, stage of development than the 'neoteinic'
forms with short wing pads, it is clear that the larger sex organs
of the nearly mature nymphs of the second form represent not
a morpho ogical, but merely a physiological phase, due to the
earlier ripening of the sex cells in a caste that never swarms and
does not leave the nest.

It is now evident that the first variety of the reproductive type,
with large brain and large sex organs, is the prototype of the
nymphs of the first form, and hence of the winged adult caste;
the second variety of the reproductive type, with smaller brain
and smaller sex organs, is the prototype of the nymphs of the
second form, and hence of the 'neoteinic' reproductive caste
with short wing pads. Two adult reproductive castes are there-
fore distinguishable in L. flavipes in nymphs with ten antennary
segments and a body length of 1.3 to 1.4 mm. It is possible
that with more careful study and better material these castes
might be distinguished at an even earlier age.

*Phase 4. Nymphs with eleven antennary segments, the third
either entire or grooved; body length 1.4 to 1.6 mm.* (figs. 12, 13).
One change to be observed under the microscope, although not
yet visible to the unaided eye, is the greater size of the head of
the worker-soldier type, which is now very evidently 'large
headed' as compared with the 'small headed' reproductive type,
(figs. 12, 13).

The mandibular muscles are stouter and larger; the greater
increase of these muscles in the worker-soldier type is evidently
related to the enlargement of the head.

The brain of the reproductive castes shows an increased bulk
and a higher degree of differentiation in the various parts, es-
pecially in the mushroom bodies and optic lobes. A similar,
although relatively smaller, increase in the worker-soldier brain
will be noted in figure 2 *b*, where the superimposed outlines of
the two types of brain are shown. In sections of individuals
of the reproductive castes with a body length of 1.6 mm., the
nerve cells in the different parts of the brain have begun to

differentiate, especially those of the mushroom bodies, antennary lobes, and the intercerebral region. The large rounded masses of cells which form the central cellular group of the mushroom bodies, known as cell group I, (Thompson '16) are now clearly defined, although mitotic figures still occur among the cells of this group. The cell groups II and III, or the nerve cells which form the peripheral part of the mushroom bodies are less clearly defined, but may be recognized.

The compound eyes of both types with a body length of 1.6 mm., are slightly larger, (figs. 20, 21); the worker-soldier eye still retains its simple undifferentiated condition, but that of the reproductive castes shows that the differentiation and the grouping of cells is proceeding rapidly.

In the frontal gland of the reproductive castes the hypodermal invagination has grown farther back and appears as a large prominent mass of epithelial cells lying somewhat above the cup shaped basement membrane (fig. 36, *f.g.*, *b.m.*); and not yet completely filling it. A very delicate but distinct fontanell nerve (*f.n.*), the former median ocellar nerve (Thompson, '16), runs from the basement membrane of the posterior part of the frontal gland into the brain, entering the protocerebral lobes in exactly the same position as in the older forms described in pt. I, namely: between the posterior roots of the mushroom bodies (*p.r. m.b.*). The hypodermal invagination of the frontal gland is smaller in the worker-soldier type, and does not extend so far backward. In figure 37, the empty basement membrane, (*b.m.*) and the fontanelle nerve (*f.n.*), are shown.

No further changes except those of growth are to be noted in this phase.

End phases

Phase 5. Nymphs with twelve antennary segments, the third segment entire and not hairy; body length 1.7 to 1.8 mm. (figs. 14, 15). The head of the worker-soldier type is still slightly larger by actual microscopic measurement than that of the reproductive types and this difference is readily noticed with a lens (figs. 14, 15, and table 1). Both types of brains are becoming more

highly differentiated and the increase in the size of the worker-soldier mushroom bodies is very marked. Practically no difference is to be noted in the compound eyes of the worker-soldier type since the last phase, but those of the reproductive castes are growing in size and complexity. The sex organs are growing larger in both types, markedly in the reproductive castes, and slightly in the worker-soldier type.

Phase 6. Nymphs with twelve antennary segments, the third entire and either bare or hairy; body length 2 mm. (figs. 16, 17). The large head of the worker-soldier type is now recognizable without a lens. Nearly half of the space within it is occupied by the large radiating bundles of the mandibular muscles. The chitinous bars of the tentorium are stouter, the cuticula covering the head and body is much thicker, and the mandibles and maxillae have a heavy yellow margin. In order to obtain consecutive sections of individuals of this age, the specimens must be kept longer (three to four hours) in paraffin.

The differences between the heads of the two types (figs. 16, 17), are now very marked, the reproductive castes being plainly 'small headed' and the worker-soldier type 'large headed.' The brain of the reproductive castes is larger and more highly differentiated than in the preceding phases, but the brain of the worker-soldier type, although it has increased in general bulk and in the size of the mushroom bodies, is still inferior to that of the reproductive castes (fig. 2 c), and shows the narrow pointed optic lobes which characterize the adult workers and soldiers. The space within the head of the worker-soldier type is filled chiefly by the large and stout mandibular muscles, which are larger than in the reproductive castes. The chitinous cuticula is thicker on the outside of the head and body, and within the head in the tentorial bars.

Except for a very slight increase in diameter, the compound eyes of the worker-soldier type of 2 mm. in length (fig. 23), are almost as simple as at the time of hatching. The eye contains more cells and these are arranged in two ill defined layers, but no differentiation into large and small cells and no grouping

of cells has occurred. A slender bundle of fibers, the optic nerve, issues from the inner surface of the eye

The compound eyes of the reproductive castes (fig. 22) have increased in diameter, the cells are highly differentiated and are arranged in groups; some of the optical cells are long and slender, with axons which run toward the inner surface.

Although it is probable that the ocelli are present in the reproductive castes at this age I have so far been unable to distinguish them.   There is little change in the frontal gland of either type since the last phases, except in size.

The sex organs, and especially the ovaries, now show very marked size differences between the two types and between the two reproductive castes, which are correlated, as before, with differences in the brains. The large brain and large ovaries of the prototype of the female nymphs of the first form, the slightly smaller brain and ovaries of the prototype of the female nymphs of the second form, and the still smaller brain and ovaries of the female worker-soldier type are very prominent and are distinguishable with a hand lens in stained specimens 2 mm. long.

We have now examined all the phases of growth within the first stage of development, or the period from the time of hatching to the first molt.   It was found that at the time of hatching two generalized types of nymphs are present, namely: the reproductive type with large brain and large sex organs, and the worker-soldier type with small brain and small sex organs.   Shortly after hatching—in the phase with ten antennary segments and a body length of 1.3 mm.—the generalized reproductive type differentiated into two groups of individuals, with large brain and large sex organs, and with smaller brain and smaller sex organs—which I have identified as the prototypes of the nymphs of the first form, with long wing pads, and the nymphs of the second form, with short wing pads, and hence of two adult reproductive castes, the winged adults, and the 'neoteinic' adults with short wing pads.

Three kinds of individuals are therefore distinguishable at an early phase: the prototypes of two of the adult reproductive castes, and the still generalized worker-soldier individuals.

From that phase onward to the end of the first stage of development, these three kinds of nymphs have grown and differentiated, but no further differentiation of the other adult castes has been observed.

This would seem to dispose of the fallacy that the so-called 'substitute' and 'complemental' royal forms of Grassi can be produced at the will of the colony by the agency of food. And now, for the two reasons that the adult reproductive castes are evidently born, and not made by feeding, and that their nomenclature is in a state of confusion, I suggest the following terms for the three adult castes found in L. flavipes and L. virginicus, namely: (1) adults of the first form, (with long wings or stubs of wings), or males and queens of the first form; (2) adults of the second form (with short wing pads), or males and queens of the second form; (3) adults of the third form (with no wing pads). or males and queens of the third form. We should then reap the advantages of continuity and simplicity. Continuity would be gained by the use of the same descriptive terms that have been applied for years,—since the time of Lespès ('56)—to the two nympha stages from which the first two castes arise; simplicity would be attained by abandoning the various lengthy and sometimes inaccurate terms now in use by different writers. For example, some of the terms applied to the first form adults with long wings are: 'perfect nsects,' 'true adults,' 'royal pair,' 'winged adults;' while Holmgren always speaks of this caste as the 'imago,' as if it were the only caste that completed its development. Some synonyms of the second form adults, with short wing pads, are: 'neoteinic nymphal forms,' 'substitute royalties,' 'complemental royalties,' etc.; and again, for the third form adults, with no wing pads, we have: 'substitute royalities,' 'ergatoid neoteinic forms,' 'larval neoteinic forms,' etc.

E. THE ORIGIN OF THE WORKER AND SOLDIER CASTES OF L. FLAVIPES

The origin of the soldier caste of Leucotermes from a worker-like form has been observed by two different writers, Lespès ('56) and Snyder ('13, '15).

Lespès describes the origin of the soldier of L. lucifugus in the following words:

Les ouvriers et les soldats ont une organisation presque identique; on pourrait dire que les deux formes ne diffèrent que par les mandibles et leurs muscles; de plus, ils subissent en même temps leur dernière metamorphose depuis le 20 juin jusqu'au 20 juillet. Ainsi que je dirai plus loin, j'ai vu cette transformation s'effectuer sous mes yeux; avant qu'elle soit terminée, il est impossible de savoir s'il va naître un soldat ou un ouvrier. La nymphe qui leur donne naissance est en tout semblable à un ouvrier sauf la taille.

The origin of the soldier caste in L. flavipes and L. virginicus is described as follows by Snyder ('13, '15). During August, nymphs that in external appearance were worker-like (with large rounded head, mandibles with marginal teeth, and broad labrum), were found in the quiescent stage that precedes a molt. The molt was observed and the newly emerged forms were identified as young soldier nymphs by the characteristic pointed mandibles without marginal teeth and the slightly elongate or oval head. Snyder further states that these newly molted soldier nymphs have fourteen antennary segments, and that the body length is over three millimeters in L. virginicus. The length of the newly molted soldier of L. flavipes was not stated, but it is probably between three and four millimeters in the larger species. Through the courtesy of Mr. Snyder I have examined the mounted heads of these newly formed soldier nymphs and have noted the presence of a large clearly defined frontal gland, which proved that internal characters are already present and are probably recognizable in those antecedent worker-soldier forms which are to become soldiers; those which are to become workers having in all probability the smaller primitive frontal gland characteristic of the adult worker.

With these facts in mind a late differentiation of the generalized worker-soldier type of L. flavipes was to be expected, and I was not surprised to find this type still generalized and undifferentiated at the end of the first stage of development. From the embryological standpoint also it would be probable that the more highly specialized coenogenetic soldier type would appear late in the ontogeny.

A thorough study of the second stage of development, or the period between the first and second molts has not yet been completed, but some whole mounts have been made and a number of stained individuals of the large headed worker-soldier type of this stage have been examined in cedar oil, with low magnification. It was hoped that this study might reveal differences in the size of the frontal gland, which should be small in the future worker, large and prominent in the future soldier nymphs.

Of two large headed worker-soldier individuals so examined, with fourteen antennary segments and a body length of about 3.75 mm., one had a clear space in the frontal gland region, between the mushroom bodies, the other a dark dense spot, which was evidently a mass of cells. These two individuals were labeled tentatively worker and soldier, and were embedded and sectioned.

The sections showed that the so-called worker individual had a frontal gland resembling that of the adult worker and also of the worker-soldier individuals of the preceding stage, in being small and delicate, and very primitive in appearance. The so-called soldier individual, however, had a large stout frontal gland of hypodermal origin, still widely open to the exterior at its anterior end, and composed of tall columnar cells with rounded distal ends. The high degree of differentiation as well as the size of this soldier frontal gland seems good evidence that it had not just appeared but that it had been forming for some time, and would therefore be distinguishable at an earlier age than 3.75 mm. Several other soldier nymphs of this age were distinguished from worker nymphs by the size of the frontal gland.

The prototypes of the worker and soldier castes therefore, although externally alike, are distinguishable by internal structural differences at the end of the second stage of development, in individuals with a body length of 3.75 mm., table 2. With further study I hope to find the complete series of phases, leading back to the generalized worker-soldier type, and forward to the workers and soldiers which are recognizable by external characters.

F. THE QUESTION OF THE ORIGIN OF THE THIRD REPRODUCTIVE CASTE, WITH NO WING PADS, IN L. FLAVIPES

No evidences whatever of a prototype for the third reproductive caste of L. flavipes have yet been observed in this study of development.

It will be recalled (Thompson, '16, and Snyder, '15 and '16), that the third reproductive caste is entirely wingless, having neither wings nor wing pads. This form is of rare and infrequent occurrence in the genus Leucotermes and practically nothing is known of its internal structure. Mr. Snyder has permitted me to quote his statement that he has found only five queens of this caste in the genus Leucotermes—three in L. flavipes, one in L. virginicus, and one in a new species—and that he knows of no record of others having been found in the United States.

DISCUSSION AND CONCLUSIONS

That the newly hatched nymphs, although externally all alike, are internally differentiated into two distinct types of individuals proves, first, that these types are predetermined at the time of hatching, and second, that the two generalized types of reproductive individuals and worker-soldier individuals are fundamentally different. There can be no question of changing one type into the other; each, from the beginning, is a distinct entity with sharply defined morphological characters.

The conversion of the 'undifferentiated' nymphs, by feeding, into large and small headed forms, and these again into the future adult castes; the production of 'substitute' reproductive forms from an ever present 'reserve' of 'indifferent larvae,' or by the alteration of forms whose development has already begun; the sudden and opportune appearance of 'substitute' forms in 'orphaned' nests; all these assumptions, which, indeed, appeal to our sense of the dramatic and marvellous, must now be regarded as possessing chiefly an historic interest.

The later differentiation of the generalized reproductive type, when it has attained a length of 1.3 mm., into the prototypes of two of the specialized adult reproductive castes results finally

TABLE 2

Leucotermes flavipes Kol.

	YOUNG OR DEVELOPMENTAL STAGES			ADULT OR FINAL STAGES	
				Sex organs developed	Post-adult
Eggs (differentiated)	Reproductive type of newly hatched nymphs 1.1 mm. Small heads. Large brain and sex organs	1. Nymphs of the first form, 1.3 mm.—1.4 mm. Small heads. Large sex organs	1. Nymphs of the first form, 1.8 mm. Small heads. Large sex organs	1. Nymphs of the first form, 3.75-4 mm. Anlagen of long wing pads → 1. Adults of the first form, 6.5-7.5 mm. Long wings	1. Enlarged egglaying queens of the first form, 7.5-14.5 mm. stubs of wings
		2. Nymphs of the second form, 1.3—1.4 mm. Small heads. Medium-sized sex organs	2. Nymphs of the second form, 1.8 mm. Small heads. Medium-sized sex organs	2. Nymphs of the second form 3.75-4 mm. Anlagen of short wing pads → 2. Adults of the second form, — 6.5-7.5 mm. Short wing pads	2. Enlarged egglaying queens of the second form, 7.5-9 mm. Short wing pads
		— — — — — ?	— — — — —	3. Nymphs of the third form, no wing pads → 3. Adults of the third form, No wing pads	3. Enlarged egglaying queens of the third form, ?-9 mm. No wing pads
				Sex organs not developed	
	Worker-Soldier type of newly hatched nymphs 1.1 mm. Small heads. Small brain and sex organs	Worker-Soldier nymphs 1.3-1.4 mm. Small heads. Small sex organs	Worker-Soldier nymphs 1.8 mm. Large heads. Small sex organs	4. Worker nymphs ?-3.75 mm. Large heads. Small frontal gland → 4. Workers 5-6 mm. Wingless	
				5. Soldier nymphs ?-3.75 mm. Large heads. Large frontal gland → 5. Soldiers 6-7 mm. Wingless	

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in the winged adult caste of the first form, with primitive ancestral characters, and the adult caste of the second form, with secondary modifications.

The still later differentiation, at 3.75 mm. or less, of the generalized worker-soldier type into the prototype of the worker with primitive structural characters, and the prototype of the highly specialized soldier with many new features is again significant.

The manner in which these castes have arisen in the individual life cycle is perhaps indicative of the way they arose phylogenetically.   The phylogeny of Leucotermes, table 3, may have begun in a primitive ancestral reproductive type with a tendency to throw off sterile or worker variations, perhaps mutants.   This tendency became fixed in the species, and the generalized reproductive type and the generalized worker-soldier type occurred side by side in every generation.   Both types still kept the tendency to vary, or mutate; as time went on, the generalized reproductive type threw off the more specialized adults of the second form, with short wing pads, etcetera, the type itself con-

TABLE 3

Phylogeny of Leucotermes flavipes
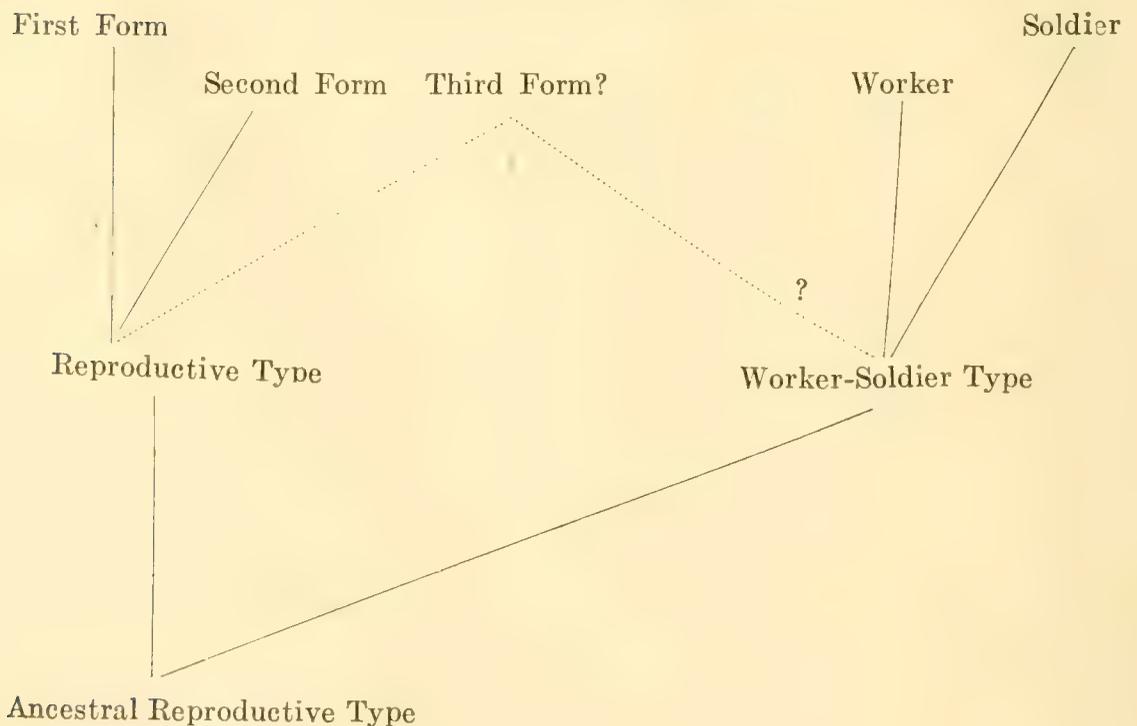
tinuing as the adult of the first form, with ancestral structure and habits. If the third reproductive caste, with no wing pads, is a true caste, it may be accounted for, according to this hypothesis, as a second mutant from the ancestral parent reproductive type. But, and I shall return again to this question, I am by no means sure that the wingless reproductive forms constitute a caste. Still later, the generalized worker-soldier type differentiated or split up into the highly specialized soldier caste and the primitive worker caste which more nearly resembles the parent form. Both soldier and worker may manifest their inherent variability by occasional fertility. Wheeler ('07) remarks: "I venture to maintain that fertile workers occur much more frequently in all groups of insects than has been generally supposed. As this fertility is merely a physiological state it has often been overlooked."

The rare and occasional winged workers and soldiers might be regarded as hybrids from one of the reproductive castes[7] with fertile workers or soldiers, or as fluctuating variations.

The reproductive adults of the third form, or the reproductive forms that are wholly devoid of wings, must next be considered. These forms are of rare occurrence in the genus Leucotermes, as stated above. There are three possibilities for the origin of these wingless and fertile individuals: first, this form may be a true caste, a mutant thrown off from the ancestral reproductive type; second, it may be a hybrid, from a worker and one of the reproductive forms; third, the individuals of this 'caste' may be fertile workers. An investigation of the internal structure and the development of this questionable caste is greatly needed.

The next question to arise is, are these facts in the development of L. flavipes in harmony with the facts observed in other genera of termites?

Bugnion has found in Eutermes lacustris that the soldier nymph is distinguishable at the time of hatching, that is, it is predetermined. Unfortunately Bugnion did not report any

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[7] Mr. Snyder has informed me that there are no known instances of fertile or winged workers or soldiers in the genus Leucotermes.

observations upon the structure of the other nymphs, but he states his belief that all termite castes are predetermined at the time of hatching. I venture to assert that, even if the future worker nymphs of Eutermes lacustris are not externally distinguishable from the reproductive nymphs at the time of hatching, the two forms have internal characters which are different.

It is evident that the various castes manifest themselves at different ages in different genera, and it is probable that there are differences in this respect within a genus. For example, Knower ('94) found that the soldier of Eutermes (rippertii?) originates late in development by molting from an antecedent worker-like form; whereas Bugnion ('12) could distinguish the soldier of Eutermes lacustris at the time of hatching.

In this connection it is interesting to recall the observation of Bobe-Moreau ('41) regarding a tiny soldier of the European L. lucifugus, which he described as just emerged from the egg. It is probable that Bobe-Moreau mistook one of the small pale newly molted soldier nymphs for a newly hatched form, since Lespès observed the young soldier nymphs of L. lucifugus emerge from a worker-like skin. But if the soldier nymph seen by Bobe-Moreau was actually newly hatched, then the suggestion offered in regard to the genus Eutermes will hold good also for Leucotermes, namely: that the castes may originate in different ways within the species of a genus.

My final conclusion is that all termite castes are predetermined in the eggs; that some castes are distinguishable, either by external or internal characters, at the time of hatching, while others may appear considerably later in the course of development. In Leucotermes the more generalized castes are the first to be manifested, the more specialized are later in their appearance.

It is a possibility that the castes of termites are mutations from the ancestral parent stock; they would therefore, if fertile, breed true. There is great need, however, of observational and experimental proof to establish this question.

There is also need of cytological study with termites. The intrinsic cause of caste determination may be as readily determin-

able as the cause of sex has been. The cause of the sterility of the worker and soldier may also be explained by cytological investigations.

Lastly, I wish to emphasize the great need of investigation, both experimental and cytological, of the eggs of all social insects. If food is not the cause of differentiation in one group of the social insects, why should it stand as a factor of unquestioned value for the other groups?

SUMMARY OF RESULTS

1. The newly hatched nymphs of L. flavipes and L. virginicus are externally all alike, but internally there are marked structural differences which divide the nymphs into two sharply defined types, the reproductive and the worker-soldier types, which are respectively the prototypes of the 'small headed' and 'large headed' nymphs of Grassi. Therefore, the fertile and sterile types are predetermined at the time of hatching.

2. The two types of newly hatched nymphs may be distinguished by the following characters: a, the bulk of the brain; b, the relative size of brain and head; c, the structure of the compound eyes; and d, the size of the sex organs.

3. The reproductive type of newly hatched nymph has a large brain, in which the mushroom bodies, optic lobes and antennary lobes are all large. The space within the hypodermis of the head is nearly filled by the brain. The compound eyes are slightly larger and more differentiated, and the sex organs are larger than in the worker-soldier type.

4. The worker-soldier type of newly hatched nymph has a small brain, with small mushroom bodies, optic lobes and antennary lobes. The space within the hypodermis of the head is not nearly filled by the brain. The compound eyes are smaller and simpler, and the sex organs are smaller than in the reproductive type.

5. The antennae of the newly hatched nymphs of both L. flavipes and L. virginicus are composed of nine segments, the third segment grooved and bare.

6. In nymphs of L. flavipes with ten antennary segments, the third segment grooved, and a body length of 1.3 to 1.4 mm., the individuals of the reproductive type are further differentiated into two groups: *a*, with large brain and large sex organs; *b*, with slightly smaller brain and smaller sex organs. These are the respective prototypes of the nymphs of the first form and the nymphs of the second form, and hence of the two adult castes of the first form, with long wings, and of the second form, with short wing pads.

7. The prototypes of the worker and soldier castes, although externally alike, are distinguishable by internal structures at the end of the second stage of development, in nymphs with a body length of 3.75 mm., and are probably distinguishable at an even earlier age.

8. The frontal gland is present, although barely recognizable, in the newly hatched nymphs, and grows larger and more complex as development proceeds. The fontanelle nerve (median ocellar nerve) is not present in the earlier phases, but is clearly seen in individuals with a body length of 1.6 mm. From the beginning, the frontal gland is larger and more differentiated in individuals of the reproductive type than in the worker-soldier type, and may be added to the list of characters by which the two types are distinguished. In individuals of the large headed worker-soldier type, with a body length of 3.75 mm. two types of frontal glands may be recognized: a small, primitive frontal gland in the worker prototypes, a large, highly modified gland in the soldier prototypes.

9. No evidences of a prototype for the third adult reproductive caste, without wing pads, have been seen in this study of the development of L. flavipes.

10. To simplify the heterogeneous nomenclature of the three adult reproductive castes I have suggested the following terms, which are in conformity with the terms applied to the oldest nymphs since the time of Lespès ('56):

1. Adults of the first form, or males and queens of the first form (with long wings or stubs of wings).

2. Adults of the second form, or males and queens of the second form (with short wing pads).

3. Adults of the third form, or males and queens of the third form (with no wing pads).

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PLATES

All figures are drawn with the Spencer camera lucida and with Spencer lenses, at the level of the stage unless otherwise mentioned.

ABBREVIATIONS

a.l., antennary lobe

b.m., basement membrane

f.g., frontal gland

f.n., fontanel nerve

fr.gn., frontal ganglion

m.b., mushroom body

md.m., mandibular muscle

n.c., nerve cord

oe., esophagus

o.l., optic lobes

ov., ovary

p.r.m.b., posterior root of mushroom body

ts., testis.

PLATE 1

EXPLANATION OF FIGURES

6 to 17   Drawn from whole mounts of the heads of nymphs of L. flavipes.   The stippled portion of the brains represents the nerve cell layer, the fibrous core is blank.   Oc. 6, obj. 16, table level, reduced one third.

6   Reproductive type, newly hatched forms, 1.1 mm., nine antennary segments.

7   Worker-soldier type, newly hatched forms, 1.1 mm., nine antennary segments.

8   Reproductive type, 1.2 mm., ten antennary segments, third segment entire.

9   Worker-soldier type, 1.2 mm., ten antennary segments, third segment entire.

10   Reproductive type, 1.3 mm., ten antennary segments, third segment grooved.

11   Worker-soldier type, 1.4 mm., ten antennary segments, third segment grooved.
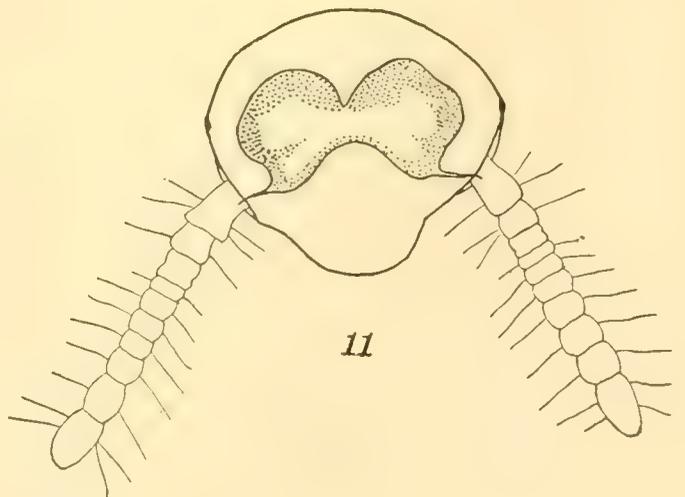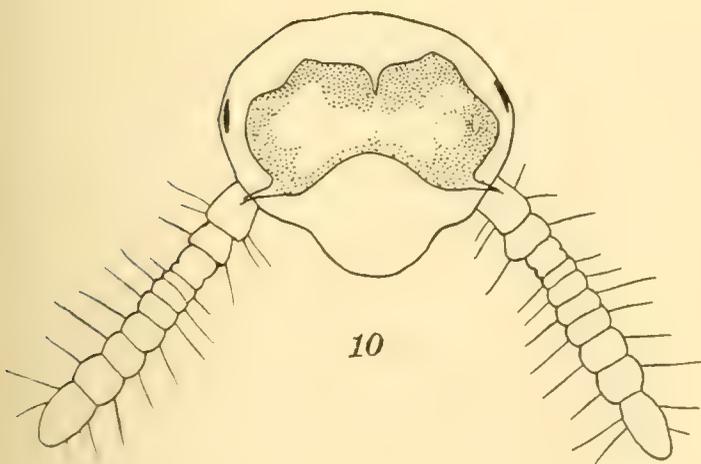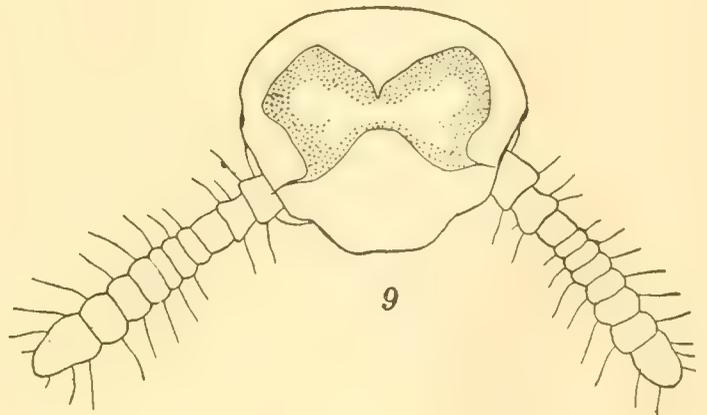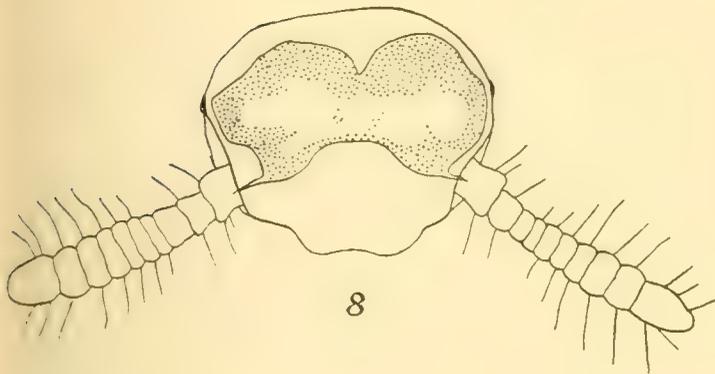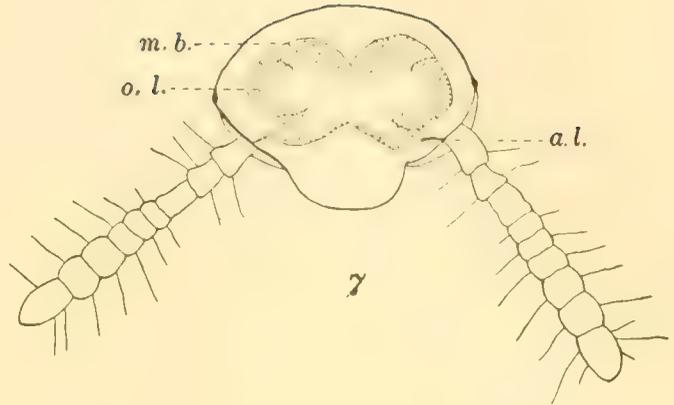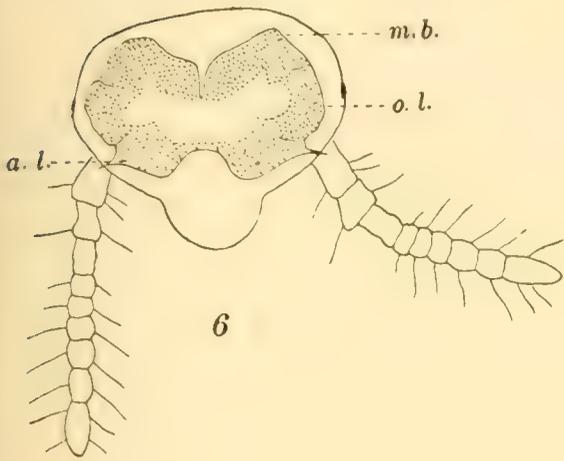
12 Reproductive type of the first form, 1.4 mm., eleven antennary segments.

13 Worker-soldier type, 1.6 mm., eleven antennary segments.

14 Reproductive type of the first form, 1.7 mm., twelve antennary segments, third segment entire.

15 Worker-soldier type, 1.8 mm., twelve antennary segments, third segment entire and bare.

16 Reproductive type of the first form, 2 mm., twelve antennary segments, third segment entire and hairy.

17 Worker-soldier type, 2 mm., twelve antennary segments, third segment entire and hairy.
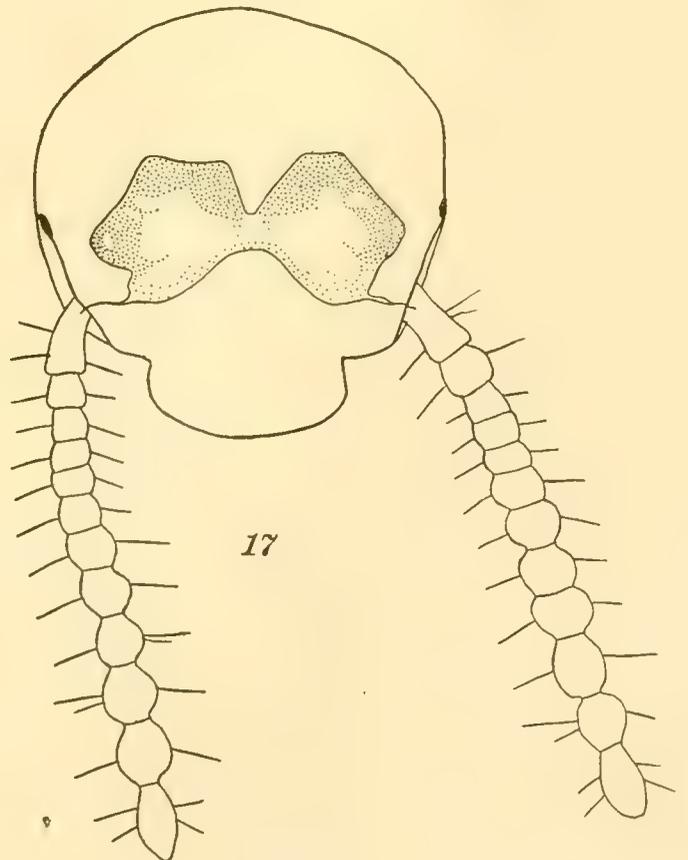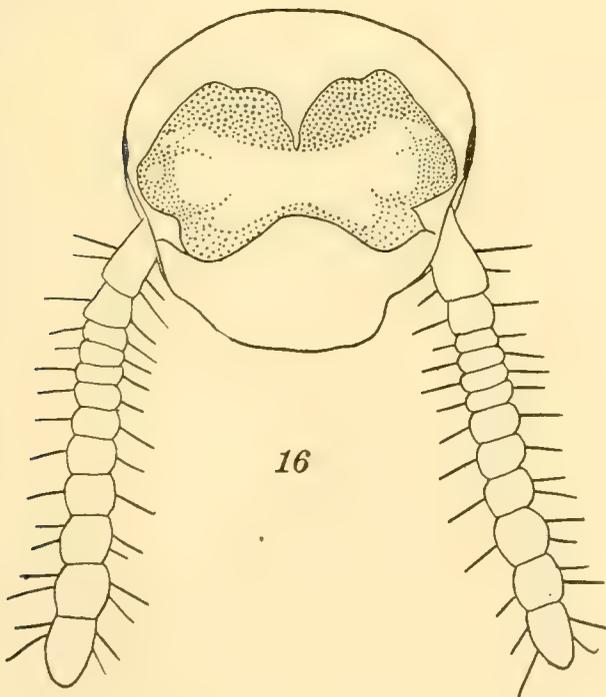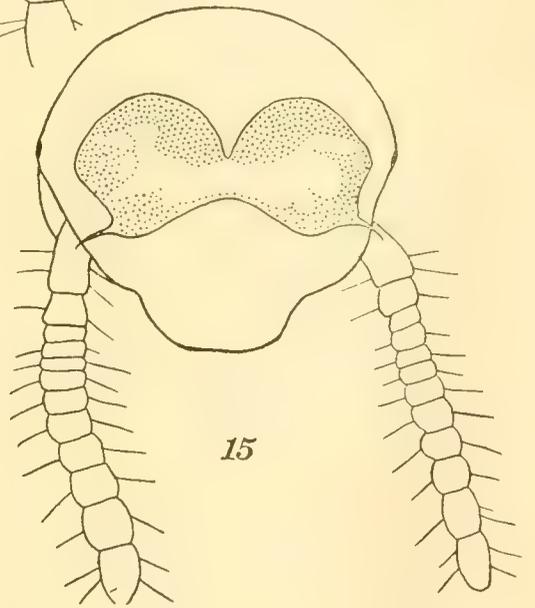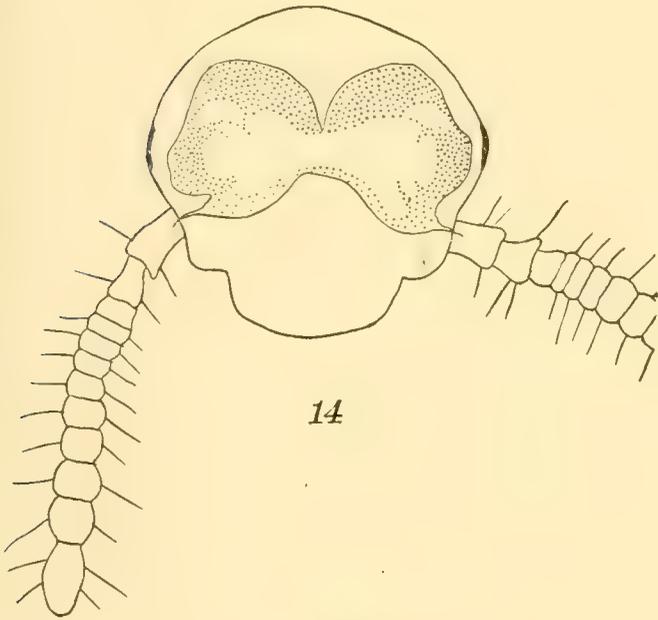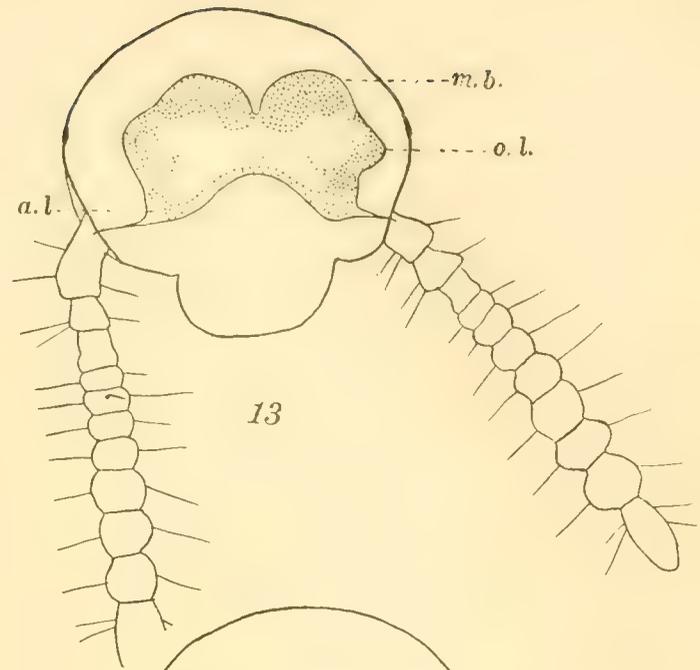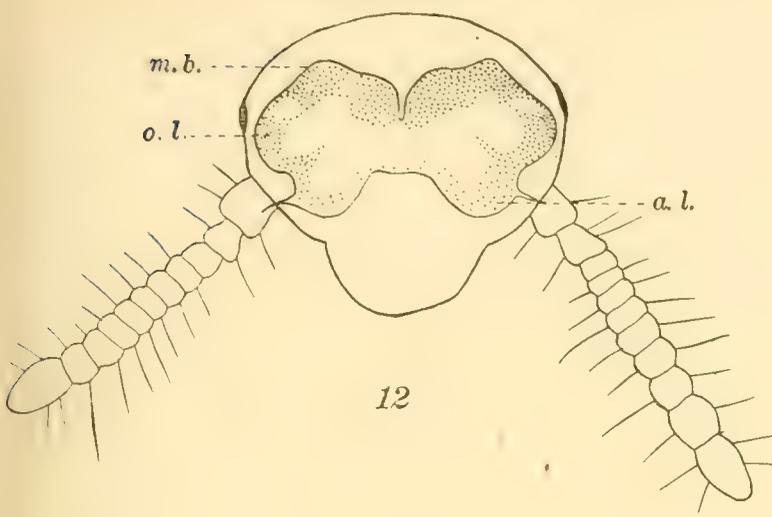
PLATE 3

18 to 23   Drawn from frontal sections of nymphs of L. flavipes. Homog. immers. 1.8 mm., Oc. 6.

18   Compound eye of the reproductive type, newly hatched nymphs, 1.1 mm.

19   Compound eye of the worker-soldier type, newly hatched nymphs, 1.1 mm.

20   Compound eye of the reproductive type, with eleven antennary segments, 1.6 mm.

21   Compound eye of the worker-soldier type, with eleven antennary segments, 1.6 mm.

22   Compound eye of the reproductive type, with twelve antennary segments, 2 mm.

23   Compound eye of the worker-soldier type, with twelve antennary segments, 2 mm.

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PLATE 4

CAROLINE BURLING THOMPSON

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PLATE 5

33 to 37 Drawn from frontal sections of the heads of nymphs of L. flavipes. Oc. 10, obj. 16, table level.

33 Anterior part of the brain of a newly hatched nymph, reproductive type; showing the hypodermal invagination of the frontal gland.

34 Middle part of the brain of a newly hatched nymph, reproductive type; showing the empty basement membrane of the frontal gland.

35 Anterior part of the brain of a newly hatched nymph, worker-soldier type; showing the first stage of the frontal gland, with no hypodermal invagination, but with the fontanel muscles.

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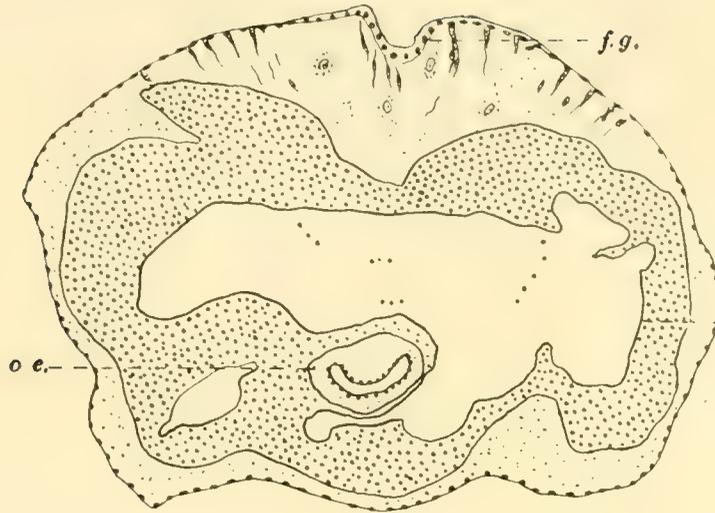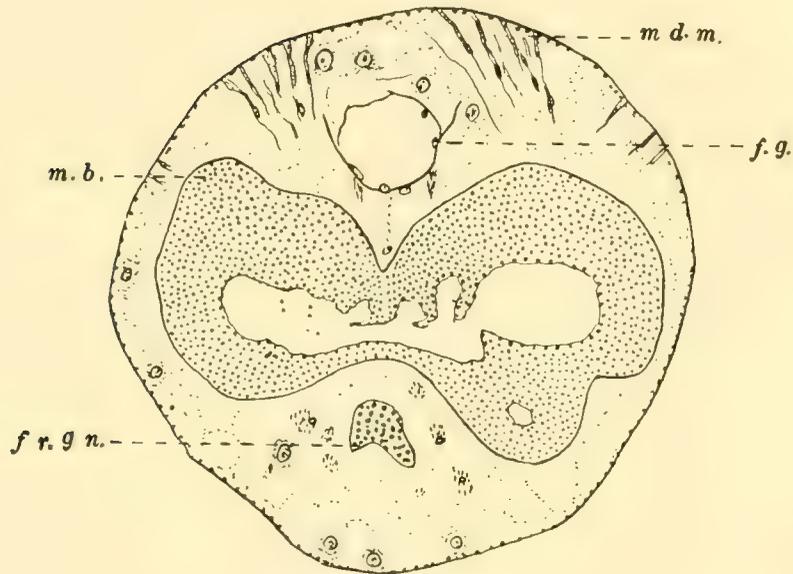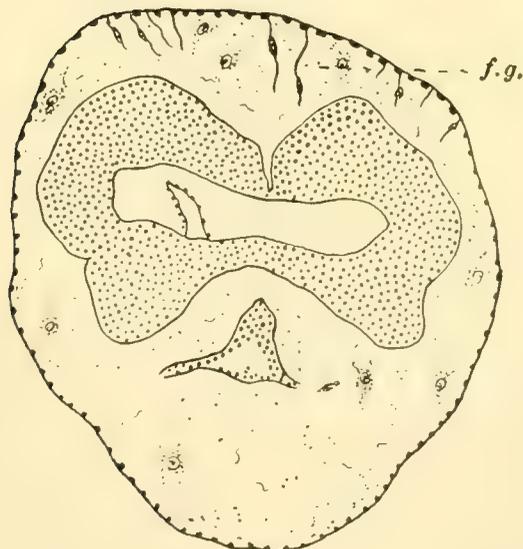
36   Middle part of the brain of a nymph with eleven antennary segments, 1.6 mm., reproductive type; showing the frontal gland with both hypodermal cells and basement membrane, and the fontanel nerve.

37   Middle part of the brain of a nymph with eleven antennary segments, 1.6 mm., worker-soldier type; showing the frontal gland, in this region consisting of the empty basement membrane, and the fontanel nerve.

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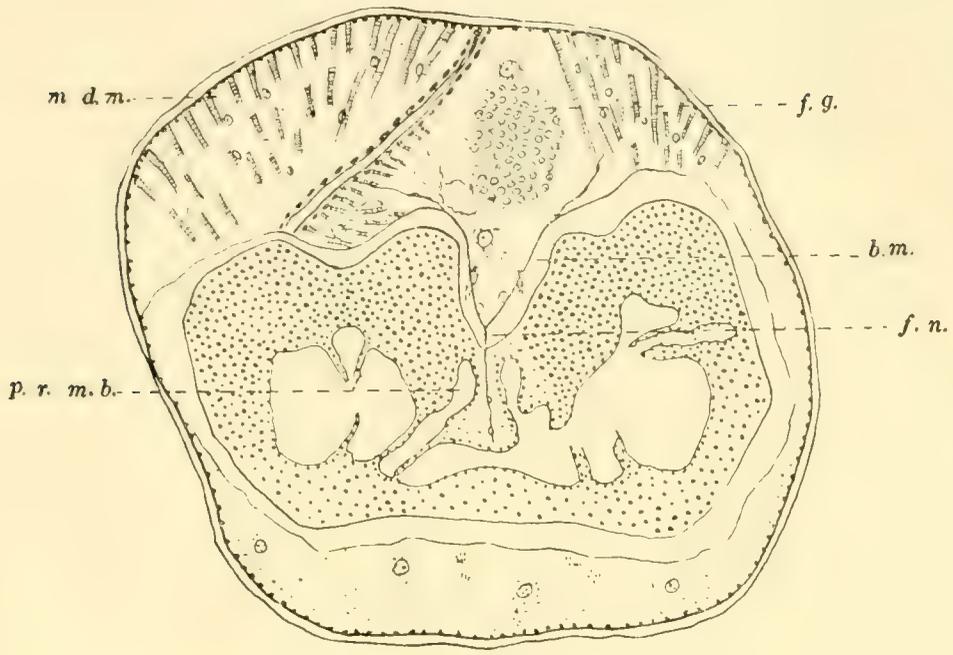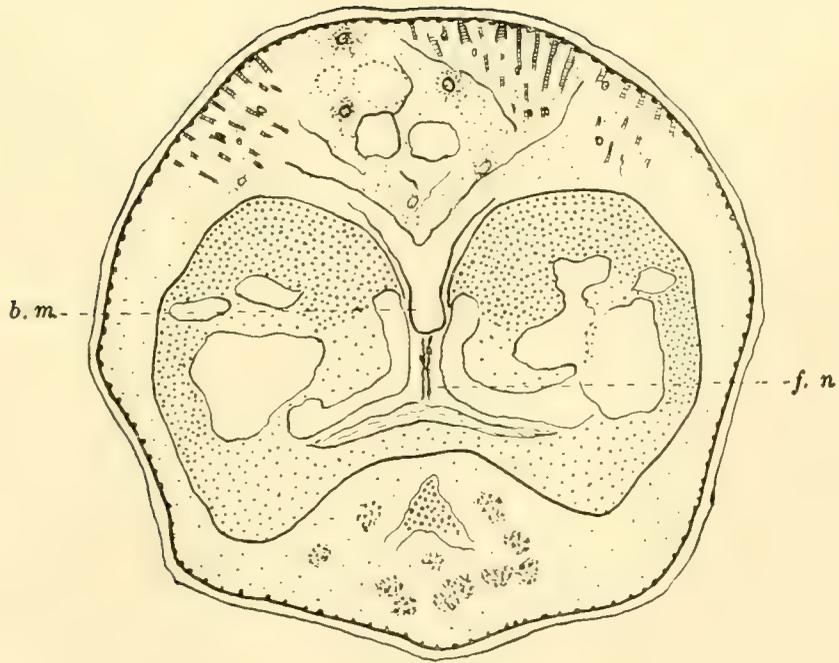
PLATE 7

38 to 42 Micro-photographs of L. flavipes taken, from slides made by the writer, by Mr. John H. Paine of the Bureau of Entomology, U. S. Dept. of Agriculture, by the courtesy of the Bureau.

38 Whole mount of a reproductive individual with ten antennary segments, length 1.3 mm., magnification 90 diameters; reduced one third.

39 Whole mount of a newly hatched nymph, with nine antennary segments, length 1.1 mm., worker-soldier type. Magnification 90 diameters; reduced one third.

40 Section of the ovary of a newly hatched nymph, length 1.1 mm., reproductive type. Magnification 470 diameters; reduced one third.

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PLATE 8

41 Frontal section of the brain of a newly hatched nymph, length 1.1 mm., reproductive type, female. Magnification 400 diameters; reduced one third.

42 Frontal section of the brain of a nymph with ten antennary segments, length 1.3 mm., worker-soldier type, male. Magnification 470 diameters; reduced one third.

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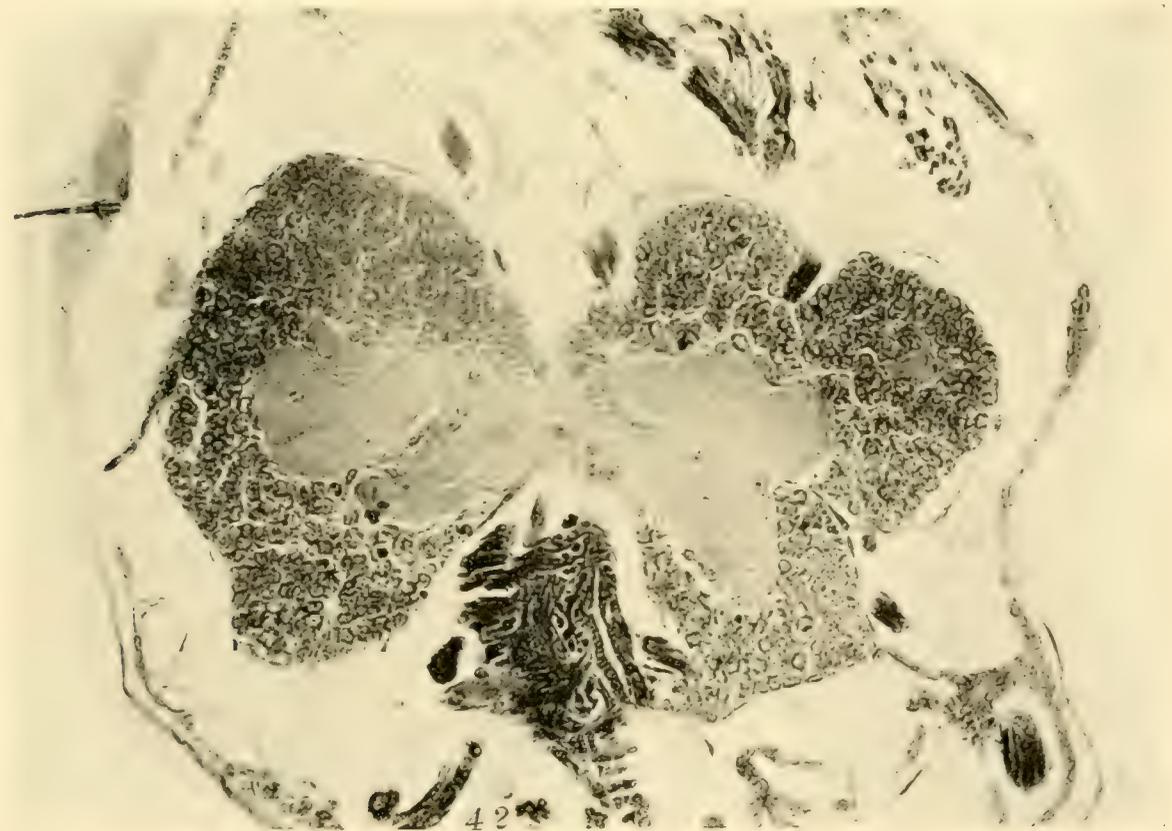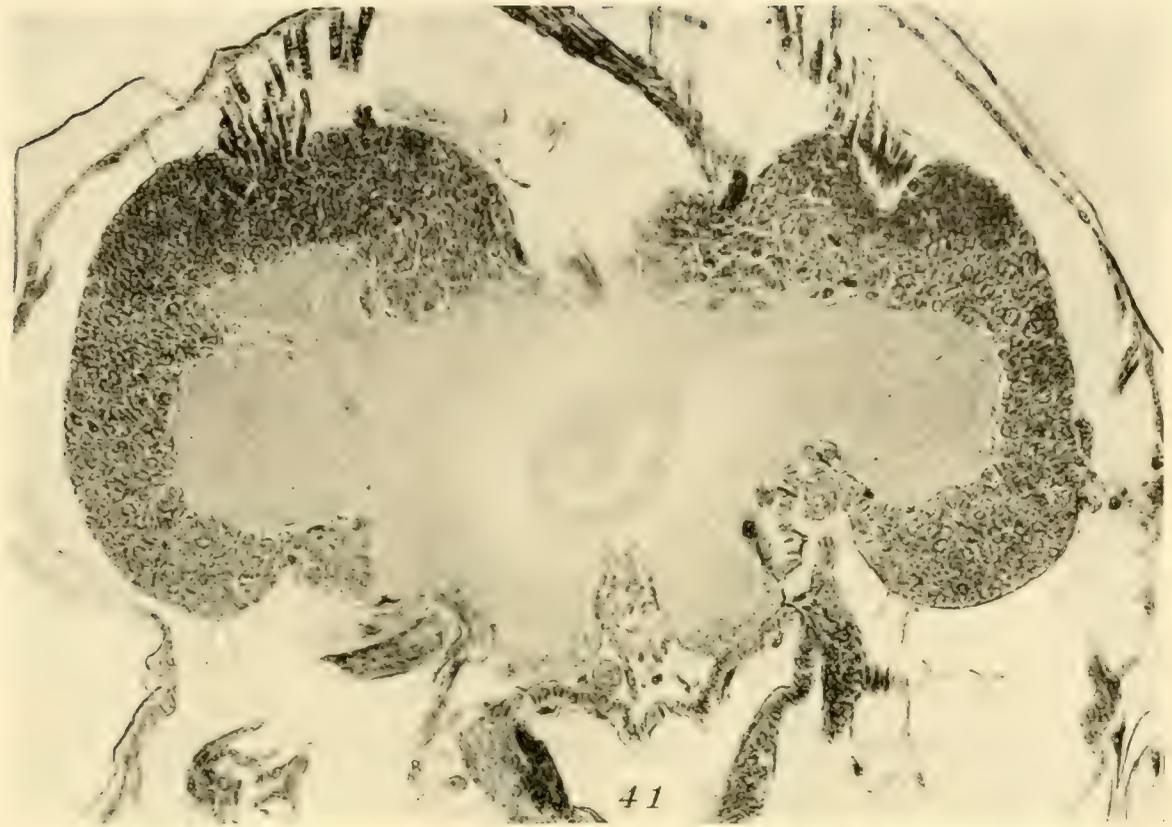
THE DIPLOID CHROMOSOME COMPLEXES OF THE PIG (SUS SCROFA) AND THEIR VARIATIONS

ROBERT T. HANCE

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TEN PLATES AND FIVE TEXT FIGURES

CONTENTS

INTRODUCTION

The terms spermatogonial and somatic have been frequently used synonymously, with respect to the diploid chromosome number, on evidence considerably more theoretical than direct. As a matter of fact comparatively little work has been done on the body cells, as the germ cells, because of their genetic significance, have, until recently, largely held the interest of cytologists. Important as is the theoretical significance of the chromosomes of the germ cells, it seems to me that the chromosomes of the body are fully as interesting. In the soma, as the various cells differentiate in to the tissues that constitute the mature plant or animal, we can see in operation the chromatin whose maturation phenomena we trace through the reproductive cells. What, we may ask, is the behavior of the chromosomes in development? Are they immutable, or do they undergo change? If changes occur, are they correlated with the character of the tissue in which the variation is found? Also in the event of change what bearing does it have on the theory of the individuality of chromosomes?

Four years ago I selected the pig for studying the problems suggested above. The methods of fixation, then in common use, proved hopeless, as the chromosomes, following the use of the usual fixatives, were clumped together beyond hope of recognition—a condition quite typical of the majority of published figures of mammalian mitoses. My work was consequently delayed until a method was discovered which preserves the chromosomes in a condition comparable to the best cytological preparations. The results obtained with this technique (Hance, '17 b) are considered to be representative of the living structures as

they compare favorably in every way with those obtained on the grasshoppers, the fixed tissue of which has been compared and checked with studies on living cells. The methods of fixation which have been developed may now be said to place mammalian tissues in the list of workable cytological materials. Probably not the least of the reasons for the few ventures in to the field of somatic cytology has been a scarcity of divisions, but this is an avoidable condition for tissues may be found showing as many mitotic figures as the best testicular material. The difficulty that investigators frequently met with in locating dividing cells in mammalian testes is entirely obviated by securing material from several animals and then selecting for study that animal which is in what I have termed elsewhere, a 'cycle of division.' The cycle holds for both testes and embryo, and I have found it also in plants.

Cytologists, who have studied the cells of the soma, may be be divided into two general groups—those who maintain that the somatic chromosome number is fixed and identical with that of the spermatogonia, and those who believe that it is variable. I believe that it may safely be said that both groups have drawn conclusions from too little evidence and, in some cases at least, this evidence has been obtained from very poor material. This is particularly true of the 'variable chromosome group,' among whom belong those, who, with Della Valle, hold the chromosomes to be unimportant structures and who believe that the supposed variations express and emphasize the lack of chromosome importance. Which of these groups is right will be considered later. Ina recent paper (Hance '18) it has been pointed out that

only when the investigator of somatic chromosomes has a sufficiently large and properly preserved number of somatic figures to study and has subjected the chromosomes to every possible analysis is he justified in taking up the cudgels for or against the theories that have developed around the many excellent studies on germinal complexes. There is a very apparent and regrettable tendency of late on the part of some cytologists to attempt merely to correlate their own findings with those of some classic work on another form, passing, at times, variations that have been found, either because they were thought unimportant, because they were supposedly pathological, or perhaps,

because they appeared impossible of interpretation. Since the physiological character of the chromosomes are as yet undetermined and since at present our only method of forming a conception of the activities of the chromatic material is through morphological studies in the broadest sense, it scarcely needs to be pointed out that uncritical wotk of the sort discribed is not likely to be productive of results.

It is quite true that when we have determined the behavior of somatic chromosomes we are not a great deal nearer the solution of development for it seems quite evident that the ultimate analysis of this problem must be largely a chemical one. But, since chemical studies of such nature are impossible at present, morphological investigations may indicate the lines which future research may advantageously follow and even may indirectly, through a careful study of the division phenomena in many tissues of a large number of plants and animals, lead to an understanding of the physiology of the cell.

TECHNIQUE

Preparation of tissue. Probably more inaccuracies in cytological work have resulted from the use of poorly prepared material than in any other way. This is particularly true of mammalian studies, and, with the possible exception of the investigations of Winiwarter, the work of other mammalian cytologists is practically valueless and will have to be carefully repeated. We have definite criteria now with which to judge our preserved material, namely the studies on the living cell (Lewis and Robertson, '16). Consequently the cytologist who attempts to unravel clumped chromosomes and to draw conclusions on the supposition that he knows the chromosomes as they are, not only invites destructive criticism of his work, but adds nothing to our knowledge and wastes our time.

The method that has given excellent results has already been described at length (Hance, '17 b). In brief it is as follows:

1. Obtain fresh specimens from as many different animals as possible so as to be sure of obtaining one or more in a 'cycle of division.'

2. Place small or finely teased pieces of fresh tissue into cold Flemming's (strong) solution to which a little urea (one-half per cent) has been added. When the bottles of fluid are surrounded with ice the temperature of the fixative is about four degrees Centigrade. Allow the tissue to remain in the fluid twenty to twenty-four hours. (Flemming's weak solution was also used successfully. See below.)

3. Wash in water for about twenty-four hours.

4. Dehydrate by very gradual steps.

5. Clear from 95 per cent alcohol in cedar oil followed by xylol.

6. Imbed in paraffin.

7. Cut the sections ten micra thick.

8. Bleach the sections for from one to twenty-four hours in peroxide.

9. Stain with iron alum haematoxylin.

This method has never failed to give good results and has been used on over sixty lots of material taken from seven mammalian species. The same results were obtained on testes, ovary and embryo.

I have studied testes obtained at the time of castration from pigs about four or five months old representing three breeds, Berkshire, Jersey red and Poland china. The first two were obtained at Philadelphia and the last from Kentucky. None of the pigs were thoroughbreds, although I was told that the Poland china was 'nearly so,' whatever that may mean. Forty-two embryos of various sizes have been fixed either entire or teased. The fixation in the case of those killed without teasing is as good as in the shredded embryos. Only a few of the specimens from this lot of material have been studied, since it was thought better to initiate this study with thorough observations on a small number of animals. It has been a matter of considerable regret that I have been unable to study any ovarian chromosomes. I recently prepared embryonic ovaries taken from foetuses about half grown, but all the material proved worthless, as the chromosomes were badly clumped. The only explanation I have to offer for this is that the system in the Philadelphia slaughter-

house in which the pigs were obtained was rather slow and the embryos were probably dead before they reached me.

Since the work on sectioned material was completed I have obtained another lot from Cincinnati. These specimens I preserved both in Flemming's weak and strong solutions to which urea had been added. The fluids were used at a temperature of four degrees Centigrade. The embryos apparently were not in a cycle of division for figures were too few to justify study. The amnion from the same pigs was fixed separately and mounted without sectioning. The amnion is quite thin and gave excellent results with the certainty of uncut cells. The slight shrinkage which occurs during the infiltration of paraffin is avoided and the chromosomes appear slightly larger in the amnionic material and in general, better separated. No differences were noted in the character of the fixation by either killing fluid. Both gave excellent results as may be judged from the photomicrographs, figures 88, 89, 90 and 93. I cannot say, at present, whether the weak solution will work equally well on thicker tissue. The chief advantage of the weak solution, as far as known now, is one of cost.

Counting and checking

When as large a number of chromosomes are involved in as small a space as are the chromosomes in the cells of the pig, drawing and counting is not easy, notwithstanding the almost perfect separation of the individual elements. Inaccuracies in drawing were avoided in the following manner: The chromosomes of a cell were carefully drawn on a 3 x 5 card and the drawing was then checked with oculars of various powers. The card was then filed away for a time. Later, it was brought out and on it the same set of chromosomes was again drawn. This drawing was checked as in the case of the first. Then the two drawings were compared and the points of difference, should any be found, were decided by comparison with the cell under the microscope. This method saved considerable time and, I believe, gave very accurate results.

Methods of measuring

The chromosomes were enlarged with a pantograph six diameters over the original magnification (which was 3400 ×). This not only made easier to measure the chromosomes but gave somewhat more accurate results. Many of the chromosomes are practically straight, so that a rule could be used in measuring. The automatic map measure was employed in obtaining the length of the curved chromosomes. The advantages of this instrument have been described (Hance, '18). It has the disadvantage that millimeters must be estimated. This, I believe, I have been able to do with an error of at the most not more than one or two millimeters. With the large magnification of 20,400 × these results appear to me to be sufficiently accurate.

Mechanical aids

The automatic tally register has been found a great convenience for counting the chromosomes. It enables the investigator to go from the drawing to the microscope without the necessity of remembering the number that has already been counted. The chromosomes are, of course, checked as counted. Before this device was tried drawings would frequently have to be counted several times to be certain that no error had been made.

An adding machine has been of the greatest assistance in this work as has also a 'Calculex,' or circular slide rule, which was used for determining percentages.

A large number of drawings of mitotic figures are presented with this paper, partly to counteract the general impression, gained from published mammalian studies, that clear figures are difficult to find, but largely to give a better idea of the appearance and relationship of the chromosomes of the various cells.

at Glenolden, Pa.   It is a pleasure to acknowledge the kindness and the interest of these various persons and their assistance in helping me get the material I needed.   Such friendly coöperation between the biological manufacturer, commercial establishment and the theoretical laboratory cannot fail, in the long run, to be productive of results beneficial to all concerned.

The many facilities placed at my disposal by Dr. McClung, and his constant interest and friendly advice and criticism have made my stay at the University of Pennsylvania not only profitable, but very enjoyable, a period always to be remembered with great pleasure.

I feel that a list of acknowledgments would be incomplete if no mention were made of my appreciation of and indebtedness to my mother and father to whose encouragement, constant interest and sympathy I owe so much.

THE CHROMOSOMES OF THE GERM CELLS

The diploid chromosome number

Only those cells have been considered in which the chromosomes were well separated (figs. 1 to 20) where the count could be made with comparative ease and accuracy.   Forty chromosomes have been found constantly in the spermatogonia of the pig.   This number has appeared in testicular material from three Berkshires, five Jersey reds and one Poland china, the material being gathered from rather widely separated regions of this country.   The drawings in figures 1 to 20, though selected for flatness to show the full length of the chromosomes are not exceptional as to clearness.   The photomicrographs in figures 88 to 99 emphasize the separation and sharp outlines of the chromosomes.

It may occur to the reader that the chromosome number may be influenced by precocious division, since, in the drawings, splits are shown in the chromosomes while they lie in the metaphase plate.   With experience the chromosomes which have divided are distinguishable by the difference of level and diameter. Furthermore, the count of forty has been made in cells in pro-

phase (figs. 1, 2 and 3) and also in anaphase (fig. 16). With the counts in these three stages checking, I feel confident of the accuracy of the result.

The structure of the chromosomes

Figures 1 to 20 show that there is some variation in the width or diameter of individual chromosomes, in any given cell. The difference within the cell is very slight and may be due to the fact that the dye is extracted more rapidly from small than from larger chromosomes. In more heavily stained material this difference is not so obvious (figs. 4 and 5) but exists to some extent. The prophases do not show this variation in diameter. In some of the metaphase chromosomes I am inclimed to believe that the extra width is due to the beginning separation of the chromatids, the split being filled with dye. It will be noticed, however, that the diameter of any one chromosome is the same throughout its length.

The presence at various stages of splits in the chromosomes is so variable that at present definite conclusions cannot be drawn. In material passed through paraffin splits are occasionally visible in a few chromosomes in prophases (fig. 3) and it is not possible to determine whether these are obliterated before metaphase is reached or not. From studies of unsectioned amnion which was much superior to the imbedded material for fine details, it seems certain that all the chromosomes of the prophase are split (fig. 81) and that this condition is maintained through the metaphase. Slight differences in fixation or staining would tend to obliterate these fine separations. A slightly oblique view of the chromosome would also tend to obscure the split.

In recent years the point of spindle fibre attachment has been found important. It is difficult in the material I have of the pig to directly determine this point as the spindles are visible in but very few instances. Indirectly it is possible to obtain some evidence as to the point of fibre attachment from the shape assumed by the chromosomes in anaphase as the form at this time would be dependent upon the point of the chromosome at which

the pull was applied. In other words, if the spindle fibre is attached to the end of the chromosome or is telomitic (Carothers '17) the daughter chromosomes pass to the poles as rods whereas if the attachment is along the body of the chromosome or is atelomitic then upon separation the daughters approach the poles as V's with equal or unequal arms depending upon whether the fibre is joined to the exact middle or to one side of the middle of the chromosome. Figure 16 shows rods and V's which have already reached the poles. Figures 84, 87 and 88 show rods V's with equal and unequal arms approaching the poles. From the material studied it may be said that the fibre attachment is in some instances telomitic and in others atelomitic. More chromosomes apparently have terminal fibre attachment than subterminal fibre attachments. I cannot present complete evidence as to the constancy of the point of fibre attachment in the pig but the similar form of certain recognizable chromosomes (the longer ones) in various cells would seem to indicate a permanency of the location of fibre attachment.

A giant cell

Figure 14 is a drawing of the chromosomes of a giant cell found among the spermatogonia. The chromosomes at the right hand end, as the cell has been placed on the plate, are somewhat crowded and indistinct, but there are at least as many chromosomes as are figured. The number is seventy-four, which is just six less than double the normal spermatogonial number. Since the chromosomes do not appear smaller than those in the normal cells and the chromosome number is nearly double that usually found, it is believed that this cell has arisen, either through the fusion of two spermatogonia or because separation of the daughter cells failed to take place after the chromosomes had divided.

The reduced number of chromosomes

Although no particular study has been made of the spermatocyte chromosomes, I have included two very clear figures at the end of plate 2. Both of these show twenty chromosomes. Those

familiar with the characteristic shape of the first spermatocyte chromosomes, as found in the grasshoppers, some of the bugs and other forms, will recognize similar forms here. These forms were not shown in the previous study of the spermatogenesis of the pig (Wodsedalek, '13).

THE SOMATIC CHROMOSOMES

The present report must necessarily be preliminary in nature and intended rather to point out certain conditions that exist, than to offer a final solution of the problem. With this in mind, two embryos were selected for the study of the chromosome conditions in the body—one embryo of eight, the other of fifteen millimeters. The smaller embryo was fixed entire, while the larger one was teased. The teasing was not sufficient to interfere greatly with the recognition of the various tissues. The fixation in both was excellent, as may be judged from the drawings, and was fully as good as was obtained in the germ cells. Only those cells were selected for drawing which were clearly uncut and in which the chromosomes were well separated and distinct. Small cells possessing a large number of chromosomes, may, even in the most perfectly preserved material, occasionally present one of two places where the number of chromosomes involved is uncertain. This will be spoken of later.

The chromosome number

It was soon found that the somatic chromosome number was not constant. The variation ranged from forty to fifty-seven, and one cell was found in which at least seventy-four chromatin bodies were counted. Ninety-one cells were counted. (See page 167.) Drawings of the various types of cells found in the embryo are given in figures 23 to 88. Although, at first, the greatest variation was thought to be confined to the blood, as more tissues were studied, it was found that the blood was not an exception to the rule and that a similar range of numbers was present in each tissue in which a number of active cells were found. The following table gives in detail the distribution of the variations in the thirteen tissues studied.

Table of chromosome numbers in the various tissues studied

The figures at the top of each column refer to the number of chromosomes and the figures in the columns indicate the number of examples found

TISSUE	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	74	NUMBER OF CELLS
Blood		2	1	2		1		2		1	1					1			1	12
Brain	3	1	2	1		3	6	3	2	3	4	2	3	4	4	1	1	1		44
Connective			1	3		2				1	1			1						9
Amnion	1	1							1		1									4
Myotome		1	1	1																3
Heart				1																1
Diaphragm					1															1
Wolffian body			1			1			1											3
Lung																1				1
Umbilicus								1	1											2
Limb buds						1				1	1	1		1						5
Optic cup															1					1
Liver						2		1					1		1					5
Number of cases	4	5	6	8	1	10	7	7	5	6	8	3	4	7	4	3	1	1	1	91

Text figure 1 illustrates graphically some of the conditions given in the above table. On this chart no one type or group of cells appears to be predominant. It seems very probable that when a larger number of cells are studied, this curve will 'straighten' out, showing approximately the same number of cells falling in the various classes.

Had the variation in number been but one or two it might have been attributed to errors in observation and drawing because of the many chromosomes in the cells. But the numbers ranged from forty to fifty-seven, which excludes the possibility of error from this source. An error of one or two chromosomes might well be made but, considering the excellence of the preparations and the care taken in checking the drawings, not one of seventeen. In recording the chromosomes my constant effort was to underestimate rather than to overestimate the number. It was frequently difficult to determine whether the chromatin body under examination was really single or composed of two parts. In all such cases, except where I was finally able to determine defi-

nitely, I have counted the doubtful element as one rather than two. This difficulty was encountered, particularly in cells possessing fifty or more chromosomes and, in many instances, cells which I have placed in, for example, the fifty chromosome class may well have fifty-one, fifty-two or fifty-three chromosomes. There are certainly, however, at least as many chromosomes in a cell as are indicated by my numbers and my drawings.

Text fig. 1   A frequency curve (heavy band) showing the group distribution of cells possessing various numbers of chromosomes as found in ninety-two somatic cells of the pig.   The thin solid line represents the range and distribution of variation for cells of the brain and the broken line does the same for the blood.   None of these curves appear to have a definite mode.   The number of chromosomes are listed on the base line while the frequencies are recorded on the axis of ordinates.

The chromosome number in uncut membranes

Since the main work of this study was completed I have obtained fresh material from Cincinnati.   The amnion of embryo pigs (18 to 20 mm.) was prepared as described (page 160) and was found exceedingly favorable for study.   The thickness varies but in the portions observed it was seldom more than three cells thick and frequently the membrane seemed to be composed of a single layer.   The certainty that the cells were uncut made the

material excellent for checking the results obtained on the earlier sectioned embryos. The clearness of the figures in these membranes is evident from figures 81 to 85 and (photomicrographs) 89 to 93 and 95.

Nearly all the cells studied were in prophase lending further support to the idea of a 'cycle of division' (page 157). Twenty-nine cells have been drawn and counted and the range of number is the same as reported for the rest of the soma. The twenty-nine cells fall into the following groups:

	NUMBER OF CHROMOSOMES											TOTAL
	40	41	42	43	44	45	47	49	50	53	58	
Number of cases	10	2	4	1	3	3	2	1	1	1	1	29

There are more cells in the forty class than found in the previous material but, as has been indicated, the frequency curve of the first cells studied was without definite mode and it may well be that the frequencies in this case are also governed by chance.

All that has been said on the structure of chromosomes on page 163 applies to the chromosomes of the somatic cells as well as those of the germ cells.

The behavior of the 'extra' chromosomes

The chromosomes of the spermatogonia have been shown to be constant in number, while those of the soma have a rather wide range. Regardless of how this variation has come about it is interesting to know how these 'extra' chromosomes the chromosomes in excess of forty) behave. Are they carried on as a part of the chromatic complex or are they thrown out to degenerate in the cytoplasm? My evidence on this point is not very conclusive, as it is difficult to find anaphase stages oriented in the proper plane and at just the right stage for counting. In one case, however, I was able to make a fairly accurate count of the chromosomes at one pole of an anaphase group. In figure 57 the plate placed toward the upper side of the page

shows at least forty-three chromosomes.   There are very likely more present, but forty-three chromosomes were readily discernible.   At the lower pole only thirty-six chromosomes were distinguishable, but the spindle axis was slightly oblique to a perfect polar view and consequently it was impossible, even to approximate the actual number of chromosomes present.   It is evident in one case, at least, that the 'extra' chromosomes behave as do the others and divide regularly.   It is rarely that I have found a cell in anaphase with the chromosomes trailing on the spindle after the others had reached the poles as might be expected if these 'extra' bodies were eliminated.   Figures 86 and 88 are thought to be two such cells.   In figure 86 there is an area surrounding a trailing chromosome that is lighter in color than the cytoplasm and which appears as the beginning of a vesicle. This vesicle might cause the disintegration of this chromosome outside of the nucleus.   The evidence for this, however, is very poor.   Evidence from another form on the constancy of the complex will be mentioned presently in support of the above statements.

The cause of the variation in number

Since the chromosomes of the male germ cells are constantly forty in number, how has the soma acquired the wide range of number that has been described?   One explanation would suggest that they are giant cells, or perhaps what may be termed partial giants, due to the division, but lack of separation of certain chromosomes, as found by Dr. Caroline Holt ('17) in the cast off lining of the intestinal wall in Culex pipiens.   This is not likely for several reasons.   In the first place the multiple chromosome cells in Culex are degenerating and abnormal, while the cells in the pig which show variation in chromosome number are perfectly normal in appearance and size, and are part of active, healthily growing tissue.   When the somatic cells are compared with the giant spermatogonium reproduced as figure 14, it is very obvious that the cells of the soma are much smaller and appear, so far as the diameter of the metaphase plate is con-

cerned, like normal spermatogonial cells. The other possibility
is that the chromosomes in the somatic cells have fragmented.
This suggested itself to me particularly, as I had discovered that
the variation in the number of somatic chromosomes in the
evening primrose, Oenothera scintillans, is due to the fragmen-
tation of certain chromosomes (Hance, '18). In the case of
Oenothera scintillans I found the chromosomes showing deep
constrictions which led to the solution of the problem in this in-
stance. The conditions in O. scintillans will be discussed later.
Although I have searched for such constrictions in the chromo-
somes of the pig, I have never found any of which I could be
certain. In several cases chromosomes have been found in
which there appeared to be a constriction but this interpretation
was always open to considerable doubt. In figure 36, to the left
of the number is one of the best cases of a thin area in a chromo-
some that I have found. One of the chromosomes in figure 85
also shows constrictions. When a large number of chromosomes
are concerned, it becomes a matter of considerable difficulty to
determine whether a chromosome has been found showing a con-
striction or whether this dent is really due to the overlapping or
approximation of two chromosomes. Although it has been im-
possible actually to demonstrate the possibility of fragmentation
through the discovery of constrictions in the chromosomes, there
are, however, indirect methods of proving that this process is
going on in the pig. If the chromosomes are breaking up we
would expect to find, as in the case of Oenothera scintillans,
that those cells showing a high degree of fragmentation would
give ocular evidence of the fact by possessing visibly shorter
chromosomes. Those cells which possess only a few more than
the spermatogonial number of chromosomes should appear like
the germ cells, while those having forty-five or more should have
chromosomes which are obviously shorter. A comparison of a
cell having many chromosomes with others in the same tissue,
or in other tissues having the spermatogonial number or very close
to it, will show that the chromatin bodies in the many-chromo-
some cells are in general, shorter. Compare figures 23 to 80 with
figures 1 to 20 and the photomicrographs 96, 97 and 98 with 99.

With the ocular evidence in favor of the breaking up of the somatic chromosomes, further evidence indicating that such a process is actually taking place may be offered. That the 'extra' chromosomes divide and behave normally has been described above. They are not as a rule at least, lost from the complex. If the extra chromatin bodies present are not due to additions to the regular nuclear equipment, but rather to the breaking up of the type chromosomes, the total length of all of the chromosomes in any somatic or spermatogonial cell, regardless of the number of separate elements possessed, should be approximately the same. The results of an investigation to determine this point follow.

THE METRIC ANALYSIS OF THE CHROMOSOMES

In studying the chromosomes, use has been made of the method first developed in the work on the evening primrose, Oenothera (Hance, '18). Measurements of chromosomes have been made before, but the discovery of the exact inter-pair relationship is, in somatic cells as far as I am aware, new, and should prove of considerable value in studying cytological data (particularly in the determination of the chromosomes involved where irregularities occur). Since these methods were worked out I find that Robertson ('15) has employed the relationship of certain chromosomes expressed in ratios in some of his Orthopteran studies. This will be referred to in the discussion. Sutton ('02) was the first to determine the paired condition of the germinal chromosomes. He pointed out that the pairs differed in length and maintained a definite and constant size from one cell generation to another, and also that the pairs, when arranged according to length formed a practically uniformly graded series. Meek ('12a, '12b and '15) has also made extensive measurements. These methods permit the accurate checking of results and eliminate, to a large degree, the personal element which so frequently enters cytological work. Relationships not optically discernible are brought out, and the application of the method to the work of others enables an investigator to check his work as regards accuracy and interpretations.

Spermatogonia

Total length of the chromosomes. The total lengths of the chromosomes in the spermatogonia at 20,400 magnification were found to range from 118.6 centimeters to 177.6 centimeters with an average of 136.9 centimeters. A glance at figures 1 to 20 will show the reason for this wide variation. Cells differ considerably in the size of the chromosomes, although it is apparent from the figures and from the measurements, that each chromosome of a cell suffers equally in any reduction or gains proportionally in an increase of size. I have reported the same conditions, rather more marked, in the somatic cells of the mosquito (Hance '17 a). This may be due to differences in nourishment, or, as in the case of the production of extra contractile vacuoles in paramaecium ('17 c), to a rapidity of division which does not permit the chromosomes time to grow to the limits of their possibilities. A graphic illustration is given in text figure 2 of the minimum, maximum and average length of the spermatogonial chromosomes.

That figure 14 is a giant cell is readily demonstrated when the chromosomes are measured. Although the total lengths of the spermatogonial chromosomes has been found to vary between 118.6 and 177.6 centimeters, the chromosomes of figure 14 measure 235 centimeters, or 58 centimeters longer than the longest set of chromosomes.

Pairs and relation of pairs. When the chromosomes of the spermatogonia are arranged according to length, it is found that they form a series which falls very gradually from the long end of the series to the short end. This may be seen in plate 9, row 1, and it is more clearly shown by the heavy black band in text figure 3. The pairing is not as strikingly obvious as in the case of Oenothera scintillans, but, considering the chances of error in drawing a large number of small chromosomes, I believe that the pairs stand out fairly well. In the table below the average lengths of the spermatogonial chromosomes are listed. Frequently the mates are found near each other in the complex but not always.
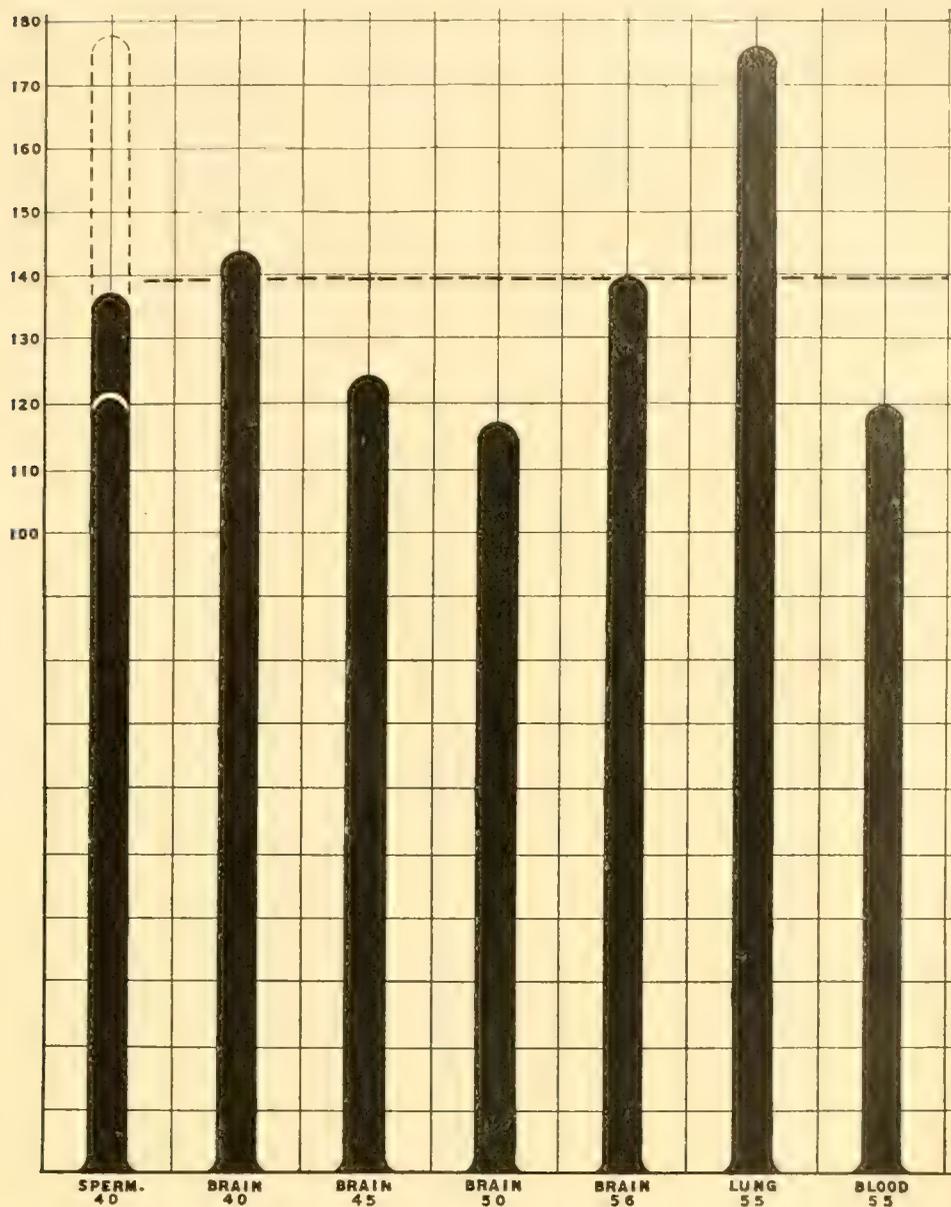
Text fig. 2 A graphic illustration of the total lengths of the chromosomes of the spermatogonia and in different body cells which have undergone various degrees of fragmentation. The figures to the left refer to the length in centimeters. The dotted addition to the pillar representing the spermatogonia indicates the greatest length found and the white line shows the minimum for these cells. The heavy dotted line across the diagram represents the average length of the chromosomes of somatic cells. Note that the total lengths of the somatic chromosomes fall within the limits found for the spermatogonia and that the average length for these cells is almost identical to the spermatogonial average.
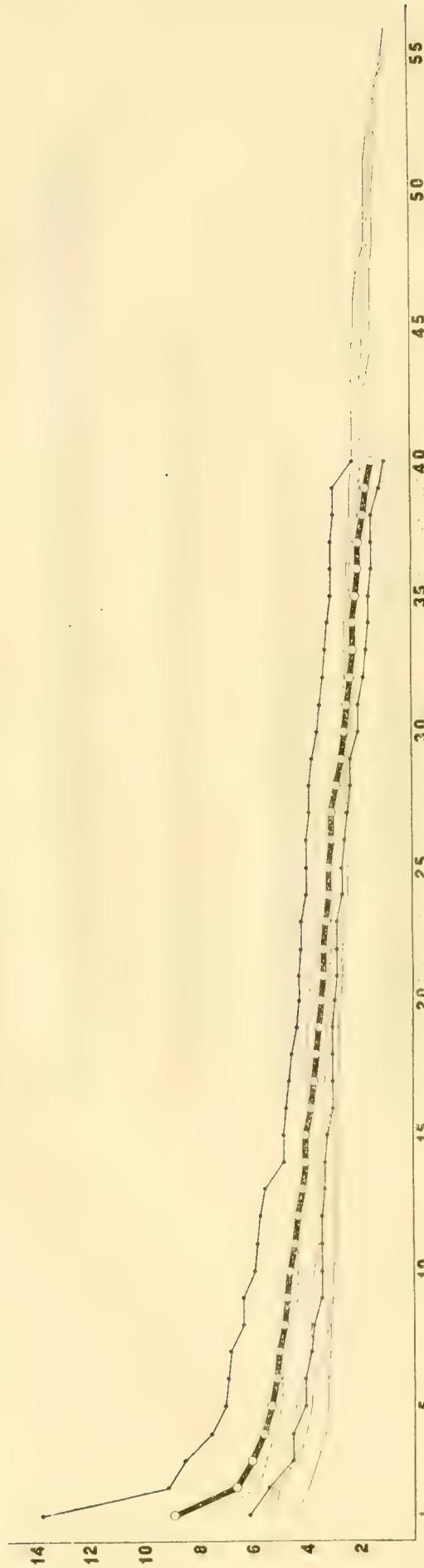
Text fig. 3 Curves illustrating the chromosome length series. The figures on the axis of ordinates refer to length in centimeters while those along the base line are the numbers of chromosomes arranged according to length. The heavy black band indicates the average length of the individual chromosomes of the spermatogonia. The lighter line interspersed with dots lying above the heavy band indicates the maximum lengths and the similar line below indicates the minimum lengths found in the spermatogonia. Other lines represent somatic chromosomes. These are described in the text.

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Table of spermatogonial chromosome lengths, pairs and relations

PAIR	CHROMOSOME	LENGTH IN CENTIMETERS	RELATION OF PAIRS IN PER CENT
1	1	8.9	
	2	6.6	
			74
2	3	6.0	
	4	5.6	
			89
3	5	5.3	
	6	5.1	
			94
4	7	5.0	
	8	4.8	
			96
5	9	4.7	
	10	4.6	
			96
6	11	4.4	
	12	4.3	
			98
7	13	4.1	
	14	4.0	
			95
8	15	4.0	
	16	3.8	
			95
9	17	3.7	
	18	3.6	
			95
10	19	3.5	
	20	3 4	
			94
11	21	3.3	
	22	3.3	
			97
12	23	3.2	
	24	3.1	
			97
13	25	3.1	
	26	3.0	
			94
14	27	3.0	
	28	2.8	
			90
15	29	2.6	
	30	2.5	
			92
16	31	2.4	
	32	2.3	
			92
17	33	2.2	
	34	2.2	
			96
18	35	2.1	
	36	2.0	
			91
19	37	2.0	
	38	1.8	
			84
20	39	1.7	
	40	1.5	

As a constant relationship between chromosome pairs had been found in Oenothera scintillans, it was of interest to determine whether such exists in the pig. That it does is shown in the above table in the column to the right. There the relationship of the pairs is expressed in terms of per cent, i.e., a shorter pair is a certain per cent of the length of the next longer pair, etc. There is a rather large increase in this percentage relationship at either end of the series. These relationships will be more readily understood from text figure 4 when the heavy black band expresses the percentage of difference between pairs of chromosomes in the spermatogonia, drawn from the figures given above. This curve shows a remarkably uniform difference in length between the pairs, except at the two extremities. In cells in which the total length of the chromosomes is short, all of these have been affected equally in the size reduction, as the percentage relationship between the pairs is the same as between the same pairs of the 'longer' cells.

Somatic cells

Total lengths of the chromosomes. The total lengths of the somatic chromosomes in the various tissues is given in the following table. As can be seen, the total lengths of the chromosomes in the somatic cells falls within the limits determined for the spermatogonia. The one exception to this statement—the fifty-chromosome brain cell measuring 117 centimeters—falls below the minimum length found for the spermatogonia by such a small amount that I am not inclined to attach any importance to it. The average length of the various cells given above is 139.3 centimeters, which is exceedingly close to the average found for the germ cells, 136.9 centimeters. The lengths from various cells are compared with those of the spermatogonia in text figure 2. It is evident that, since the total length of the chromosomes in the various classes of cells is approximately the same, the increased number of chromosomes must be due to fragmentation.

Figures 23 to 80 show that any tissue may have metaphase plates that vary as much in total diameter as do those of the
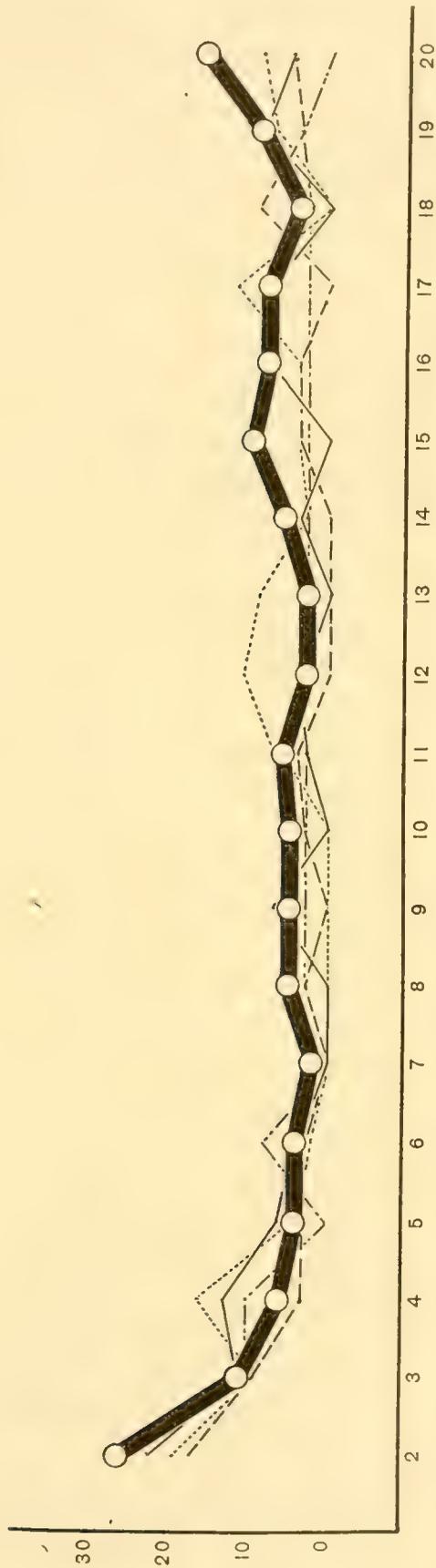
Text fig. 4 This curve illustrates the length difference between chromosome pairs expressed in percent. The numbers to the left refer to per cent and the numbers along the base refer to pairs of chromosomes. The heavy black band indicates the percentage difference between chromosome pairs in the spermatogonial cells and the other lines represent similar relations between the first twenty pairs of chromosomes in highly fragmented cells.

spermatogonia. The measurements given above bear this out. It may be pointed out that, although considerable variation exists between the total lengths of all of the chromosomes in the various cells, this variation is not greater in one tissue than in another and falls within the limits determined for the spermatogonia.

On plate 10 the chromosomes of a number of cells in various degrees of fragmentation are arranged according to size. It is obvious that the somatic chromosomes, particularly at the long end of the series, are shorter than those in the same column in the row devoted to the spermatogonial chromosomes or to the somatic cells possessing forty chromosomes. The conditions illustrated on this plate will be referred to later.

Table of total chromosome lengths in the cells of various tissues and in cells possessing different numbers of chromosomes

TISSUE	NUMBER OF CHROMOSOMES	TOTAL LENGTH OF CHROMOSOMES IN CENTIMETERS
Amnion	41	129.2
Blood	55	129.8
Brain	40	143.9
Brain	45	124.1
Brain	50	117.0
Brain	56	139.9
Limb bud	52	152.6
Liver	53	158.4
Lung	55	176.1
Myotome	43	
Average		139.3

The length of the chromosome fragments. In my studies on Oenothera scintillans I considered all the chromosomes which, when arranged according to length, fell below the shortest chromosome in the normal diploid series, to be portions or fragments of longer chromosomes and I found them to be remarkably alike in length. When the chromosomes on plate 9, to the right of chromosome 40, are studied, it is evident that they, too, vary but little. By actual measurements they aver-

age 17 millimeters, with one extreme measuring 9 millimeters and the other 25 millimeters. The majority approach very near the average length. Recalling that the longest chromosomes of the series average 8.9 centimeters, it seems justifiable to consider the chromosomes below chromosome 40 in the length series to be very nearly of even size in the majority of cases.

Discussion of the results obtained by measuring the chromosomes

The conditions in Oenothera scintillans. In order that the metrical studies of the chromosomes of the pig may be better understood a synopsis of similar work on Oenothera scintillans (Hance '18) is given below. As the chromosomes in this Oenothera were much less numerous than in the pig, more clear cut and definite results were obtained, and they aided greatly in the interpretation of the observations on the latter.

1. In material collected from three generations of Oenothera scintillans the somatic chromosome number varies from 15 to 21. This variation is not correlated with any particular tissue, but exists throughout the plant.

2. The type or fundamental number of chromosomes is 15.

3. The 'extra' chromosomes divide regularly.

4. Each pair of the type (15-chromosome) group differs from the next pair in the series by 9 per cent, or a shorter pair is 91 per cent the length of the next longer pair. This is apparently a constant relation.

5. The 'extra' chromosomes have been shown to be fragments of the type chromosomes, and the total length of the chromosomes in the various classes of cells has been found to be practically identical. The fragments are very nearly of even length.

6. With the aid of the paired conditions of the chromosomes and of the relation between the pairs, it has been found possible theoretically to reunite the fragments with the chromosomes from which they have separated.

7. Fragmentation in the cells studied, has been confined largely, but not exclusively, to the longer chromosomes.

8. Fragmentation does not occur in the germ line.

The conditions in the pig. With the increase in the number of the chromosomes the difficulties of analysis are increased. It is consequently necessary to know how far we may trust the accuracy of the present work before it is possible to draw conclusions from the results. If the majority of the results obtained in the present study can be shown to be safely comparable to those obtained on O. scintillans, it will be justifiable to assume that the conclusions reached for the earlier and more favorable form will be applicable to the pig.

A very interesting relation exists as regards the degree of fragmentation in the two forms. Considering only the cells in which the highest number of chromosomes is found in both the Oenothera (21 chromosomes) and the pig (57 chromosomes) in comparison to the type or fundamental numbers (15 and 40 respectively), it becomes evident that the evening primrose has suffered a fragmentation of 40 per cent of its chromosomes and the pig of 42.5 per cent. While future work may show that this percentage of fragmentation may mean nothing and is merely a coincidence, for the present it is suggestive.

To review briefly the results obtained in the study of the chromosomes of the pig:

1. The spermatogonial number is 40.

2. The somatic number as determined in the present study ranges from 40 to 58.

3. The 'extra' chromosomes divide as do the type chromosomes.

4. A difference averaging 12 per cent has been found to exist between spermatogonial pairs. If the relationships of the length of the pairs on either end of the series be omitted the average per cent is but five.

5. The total length of the chromosomes of any somatic cell, regardless of the number of chromosomes or of the tissues of which it is a part, falls within the length limits found for the spermatogonial chromosomes.

6. The 'extra' chromosomes, for reasons set forth above, must be fragments of the normal chromosomes.

7. The fragments are very nearly of even length.

8. Fragmentation does not affect the germinal chromosomes.

A comparison of the conditions in the two forms. When the summary of the observations on the pig is compared with the preceding one on Oenothera scintillans it is plain that, with one or two exceptions, the words pig and Oenothera could be interchanged without affecting the truth of the statements in the slightest. The relationship between the pairs differs slightly, but is, however, fairly constant in both species. The chief discovery in the case of the Oenothera is that it is possible to reunite the fragmented chromosomes and thus determine, with a fair degree of accuracy, which chromosomes have broken up. Is this method of procedure applicable to the chromosomes of the pig? Can the chromosomes which have fragmented be located? The chance of even approximate accuracy in attempting to reunite the fragmented chromosomes of the pig is so slight that it would be unwise to base any conclusions on the results. This is true for the following reasons: 1) The large number of chromosomes and the consequent and very probable slight inaccuracies in drawing are prohibitive of the success of such a study. 2) The very slight differences, in the majority of instances, between the chromosome pairs present no definite criterion on which to base judgment as to which chromosomes are out of place in the series and consequently with which fragments they should be united.

It is possible, in a more direct way, however, to determine which chromosomes of the series are breaking up. The method is not so completely satisfactory as the one used with the evening primrose, but serves to show, in a general way and with a considerable degree of accuracy, which end of the chromosome series is breaking up. In plate 9 it will be recognized at once that, in general, in those somatic cells (row 3 to 12) possessing a large number of chromosomes, the chromosomes at the beginning of the series are shorter than the chromosomes of the spermatogonium (row 1) and of the unfragmented brain cell (row 2) in the same columns. There are exceptions to this which tend to show that, in breaking up, the chromosomes may not follow a definite plan beginning with the long chromo-

somes and proceeding down the series. In row 6 a 41-chromosome cell is represented. Here the first two chromosomes are very clearly entire, judging their lengths by those of the first two chromosomes of row 1. It appears very probable that either chromosome 3 or 4 has been the one to lose the portion represented by chromosome 41. In row 11 the first two chromosomes are long, but so, also, are the other chromosomes in this cell, and when considered from this point of view these chromosomes are relatively very nearly as short as the first pair in row 10. The chromosomes in row 11 measure 176.1 cm., while the total length of those in row 16 is 153.6 cm. Row 11 is therefore 8.8 per cent longer than row 10. If the total lengths of the chromosomes vary by 8.8 per cent then, according to the statements made above that in reduction (for whatever reason) each chromosome suffers proportionately, any corresponding pairs from either of the cells should differ by very nearly the same amount. It is, of course, not expected that exact results may be obtained in all cases for reasons pointed out earlier. Consider for example the pairs composed of chromosomes 1 and 2 in rows 10 and 11. These chromosomes average 5.1 cm. in length in row 10 and 6.7 cm. in row 11. and consequently differ by 7.6 per cent. In this instance there is a discrepancy of 1.2 per cent. When the next pair in each cell is compared it is found that the difference is 8.8 per cent exactly the same as the variation between the total lengths. This is sufficient, I think, to indicate that the difference in total length is due to a proportional reduction of the chromosomes of row 10 and that the corresponding pairs in each of the rows are comparable. Furthermore it is evident that the longer chromosomes in both rows have in all probability lost a portion of their length since they are shorter than the corresponding 'typical' pairs.

The fact that it is the members of the long end of the chromosome series which are breaking down becomes more evident in text figure 3. For the sake of clearness the graphs for only three greatly fragmented cells have been drawn. These are represented in light unbroken lines. Note that in all three cases the curves start out below the curves representing the

minimum lengths found for the spermatogonial chromosomes. One cell enters within the spermatogonial minimum curve at chromosome 3 and continues, either on it or very close to it, for a considerable distance. This, however, is a 'longer' cell than the other two and would be expected to rise above the type minimum more quickly than the others. *It does not, however, rise noticeably above the minimum curve at the long end of the series,* notwithstanding its extra total length. The other two curves do not rise above the minimum curve until chromosome 27 is reached. These three cases, which are typical for others, most certainly show that the chromosomes at the long end of the series have been reduced considerably in length. This becomes especially clear when the broken lines in text figure 3 are followed and contrasted with the results mentioned above. These curves represent one cell with unfragmented chromosomes and another in which there is a single fragment. The curves lie within the extremes of the spermatogonial lengths for the full distance, and the curve representing the chromosomes of the unfragmented cell coincides with the spermatogonial average curve for a great part of its length.

Hence it seems entirely justifiable to claim that, although the exact chromosomes which have broken up cannot be ascertained, as was done in the case of Oenothera scintillans, the same general conditions hold true, namely, that the chromosomes of the long end of the series have fragmented. This, of course, does not exclude the possibility that some of the shorter chromosomes have broken up. Since this was discovered in Oenothera scintillans, and since the other conditions in both forms are so comparable, it seems probable that the fragmenting of some of the shorter chromosomes may occur in the pig. Neither has it been possible to show that a chromosome has not lost more than one piece. For various reasons, however, I am, at present, inclined to think that a chromosome does not lose more than one piece or, if it does, that the condition is rare.

The point of fragmentation. That the fragments should be so nearly of even length in the two forms studied is very suggestive, and would seem to indicate that one or two chromomeres have

broken off and that the loss is limited to that amount of chroma-
tin. Since the fragments are fairly uniform in length, the
chromosomes must be reduced by more or less equivalent
amounts, and consequently we should not expect to find the
percentage relation between the pairs showing any marked
variation from that found between the spermatogonial pairs.
This is shown graphically in text figure 4 where the percentage
relations of the first twenty pairs of four highly fragmented cells
are plotted. As can be seen, the curves vary but slightly from
the spermatogonial curve.

It has been pointed out that the long chromosomes are the
chief ones involved in fragmentation. A study of the longer
chromosomes Plate 10, rows 1 and 2 (unfragmented cells) reveals
that several are J-shaped or formed as an unequal armed V.
Assuming that the point of spindle fiber attachment is located
at the apex of the J (page 163) the possibility that fragmenta-
tion has occurred at this point immediately suggests itself since
the short arm of the chromosome is in many cases approximately
the length of the chromosomes (the fragments) lying above 40
in the fragmented series. It will be noted on Plate 10 that
there are several J-shaped chromosomes in those rows represent-
ing cells in which little or no fragmentation has occurred. With
an increasing degree of fragmentation the number of curved
chromosomes becomes less and less. In cells with high num-
bers of chromosomes those lying in the same columns as the
curved chromosomes of rows 1 and 2 are generally rod-shaped.
While this is but circumstantial evidence it offers a logical and
interesting explanation of the process as occurring in these cells.
It is, of course, but a tentative theory which may be proved
or overthrown by further work.

DISCUSSION

The work of other mammalian cytologists

The amount of published cytological work on the mammals
is neither large nor reliable, when compared to that which has
been done on other forms as, for instance, the grasshoppers and

the bugs. Since the present paper deals with the diploid number alone, I will consider only that part of the reported mammalian investigation. It is rather interesting to note the elaborate descriptions of the resting and prophase conditions and the brief consideration and few figures of the chromosomes themselves. The inference is rather obvious, and it is partly to counteract the impression that sufficiently clear, and easily studied chromosome complexes are scarce in mammalian material, that I have presented the large number of figures at the close of this paper.

The investigation of the most importance to the present study is Wodsedalek's work on the pig ('13). He has reported 18 chromosomes in the spermatogonia and 20 in the ovary. He also found two classes of embryos, one with 18 and the other with 20 chromosomes. He figured two X-chromosomes. The discrepancy between Wodsedalek's count of 18 chromosomes and my own of 40 chromosomes in the same type of tissue is rather large, and I feel sure, has been due largely to the difference in the methods of preparing the material. In a paper on "The fixation of mammalian chromosomes" ('17 b) I have described the result of fixing mammalian tissue with various fluids, and also the effect of fixing stale tissue (which, indeed, may not have been removed from the body for more than 15 to 20 minutes). Wodsedalek's figures are practically identical with those I have found in either poorly fixed tissue or in tissue that was dead when preserved. The chromosomes are clumped and undecipherable, as is evident in the one spermatogonial and three embryonic diploid cells figured in Wodsedalek's paper. A very interesting condition seems generally to occur when chromosomes clump together as the result of poor fixation. The number is reduced to approximately one half through the fusion of chromosomes lying along side of each other—probably mates. In the case under discussion, Wodsedalek's count is two less than one half of the count I have made. In this condition, such chromosomes as can be distinguished at all, are at least double the width of those found in properly fixed material. It is very evident that such poorly preserved tissue should never be accepted as avail-

able for study, since any conclusions based on such material are not only open to criticism, but are very probably wrong. A form possessing so large a number of chromosomes as the pig enclosed in so small a compass must, indeed, be prepared with the greatest care if results of any value are to be obtained.

It is possible to show more conclusively that the diploid chromosomes of Wodsedalek's pig possess the same amount of chromatin as I have described for the number of individuals studied. If a fusion between adjacent chromosomes, or perhaps between pairs, has occurred, the total length of the chromosomes in Wodsedalek's figures should be about one half the length I have found. Furthermore, the chromosomes (provided the magnification was the same) should average about twice the width of those figured in this paper. It seems probable that, however much the widths may have been effected through poor fixation, the lengths may not have been greatly disturbed, or, if they were shrunken, the shrinkage would affect all alike. Consequently I believe that the measuring of these chromosomes may be relied upon to give fairly accurate results. The chromosomes of the four figures in his paper were copied and enlarged six times over the published size as carefully as possible with the following results.

The length of the chromosomes in the figures of the diploid complexes published in Wodsedalek's article on the spermatogenesis of the pig ('13)

TISSUE	FIGURE NUMBER	NUMBER OF CHROMOSOMES	TOTAL LENGTH $\times$ 6 IN CENTIMETERS
Spermatogonium............................	18	18	56.5
Oogonium.................................	60	20	49.2
Mesonephros..............................	59	18	44.3
Mesonephros..............................	62	20	47.6
Average.....			49.6 or approximately 50 cm.

The magnification used by Wodsedalek was about one third less than that I have employed. Consequently one third must

be added to 50 cm. to bring it up to a magnification corresponding to that used in the present work—50 + 16 = 66 cm.

The total average length of the spermatogonial chromosomes in my material—136.9 cm.

The total average length of the dipoid chromosomes in Wodsedalek's material—66.0 cm.

The length of the latter chromosomes is 48 per cent that of the upper lot, or very nearly one half—66 cm. × 2 = 132 cm. This is so near the average length of the spermatogonial chromosomes found in my work as to be practically identical. In the same way the widths may be determined. I was hampered again by being unable from the figures to determine certainly the limits of the chromosomes, but I think that any error that may have been introduced has been largely checked by averaging the results.

Average chromosome width—12 mm.; + $\frac{1}{3}$ magnification—16 mm. Average chromosome width in my material—7 mm.

The first set is a trifle more than double the width of the latter, not a sufficiently great difference, however, to be significant, considering the chances of error. Text figure 5 illustrates the above results graphically. At the right is the figure of the spermatogonial chromosomes in Wodsedalek's paper, and a column drawn to scale from the above calculations illustrating the average total length and average width of the chromosomes. To the left are corresponding figures representing the present work. This column is obviously a little over twice as high and one half as wide as the column to the right. The area of the left hand column (as reproduced) is 468.5 square mm.; of the right hand column, 441 square mm. It is safe to conclude therefore that both Wodsedalek and myself have been dealing with the same amount of chromatin, and the difference lies wholly in the difference of the preservation of the chromosomes; those in the former study being clumped and undecipherable, while those reported here are well separated.

Though I have little, direct proof to offer at present, I am inclined to believe that other features of the chromosome history reported in the earlier paper, particularly as regards the num-

ber of chromosomes in the first and second spermatocytes, and the secondary reduction phenomena in the second spermatocytes, were influenced by the same conditions which affected the number of spermatogonial chromosomes. The first spermatocyte cells that I have counted have had twenty chromosomes, and the secondary spermatocytes—although I have not found
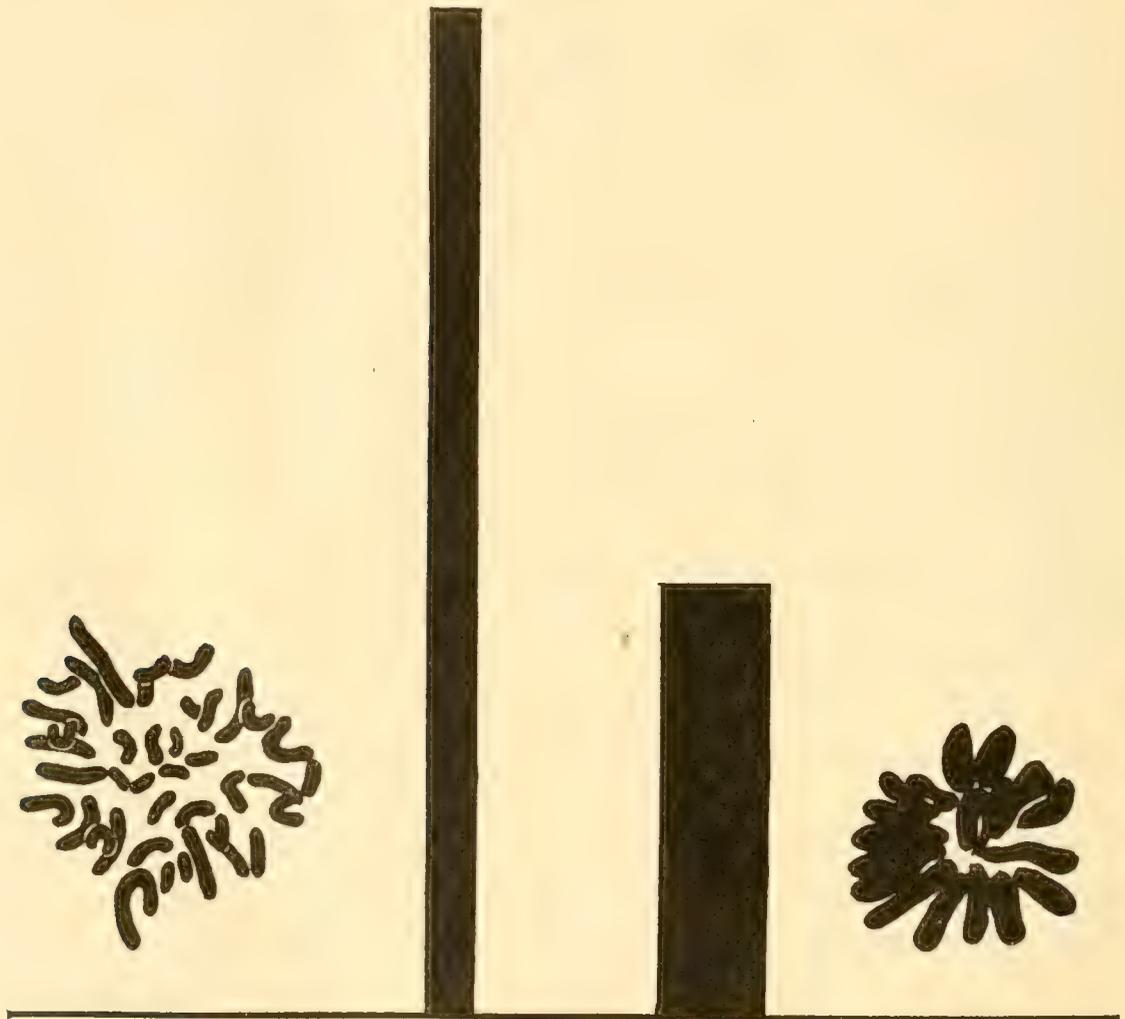
Text fig. 5 A diagrammatic comparison of the amounts of chromatin in pig spermatogonia studied by Wodsedalek and myself. The metaphase plate to the left shows forty chromosomes (× 3400), the column next to it represents the total length of the chromosomes and average width or diameter of the spermatogonial cells. The column and chromosome group (showing presumably eighteen chromosomes (× 3495)) to the right illustrates the same features for the spermatogonial figure published by Wodsedalek. It is evident that the pillar to the left is about twice as high and one half as wide as the one to the left, both consequently possessing the same area. See text.

any in which an accurate count could be made—have shown no tendency to a secondary or pseudo-reduction. Neither have I, in the present study, seen any bodies comparable to the X-chromosomes shown in Wodsedalek's figures. In poorly fixed material obtained some years ago I have seen similar bodies. This problem I hope to take up shortly.

Other cytological work carried out on mammals includes studies on man by Flemming ('98), Farmer and Moore ('05), Moore and Arnold ('06), Duesberg ('06), Guyer ('10), Montgomery ('12), Winiwarter ('12), Jordan ('14) and Wieman ('13 and '17). The diploid number as given by these workers varies from 22 to 47 or 48. The rabbit is claimed to have chromosomes ranging in number from 22 to 80. This form has been studied by Flemming ('98), Winiwarter ('00), (who believes that the somatic number ranges from 42 to 80), Barratt ('07) and Bachhuber ('16), Miss Stevens, in 1911, reported 56 chromosomes for the guinea pig. Vom Rath, in 1894, found the somatic chromosomes in the dog to range from 8 to 64, and pointed out that the various numbers were always multiples of eight. Winiwarter and Sainmont ('09) described 36 chromosomes for the sex and somatic cells of the cat. In the opossum Jordan ('11), found 17 chromosomes in both germ and somatic tissue. Schoenfeld's work on the bull ('07) is largely a study of the prophase behavior, and the number of chromosomes is not given. Wodsedalek found 37 chromosomes in the horse ('14) and 51 chromosomes in the mule ('16). Yocum ('17) has reported 20 chromosomes as the reduced number in the mouse. He has not counted any spermatogonial complexes. His drawings show the chromosomes to be much better separated than is the case in the majority of published mammalian studies.

If we may judge the accuracy of the count from the character of the published figures accompanying the above papers, I believe that we are justified in accepting but few of the writers' conclusions. The chromosomes are massed together, as shown in the case of Wodsedalek's work on the pig, and it seems very likely that fusion of the chromosomes has occurred in the various forms mentioned above, as has been shown to have occurred

in the previous work on the pig. In a study of the tissues from seven mammals in connection with work on "The fixation of mammalian chromosomes" (Hance, '17 b) the chromosomes of none of the forms studied, when killed and treated in the proper way, were clumped together or variable in width. They did not look like amorphous lumps of stainable material which is the appearance of many of the mammalian chromosomes in published figures. Furthermore, the number was always high, in all cases about 40 or more. With a large amount of material from so many mammals, I think that I have a fair basis for the criticisms I have made of the mammalian cytological studies up to date. Winiwarter's work stands out above that of other workers, if we may judge the excellence of his fixation from the clearness of his published figures. The diploid chromosomes he figures for the cat are very comparable, in form and number, to those I have found in this animal. I have already suggested ('17b) that when a study of human cytology is repeated on properly preserved and freshly killed tissue, the number of chromosomes will be nearer that reported by Winiwarter than by other investigators. The variations in the somatic chromosome number which he has found in the rabbit are very suggestive from the point of view of the present work and will be considered later. Miss Steven's figures of the spermatogonia of the guinea pig ('11), although she found but few clear cells, appear to justify her count of 56. Wieman's prophase figures, in his paper on the chromosomes found in a human embryo appear considerably better than do his later figures on the spermatocytes ('13 and '17). Wodsedalek's figures of the chromosomes of the mule ('16) are somewhat better, as regards separation, than those in his papers on the horse and the pig, but all leave much to be desired in the way of regularity of chromosome outline and differentiation of chromosomes.

Reported cases of fragmentation

The most familiar case of variation in somatic chromosome number is that of Ascaris megalocephala reported by Boveri. Here the fertilized egg starts with two chromosomes, and the

cell, which may be considered as the anlage of the future germ cells, retains the two chromosomes intact, while the chromosomes of the cells forming the body tissues break up into as many as sixty fragments. This fragmentation is said to be accompanied by a chromatin diminution. If this be true, the quantity of the chromatin in the cells of the soma is probably not the same as it is in the germ line, contrary to what I have found in the pig and in Oenothers scintillans. A metrical study of the chromosome behavior in Ascaris would be of considerable value. Nachtsheim ('13) found 32 diploid chromosomes in the bee, while Petrunkewitsch ('13) found 64 in the blastoderm of fertilized eggs. Hoy ('16) points out that "though the reports of several investigators tend to show that the number of chromosomes varies in the bee, the number is always 8 or a multiple of this." (Nachtsheim found that the number in the oogonia was 16 and in the oocytes 8. He considers that oogonial chromosomes are double, thus accounting for the 32 chromosomes found in the soma.) This condition is from one point of view, comparable to that found by Dr. Caroline Holt ('17) in the cells of the cast off intestinal epithelium of the mosquito, where the number varied from 6 to 72, always in multiples of three. These are degenerating and consequently pathological cells, and differ in this respect from the normal cells of the bee.

In the Hymenopter, Nematus ribesii, Doncaster ('07) found the diploid and the haploid chromosome number to be 8 and 4 respectively, while he counted 16 chromosomes in the ovarian sheath. He considers that the chromosomes of the germ cells may be compound and consist of a number of smaller units which become separated in the somatic cells.

I am glad to have this opportunity of correcting two regretable errors that were accidentally inserted in my paper on Culex pipiens ('17) in regard to Seiler's work on Phragmatobia ('14). In one place I coupled his name with Taylor's as having worked on the mosquito, when the other name should have been Lohman, and, in referring to his work on the moth, said that he found the somatic chromosome number to be the same as the diploid number of the germ cells. The diploid number is 56

although 58, 61 and 62 chromosomes have been found in the soma. This has been brought about by the fragmentation of the X and Y chromosomes. It is confined to these bodies, and Seiler points out that the breaking up of the sex chromosomes in somatic nuclei has been observed quite often.

The extra number of chromosomes found in giant cells is not due to the same causes as the above, and they are found in the germ tissue as well as in the soma. I have shown the chromosome relation of a giant cell to the normal cells.

The work of Della Valle ('09) and his conclusions in regard to the variability of chromosomes, is so obviously faulty that I shall not consider them here, particularly since Dr. McClung has discussed his observations and theories at length in a recent paper ('17).

From the above synopses of reported cases of chromosome variations it is evident that the somatic number has been discovered to be different from the spermatogonial number in a few cases and that this is largely due to the breaking up of the major or, what I have termed, the 'type' chromosomes, and in certain cases the same chromosomes are always affected while the others remain intact. If the various workers had measured the chromosomes in the tissues studied we should have more information in regard to the constancy of the chromatin elements.

The metrical study of chromosomes

Not very much work has been done in the way of measuring chromosomes, and some of the studies along this line have not produced results of value. Among these I think we may place the work of Meek ('12 a, '12 b and '15), whose work was exceedingly carefully executed, but whose conclusions had to be retracted eventually as they were too sweeping and based on insufficient data.

Farmer and Digby ('14) found that the "total amount of chromatin substance in the nucleus of each of two types of hybrids known as Primula kewensis is the same. The nuclei of the one form of hybrid contains twice as many chromosomes as

the nuclei of the other type, but the increase in number is associated with a corresponding diminution in size."

Meves ('11) found a graded series of chromosomes in the salamander He did not believe that his results showed that pairing exists, but it appears to me that the pairing in his material is as marked as can be expected, considering the possibilities of error through foreshortening and in drawing. This becomes especially evident when the exceedingly long chromosomes of the salamander are recalled.

Robertson ('15) made use of a constant length relation between certain chromosomes to determine the amount of deficiency of chromosomes which had lost a portion of their length, and for the relation of the chromosomes of one type of cells to that of another ('16). The relation of chromosomes in the Orthoptera is evidently as constant as in the case of the two forms I have studied. In a very few measurements of the chromosomes of grasshoppers that I have made, the relationship between pairs is very similar.

I feel certain that many valuable results will be obtained through the measuring of chromosomes and the study of their relation to each other and to the chromosomes of closely related groups. Other applications of these methods have been pointed out in my paper on Oenothera scintillans ('18). In a preliminary survey of the difference in length between the various chromosome pairs of several plants and animals, I have found this relation (between pairs) to be practically constant in any one group and, furthermore, in the few examples thus far examined, it does not vary greatly between widely separated groups. What this means is, of course, impossible to imagine or predict at present. It is exceedingly suggestive, however, of a physical or physiological phenomena which future discoveries may show to be of fundamental importance.

Chromosomes in development

At the beginning of this paper it was pointed out that an investigation of somatic chromosomes was really a study of the "behavior of chromosomes in development." We have not, I

fear, progressed very far toward the solution of the problem, but several points have been developed which are of some interest and may form a basis for future work along these lines. The fact that the chromosomes may fragment in the soma has been shown to be meaningless, so far as disturbing the quantity of chromatin is concerned, in both the pig and Oenothera scintillans. A definite and apparently constant relation between the chromosome pairs has been discovered which will be of great value in analysing unusual chromosome conditions, such as fragmentation, multiple chromosomes, and the total loss of parts of chromosomes.

Contrasted to the breaking up of chromosomes we have the fusion of certain elements, as described by McClung ('17), Robertson ('16) and Woolsey ('15) and the loss of a portion of certain chromosomes; Carothers ('13 and '17), Robertson ('15) and Wenrich ('16). In these cases the chromosome phenomena link up closely with the taxonomic peculiarities. With the exception of those cases in which certain chromosomes are deficient, the total length of the chromosomes of the cells where these other phenomena have occurred, is, as expected, the same as in the cases where the chromosomes have not fused. The fusion has come about simply as an end to end union. What these phenomena may mean or how or why they have occurred is as much of a riddle as is the breaking up of chromosomes.

A great mass of unquestionable work on plants and animals has demonstrated that the somatic number is the same as the diploid number of the germ cells. To mention but a few—the studies on the squash bug (Morril, '13), Hoy ('16), those on many of the Oenotheras (Gates '15), the work on the mosquito and the Drosophila (Metz, '14 and '16, Whiting, '17 and Hance, '17) have all irrefutably shown that the two types of cells are alike in chromosome numbers.

What, then, does the fact mean that, on the one hand, we find the somatic chromosomes breaking up, while, on the other, they are constant in form and number? In the case of Oenothera scintillans I have pointed out that this is the most unstable of all the 'mutants' of the Oenothera series and never breeds true.

It may be that this breaking up of the chromosomes is correlated with the genetic impurity of the plant. The mosquito is without doubt a 'pure' form, and its somatic chromosomes are constant in number. It is difficult to see how Ascaris can be anything but a homozygous form, and yet the somatic chromosomes are highly fragmented. The pig is probably not pure, considering the length of time it has been domesticated, and its somatic chromosomes show a variation in number. The evidence would seem to be rather evenly balanced at present and no conclusions can be safely drawn.

I think, however, that it may be said with some assurance that the present work, and such previous work as is reliable, has tended to show that the chromosome equipment is the same in the various cells of the body, and when fragmentation occurs, it has not been found to affect one tissue more severely than another. In other words, no tissue specificity of the chromosomes has been found.

The fact that the chromosomes of the majority of somatic cells are more or less fragmented would lend some support to the theory that a germ line or cycle exists, the cells composing which are set off at an early period in the history of the individual. While it is not impossible (from the evidence of the present work) that the germ cells may come from already specialized tissues (Hargitt, '17), it seems rather improbable that cells in which the chromosomes, in all probability, have broken up should be able to reform the typical germinal complex. In view of the present work it seems very likely that the anlagen of the future reproductive cells are set off or guarded from the processes which are taking place in the soma, early in the life history of the animal. Otherwise, since, in the majority of somatic cells studied, the chromosomes break up to a greater or lesser degree, we should have to postulate, either that the chromosomes have the power to recombine as stated above, or that certain cells of some tissue do not undergo the changes common to the rest of the soma. The latter supposition is essentially the germ cycle theory.

The individuality of the chromosomes

It is not my purpose to enter into a full discussion of the theory of the individuality of chromosomes, as Dr. McClung has thoroughly reviewed this subject in a recent publication ('17). I wish merely to add the proof of the present work to that which has already been done. What follows may have more weight when it is said that I began these investigations with the purpose of disproving the hypothesis and was forced into the ranks of the 'believers' by the unquestionable results obtained.

It has been shown that in the somatic cells the chromosomes which are unfragmented agree in length, chromosome for chromosome, with those of the spermatogonia, and the relation between the pairs is the same. When the chromosomes break up, the fragments behave as entire chromosomes at division, and the total length of all the chromosomes of the cell is the same as in the unfragmented cells and the spermatogonia. The quantity of chromatin is undisturbed. What I have said in regard to the chromosome situation in Oenothera scintillans, in respect to the individuality hypothesis, may well be said for the similar conditions in the pig.

Since no chromatin appears to be lost in the processes that are going on in O. scintillans and as the fragments divide as regularly as they did before they were separated it would seem that at least the spirit of the theory of the individuality of chromosomes was maintained. If the theory of the individuality of chromosomes can only recognize a strict morphological continuity then the chromosomes in scintillans lose their individuality through breaking up. If the theory is broader in its scope and admits an individuality not only of whole chromosomes but of the chromatin or chromomeres then the scintillans situation falls within its limits. It is the latter interpretation of the theory that I believe cytologists hold or are tending toward today.

SUMMARY

Technique

1. An improved preservation of mammalian tissue has been obtained through the use of Flemming's (strong and weak) solution, to which has been added a little urea and which is

used at a temperature of four degrees Centigrade.   The pieces of tissue fixed must be very small and absolutely fresh (p. 158).

Observations

2. The number of spermatogonial chromosomes is 40 (p. 162 figs. 1 to 20).

3. The reduced or first spermatocyte number of chromosomes is 20 (p. 164, figs. 21 and 22).

4. The somatic chromosomes range in number from 40 to 58—one cell with 74 chromatin bodies has been found (p. 165, figs. 22 to 80).

5. The 'extra' chromosomes divide as do the type chromosomes (p. 168, fig. 57).

6. The variation in number is caused by the fragmentation of certain chromosomes (p. 169).

Analysis of the chromosome conditions

7. The total length of the chromosomes ($\times$ 20400) of the spermatogonia varies from 118.6 cm. to 177.6 cm.; average, 136.9 cm. (p. 172).

8. When the chromosomes are arranged side by side according to length, they form a series which falls very gradually from the long end to the short end of the series (p. 172).

9. In such a series the chromosomes are found to lie in pairs (p. 172).

10. A fairly constant difference in length exists between the pairs (p. 176).

11. The total length of the chromosomes in the somatic cells ($\times$ 20400) ranges from 117 cm. to 176.1 cm., showing these cells to be within the spermatogonial limits (p. 176).

12. No tissue specificity of chromosome behavior has been found (p. 165).

13. The chromosome fragments are very nearly of even length suggesting that the chromosomes lose a portion constantly represented by one or two chromosomes (p. 178).

14. The conditions found in Oenothera scintillans and the pig agree in practically all details (p. 181).

15. The chromosomes at the long end of the series are the ones that are chiefly concerned in fragmentation (p. 183).

16. Fragmentation does not enter the germ line.

Discussion

17. Much of the cytological work that has been done on mammals is believed to be faulty due to improper fixation (p. 184).

18. Previous studies on the pig are reviewed and it has been demonstrated that, although only 18 chromosomes were reported, the actual amount of chromatin present is probably the same as found in this investigation. The chromosomes as reported are fused and clumped together in the earlier work; due to poor fixation (p. 185).

19. The various types of chromosome variation (multiples, deficient chromosomes, the loss of chromosomes and fragmentation) that have been studied recently are briefly discussed (pp. 190, 194 and 232).

20. It is pointed out that although many of these peculiarities are closely linked with taxonomic differences, the cause of these variations is still obscure (p. 194).

21. The additional evidence that the present work affords the theory of the individuality of chromosomes is commented upon (p. 196).

Results of the investigation in brief

The chromosomes of the soma may fragment but the fragments continue as part of the complex. The total amount of chromatin in the somatic cell in which fragmentation has occurred is the same as in the spermatogonia. There is no fragmentation in the germ cells, and the chromosome complex remains unchanged.

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Figures 1 to 88 were drawn with the aid of a camera lucida using a Zeiss 1.5 mm. apochromatic objective and a 12 × compensating ocular. This gave a magnification at table level of 3400 ×. The pencil drawings were then enlarged to twice the original size with an accurate pantograph. They are reproduced two-fifths off at a magnification of 4080 ×. In all cases where chromosomes are believed to have divided the mates are connected with a bar except when the separation appears as only a slight slit.

Figs 1 to 80 have been drawn from material fixed in Flemming's strong solution. See page 217 for complexes found in material preserved in Flemming's weak solution.

PLATE 1

EXPLANATION OF FIGURES

Spermatogonia from three breeds of pigs

FIGURE NUMBER	STAGE	BREED	ANIMAL
1 and 2	Prophase	Jersey red	a
3	Metaphase	Berkshire	f
4 and 5	Metaphase	Berkshire	f
6, 7 and 8	Metaphase	Jersey red	a
9	Late prophase	Jersey red	c
10	Metaphase	Jersey red	a
11 and 12	Metaphase	Poland China	

PLATE 2

Spermatogonia and first spermatocytes

FIGURE NUMBER	STAGE	BREED	ANIMAL	NUMBER OF CHROMOSOMES
13	Metaphase	Berkshire	f	40
14	Giant cell spermato-gonium	Berkshire	f	74 or more
15	Metaphase	Berkshire	f	40
16	Anaphase	Berkshire	f	40 at pole to the left in-cluding chromosome lying between poles. Poles displaced in drawing
17, 18, 19 and 20	Metaphase	Jersey red	a	40
21 and 22	Spermatocyte I. Meta-phase	Jersey red	a	20
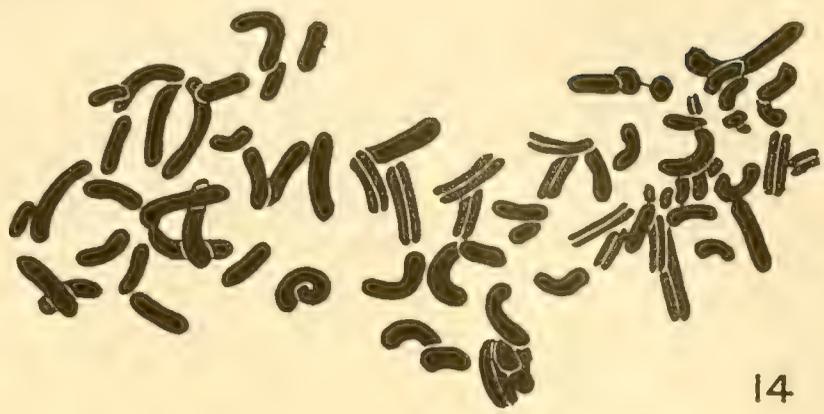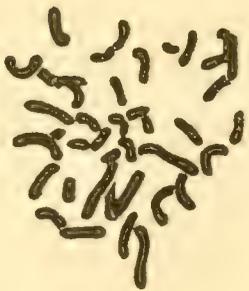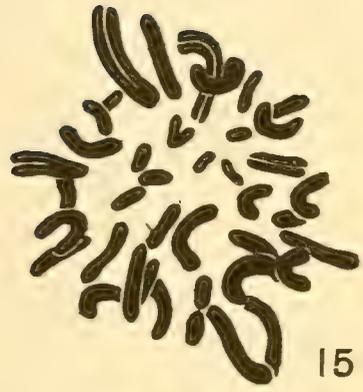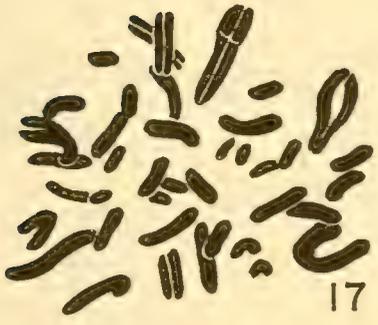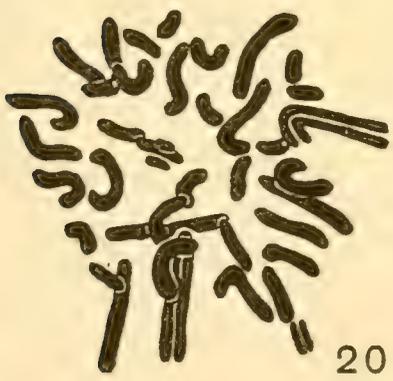
PLATE 3

EXPLANATION OF FIGURES

Polar views of metaphase stages from the brain of a 10 mm. pig embryo

FIGURE NUMBER	NUMBER OF CHROMOSOMES
23	40
24	42
25	43
26	46
27	48
28	47
29	49
30	50
31	50
32	52
33	54
34	54
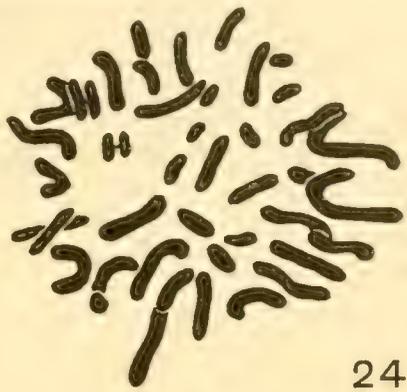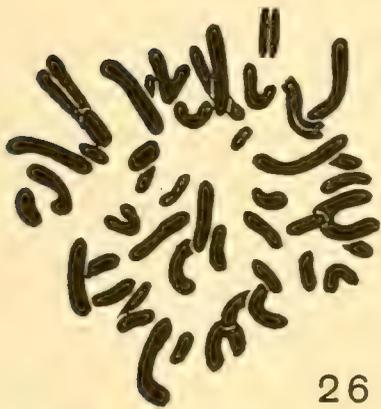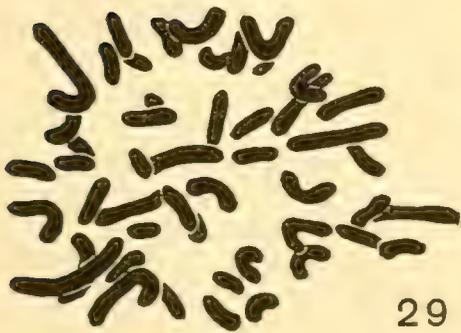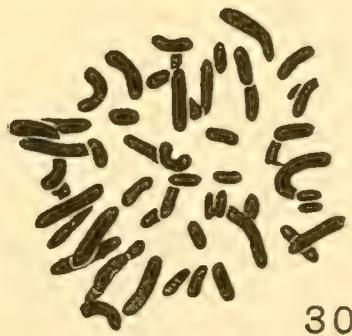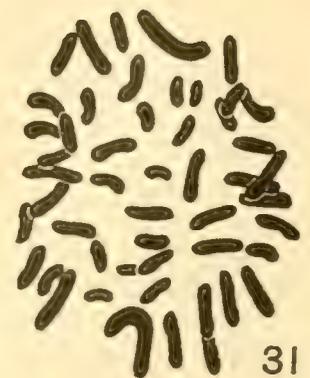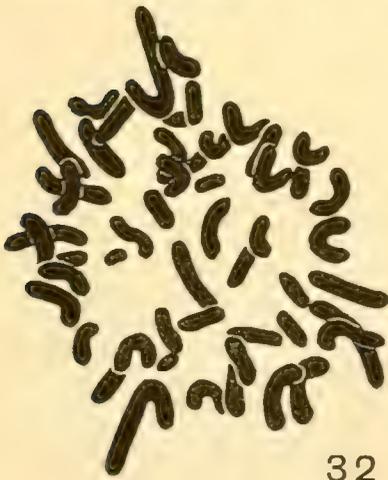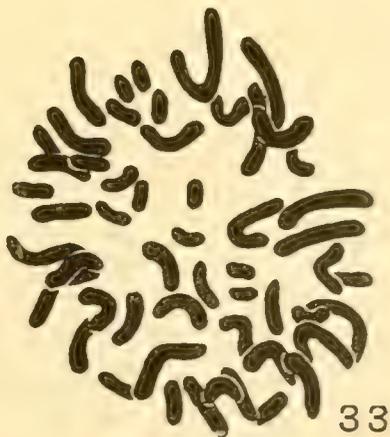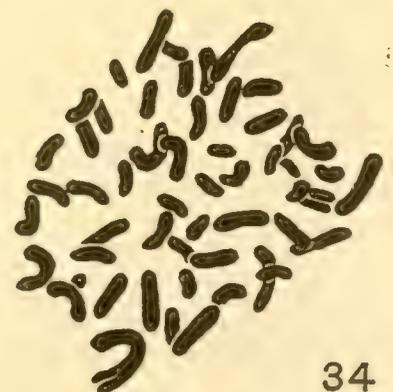
EXPLANATION OF FIGURES

Polar views of metaphase stages from blood cells of pig embryos
35 to 45 From a 10 mm. pig embryo
46 From a 15 mm. pig embryo

FIGURE NUMBER	NUMBER OF CHROMOSOMES	REMARKS
35	41	Count not certain
36	41	Count not certain—note thin section in chromosome to left of number. May be possible point of fragmentation
37	42	
38	43	
39	44	
40	45	
41	47	
42	47	
43	49	
44	50	
45	55	
46	73	Very uncertain—the number of chromosomes is at least as many as indicated
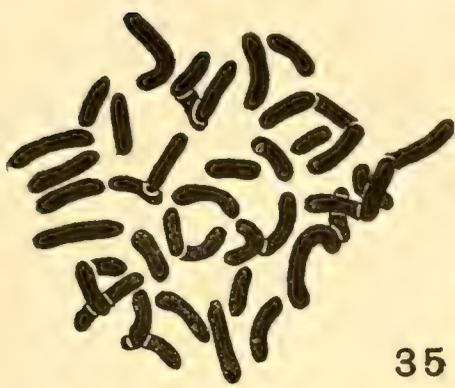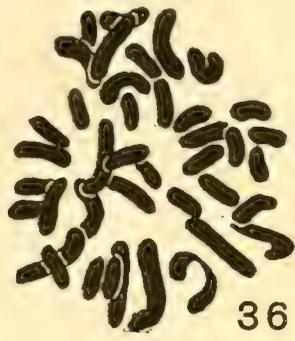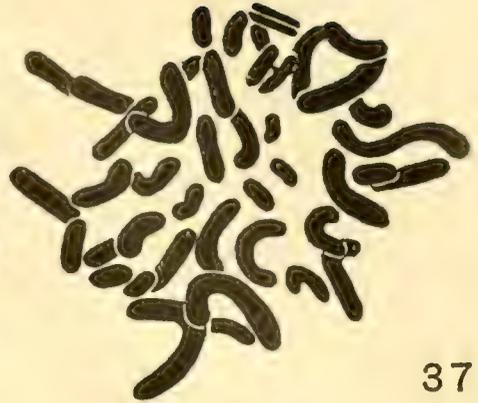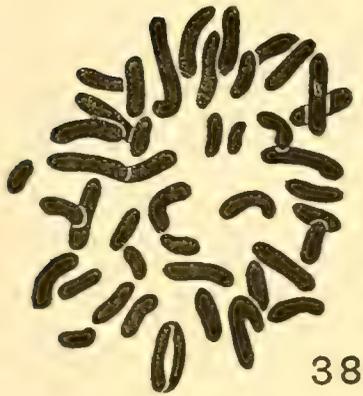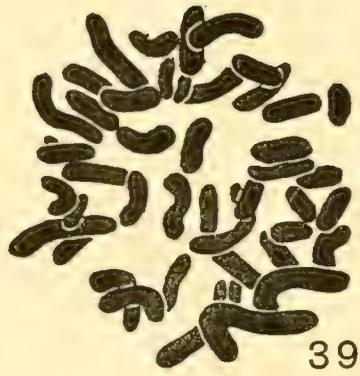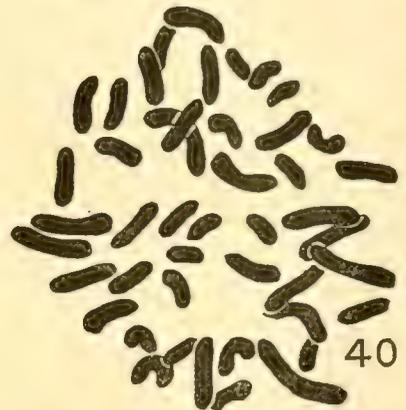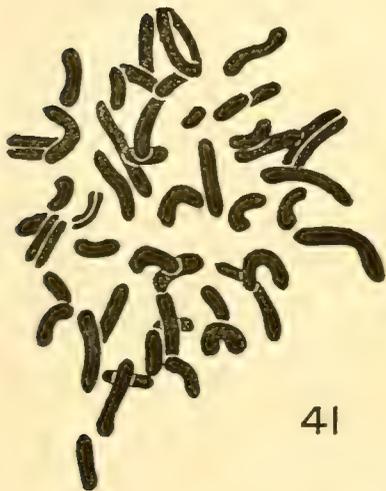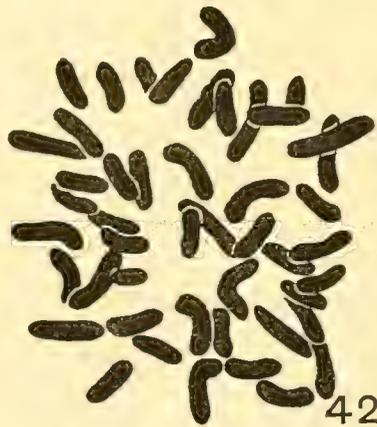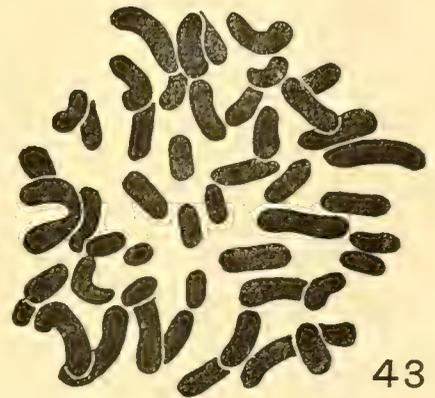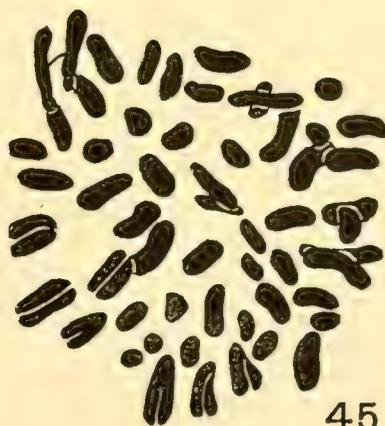
209

FIGURE NUMBER	NUMBER OF CHROMOSOMES	REMARKS
47	42	Count not certain—probably higher
48	43	
49	43	
50	45	Count not certain
51	47	
52	45	
53	49	
54	50	Count not certain
55	51	Count not certain
56	53	

57   The upper pole shows 43 chromosomes.   Though the count is not certain there are at least as many chromosomes as figured.   Since there are this number it is evident that the fragments must divide.   The lower pole shows but 36 chromosomes but it was turned at an angle which made accurate drawing impossible.
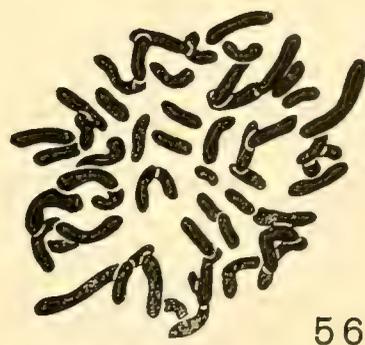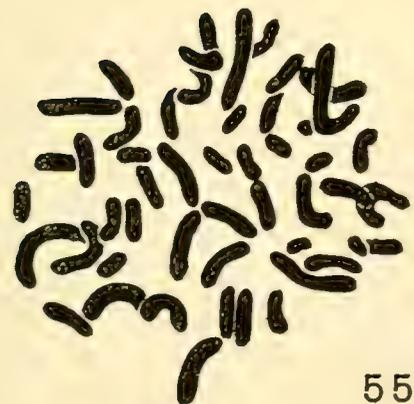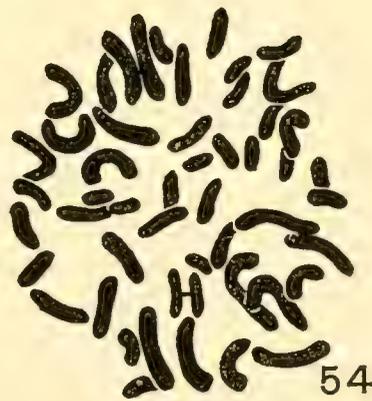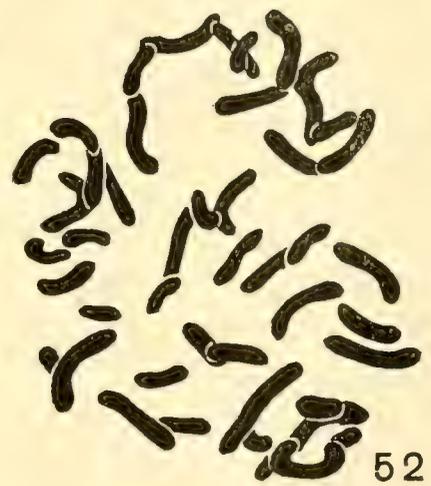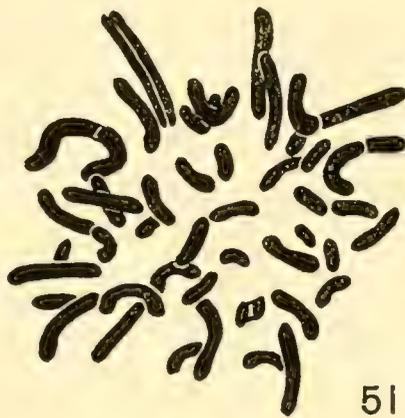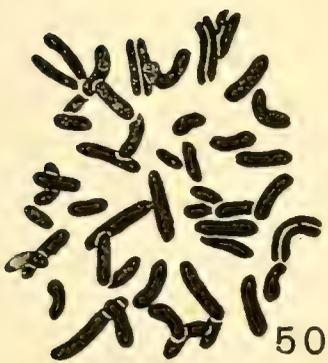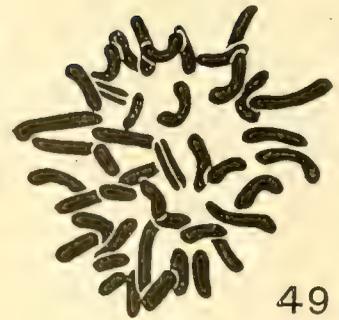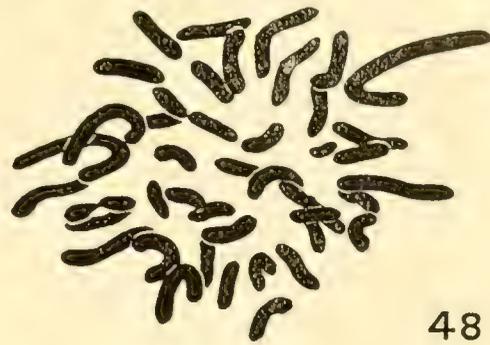
FIGURE NUMBER	TISSUE	NUMBER OF CHROMOSOMES	REMARKS
58	Diaphragm	44	Count uncertain
59	Limb bud	45	Count uncertain
60	Limb bud	48	
61	Limb bud	49	
62	Limb bud	50	
63	Limb bud	52	
64	Myotome	44	
65	Myotome	41	
66	Heart	43	
67	Myotome	43	
68	Mesoderm	54	Probably a giant cell
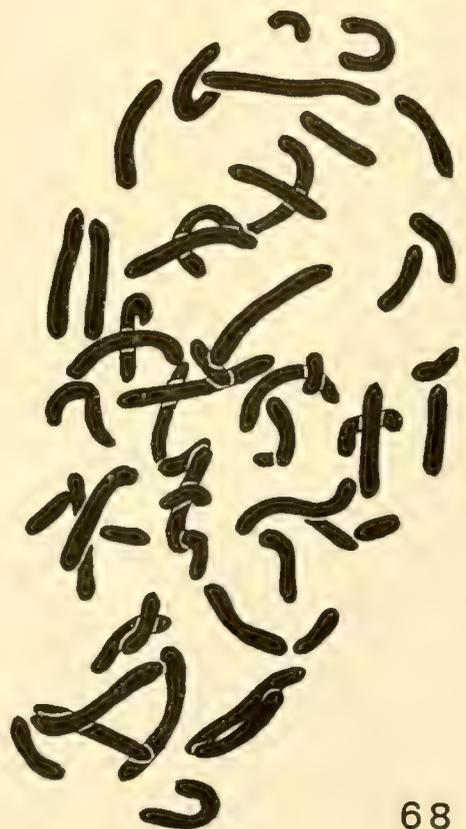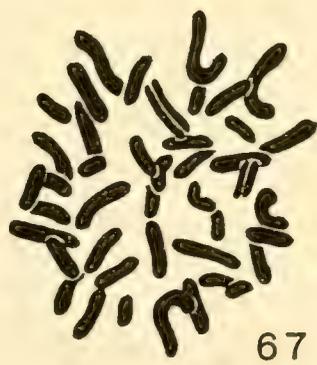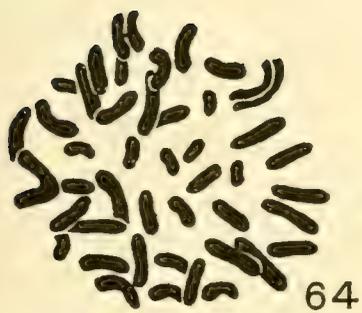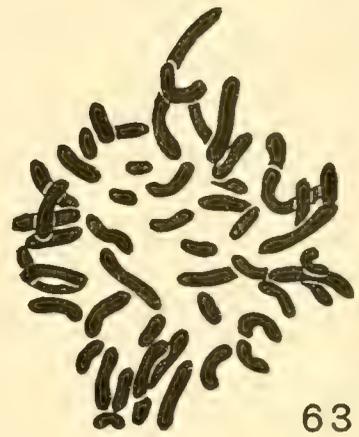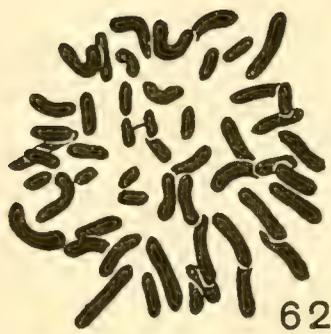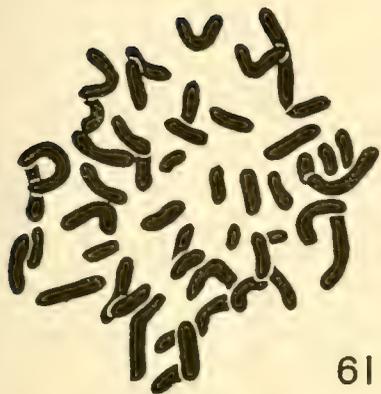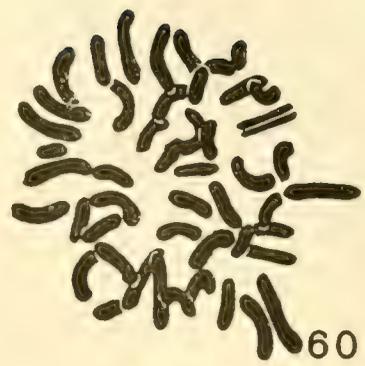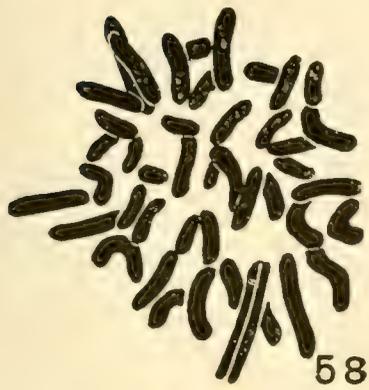
PLATE 7

Polar views of metaphases in cells of various tissues
69 and 70   From 15 mm. pig embryo.   Other figures from 8 mm. pig embryo.

FIGURE NUMBER	TISSUE	NUMBER OF CHROMOSOMES	REMARKS
69	Amnion	40	
70	Amnion	41	
71	Amnion	50	
72	Liver	47	
73	Liver	53	
74	Liver	45	
75	Wolffian body	41	
76	Wolffian body	45	
77	Wolffian body	48	
78	Lung	55	
79	Optic cup	53	Count uncertain
80	Umbilicus	46	
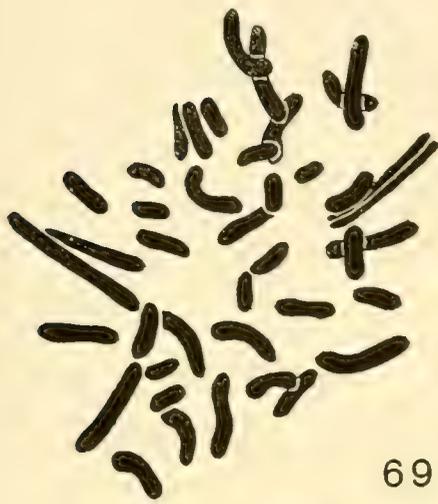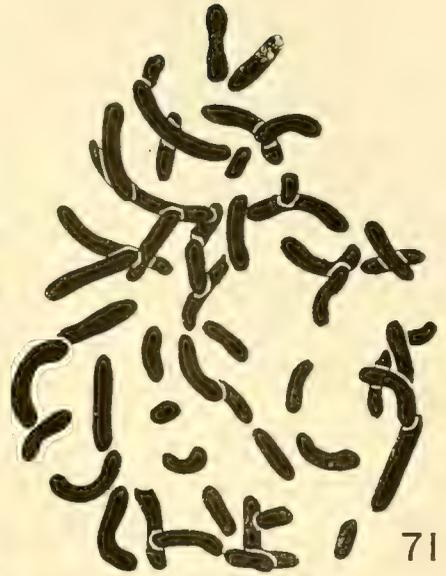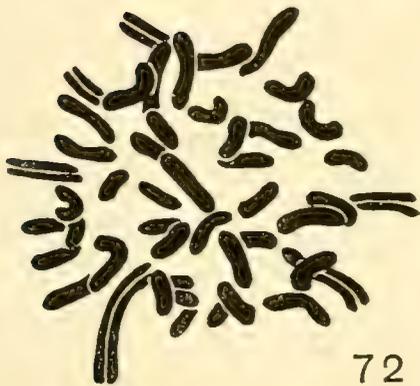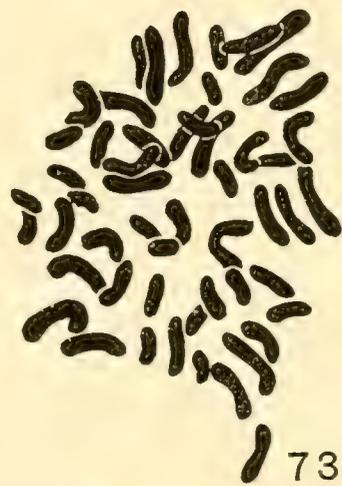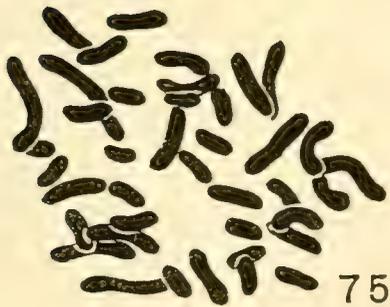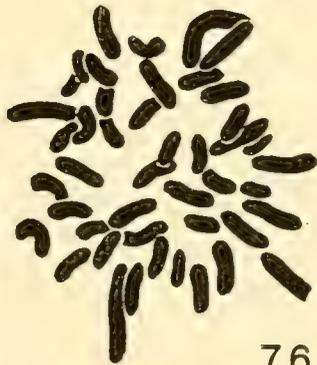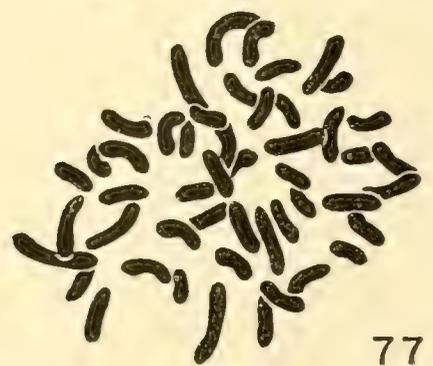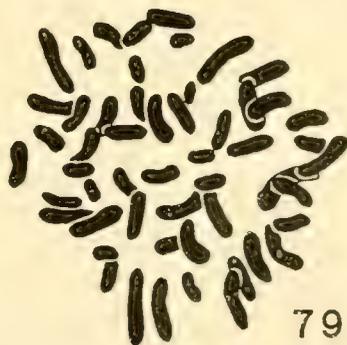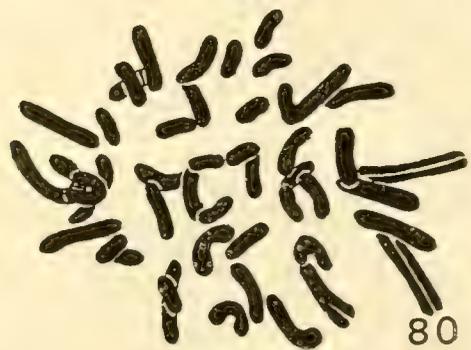
PLATE 8

81, 82, 84, 85 and 88   Drawn from amnion fixed in Flemming's weak solution.
83, 86 and 87   Drawn from amnion killed in Flemming's strong solution.

FIGURE NUMBER	NUMBER OF CHROMOSOMES
81	40
82	53
83	40
85	44

84, 86, 87 and 88   Show the structure of the chromosomes as they pass the poles and are discussed in the text on page 163.

81

82

83

84

85

86

87

88

217
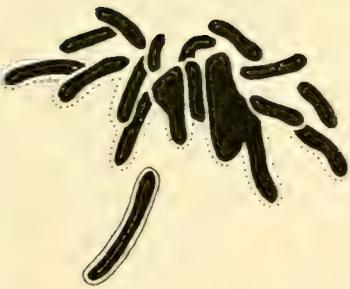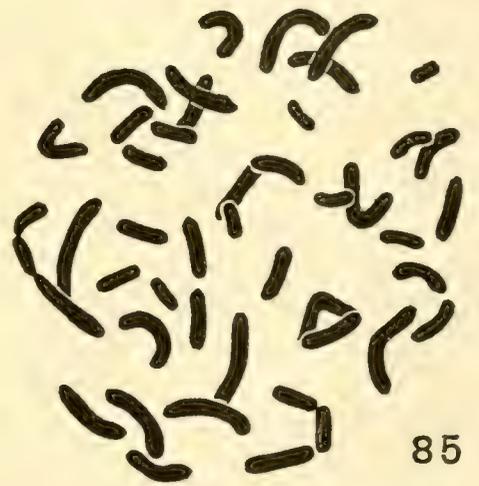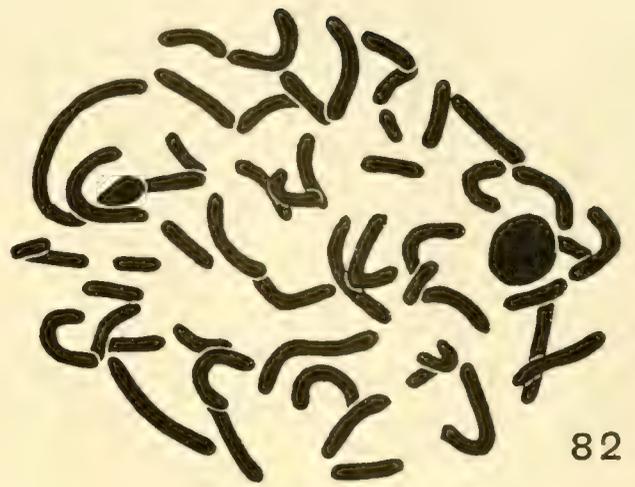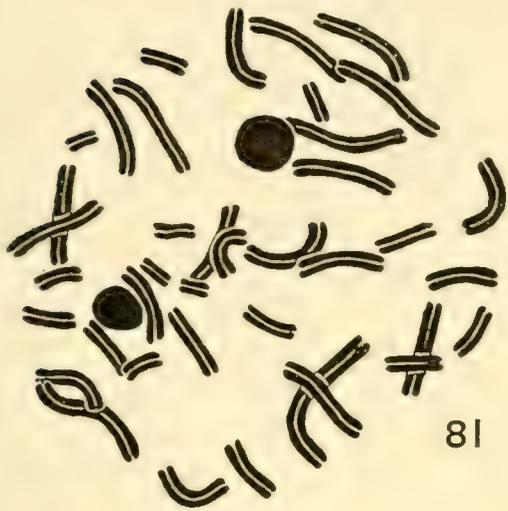
PLATE 9

In preparing these photomicrographs a Zeiss 1.5 mm. apochromatic objective and a 4 X compensating ocular were used. The magnification at the level of the plate was about 1000 diameters. Seeds Process Plates were used. The photographs were made with the aid of artificial illumination. The photomicrographs appear in reproduction at the original magnification.

FIGURE NUMBER	TISSUE	NUMBER OF CHROMOSOMES	SAME CELL AS FIGURE NUMBER
89	Amnion		
90	Amnion		
91	Amnion	53	82
92	Amnion	45	
93	Amnion	50	
95	Amnion	50	
96 and 97	Spermatogonia	40	4 and 5
98	Brain	42	24
99	Blood	55	45

89, 92, 93 and 95   Fixed in Flemming's weak solution.

90, 91, and 96 to 99   Killed in Flemming's strong solution.

94   Illustrates the results obtained in poor fixation. This is a photograph of a group of poorly fixed spermatogonial cells in the testes of a pig.
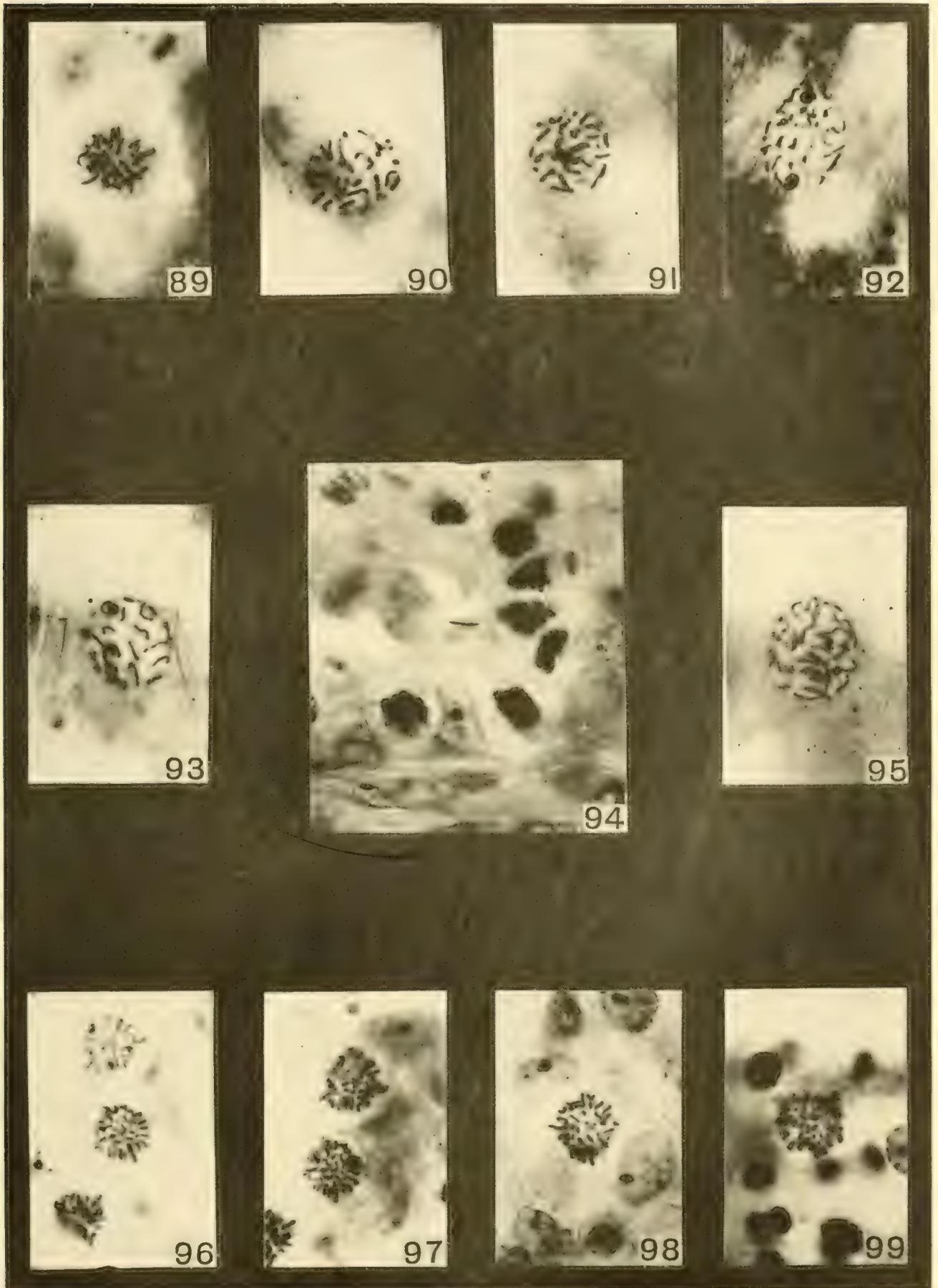
PLATE 10

See table giving the length in millimeters of the chromosomes.

The chromosomes were enlarged to 20400 diameters before being measured. Under 'figure number' is given the number of the complex as it appears on previous plates. The number of the individual chromosomes as they appear on plate 10 is given in the columns to the left.

FIGURE NUMBER	5	23		31		70	67	63	45	56	78	73
Tissue	Spermatogonium	Brain	Brain	Brain	Brain	Amnion	Myotone	Limb Bud	Blood	Connective	Lung	Liver
Row Number	1	2	3	4	5	6	7	8	9	10	11	12
1	70	76	65	49	55	83	54	52	44	52	68	50
2	67	58	47	35	53	75	45	47	39	50	65	47
3	56	55	46	35	43	50	43	46	36	46	55	46
4	50	52	40	35	40	48	40	46	33	45	49	45
5	49	49	40	30	38	45	40	44	32	44	45	43
6	46	49	38	29	38	42	39	41	32	40	43	42
7	46	46	37	29	33	40	38	40	11	38	43	41
8	45	45	36	29	32	39	37	40	31	38	42	41
9	42	43	34	29	31	37	35	39	30	38	40	39
10	41	43	34	27	31	35	36	38	30	37	40	38
11	40	43	34	27	30	35	34	37	29	35	38	37
12	40	43	34	26	30	34	33	36	29	35	38	37
13	36	40	33	26	30	34	31	35	29	35	37	36
14	35	40	33	26	30	32	30	35	28	33	37	35
15	35	40	31	26	30	32	30	34	28	34	37	34
16	32	39	31	26	29	31	30	33	28	34	35	34
17	31	39	30	25	28	31	30	33	28	33	35	34
18	31	39	30	25	28	31	29	33	27	33	35	34
19	31	39	28	25	28	30	28	32	27	32	34	34
20	31	38	28	25	27	30	27	32	27	31	33	34
21	30	38	28	24	27	30	27	31	26	31	33	32
22	30	33	28	24	27	29	26	30	26	30	33	32
23	30	33	26	24	26	28	26	30	25	30	33	32
24	30	30	26	23	26	28	26	29	25	30	33	31
25	30	29	25	23	26	27	25	28	25	29	32	31
26	28	28	25	22	25	26	25	28	25	28	31	31
27	28	28	24	22	25	26	25	28	25	28	30	31
28	28	28	23	22	25	26	25	27	25	27	30	29
29	23	27	23	22	25	24	24	27	25	27	30	28
30	21	26	21	22	25	23	24	26	24	26	30	27
31	21	25	21	20	23	23	23	26	24	25	30	26
32	21	25	21	20	23	23	23	26	24	25	29	26
33	20	23	21	20	22	22	23	26	23	25	28	25

PLATE 10

THE DIPLOID CHROMOSOME COMPLEXES OF THE PIG

ROBERT T. HANCE
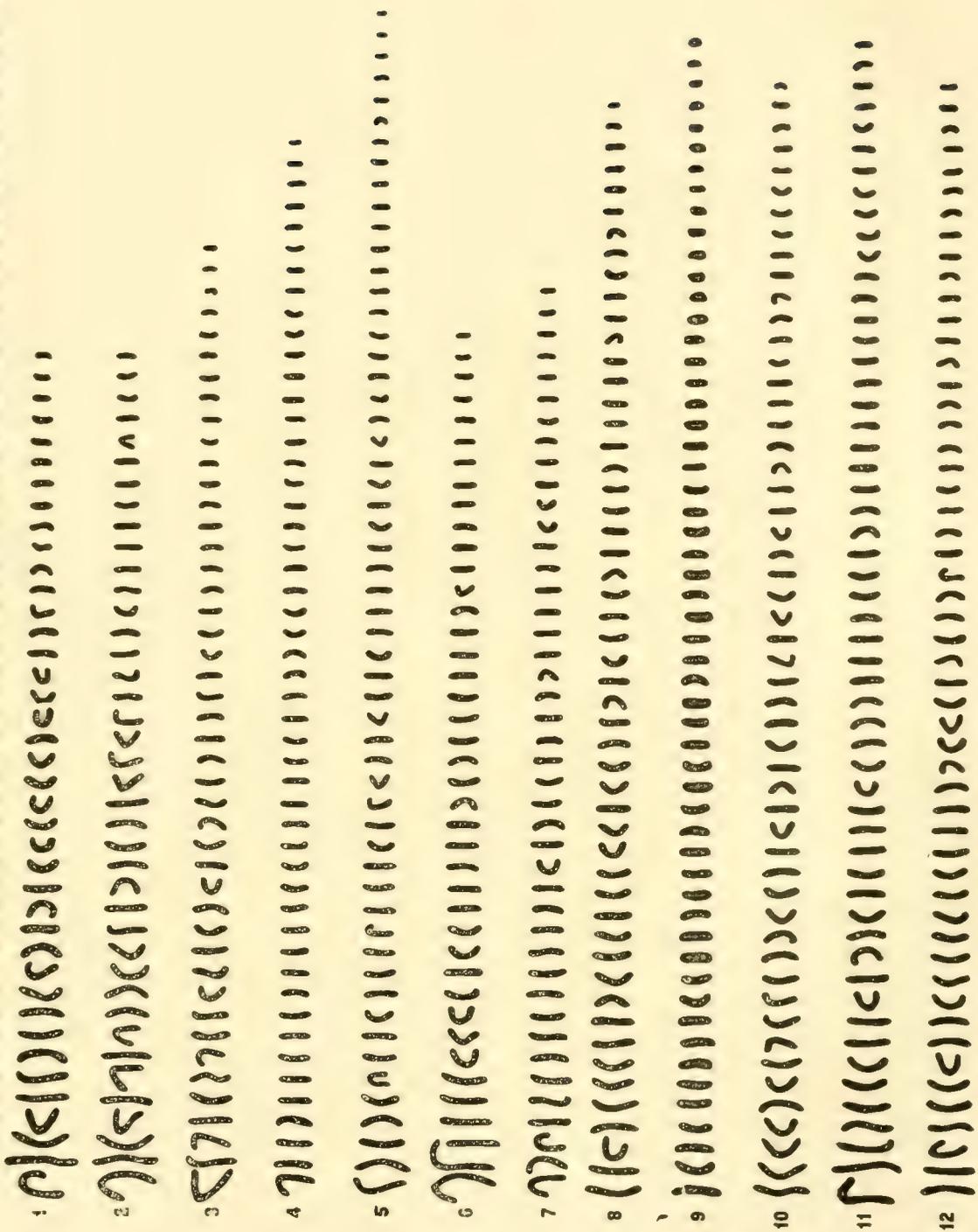
FIGURE NUM-BER	5	23		31		70	67	63	45	56	78	73
Tissue	Spermato-gonium	Brain	Brain	Brain	Brain	Amnion	Myotone	Limb Bud	Blood	Connective	Lung	Liver
Row Number..	1	2	3	4	5	6	7	8	9	10	11	12
34	20	23	20	20	22	22	22	25	23	25	27	24
35	19	23	18	20	22	21	21	25	22	24	27	24
36	19	23	18	20	21	20	20	25	22	24	27	24
37	19	22	18	19	20	19	20	23	21	23	27	24
38	17	20	18	19	20	18	20	23	20	23	26	23
39	16	20	18	19	19	17	18	23	20	23	26	23
40	15	18	17	19	19	15	18	22	20	22	25	23
41			17	18	19	12	17	22	19	22	25	23
42			16	18	19		16	21	19	22	24	22
43			14	18	19		14	21	17	22	24	22
44			13	17	18			21	15	22	23	21
45			13	17	18			21	15	21	23	21
46				17	18			19	15	21	23	20
47				17	18			19	15	20	23	20
48				16	16			19	14	17	22	20
49				14	16			19	14	17	22	19
50				10	15			19	14	17	21	18
51					15			15	13	17	21	18
52					14			12	13	16	20	17
53					14				12	12	18	16
54					12				12		18	
55					10				11		17	
56					9							

OBSERVATIONS ON THE EMBRYONIC DEVELOPMENT OF THE MANTID PARATENODERA SINENSIS[1]

HAROLD R. HAGAN

TWO TEXT FIGURES AND THREE PLATES

While engaged in the study of the general embryology of insects I became interested in a peculiar dorsal organ and the method of revolution of the large imported mantis, Paratenodera sinensis Sauss. There are many other interesting factors in the development of this insect which must be passed over in the present paper.

My sincere thanks are due to Prof. W. M. Wheeler, under whose direction this work was prepared, for his many helpful suggestions and his criticism of the text. I am also indebted to the kindness of Prof. C. T. Brues for aid in securing the illustrations.

The ootheca is deposited in September, the insect hatching the following spring. The construction of the ootheca and the emplacement of the eggs are similar to those of Mantis religiosa so well described by Giardina ('97–'00).

The earliest development of P. sinensis unfortunately cannot be presented at this time as the material was secured from Philadelphia through the mails and when received several days had elapsed between oviposition and laboratory treatment of the first oothecae. It is probable that the very earliest stages are quite similar to those of M. religiosa described by Graber ('90) and Giardina (97b). The latter especially has considered at length the very earliest stages including the fertilization of the egg, oviposition, cleavage and the formation of the ventral plate. My material covers succeeding stages.

The egg. The egg is elongate-oval, with an evenly rounded posterior end and tapering slightly to a somewhat smaller, more pointed anterior end. The shape is generally cylindrical but the anterior end is usually a little compressed laterally due to the pressure of the adjoining eggs in the ootheca. Distinct though not strongly concave and convex surfaces denote the dorsal and ventral surfaces respectively. A prominent button-shaped micropyle almost covers the anterior end of the egg. The length averages 4.5 mm. and the diameter near the middle of the longitudinal axis is about 1.3 mm.

The embryonic rudiment. The ventral plate is remarkably short when compared with the size of the egg. It varies, according to a number of measurements, from 0.46 mm. to 0.59 mm. in diameter. In surface view it consists of a sharply defined circular area of closely compacted cells on the ventral side of the egg, about two-thirds the distance from the anterior pole. When the egg is viewed from the lateral aspect this area appears flattened or even as a very slight depression in the yolk.

After gastrulation the germ band elongates rapidly, involving the entire ventral plate; the anterior end develops laterally also to form the rudiments of the cephalic lobes, while the posterior end assumes a lengthened form, giving the whole a shape roughly suggesting the letter T (fig. 1). The antero-posterior axis of the germ band corresponds with the same axis of the egg and lies in the same direction. The anterior end of the embryo is directed toward the anterior end of the egg throughout its entire embryonic history. On arriving at this stage, however, the embryonic rudiment undergoes successively a number of intermediary steps. The first change noticed is the lengthening of the circular or slightly oval ventral plate with apparently a slight contraction of its width. This movement is accompanied by activity in the antero-lateral region where the cephalic lobes are beginning their development. The ventral plate at this point appears somewhat U-shaped in outline, the base of the U corresponding to the posterior end. This appearance is quickly passed because of the continued elongation of the germ band and the active growth and extension of the rudiments of the cephalic lobes.

During this time the posterior and lateral margins of the germ band undergo a folding which initiates the growth of the embryonic envelopes. This fold extends laterally forward along the edges of the germ band, and extension toward the midline of the germ band proceeds until the edges unite, forming the amnion and serosa.

Further development consists in the enlarging and rounding of the cephalic lobes, accompanied by a continuation of growth at the posterior end of the germ band. The region immediately caudal to the lobes retains its width, while after the embryonic rudiment has attained a length of about 0.6 mm. to 0.7 mm., extension posteriorly is apparently retarded slightly, and a lateral development of the posterior third of the germ band takes place. This area becomes a little wider than the middle third. In this sequence of growth we find three distinct developments; first, a marking off of an anterior third of the germ band which will subsequently give rise to the eyes, brain, antennae, etc.; second, a middle third from which the mouthparts apparently arise; third, a posterior part destined to become the thoracic region and from which the thoracic appendages are later developed. While three quite distinct regions may be observed, they cannot be considered as macrosomites in any sense, for they are identified by minor external conformations only and are not separated by any process of segmentation. The posterior termination of the thoracic region consists of a rounded area of cells whose activity will be discussed presently.

During the development just described the embryonic rudiment has lengthened from about 0.65 mm. to a little over 0.9 mm. Up to this point the entire area has revealed no differentiation in structure; there is what appears in external view simply as a peculiarly shaped homogeneous mass of cells.

The indusium. A peculiar serosal structure has been observed in this and in several succeeding stages which resembles very closely an early stage of the indusium of Xiphidium with which I think it should be homologized. According to Wheeler ('93) the indusium of Xiphidium becomes a large three-layered organ enveloping practically the entire egg. Subsequent study has

not shown that in any other insect the indusium attains such development. On the other hand, we should expect this organ to be present in more or less rudimentary form in a few, at least of the other orthoptera. Indeed, such is the case in Orchelimum vulgare where the development of the organ closely approaches that of Xiphidium, while in Stagmomantis a small group of cells immediately anterior to the germ band probably represents a more rudimentary type of indusium than I figure for Paratenodera sinensis. Two well developed indusial structures have been described by Muir and Kershaw ('12) for one of the Homoptera. They find that the embryo of Siphanta acuta possesses an outer indusium adhering to the inner surface of the serosa. It completely surrounds the yolk and embryo. A second indusium is closely applied to the yolk and is fused to the edges of the amnion. It does not cover the amnion or the embryo. That the structure in the mantids has escaped notice so universally can be readily understood if it chanced to occupy a position similar to its location in P. sinensis where it is usually torn off with the embryonic envelopes while the embryo is being prepared for staining and mounting.

As early as the stage shown in figure 10 there is a very compact mass of cells lying in the serosa just above, or a little posterior to the cephalic third of the ventral plate. In the later stages (figs. 11, 12) this area is shifted posteriorly until it lies over the last mandibular or the first thoracic metamere. The cells of the serosa are very much flattened so that each covers considerable area, while the indusium remains compact and distinctly columnar as is seen in sagittal section (text fig. 2, A). The nuclei are uniformly situated at the bases of the cells which, at their upper ends, appear alveolar in structure and apparently are attached firmly to the vitelline membrane. This is the same stage as seen in figure 2.

It has been shown in Xiphidium that, after segmentation occurs, the embryo moves toward the posterior pole in its invagination and passage through the yolk to the dorsal surface of the egg. This movement separates the embryo from the indusium and leaves the latter free. It is only after such separa-

tion that the indusium begins its rapid growth and envelopment of the yolk. In P. sinensis the indusium is not further developed. I offer the suggestion that it is simply pulled over the embryonic rudiment as the amnio-serosal fold progresses to form the embryonic envelopes. Accepting my idea of homology, therefore, readily explains the presence of this structure and offers an explanation of its unique position in the serosa. The organ persists in place, apparently firmly attached to the vitelline membrane, until sometime after completion of revolution, when it frees itself and is ingested by the embryo during the formation of the dorsal walls. This structure is clearly not to be called the dorsal organ, though it may become part of it, as will be evident presently. Another organ later makes its appearance which answers very satisfactorily to Wheeler's ('89) definition of a dorsal organ. "The term 'dorsal organ' has been applied to the peculiar thick lump of cells resulting from the concentration on the dorsal yolk of the remains of either the amnion or serosa, or of both, preparatory to their sinking into the yolk and being absorbed."

Definitive segmentation and the appendages. Referring again to the small area of cells posterior to the thoracic region, active growth takes place as soon as the lateral development of the thoracic region has proceeded to its completion for the present, as shown in figure 2. As the definitive thoracic segments are becoming visibly outlined by the grouping of the cells, this posterior mass is rapidly developing caudally to form the abdominal segments. It is during this extension that the antennal, buccal, thoracic and abdominal rudiments first make their appearance.

The antennal rudiments originate as the result of a rapid multiplication of cells in a rather restricted area on the hind margin of the cephalic lobes, a little less than half way between their margins and the median line. Immediately behind this area a strip of clear cytoplasm is apparent, making the antennal proliferations even more noticeable. This very rapid cell division crowds a large number of cells with little cytoplasm into a compact mass. As growth continues, the tendency is appar-

ently a bulging of this area, and the swellings thus produced develop later into the antennae. Soon the labrum can be distinctly recognized as a conical evagination a little anterior to the proximal edges of the bases of the antennal rudiments. It is distinctly an unpaired organ, but a line of bifurcation is visible, even after the revolution of the embryo, indicating its primitive paired condition. This is also the view held by Viallanes ('90) for M. religiosa. The protuberances produced on the buccal and succeeding areas arise in a manner quite similar to those of the antennae. The development of the swellings in the thoracic region proceeds much more rapidly and they are at all times more conspicuous than those of the buccal or abdominal areas. My preparations show that the antennal rudiments appear first, followed by the rudiments of the thoracic appendages, then appear practically simultaneously the rudiments of the labrum and the maxillary appendages. This is not entirely in conformity with the views taken by Vaillanes ('90) and Giardina ('99) in their studies of Mantis religiosa.

As posterior growth of the embryo progresses, the definitive segments of the abdomen become more clearly recognized in antero-posterior sequence. By the time that the first five abdominal segments are recognizable, the terminal enlargement undergoes a change, developing into the telson, and appears ventrally flexed by the time two more segments are visible, (fig. 3). The abdominal appendages arise as evaginations in a manner analogous to the others. They consist of a pair of finger-like pleuropodia on the first abdominal segment and of merely prominent swellings on the remaining segments. The telson, however, has prominent projections which undergo marked changes during development. In Stagmomantis they are all, except the pleuropodia, much more developed, resulting in a series of bladder-like organs. The first abdominal pair consists of conical finger-like processes which are quite alike in both Paratenodera and Stagmomantis. The development of the structure of the telson I hope to make the subject of a future paper.

The stomodaeum is discernable shortly before the flexing of the telson, while the proctodaeum is visible immediately after.   The median groove is now a deep furrow and corresponds with the median longitudinal axis of the embryo.   In the meantime the brain has assumed a more definitive outline and the optic plates are differentiating, the nuclei becoming numerous and closely compacted to form a dark band around the lateral margins of the cephalic lobes.   The tritocerebrum is recognized as a broad metamere anterior to the mandibular metamere (fig. 3).

Revolution of the embryo.   Up to this point the embryo has been lying on the ventral side of the egg with its longitudinal axis in a straight line.   Now a most curious movement may be observed.   Its long axis suddenly becomes curved.   The head and telson are still directed toward their respective ends of the egg, but the middle of the embryo is bent toward the lateral margin (text fig. 1).   This movement is followed by the anterior end of the embryo moving to the side, resulting in slightly straightening the median line.   The head is now pointing diagonally forward, the thoracic region still exhibiting a slight bending, while the telson alone lies in its original place.   The entire embryo then passes laterally around the yolk, maintaining its superficial position, and finally comes to rest on the dorsal side of the egg, the ventral side of the embryo up, while the dorsal side retains its original proximity to the yolk.   During this movement, the telson may move rapidly enough to catch up with the anterior end, causing the axis of the embryo again to coincide with that of the egg during the remainder of the migration.   This is not always so, and the anterior end of the embryo frequently arrives on the dorsal line slightly in advance of the more posterior parts.   At different times, during this movement, depending also largely upon the individual case, the median line of the embryo has again become straightened longitudinally. This sometimes occurs shortly after the movement is initiated. In the present study it was found that about three-fourths of the embryos passed around the right side of the egg, but this must not be considered, for the present at least, as indicating any preference in orientation, but rather as an accidental observation

which might not be supported by tabulating a larger number of cases. The embryos passing around the left side of the egg presented no discoverable differences from the former class. Embryos of eggs adjoining each other in the ootheca passed around the same or opposite sides of the yolk mass indifferently. The gravitational position of the ootheca had no influence in regulating this movement as was determined by a study of a number of oothecae in various positions.

This movement was incidentally mentioned by Giardina ('00) in his general account of the development of Mantis religiosa.
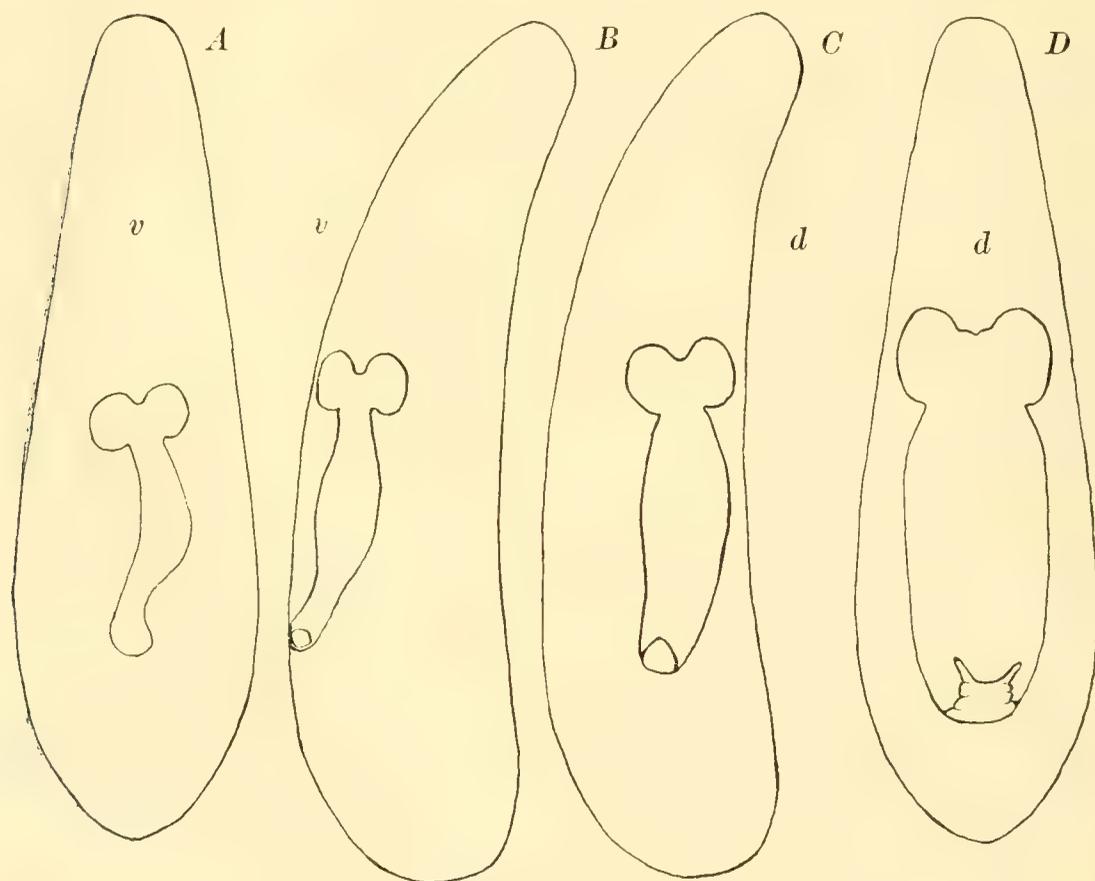
Text fig. 1 Diagram to illustrate the movement of the embryo from the ventral to the dorsal surface of the egg. A, ventral yolk, Embryo with characteristic curvature which immediately precedes revolution. B, lateral aspect showing embryo about one-third around. C, same view, but embryo about two-thirds advanced to dorsal yolk. In this instance the posterior end of the embryo has moved faster than the cephalic end so that its axis is parallel with that of the egg. D, dorsal yolk. The embryo remains ventral side up until after emergence from the ootheca. d, dorsal surface; v, ventral surface of the egg.

It is unique among observed insect embryos in at least two respects: first, the embryo reaches the dorsal yolk by means of a lateral or sidewise migration instead of proceeding head first or tail first as is commonly the case: second, the embryo, after arriving in position on top of the egg, remains there for the rest of its embryonic growth and even on hatching works its way out of the ootheca from this position. The usual revolution of hexapod embryos, when such a movement occurs, is followed later by some sort of reversal during which the embryo returns to its original position.

Growth during revolution. Growth continues during the foregoing movement, and very early there can be perceived a tendency toward a general thickening of the entire embryo. It becomes broader, covering a larger proportion of the yolk; it also elongates. By the time it is in a mid-lateral position; the antennal, maxillary and thoracic appendages, instead of appearing as mere finger-like projections, have become considerably elongated and exhibit, as well, definitive segmentation, though this is far from complete. The first abdominal segment has developed a pair of elongate conical processes, which are primitive appendages. The tracheal invaginations are unusually distinct and the telson is undergoing several modifications. The abdomen shows ten definitive segments and with the telson, eleven in all. The last three are ventrally flexed.

When the embryo reaches the dorsal surface of the egg it appears as in figure 4. We note at once the rapid development that has taken place in the whole embryo and particularly in the appendages. The procephalon is clearly outlined, the optic plates are heavily pigmented and the tritocerebrum is drawn up under the procephalon. The buccal segments are likewise drawn forward into a more compact mass. The thoracic region is as broad as the cephalic area, and all of the appendages show greater or less signs of segmentation. The first abdominal appendages are still relatively long and project diagonally outward. The following pairs are certainly reduced in size. In fact they rapidly flatten out and a little later apparently form the margins of the sternites. As a result of the disappearance

of these appendages as swellings it can be seen that each of the two sternites is composed of two sclerites. One is adjacent to the median furrow and the other, lateral to it, is composed of the tissue which formerly constituted the appendage. The two are separated at first by a very pronounced suture, but in succeeding stages this is gradually obliterated.

A description of the development of the appendages of the cephalic and thoracic regions would be simply a record of growth by successive slight modifications to form the nymphal structures. The chordotonal organs are now visible externally in the tibiae as deep pits, although they, too, become obsolete some time before hatching (fig. 4). The appendages entering into the formation of the gonapophyses undergo profound modifications from now on throughout embryonic development.

The dorsal organ. The growth of the dorsal body wall follows the fusion of the embryonic envelopes and their subsequent rupture immediately over the ventral surface of the embryo. An examination of the serosa at this time shows that a contraction of its cells, particularly in the region of the indusium is taking place. The fused part of the amnion and serosa contract and fold back over the margin of fusion. The continued contraction of the ventral serosa (dorsal to the embryo) pulls the remainder of the amnion back over the yolk, leaving the embryo free in the place between the dorsal yolk and the vitelline membrane. The yolk is now enclosed by the embryo in the postero-dorsal region, by the amnion on the posterior, postero-lateral and antero-dorsal areas, while the serosa covers the remainder. As the body wall now grows down over the yolk, the amnion is drawn, by contraction of the serosal cells, along the future mid-dorsal line of the embryo. Growth of the body wall proceeds from the posterior and postero-lateral edges in advance of the anterior regions. This results in fusion along the mid-dorsal line of the embryo and the completion of the tergites successively from the caudal end forward.

The contracted serosa lags behind the forward growth of the body walls and a large fold of it covers the proliferating margins of the embryo. Two peculiar organs are now apparent in the

serosa, evidently taking up much of the slack in the posterior serosa and, what is perhaps even more important, they are exerting a pull on the amnion in the cephalic area which encloses a large amount of yolk which is to be ingested before closure of the mid-dorsal line. The embryo itself, in its forward growth, is assisting in forcing this mass of yolk back, as it now occupies over two-thirds of the dorsal surface of the egg. The lateral extension of the optic plates, the further differentiation of the buccal segments and nerve centres, with the consequent thickening of this whole cephalic portion of the body, tend to push the yolk back into a less crowded region of the egg.

The two organs now visible in the serosa are composed of its cells each arranged like a cup around a hollow core. They are tall and columnar in section (text fig. 2, B, C, D, F). Their sole purpose is evidently to further the contraction of the serosa in order to expedite its ingestion within the body cavity with the last of the anterior yolk. The first one has the lumen opening out toward the vitelline membrane. I am convinced that this is the indusium because it has the same cellular structure, occupies the same position as maintained by the indusium in the preceding stage, and finally, because I am unable to find the indusium in any other situation. The second contraction organ is immediately posterior to the first and is composed of cells identical in appearance to those of the remainder of the serosa proper. Its lumen opens toward the dorsal wall of the embryo. As the completion of the dorsal body-wall of the embryo continues, the anterior yolk is pushed posteriorly and ingested in the dorsal thoracic region. The dorsal organ is composed of the serosa, its contraction organs and the amnion. The anterior portion of the amnion is probably the last part to be absorbed, as it immediately precedes the growth of the ectoderm and the mesoderm over the dorsal surface of the head and thorax of the embryo and the rapidity of its movement is therefore dependent upon the rate of growth of these embryonic tissues.

A late embryonic envelope. Just before hatching the embryo secretes the chitinous epidermis (fig. 9). Each of the appendages thus becomes individually incased so that they are all separated

completely from actual contact with each other or with the body
except at the points of articulation. This covering cannot be
the vitelline membrane or the chorion, as either of these would
appear as a simple sac enclosing the body and appendages.
According to Phil and Nellie Rau ('13) this covering is a simple
sac enclosing the embryo and binding the appendages (except
the prothoracic legs) to the body. They suggest that the first
pair of legs, project through this envelope and are therefore free.
I find that Muir and Kershaw ('12) sometime ago pointed out
the common error of supposing this cuticle to be amniotic in
origin. This membrane exhibits ridges, pits and spines on its
exterior surface due to the conformations of the hypodermis
which secretes it. The front of the head is produced considerably
to form an organ which aids by its alternate contraction and
expansion, in the escape of the animal when hatching and emerg-
ing from the ootheca. In function it is clearly analgous to the
ptilinum of the higher Diptera. Over this area the envelope
is very materially thickened, possibly for protective purposes.
At the posterior end the tips of the envelope covering the cerci
are connected, each by a slender thread, to the egg membranes.

The first ecdysis. Hatching is accomplished by alternately
contracting the dorsal and ventral parts of the body with a sort
of undulatory motion. Shortening the ventral surface pulls
forward the dorsal segments, and reversing the movement ad-
vances the ventral parts and the appendages. The return of
the segments to their former positions is prevented by their
strongly chitinized hind margins and the posteriorly directed
spines and bristles, particularly on the legs and cerci. At this
time the partially flexed portion of the abdomen becomes straight-
ened posteriorly.

After hatching, the insect is suspended by the two threads
attached to the posterior part of the epidermis. While hatching
it has increased in length and, almost as soon as it is hanging

Text fig. 2 The dorsal organ and the indusium. A, sagittal section of the
embryonic rudiment through the appendages and the indusium. This stage
is a little later than that of figure 2. The embryonic envelopes are slightly dia-
gramatic, being shortened and not following the outlines of the embryonic rudi-

ment. × 65. B, the indusium, sagittal section, at a stage corresponding to those of figures 7 and 8. The dorsal organ is forming. × 127. C, the dorsal organ in sagittal section at a slightly later stage. The amnion covering the anterior yolk is yet to be added. This stage is figured in surface view in figure F. The indusium seems to have a definite function for the first time in its accumulation of a large amount of serosal protoplasm for ingestion × 144. E, the indusium in cross section. Same stage as figure C × 100. E, cross section through the posterior portion of the serosal contraction organ of figure C. The ectoderm and endoderm of the dorsal wall of the embryo are clearly distinguished. × 110. F, ventral surface of the egg showing anterior yolk, the formation of the dorsal organ and the growth of the dorsal walls of the embryo. × 21.
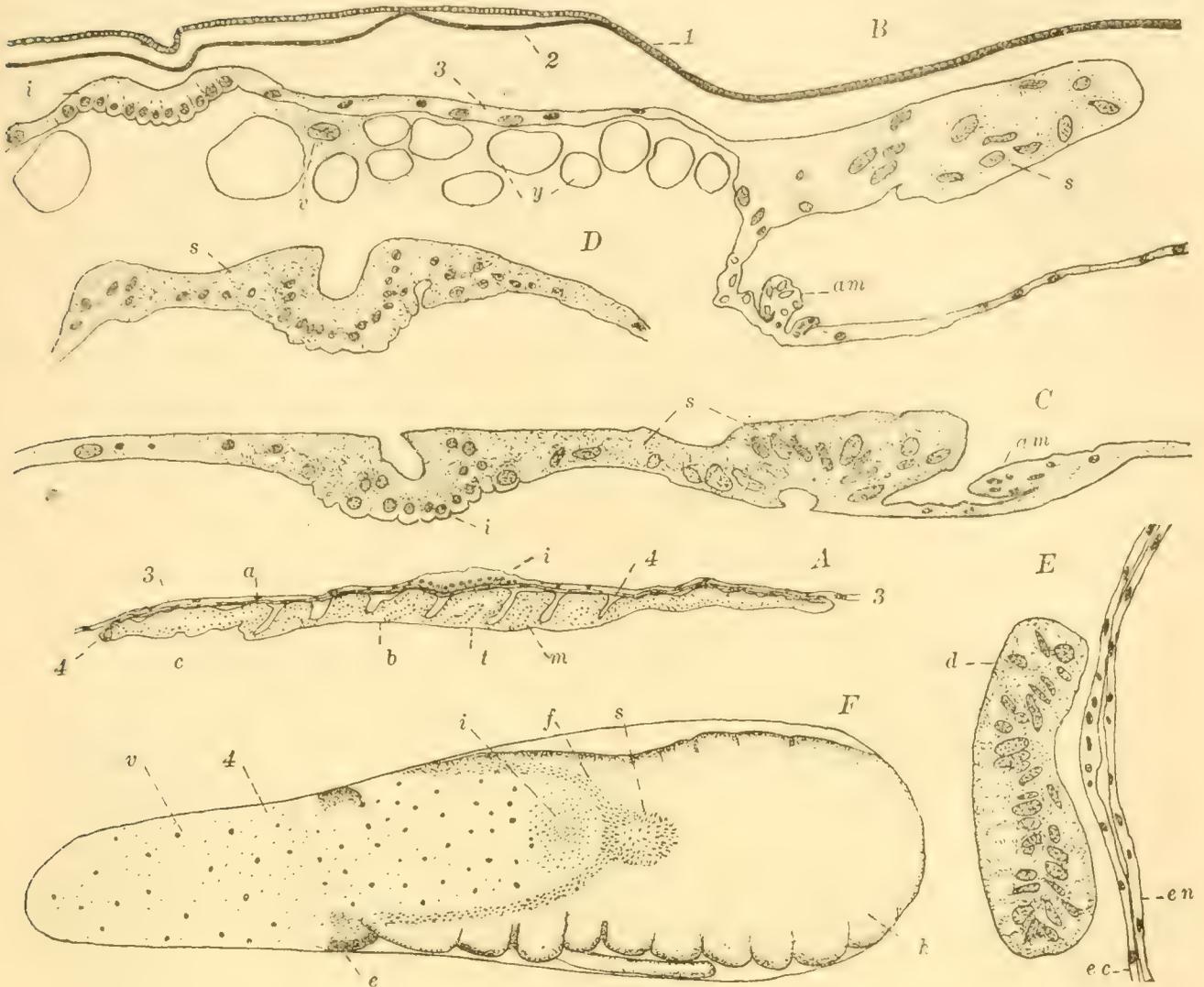
A, antennal rudiment; *am*, amniotic protoplasm; *b*, second maxillary rudiment; *c*, cephalic region; *d*, dorsal organ; *e*, eye; *ec*, ectoderm; *en*, endoderm; *f*, lateral folds of serosa; *h*, chitinous secretion; *i*, indusium; *m*, mesoderm; *s*, serosal protoplasm; *t*, prothoracic limb rudiment; *v*, vitellophag; *y*, yolk; *1*, chorin; *2*, vitelline membrane; *3*, serosa; *4*, amnion.

free from the ootheca, it is able to rupture the membrane which has now become too small for it. A longitudinal slit occurs over the prothorax which is literally "popped" out through it. Observers of this phenomonon have uniformly described the humped position of the embryo as the cause of the rupture of the cuticle. My study has led me to the conclusion that we are dealing merely with an attitude of the body as a result of the dorsal split in the cuticle which permits the protrusion of the prothorax through the opening. Continued movements, not only of the body walls but also of the body fluids, force more and more of the animal through this slit. The prothorax is greatly elongated by this means and appears as a hump or loop over the head and thorax. Alternating saw-like movements of the limbs soon release the head and thoracic regions; then the envelope is worked backward off from the abdomen and the limbs are freed in succession. The abdomen is entirely uncovered before the tarsi of the metathoracic legs. In thirty to fifty minutes after suspension the nymph is running about actively and is approximately three times the length of the egg from which it hatched.

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PLATE 1

1 Embryonic rudiment showing the indusium in the serosa. × 22.

2 Slightly later stage showing the cephalic, buccal and thoracic regions and the posterior extension which later developes into the definitive abdominal segments. × 25.

3 The optic plate, labrum and antennae are sharply defined as are also the following appendages back to the fifth abdominal segment. The telson is flexing ventrally. The tritocerebral area is visible beneath the antennae. The stomodaeum and proctodaeum have made their appearance. This stage is slightly earlier than figure 13. × 26.

4 A considerable later stage. The tritocerebral area and also the buccal region are drawn up more nearly into position. The spiracles are plainly visible even earlier than this. The chordotonal organs are indicated as pits on the definitive tibiae. × 27.

5 Slightly older than figure 4. The abdominal appendages can no longer be seen. × 27.

6 A somewhat later stage showing the telson almost straightened. The shaded portion on the right of the figure is the dorsal wall which is now beginning to enclose the yolk. Segmentation is very distinct. × 26.

7 and 8 Succeeding stages. The condition of the embryo in its dorsal growth corresponds closely to text figure 2, *F*. 7, × 22. 8, × 26.

9 The embryo a short time before emergence. The epidermis can be seen more or less loosely adherent to the embryo particularly in the postero-dorsal region. × 14.5.
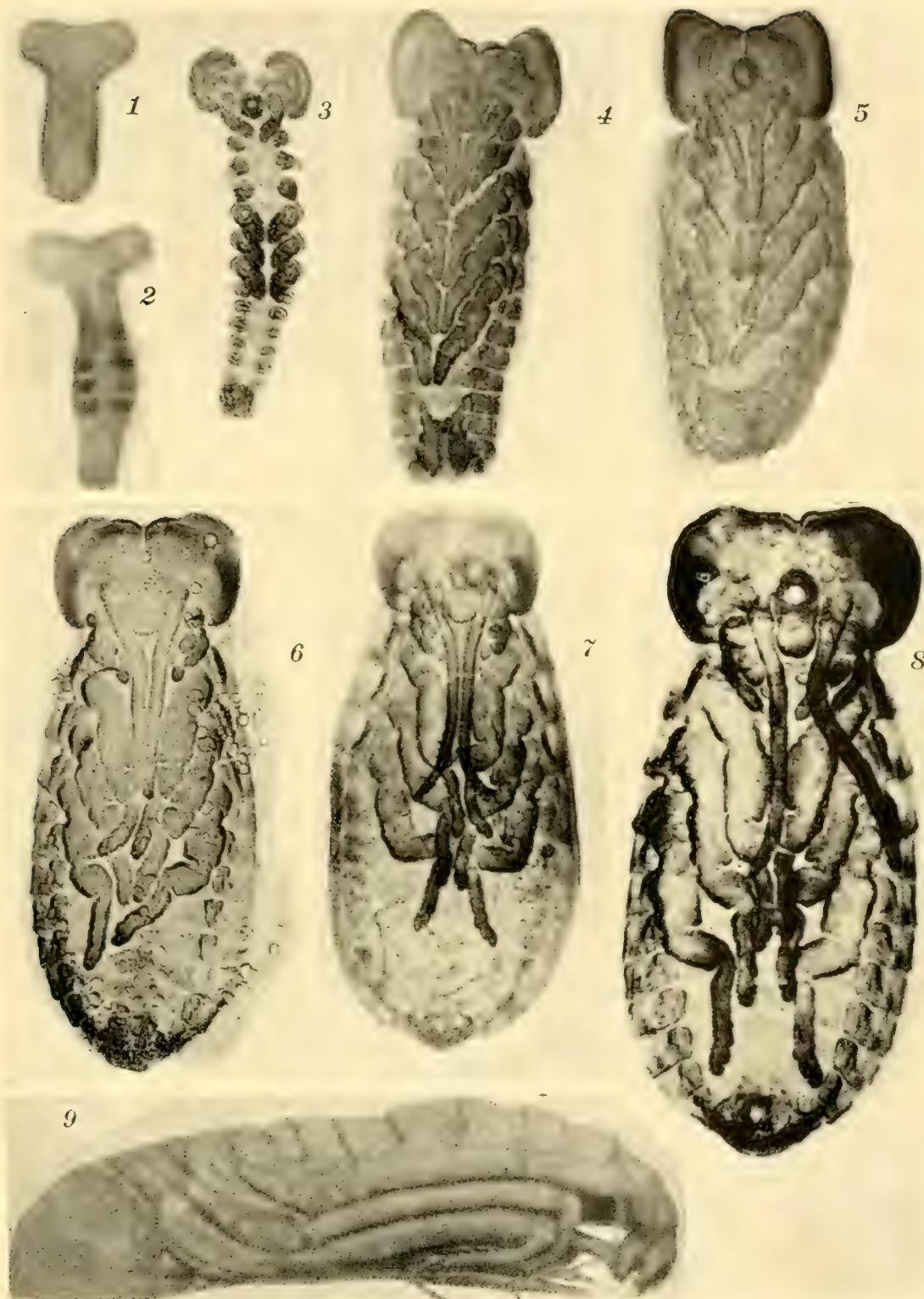
10 Surface view of ventral yolk showing the embryonic rudiment. Cephalic, mandibular and thoracic areas recognizable. × 20.

11 Same view, later stage embryo. Cephalic lobes, rudiment of appendages and abdominal growth are the characteristics of this stage. × 26.

12 Same view. The peculiar bending of the embryonic axis preceding revolution to dorsal yolk. (The egg is tipped slightly to the right). × 25.

13 Lateral view showing the embryo passing around the right side of the egg. (Egg is below normal size.) × 25.

14 Lateral view. This embryo is passing around the left side of the egg. × 27.

15 Dorsal view. This stage is one shortly after the embryo has come to rest on the dorsal yolk. × 27.
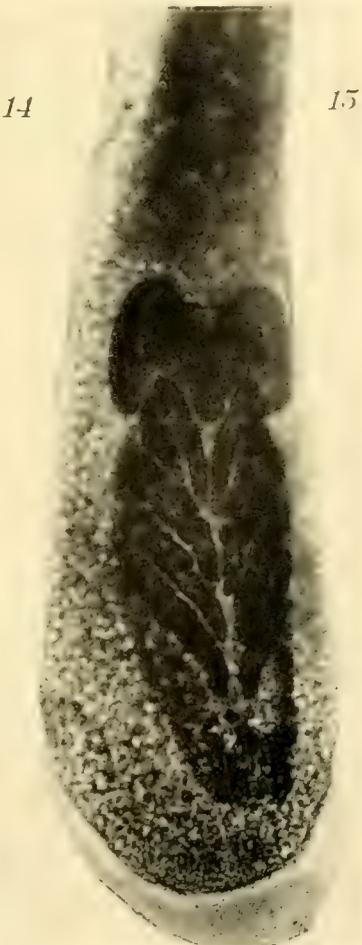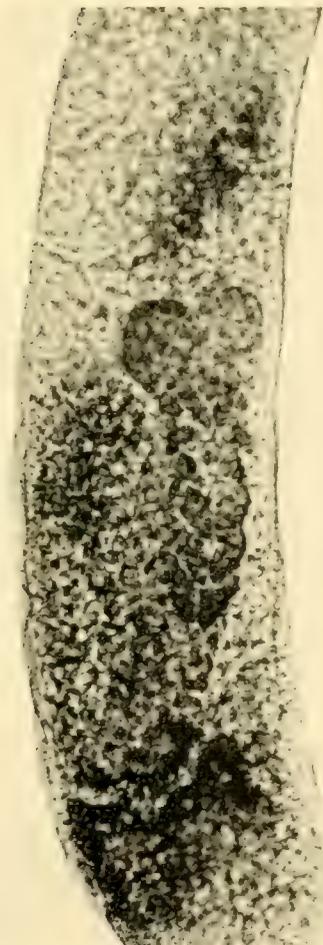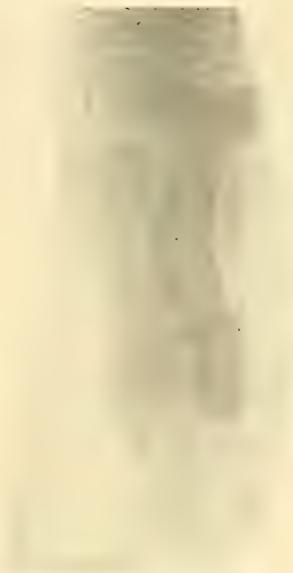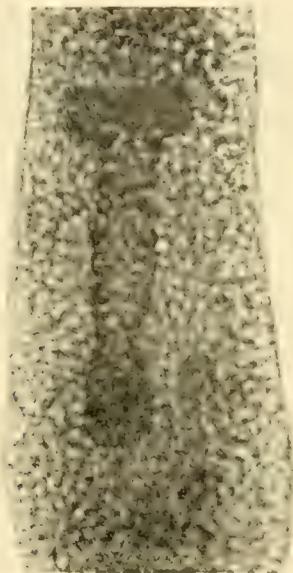
EXPLANATION OF FIGURES

16   Embryos hatching from an ootheca.   At first they hang suspended by the threads attached to the hypodermis but almost at once undergo their first ecdysis which frees them.   (Natural size.)

17   First nymphal stage with first ecdysis almost completed.   Removal of the epidermis from the metatarsi seems to be most difficult.   × 4.

18   The adult female mantid, Paratenodera sinensis.   (Natural size.)

ZEUGOPHORA SCUTELLARIS (SUFFR.)

B. H. GRAVE

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TWELVE FIGURES

The literature upon the beetles of the genus Zeugophora, except for descriptions of species, is very meagre. The life history of only one species is known and there are no published figures of the larvae and pupae of any species. Kaltenbach, however, gives a good brief description of the larva of Z. flavicollis.

The observations recorded in this paper were begun in the fall of 1912 at the University of Wyoming. The work was continued during the year but was interrupted the following June. After an interval of two years new material was secured which permitted completion of the work under laboratory conditions.

During my stay at the University of Wyoming I became interested in some larvae which were feeding upon the leaves of the cottonwoods which border the walks of the University campus. The leaves were badly discolored and as much as one-third or one-half of the green tissue of many of them had been destroyed by these larvae. It was for the purpose of identifying the beetle that I began the study of its life history which is briefly as follows:

LIFE HISTORY OF ZEUGOPHORA SCUTELLARIS

During the summer months the larvae work in the leaves beneath the epidermis, eating out the pulp and causing blackening of the parts affected. As stated above, a large part of the chlorophyl-bearing tissue may be destroyed in this way by the end of summer, thus rendering the leaf ineffective as a starch-

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making organ. The larva may therefore appropriately be called a leaf miner. Late in the season, at the time the leaves fall, it emerges and enters the ground. After burrowing to a depth of between one and one-half and two and one-half inches below the surface, it excavates a little spherical cavity in which it coils up for the winter sleep.

About the last of May of the following spring (May 25–June 15) the larvae transform into pupae. As yet I have no definite records concerning the date of emergence of the adult under natural conditions, but the indications are that the duration of the pupa is about three weeks or possibly a month in cool weather. Under laboratory conditions it is about three weeks or less. The first beetles may therefore be expected to appear by the middle of June. There is reason to believe that they appeared as early as June 10 in the year 1913, which was a rather early spring for that locality. The number of pupae in the soil was considerably diminished before June 15 and the supposition is that they had metamorphosed. I found no imagoes at that time, however, probably because I did not know what kind of beetle to look for.

It has not been possible for me to return to Wyoming during the past three years so that it is not known whether the eggs are laid en masse or singly, upon the twigs and leaves. The fact that only one larva, as a rule, attacks a leaf might lead one to suspect that the eggs are laid singly. A number of beetles which were hatched from breeding boxes were kept in captivity and one of them laid eggs ten days after it emerged. It seems likely therefore that under normal conditions the eggs are laid upon the leaves and twigs during the latter part of June and the first part of July and that the larvae enter the leaves soon after, and begin their destructive work.

The adult beetles, as might be expected, feed upon the leaves of the cottonwood. They swallow the softer parts and discard the fiber.

EXPERIMENTAL RESULTS

During the fall of 1912 the larvae were discovered to enter the ground. Infested leaves were placed in breeding boxes stocked with sandy soil. As the leaves dried the larvae emerged and entered the soil in large numbers. It was thus possible to keep the larvae in the laboratory over winter.

Again, after all the leaves had fallen I dug beneath the trees and found the larvae in their burrows, and later digging, during the month of February, showed that they pass the winter as larvae.

The larvae which were kept in the laboratory were neglected and many of them died from lack of water. A few transformed
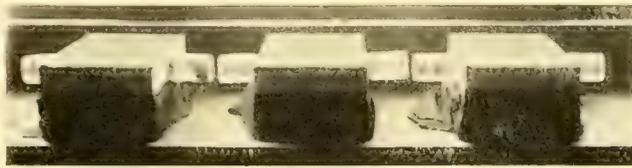
Fig. 1   Photograph of breeding boxes. They consist of a box 14 × 18 × 4 inches, and contain sandy soil. Each box has a cap similar in all respects to the box, except that it is roofed over by black cloth, to permit good ventilation. Pint mason jars are screwed into holes in the sides of the cap. These jars permit light to enter, and insects upon hatching are attracted to them. The line of junction between the box and its cap is covered over by a band of black opaque cloth, which is tacked to the cap only, and hangs down over the seam.

to pupae as early as the first of March or the last of February, but if any beetles emerged from these pupae, they escaped. All other attempts to secure the adult insect during this year were likewise unsuccessful.

During the present year much better results have been attained. Larvae received from Wyoming by mail, were allowed to enter the soil of breeding boxes (fig. 1). They were watered once per week during the year. On March 1 they were still larvae. On March 20 one half or more had changed to pupae. On April 4 the first adult beetle appeared. These data indicate that the duration of the pupa under laboratory conditions is about three weeks. Other evidence on this point confirms the above statement. Six larvae were placed in a breeding box on

April 15. Two of them changed to pupae before April 20. One of these pupae emerged as an adult on May 7, making the duration of the pupa about three weeks or less.

Since the first pupae were found, under natural conditions, on May 25, and since the number of pupae in the soil was considerably reduced before June 15, it would seem that the duration of the pupa under natural conditions is also about three weeks.

The following table gives a record of the date of appearance, and measurements of beetles as they emerged from the breeding boxes.

DATE OF EMERGENCE	NUMBER OF BEETLES	MEASUREMENTS
		mm.
April 4	1	3.5
April 5	1	4.5
April 7	1	4.5
April 11	2	3.5 and 4.5
April 12	1	4
April 15	2	3.5 and 4.5
April 17	1	4.5
April 18	1	3.5
April 25	1	3.5
April 26	1	4.5
May 7	1	4.5

It will be noted that there are two sizes. Those marked 3.5 mm. varied between three and one-half and four millimeters while those marked 4.5 mm. varied between 4.5 and 5 mm. In all probability the larger ones are more nearly normal. The disparity in size is apparently not due to a difference in sex. The table also shows that beetles emerged during the period of a month under laboratory conditions.

Under normal conditions the first pupae were found on May 25 and the last larvae about June 15. It is apparent therefore that beetles may continue to emerge for a considerable period, and this fact should be taken into account in attempting to spray for the eradication of the pest.

MOULTING AND DEVELOPMENT OF PIGMENT

Incidentally some observations were made on moulting and the development of pigment. On April 15 the contents of one box were examined. A half dozen larvae, the same number of pupae, and two beetles were found. These two beetles were just emerging from the pupa case. They had freed all parts except the tarsi and a portion of each antenna. They were pulling and hauling energetically to free the feet. The remains of the pupa cases were crumpled into a shapeless film entangling the feet.

At this time the black pigment had not yet appeared. The beetles were almost uniformly straw-color except the elytra and antennae which were slightly brownish. On being placed in a lighted room they attained the black pigment within twenty four hours. It may be that light and oxygen are factors in the production of pigment, but pupae exposed to light do not develop pigment. All beetles were quite dark when they emerged from the soil, although the black became more intense later. They seem to remain in the ground a day or so after moulting the pupa skin, possibly longer.

DETAILED DESCRIPTION OF LARVA, PUPA AND IMAGO

Kaltenbach in his Pflanzenfeinde ('74, p. 544) gives a good brief description of the larva of Z. flavicollis. He states also that the metamorphosis takes place in the earth and that the summer brood appears in May of the following year. He gives no figure of the larva and his is the only reference in biological literature to the early stages of the Zeugophora. Up to the present time there has been no published description of the pupa of any member of this genus. Kaltenbach says of Z. scutellaris "Wurde von Apotheker Hornung und Dr. Suffrain auf Populus nigra gefunden. Ersten Stände unbekennt."

THE LARVA

The larva, when full grown, measures between 5 and 8 mm. n length. Seven millimeters may be considered the normal length for the species. It may be supposed that the larvae continue to feed and grow until the supply of food is cut off by the falling of the leaves. They seem to be unharmed by the autumn frosts.

The color of the larva varies from yellowish or straw to white, except the head, which is brown.

The body is flattened (depressed) in the dorsiventral plane. Twelve thoracic and abdominal segments can be counted. The abdomen ends in a slender tip which may represent an additional segment. The first thoracic segment consists of two annulae. The segmentation throughout is deeply marked and the strong lateral prolongations of the abdominal segments seem to be characteristic.

Thoracic appendages are wholly undeveloped or do not appear on the surface.

The antennae are well developed but short, and are directed straight forward. Each consists of four segments, counting a short, thick basal segment.

The mouth parts are best described in connection with the figures (figs. 5 and 6). They are similar to those of other biting insects except that each part has interesting characteristic features. The labium consists of the mentum, sub-mentum and gula, but it bears no palpi. The maxillae appear to be immature and the typical parts are not clearly defined in preparations examined. The three-jointed maxillary palpi are well developed. The mandibles have a smooth knife-blade-like cutting edge and are beautifully hinged at two points, one dorsal and the other ventral, as shown in figures 5 and 6. The external and internal mandibular muscles are shown in figure 5. It is to be noted that the muscle fibers are not inserted directly into the mandible but are joined to it by a tendon of connective tissue. The labrum has a scalloped or incised edge and consists of two lobes, as shown in figure 5.

The entire body is sparsely bristled, as indicated in figures 2 and 3. There are two pairs of bristles on the lateral margins of the head. The ventral surface of the head bears four pairs. The first thoracic segment has three pairs of bristles situated dorsally, and two laterally. Four longitudinal rows of bristles occur on the dorsal surface of the larva and two rows on the ventral aspect (figs. 2 and 3). Each lateral prominence is tipped by a strong bristle. Figure 4 shows the arrangement of the bristles on the abdominal segments as seen in cross section. Most or all of these bristles apparently assist in locomotion.

In pointing out the characteristic features of the larva which are most readily recognized one should perhaps call attention to the depressed body, the strongly developed lateral projections of the abdominal segments, and the arrangement of the bristles, all of which can be seen at a glance.

According to Professor Aven Nelson of the department of botany at the University of Wyoming, the larva prefers Populus acuminata but is also found upon other species in the West. He writes as follows:

I beg to state that the larva seems to prefer Populus acuminata (Rybd.), the Colorado cottonwood, though I am inclined to think that it occurs in the leaves of other species. The species native along our streams is P. augustifolia (James), and in some of the lower altitudes of the state there is also a broad-leaved cottonwood, P. occidentalis. The latter I am quite sure is infested with the beetle but I am not sure that P. augustifolia is.

In Europe, according to Suffrain, the adult beetle Z. scutellaris feeds upon Populus nigra.

THE PUPA

The pupa is strikingly like the imago in anatomical features, though the parts are less sharply and gracefully outlined. This, of course, is the usual condition of pupae in general, and is to be expected in an immature and quiescent insect (see fig. 7).

The pupa is yellowish or straw colored. At first there is no indication of the dark pigment of the adult but before the final metamorphosis the antennae and elytra begin to take on a

brown color. The head and prothorax are bent sharply forward and the antennae pass backward and dorsally beneath the pro- and meso-thoracic legs. The mouth parts approach those of the adult beetle in structure but the mandibles are larger and have a smooth outline, without teeth. The adult toothed mandibles can be seen within, however, as the time for the emergence of the beetle approaches.

The characteristic prothoracic humps are evident. The broad tarsal pads which are so striking a feature of the imago are clearly outlined in the tarsi of the pupa. The tip of the abdomen has a complicated system of plates, most of which are shown in figure 7. The larval skin normally remains attached to the tip of the abdomen but it can be removed without difficulty. Three bristles are situated in a triangle over each eye and four pairs of large symmetrically placed bristles extend backwards from the tip of the abdomen, as shown in the figure. There are numerous other bristles on the body but they are smaller and seem to be of another character.

When disturbed in its burrow the pupa moves its abdomen actively and wriggles about more or less.

The characteristic features seem to be the broad tarsal pads, the thoracic prominences, and the bristles over the eyes and on the tip of the abdomen.

THE IMAGO

For the original description of Zeugophora scutellaris reference should be made to Entomologische Zeitung zu Stettin, vol. 1, 1840, page 99, by Suffrain. The species was redescribed by Lacordaire in his "Monographe des Colleopteres Subpent meres Phytophages," vol. 1, 1845, p. 236. Another short description of the beetle is given in "Proceedings of the Academy of Natural Sciences of Philadelphia," 1873, page 23, by G. R. Crotch (and M. A. Cantab). These descriptions do not agree in all particulars. Suffrain gives the color of the lighter parts as bright yellow, while Crotch describes it as testaceous yellow. There is a considerable reddish tint in the specimens with which I worked.

Suffrain gives the size as 2 to $2\frac{1}{6}$ lines which is practically 4.5 mm. Judging from my material the adult beetles vary considerably in size, but two sizes clearly predominate, the smaller ones measuring a scant 4 mm., while the larger ones measure 4.5 mm., or slightly above.

The prothorax has the conical prominence on either side, which is so characteristic of the genus Zeugophora (fig. 8).

The color markings are striking; the head, prothorax, mesothorax, scutellum and legs are straw or amber, while the metathorax, abdomen and elytra are black. The antennae are composed of eleven segments, the first three of which are usually amber or straw, the fourth brown and the remainder black. The eyes and mandibles are black.

The most characteristic structural features, aside from the prominence on the prothorax, are found on the legs. There is a pair of spurs on the distal end of the tibia (figs. 10 to 12). The tarsus is four-jointed, the penultimate segment of which bears a very large cloven or bifid pad, upon which the insect walks. This pad is covered by a dense coating of long bristles (figs. 10 to 12). The elytra are coarsely punctate and the entire body is sparsely pubescent except the tarsi, which are hairy or bristly.

THE EGG

Eggs were secured from only one insect, although a considerable number were kept in captivity and fed upon leafy branches of the cottonwood. The egg is microscopic and appears to be surrounded by an extra envelop and filament similar to that of the decapod-crustacean's egg. If this observation is correct it appears to be unusual.

Judging from the fact that only one larva inhabits a leaf I have thought that the eggs may be laid singly, although this is mere supposition. Kaltenbach claims to have seen two or three larvae of Z. flavicollis in a single leaf in Sweden.

ACKNOWLEDGMENTS

I wish to acknowledge my indebtedness to Prof. S. K. Loy of the Department of Chemistry at the University of Wyoming, who collected living material and sent it to me, making it possible to complete the study of the life history.

I am indebted also to Prof. L. O. Howard and his staff, who identified the species and encouraged me to finish the work; to Prof. Aven Nelson of the University of Wyoming, who determined the species of trees upon which the larva feeds.

During the present summer Prof. A. S. Pearse and Prof. W. S. Marshall at the University of Wisconsin have given me every convenience and shown me every courtesy, for which I wish to express appreciation.

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PLATE 1

2 Dorsal aspect of larva outlined by camera lucida showing general appearance. Note especially the number and arrangement of bristles and the sharp divisions between segments. The dotted line in the head represents a suture. The heavy black line is a chitinous bar in the dorsal wall of the head.

3 Ventral view of larva showing bristles, etc. The four pairs of bristles which occur on the ventral surface of the head are not indicated. The two black lines are chitinous bars, supporting the mandibular articulation.

4 Diagrammatic cross-section of an abdominal segment showing arrangement of bristles and outline of depressed body.

5 Drawing of head as seen from above, viewed as a semi-transparent object. *A*, Antenna; *C*, Clypeus; *L*, Labrum; *M*, Mandible; *Ma*, Mandibular articulation; *EM*, External mandibular muscle; *IM*, Internal mandibular muscle; *T*, Tentorium; *S*, Suture line between front and genae.

6 The head as seen from below, highly magnified. *A*, Antenna; *L*, Labium; *M*, Mandible; *MA*, Ventral mandibular articulation; *MAX*, Maxilla; *CB*, Chitinous bar supporting the mandibular articulation.
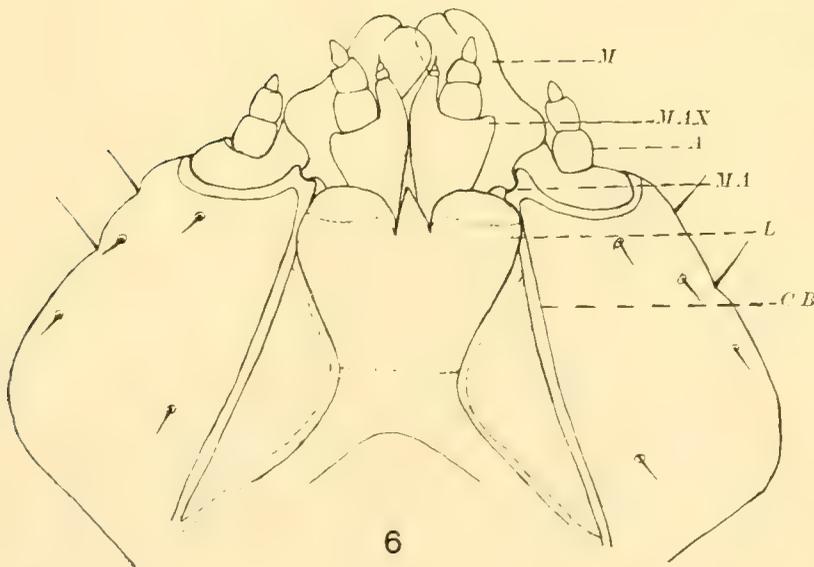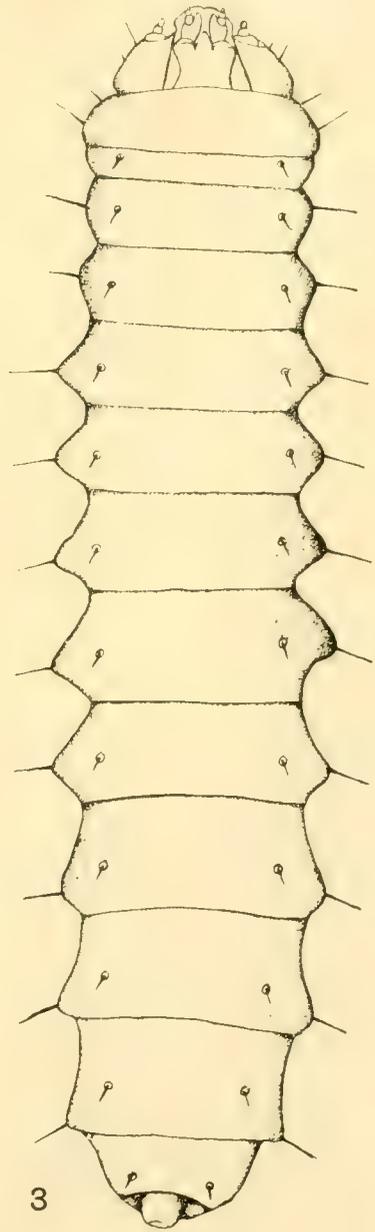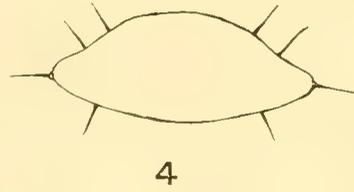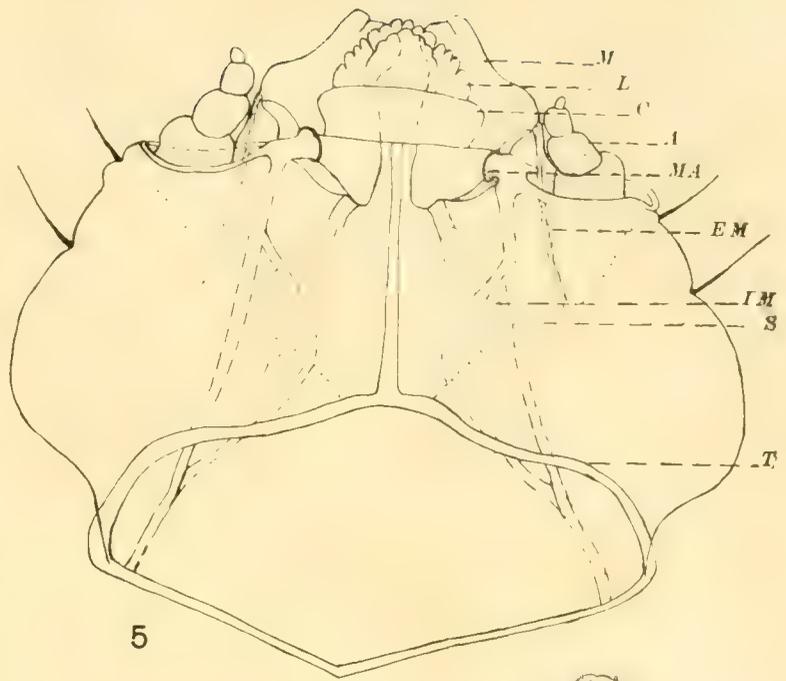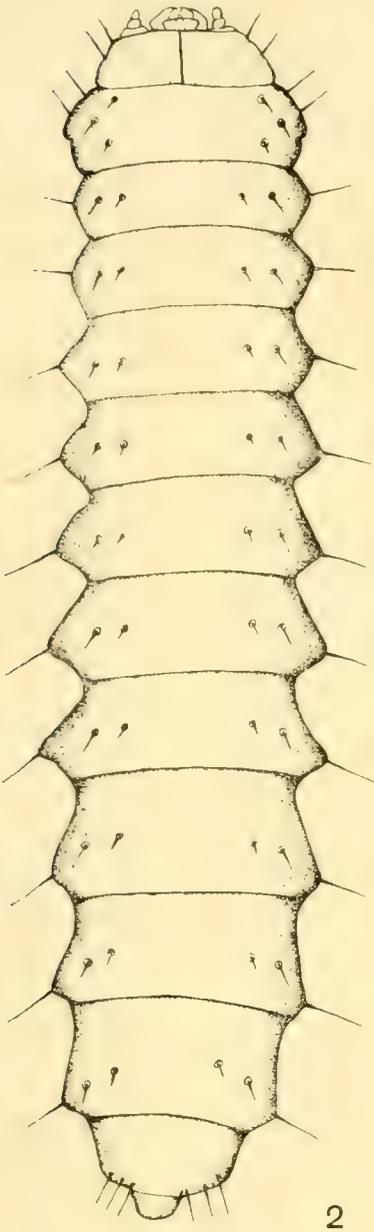
PLATE 2

7   The pupa.   Note conical prominence on prothorax.
8   The beetle.   Pits of elytro and coloration not shown.
9   The antenna.
10 Entire prothoracic leg as seen from the side.
    Note spine on the tibia and the bristled pulvillus.
11 Tarsus of prothoracic leg as seen from above, showing bifid pulvillus.
12 Tarsus of methathoracic leg as seen from the side.

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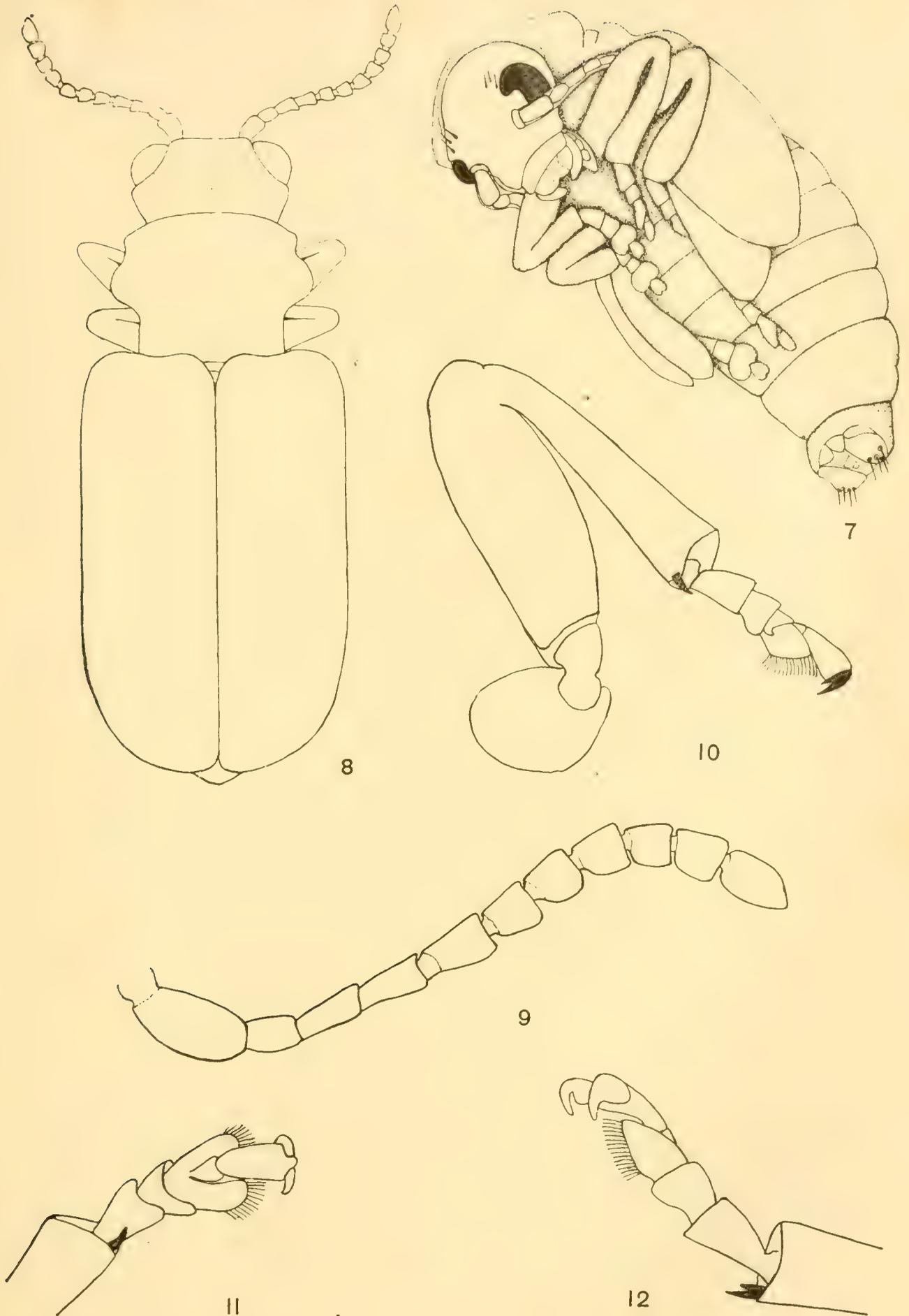
OOGENESIS IN THE WHITE MOUSE

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New York*

FIFTY-TWO FIGURES

CONTENTS

INTRODUCTION

In the last two decades or so there has been produced a vast
amount of cytological work which has had as its object the
description and explanation of the structure, behavior, and
nature of the chromosomes. Particular attention has been paid
to the maturation divisions in the two processes oogenesis and
spermatogenesis and to the stages leading up to these. Two
facts seem to stand out clearly enough:—that by these two

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rapidly succeeding divisions the amount of the chromatin and the number of the chromosomes in the mature germ cells is reduced to half that found in the somatic cells. There have arisen in the interpretation and explanation of these facts differences of opinion, some of which will be taken up later in a discussion of synizesis and synapsis.

Of the two processes, spermatogenesis has received more attention; it is completed in less time and in many forms a single adult testis will show all the stages of development from spermatogonium to spermatozoon. Oogenesis, on the other hand, is extended over a long period; in mammals at least and in many other forms as well, the process may start in the young embryo and continue into adult life. The formation of the deutoplasm—vitellogenesis—with the consequent enormous growth in size of the egg-cell, further complicates this process. Material for the study of the development of the male germ cells is easier to obtain, then, and is more convenient to work with. Nevertheless, oogenesis has not been neglected.

In 1900 v. Winiwarter published his paper on oogenesis in the rabbit and man, and in 1908 there appeared his monographic work in collaboration with Sainmont on the organogenesis of the ovary and oogenesis in the cat. These two studies have had an important influence on the work since done along similar lines. An instance which might be mentioned is that the terminology, first proposed by von Winiwarter and later slightly modified by both authors, has been adopted by a large number of those who have made a study of oogenesis and particularly, perhaps, of spermatogenesis.

Von Winiwarter and Sainmont state that they found the cat a more favorable form to work with than the rabbit because the period of gestation is longer (sixty days instead of twenty-eight); the development of the animal is slower and consequently that of the ovary is more gradual, which made it easier to obtain the various stages. On the other hand, it seemed to me that it might be desirable to make a study of a form which had a short sexual cycle, which could be readily obtained in large numbers, and which could be easily bred. The white or albino mouse

was selected for this work as it seemed to offer a number of advantages. The sexual cycle is completed in about sixty days (Kirkham, '16) of which the period of gestation occupies twenty or so. For this reason it is possible to secure a complete series of ovaries from birth to sexual maturity, as well as during embryonic life, without involving an excessive amount of material. The small size of the ovaries of even adult mice reduces the manipulation, a consideration not to be despised when making a large number of preparations.

Von Winiwarter and Sainmont state—reserving a full description for a later chapter which, as yet, has not appeared—that the definitive ova are formed after birth in the young kitten about three and a half or four months old, shortly before sexual maturity. This new formation of egg-cells takes place by a differentiation of cells from the germinal epithelium of the ovary. If this post-natal formation is of more than an exceedingly limited and special significance, it will be found to occur in other forms. Accordingly, in a form like the mouse which has a short sexual cycle, making it fairly easy to secure a complete series of ovaries between birth and sexual maturity, it should be possible to confirm or disprove such a new formation of egg-cells.

In this study, the emphasis is laid on oogenesis, which is used to mean the development of the definitive germ cells or ova from undifferentiated cells into primary oocytes in mature or nearly mature Graafian follicles. Further development, that is, maturation, will not be discussed, being beyond the limits planned for this study. My results agree with those of von Winiwarter and Sainmont in that, in the mouse, this process of oogenesis or differentiation of the definitive ova takes place after birth in the period before sexual maturity. Furthermore, in the mouse, as they found in the cat, the germ cells formed before birth during embryonic and foetal life all degenerate and have nothing to do with the development of the definitive ova. The development of these cells, however, and the morphogenesis of the ovary will be briefly described in order that the process of oogenesis proper may receive the setting necessary for its appreciation and understanding.

MATERIAL AND METHODS

The material on which this study is based consists of a rather complete series, serially sectioned, of ovaries of white mice ranging from embryos of 10 mm. length to adults—in all nearly 300 ovaries. From birth to two days afterwards, the ovaries were fixed at intervals of two to three hours; from two to forty days, at intervals of one day; and from forty to sixty days, at intervals of five days. A number of ovaries from adult mice, pregnant and not pregnant, were also fixed. The series of embryonic and foetal ovaries ranges from those of embryos 10 mm. in length, at intervals of one millimeter, to foetuses 25 mm. long, or practically full term. These embryos, according to Kirkham's table ('16 a) range from about fourteen to twenty or twenty one days post coitum.

For fixation, Hermann's, Flemming's, Carnoy's (6–3–1, with and without the addition of mercuric chloride), picro-aceto-formol (Bouin's), and sublimate-acetic were used, each ovary of a mouse being treated in a different manner. It was found that Hermann's and Flemming's fluids preserved the nuclear structure excellently in the interior of the younger ovaries, but that the outer two or three layers of cells were over-fixed, presenting the glassy, homogeneous appearance with lack of detail characteristic of cells over-fixed with osmic acid. Carnoy's fluids gave excellent preservation of the nuclei, but in a number of cases the sections, when stained with iron hematoxylin, showed a solid black band or border, one to three cells deep around the ovaries, completely obscuring detail. Picro-aceto-formol was the most useful and successful fixing fluid for preserving the outer layers of cells—particularly the germinal epithelium. I might add that where there was any tissue in contact with the ovaries (a bit of the peritoneal wall was sometimes snipped off with the smaller ovaries) or where the periovarian capsule was thicker than usual, due to its penetration by the oviduct, Flemming's fluid preserved the outer cells very well, the extraneous tissue becoming over-fixed and saving the ovary itself from that fate. The figures from Flemming and Hermann material are of cells thus protected.

Iron hematoxylin (Heidenhain's) was used for staining most of the sections, and for bringing out the details of the chromatin it can hardly be improved. Flemming's triple, usually one of the shorter methods, was used with Flemming fixed material in a number of cases, and proved very useful in bringing out the nucleoli and the idiosome.

A large number of ovaries of different ages were also fixed and stained for mitochondria. Benda's own method was used, with varying success. More reliable and certain, and better in several ways, is the following method: the tissue is fixed in Benda's fluid (Flemming's, with about one drop of glacial acetic acid to each 10 cc.) or Helly's, or Zenker's fluid (with the acetic acid reduced as above) and then mordanted in 2–2.5 per cent potassium dichromate for two to four weeks, the longer time being perhaps preferable. Sections are stained with Weigert's copper hematoxylin. Tissues fixed in Benda's fluid with no subsequent mordantage, and stained with copper hematoxylin also gave very good results. By this method the mitochondria are stained black or blue-black and stand out very clearly against the yellow background of the cytoplasm.

MORPHOGENESIS OF THE OVARY

In the development of the cat's ovary, von Winiwarter and Sainmont ('08) describe two proliferations of cells from the germinal epithelium before birth, forming medullary and cortical cords respectively, and lay some emphasis on these as separate down-growths. The germ cells and follicle cells of the first proliferation and the germ cells of the second all degenerate by the time the kitten is a few months old. At the age of about three and a half or four months, by a renewal of the activity of the germinal epithelium, a third proliferation of cells occurs, from which develop the definitive egg-cells or ova, making up the definitive cortex of the ovary. Kingsbury ('13) does not distinguish between a first and a second proliferation of cells in the embryo, considering them parts of one continuous process. From the cells which arise first from the germinal epithelium are formed the 'medullary cords,' and the 'cortical cords' or tubes of

Pflüger are formed later, outside of or peripheral to these; and medullary are not to be sharply distinguished from cortical cords. Kingsbury also found no evidence of a third or post foetal proliferation of cells shortly before sexual maturity.

In the mouse there is a single continuous proliferation of cells from the germinal epithelium which extends up to about birth. This proliferation of cells does not take the form of tubular 'down-growths,' as in the cat and other forms, but the cells are in irregular masses just beneath the epithelium. Later, as the ovary grows in size and the stroma cells increase in number, these latter tend to wander in and break these masses of cells into short, thick structures which are, however, imperfectly and incompletely separated from each other (fig. 9). The small size of the ovary may be responsible for this pattern in the development; there is not enough room for the formation of the pronounced cords or egg-tubes found in other larger forms. These groups of cells are not at first separated from the germinal epithelium, but with the development of the tunica albuginea these cell-masses become cut off about the time of parturition. The development of the tunica will be taken up later. As was stated above, this process of cell-formation is a continuous process in the mouse; there is no distinction to be made between a first or medullary and a second or cortical proliferation. The inner regions of these cell-masses or groups might be termed 'medullary,' and what might be called 'medullary follicles' are probably formed by the separation of some of the germ cells with their follicle cells from these masses. These 'medullary follicles' would contain cells formed first from the germinal epithelium, which consequently are further along in development than those more peripherally located.

There is a small amount of stroma in the ovary at this time, formed by the differentiation and multiplication of mesenchymal cells which have wandered in at the hilum. Blood vessels are present as a few capillary loops. The stroma cells, as mentioned above, grow peripherally in strands and partially separate the cell-masses from each other (fig. 9). These stroma cells, on reaching the base of the germinal epithelium, begin to grow

around under this as the developing tunica albuginea, partially separating these masses from the epithelium. These groups of cells retain a partial connection with the epithelium until about birth when the tunica is practically complete, although not very thick. At this time, the tunica is made up of one or two layers of spindle-shaped or fusiform cells. Even in the ovary of the adult mouse the tunica is not a very dense structure, consisting of a few layers (3 to 6) of flattened or fusiform cells. It seems to be continuous with the stroma in places where the latter is radially arranged, and hence appears thicker in some regions than in others. As will be seen later, the tunica has a part to play in the development of the definitive ova.

The cell-masses are made up of two kinds of cells—those with large round nuclei and a relatively small amount of cytoplasm, the 'primitive germ cells or oocytes,'[1] and smaller cells with smaller round or oval nuclei, the 'indifferent cells,' which are to be regarded as the future follicle cells. In embryos or foetuses of 23 mm. length (ca. nineteen days post coitum) a few egg-cells in the central part of the ovary possess follicles made up of a single layer of these cells. As they surround an oocyte at first, they are somewhat flattened, perhaps as a result of an increase in size on the part of the germ cell: the follicle cells may be stretched out a little before dividing to keep pace with the oocyte in its growth. This follicle-formation proceeds toward the periphery, and is quite rapid from this time up to and after birth (fig. 10). By three days after birth all the germ cells in the ovary proper are surrounded by follicles, which, in the central part may be two-layered, of cuboidal cells, and peripherally just under the epithelium are made up of a single layer of flattened cells. This condition persists for a few days, the centrally located follicles gradually growing in size (fig. 11). In young mice of 14 days, the central follicles have from three to five

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[1] In this paper I use the terms 'primitive oocytes or germ cells' to refer to the cells formed from the germinal epithelium in the first or embryonic proliferation; 'definitive oocytes or germ cells' will be applied to the egg-cells arising from the germinal epithelium in the proliferation of cells between birth and sexual maturity. The terms 'primordial germ cells' and 'primary gonocytes' have been used by authors in another sense and would not be appropriate here.

layers of cuboidal cells and fill up the interior of the ovary, constituting the bulk of it (fig. 12). Clearly marked off from these is a superficial layer of egg-cells two to five deep, in primary follicles, made up of a single layer of flattened cells. The large central egg-cells with their follicles are those which have arisen by the proliferation of cells from the germinal epithelium before birth, as described above; the oocytes in the primary follicles have been formed from the epithelium by a proliferation beginning about birth or shortly after—a process to be described below. Cavities begin to appear in the larger follicles between fifteen and eighteen days post partum, and a degeneration of the egg-cells sets in at about the same time. The evidence shows that all these germ cells formed before birth degenerate and are resorbed, none of them developing into definitive ova. This degeneration takes the form of atrophy and resorbtion in some cases, but in others there may occur atresia folliculi, accompanied by the formation of a first polar body and a degenerative fragmentation of the egg-cell, simulating more or less closely a parthenogenetic cleavage (Kingery, '14). ·

During the early development of the ovary the cells of the germinal epithelium vary in shape from rather tall cuboidal to somewhat flattened cells, a large number being more or less rounded. The nuclei are large, nearly filling the cell, and the cytoplasm is scant in amount. After birth certain of these cells begin their development into oocytes. They begin to grow and enlarge in situ in the epithelium. At first more or less spherical, as they become larger than the other cells of the epithelium they project from the surface of the ovary. These small protuberances are very noticeable between three and thirty days after birth (figs. 35, 36, 38, 1 to 3.) These cells soon become oval, however, with their long axes tangential to the surface of the ovary. As they enlarge, the adjacent epithelial cells are crowded to either side (or end) and are flattened against and around the egg-cells (figs. 35, 36.) As the egg-cells grow still larger, the bulging on the surface becomes more marked. In the course of further development some of these flattened cells extend up over (outside of) and under (inside of) the oocyte so

that the latter is completely surrounded by a layer of flattened cells, a primary follicle, while still in the germinal epithelium (figs. 2, 3, 38). As growth proceeds, the other cells of the germinal epithelium extend up over this oocyte in its primary follicle which is in this manner 'left behind' in the tunica albuginea under the epithelium. The cells of the epithelium then unite over the egg-cell in its follicle, closing over it (cf. figs. 1 to 6, which are designed to show this).

These primary follicles remain in the tunica albuginea at first, but as development goes on, they come to lie in the stroma
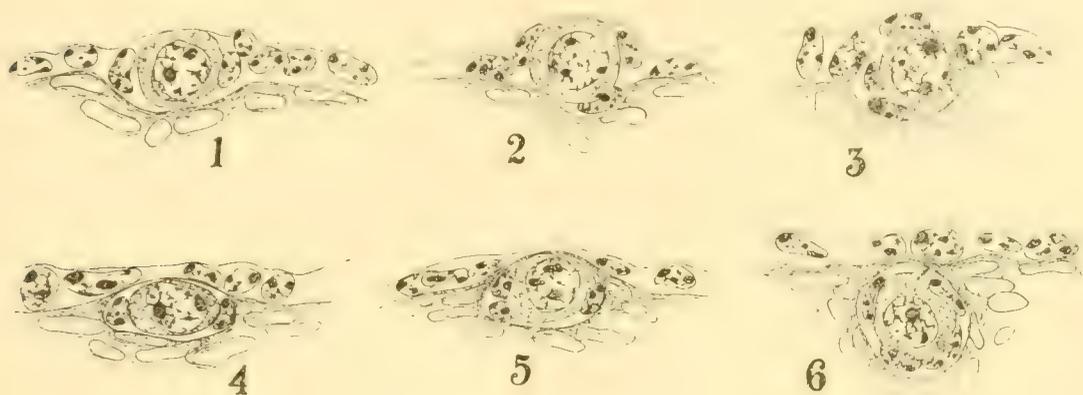
Text-fig. 1 to 6   These are to show the development of the follicle of a definitive germ cell and the way in which the oocyte migrates from the germinal epithelium into and through the tunica albuginea. In the figures, the free surface of the epithelium is up and the tunica and ovary down. Figure 3 is from an ovary of a mouse three days old, while the others are from the same ovary of a mouse twenty days after birth.   × 625.

beneath it. The cells of the tunica, not a very dense layer of tissue, are apparently active in this migration of the follicles. They separate underneath (central to) and close up over (peripheral to) the follicles, and by a continuation of this process, these gradually pass through the tunica and reach the stroma beneath (figs. 3 to 6). New egg-cells in follicles are being continually added outside these, the later formed being, of course, younger than those more deeply located, which, in turn, are more peripherally situated than the cells originating during embryonic life, the primitive germ cells. These latter, as mentioned above, make up the bulk of the ovary for the first twenty

or twenty-five days after birth (figs. 11, 12). As they degenerate, they are gradually replaced by the definitive ova whose follicles have by this time begun to enlarge.

In the course of the degeneration of the primitive oocytes (those of embryonic origin) the space they occupied in the center of the ovary becomes filled with stroma and blood vessels which, in their growth, form the definitive medulla (figs. 11 to 13). This is well formed by about twenty-eight days after birth. What may now be termed the definitive cortex is filled with follicles containing oocytes of postnatal formation (definitive germ cells) and follicles of embryonic origin (primitive germ cells) which project into it from the medulla. Thus, the definitive medulla of the adult ovary includes the embryonic medulla and a large part if not all of the embryonic cortex. The definitive cortex is exclusively of post-partum formation.

The origin of the rete ovarii is still an open question. Von Winiwarter and Sainmont ('08) describe the rete of the cat as arising from the organ of Mihalkowics which forms a net-like structure of solid cords of cells which later acquire lumina. This net-work of tubules retains its connection with the uriniferous tubules (epoophoron) and forms a new connection with the inner ends of the medullary cords. The organ of Mihalkowics is described as arising from the capsules, of the glomeruli in the cephalic part of the mesonephros.

Felix ('12) states for man that, in the degeneration of the mesonephros the glomeruli, corpuscles, and secretory parts of the uriniferous tubules are the first to go, and the collecting tubules persist. The 'rete blastema,' a product of the epithelial nucleus which itself has arisen from the germinal epithelium, forms a net-work of cords. These cords, which are solid at first, but acquire lumina about birth, form the rete ovarii. They connect up with the collecting tubules of the mesonephros.

Kingsbury ('13) suggests that, in the cat, the rete may arise partly from the mesothelium at the cephalic end of the ovary and partly from ingrowths from the mesonephros.

In the mouse the mesonephros has a relatively slight development and degeneration sets in early. I did not make a special

study of the origin and development of the rete ovarii in the mouse, reserving it for a possible future work. The suggestion is ventured that it apparently arises by ingrowths from the mesonephros. The rete is found as a constant structure in the adult ovary.

An interesting feature of the ovary of the mouse is the marked development of a peritoneal fold, forming a more or less complete covering or capsule for the organ (figs. 8, 9, 10, 12, 13). Huber ('15) mentions, but does not describe, a similar 'periovarian capsule' or 'bursa ovarica' in the rat. Van Beneden ('80) described in the bat a periovarian capsule which he said was closed, with no communication with the peritoneal cavity. Sobotta ('95) found in the mouse a connective tissue capsule which completely surrounds the ovary. He states that the space between capsule and ovary is filled with a clear serous liquor and that during heat the capsule becomes enlarged. Similar structures have been described for other mammals (Schmaltz, '11).

My material shows that this membrane arises as a fold of the peritoneum which grows over and encloses the ovary. The oviduct is in the fold as it develops, so that when the capsule is completely formed (embryo of 23 mm. length) the fimbriated end is inside, opening toward the ovary. This capsule is a delicate membrane made up of two layers of peritoneal epithelium between which is a small amount of connective tissue in which a few small blood vessels run. This was found to be apparently a complete capsule in rather more than half the cases examined; in the others there was an opening into the peritoneal cavity close to the hilum of the ovary, near where the oviduct penetrates the capsule.

OOGENESIS

As has been described above, there are in the ovary of the mouse, two proliferations of cells from the germinal epithelium, one during embryonic and foetal life, and the second extending from birth, or a day or so after, nearly to sexual maturity. The second proliferation constitutes oogenesis proper; but it is

advisable to take up the development of the first or 'primitive germ cells' because of the part they play in the morphogenesis of the ovary, and because von Winiwarter and Sainmont have described the history of these cells in the cat with so much attention to detail, as 'oogenesis.'

It was stated above that this first proliferation of cells from the germinal epithelium is a continuous process, but it should be understood that the rate of proliferation is not uniform. It is well marked in embryos from 12 to 22 mm. length (ca. fifteen to nineteen days post coitum), when it becomes slower. From this time on it is rather slow and at about three days post partum it has ceased altogether and the second proliferation has begun. This second state of activity on the part of the germinal epithelium is manifested rather slowly, and it is over shortly before sexual maturity. It may be that the two proliferations in the mouse are parts of one process, the second (post partum) being a continuation of the first; the discussion of this will be taken up later.

The earlier stages of the nuclear development of these germ cells of the first or embryonic proliferation, the 'primitive oocytes,' are usually passed through by the cells while still in the germinal epithelium. After a number of cell-divisions a cell begins to differentiate. The resting stage after each mitosis von Winiwarter calls 'protobroque.' After the last oogonial mitosis in the mouse, the cell returns to this stage (fig. 14). The nucleus shows a delicate network of chromatin on a reticulum of linin. At the intersections the chromatin is massed in larger granules, among which are two or three nucleoli, one of which is usually larger than the others. With Flemming's fluid and triple stain, the red nucleoli stand out distinctly from the blue-violet reticulum of chromatin. No idiosome is seen at this stage, and the mitochondrial content is made up of granules sparsely scattered through the cytoplasm. Cells in this phase are found in the germinal epithelium, in embryos from 13 mm. length up to birth.

There is no indication of the stages 'poussieroide' or 'deutobroque' of von Winiwarter and Sainmont ('08). In the case of the former, the suggestion might be ventured that this ap-

pearance (to judge from their figures) is an artifact, due to over-fixation with the osmic acid fixer used (Flemming's). The cells are described as being in the germinal epithelium, and I have found that over-fixation is regularly the fate of the cells in the outer layers of the ovary.

In the mouse, the cells pass from the 'protobroque' to the 'leptotene' stage, which is of short duration. The chromatin net-work gradually becomes heavier in places and breaks down in others, so that the result is the formation of long slender bands or threads, connected by delicate cross-bars (figs. 15, 16). These cross-bars disappear later and the chromatin is then arranged as a tangle of long slender threads (fig. 17). At least one nucleolus is present, frequently hidden in the knot of chromatin bands. An idiosome is not yet distinguishable. It is difficult to say whether the chromatin threads have any definite arrangement; in some cells they seem to have more or less the form of a horse-shoe, with their ends all toward the same side of the nucleus. This disposition, however, does not seem to be universal. Cells in this phase are usually found in the germinal epithelium, but many are also encountered beneath this in the ovary.

This stage soon passes into the following in which the chromatin threads undergo a contraction to one side of the nucleus—synizesis (fig. 18). This contraction is not extreme in the mouse and the chromatin bands can usually be distinguished, a few extending out of the tangle. The nucleoli are hidden among the threads and it is difficult to make them out; one at least, however, is present at this time. No idiosome was distinguished, so it is hard to say with certainty whether the contraction of the chromatin is toward that side of the cell. The mitochondria have increased in number and are massed around the nucleus. Cells in this stage are most frequently found in the ovary under the germinal epithelium.

The next stage, 'pachytene,' is to a certain extent synchronous with the preceding. During synizesis the long slender threads of chromatin begin to shorten and thicken. As this progresses, they assume, at the same time, a moniliform appearance, as if

made up of granules strung together (figs. 18, 19). At first a fairly regular arrangement as loops is seen in some of the cells, a persistence of the disposition in the preceding stage. This definite arrangement of the chromatin threads, if indeed it be of constant occurrence, is soon lost, and with the further shortening and thickening of the bands, they become irregularly arranged in the nucleus. At least one nucleolus is visible, at first among the ends of the chromatin threads and later almost anywhere in the nucleus. I was unable to distinguish an idiosome at this stage, but the mitochondrial granules, sometimes with a few rods among them, have begun to accumulate in the still rather scant cytoplasm at one side of the nucleus, forming a well-marked crescent-shaped mass. Later, when the idiosome is clearly distinguishable, it occurs in the center of this group of mitochondria, so it is safe to assume that this crescent marks its location. When a definite arrangement of the chromatin threads can be made out, their ends are directed toward this accumulation of mitochondria.

This pachytene stage is of rather long duration—in fact, cells in this phase are found in the ovary up to a couple of days after birth. It passes into the 'diplotene' stage of von Winiwarter. That is, some of the thick monliform threads of chromatin begin to split longitudinally (fig. 20). Y-shaped and ring-shaped chromatin segments are formed as a result, depending on whether the bands split at one end first or in the middle. In threads split at one end, the arms of the Y occasionally become twisted around each other, in the 'chiasmatypie' of Jansens, to which some have attached such importance in the explanation of the behavior of certain characters in inheritance—in 'crossing over,' for example. One or two nucleoli are visible during this stage, which is relatively short and blends with that following. Cells in this phase are found in ovaries of embryos about 22 mm. long, and as late as ovaries of mice a day or so after birth. Follicle formation begins at about the time the cells enter this stage. Practically all the early diplotene cells are in primary follicles, made up of a single layer of flattened cells. Later

diplotene germ cells, and those in the following stage (dictyé or dictyate) are in follicles more advanced in development.

The idiosome is clearly distinguishable for the first time in the diplotene stage. A mitochondrial technique is apparently the best method for bringing this out, but it is visible after Flemming's or Hermann's fluids. It appears as a deeply staining body in the cytoplasm, close to the nucleus. At first it is hidden in the crescent-shaped mass of mitochondrial granules, but when these become scattered through the cytoplasm, as happens shortly, the idiosome stands out clearly. Only occasionally are centrosomes seen in it.

There is a certain overlapping of this stage and the next, the 'dictyé' or 'dictyate.' In many cells, while some chromatin threads are splitting, others begin to thin out and lose their character of threads or bands. The chromatin becomes arranged in irregular masses and granules at the intersections of a network or reticulum made up, partly of chromatin and partly of linin (figs. 21 to 23). One large nucleolus and two or more smaller ones are usually present. The chromatin and linin are frequently in close relation with the large nucleolus which stains much less intensely as it enlarges. The idiosome is plainly visible in the cell near the nucleus, and the mitochondria, mostly granules, but with beaded rods and threads appearing in increasing numbers, are evenly scattered throughout the cytoplasm.

Von Winiwarter and Sainmont ('08) consider this stage, dictyé described and named by the former in his work on the rabbit a form of degeneration and not a normal step in development. R. Van der Stricht ('11) who employs von Winiwarter's terminology, states that this is not always a stage marking degeneration, but is frequently normal, appearing in maturing Graafian follicles. In the mouse, this stage bears a resemblance to the later phases of the development of the definitive oocytes (figs. 44 to 46), and is to be correlated with the marked growth in size of both nucleus and cell. This growth, together with the fact that these cells degenerate without forming ova, undoubtedly are important factors in producing these nuclear changes. This degeneration

and its bearing on the origin of the definitive ova will be discussed later.

Oogenesis proper, the second or post-foetal proliferation of cells from the germinal epithelium, begins within two or three days after birth and extends nearly to sexual maturity; that is, the process lasts about thirty-five or forty days. At birth the first proliferation has become so slow that the cells are differentiating in the epithelium instead of in the ovary underneath, and pachytene and diplotene cells are seen in the epithelium itself. This condition does not last, however, as the process apparently stops entirely within three days after birth. That is to say, the second proliferation is an indication of a renewed activity on the part of the germinal epithelium, and may be considered as beginning where the first left off. Whether the two are to be considered as parts of one continuous process is a question which will be discussed later.

Since the developing definitive oocytes pass through the early stages of their differentiation in the germinal epithelium, it is somewhat difficult to determine the correct seriation of stages. Criteria employed are the relative sizes of the nuclei and of the whole egg-cell in a single ovary, and the size and appearance of the cells and nuclei in ovaries of different ages.

The formation of egg-cells from the epithelium is most rapid during the time from three to twenty-five days post partum, and in a single ovary of this period practically all the early stages may be seen. Advantage has been taken of this fact in making the drawings: a number have been drawn from one ovary (eleven days post partum). It has been possible thus to show relative size very clearly, and the cells have all had the same fixation and stain. However, cells from a number of other ovaries, differently fixed and stained, have been drawn; accordingly there is no ground for a possible contention that my results are due to any one special method. This process goes on practically up to sexual maturity although more slowly in the latter part of this period, and is completed forty or forty-five days after birth. At this age the majority of female mice are sexually mature, and the ovaries contain all the oocytes which will be differentiated.

The development of these germ cells, the definitive oocytes, is marked chiefly, perhaps, by the utter absence of the complicated chromosomal history which is usually associated with this process. In the description to follow, it seems convenient to speak of three stages in the development, 'a,' 'b,' and 'c;' these are, of course, more or less arbitrary, and are not sharply distinguished, one passing almost insensibly into the next.

Apparently any cell in the germinal epithelium is a potential egg-cell. From the cells of this epithelium develop oocytes, follicle cells, and residual germinal epithelial cells; and at first there is no way of distinguishing the different kinds of cells or their potentialities. At first neither the mitochondrial content nor the nuclear structure is distinctive or characteristic of any one of these possible lines of development. It is not until one of these cells, in the course of its differentiation, begins to grow in size that a germ cell can be distinguished from other cells in the epithelium. It would be difficult to determine just what the factors are which determine the line of differentiation any cell of the epithelium will take.

To digress for a moment: Jenkinson ('13) states that the "oocytes of the outermost layer often lie practically in the epithelium," but he thinks that they have developed from the 'primordial germ cells' which have migrated in at the hilum of the ovary. There can be no doubt whatever that in the mouse the oocytes start their development in the epithelium. Figures 35, 36 and 38 have been drawn with the adjacent cells of the epithelium to bring out this point. In figure 35, in which, through shrinkage, the epithelium has been slightly torn away from the ovary, it is shown conclusively, I think, that the egg-cells are actually in and a part of the epithelium and not merely crowded against the basal side of it.

Stage 'a.' The cells of the germinal epithelium have, of course, been dividing during the growth of the ovary, keeping pace with its increase in size. This might be termed the "multiplication period' (figs. 25 to 27). Beginning about three days after birth, certain of these epithelial cells commence to grow in size, and from then on they may be considered oocytes (primary) since

they develop into the definitive ova. These cells of the germinal epithelium, after each mitosis, return to a condition, the resting stage between divisions, which resembles that described as 'protobroque' by von Winiwarter (fig. 24). It is from this point, after the last 'oogonial division' that the egg-cells in the mouse begin their further development. The cells in this stage are scarcely distinguishable from those in the resting condition between mitoses, and also resemble the 'protobroque' cells of the embryonic proliferation already described, although they may be considerably smaller (figs. 24 and 14). The nucleus is made up of a delicate network or reticulum of chromatin on a linin frame-work; small clumps or granules of chromatin are located here and there at the intersections of the net-work. The reticulum may be poorly defined and the granules of chromatin appear more or less isolated in the nucleus. Two to five nucleoli may be present, although the more usual number is two or three. Flemming's fixation and triple stain, or some similar technique, is necessary to distinguish these nucleoli from the larger clumps of chromatin, with which they may be confused in iron hematoxylin preparations. One nucleolus is usually larger than the others and is sometimes oval or elongated (figs. 28, *d* and 29, *d*).

Stage 'b,' (figs. 29 to 33). This stage is marked by a slight increase in size of both oocyte and nucleus. The chromatin granules may increase in size, and the reticulum becomes heavier. There seems to be an actual increase in the amount of chromatin: as the whole chromatin network is heavier or coarser (figs. 29 to 34) from two to five nucleoli may be present, although, as in the preceding stage, two or three seems to be the more usual number.

Stage 'c,' (figs. 34 to 46). The next phase is marked by a gradual change in the nuclear structure. The whole cell is growing markedly in size and, as the nucleus enlarges, the reticulum of chromatin becomes attenuated and loses to a certain degree its character as a network. Apparently the cross-bars break and the chromatin 'flows back' to the longer strands, forming threads or strands with granules of chromatin on them (figs.

36 to 40). The chromatin loses more and more its staining reaction and the nucleus appears filled with more or less isolated strands and granules of faintly staining chromatin. These strands are eventually arranged as smaller masses and threads of irregular form, with granules scattered here and there, and with a faint, irregular, incomplete reticulum of linin. Two or three nucleoli are usually visible, one frequently larger and less intensely stained than the others (figs. 41 to 46).

This condition of the nucleus, with the chromatin widely scattered in irregular granules and strands and with two or three nucleoli, resembles to a certain extent the 'dictyate' stage de-
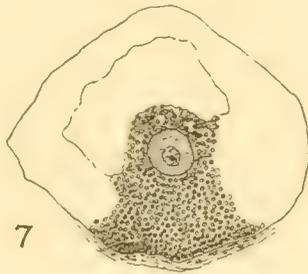
Text fig. 7   A definitive oocyte in a mature or nearly mature Graafian follicle. This is a sketch of the oocyte whose nucleus is shown in figure 46.   The follicles of the mouse do not attain the large size nor the marked vesicular character of those of many other mammals.   × 44.

scribed above for the primitive germ cells. This is not surprising, perhaps, when it is considered that the 'dictyate' stage of the primitive germ cell and stage 'c' of the definitive oocyte are each correlated with the enormous growth of the germ cell and its nucleus. Compare, for example, figure 36 with 46; the latter oocyte is so large that only the nucleus is shown in the drawing. This oocyte (fig. 46) is in a nearly mature Graafian follicle (text fig. 7) and is almost ready for maturation.

As the oocytes enter stage 'b,' they begin to grow in size and, although at first more or less rounded, they become more oval, with their long axes tangential to the surface of the ovary; but their shape is apparently dependant on pressure and the effect of the adjacent cells. Oocytes in stages 'a' and 'b' are found in the germinal epithelium, and occasionally cells of stage 'c'

occur here also (figs. 35, 36, 38). Usually however, oocytes in stage 'c' are in primary follicles or in Graafian follicles of various stages of development, up to the nearly mature primary oocyte in the adult or sexually mature ovary (figs. 4, 5, 6, 40, and 7). The manner in which the oocytes penetrate the tunica albuginea in leaving the germinal epithelium and the formation of the follicles have already been discussed (p. 384–385).

An idiosome is not visible in stage 'a,' and becomes well marked only in cells of stage 'b.' Centrosomes are not infrequently clearly distinguishable in the idiosomes (figs. 32, 38). The mitochondrial content of the cells of the germinal epithelium is very scanty, and consists of a few granules close around the nucleus (fig. 47). As the oocyte begins to differentiate and grow in size, the mitochondria increase in number and become located chiefly, perhaps, in the two ends of the cell, now somewhat oval in shape (fig. 48). They are arranged near the nucleus and, as differentiation proceeds, they tend to accumulate more in one end or side of the cell, forming a more or less crescent-shaped mass which marks the place where the idiosome develops (figs. 48 to 50). At about the beginning of stage 'c' the mitochondrial mass becomes dispersed and the granules are scattered quite uniformly through the cytoplasm, accompanying the growth of the cell (figs. 50, 51). There is no indication of a peripheral condensation of the mitochondria such as described for the cat's egg by R. Van der Stricht ('11).

The oocytes remain in this stage 'c,' which somewhat resembles von Winiwarter's 'dictyé,' until ready for maturation. There can be no doubt of this. Egg-cells have been followed in their development up into mature or nearly mature Graafian follicles in sexually mature and adult ovaries, and the nuclei are all in this phase (figs. 44 to 46, 7). The further development of the oocytes, the maturation divisions and the formation of the mature ova, was not investigated, as beyond the scope of this work.

DISCUSSION: LITERATURE AND OBSERVATIONS

The monographic work by von Winiwarter and Sainmont ('08) on the organogenesis of the ovary and oogenesis in the cat has had its influence on studies since made along similar lines, and as has been mentioned above, their revision of von Winiwarter's terminology has been adopted by a large number of authors. But so far, no one, apparently, has called attention to a feature of their work which seems to me to be rather inconsistent and illogical. Their account of oogenesis is presented with great care and attention to detail, but, as they themselves state, all of the cells which go through this process degenerate, and not one develops into a definitive ovum.[2] It is not quite clear just how the course of development of such cells, all of which degenerate, constitutes oogenesis. If oogenesis be used in a broad sense to mean the course of development of egg-cells, with no regard to their ultimate fate, then perhaps, the application of the term is justified here. However, if oogenesis is taken to mean the development of the definitive ova, von Winiwarter and Sainmont have not described such a process.

They state that the definitive ova are formed by a renewed activity of the germinal epithelium shortly before sexual maturity.[3] They merely mention this and say a full description will follow later. In a note appearing in 1908 at about the same as their memoir (von Winiwarter and Sainmont '08[a]), they discuss very briefly (four pages) the post-foetal formation of ova in the cat. They state here that the question of a new formation

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[2] "Au second chapitre du présent mémoire nous avons démontré que les cordons corticaux ou tubes de Pflüger ainsi que toutes les formations auxquelles ils donnent naissance (ovules, follicules primordiaux, follicules de de Graaf développés) ne sont que productions transitoires, au même titre que les cordons médullaires." (Von Winiwater and Sainmont, '08, p. 165.)

[3] "Pendant que ces dernières modifications se déroulent l'activité de l'assise épithéliale entre une dernière fois en jeu pour aboutir aux invaginations épithéliales. . . . . Ces invaginations, jointes aux cellules folliculeuses de zone corticale primitive, aboutissent à la formation de la zone corticale définitive de l'ovaire, à laquelle, seule, sera réservée la production des oeufs définitifs. Son histoire appartient a un chapitre ulterieur." (Von Winiwarter and Sainmont, '08, p. 259–260.)

of ova has not been settled one way or the other for two reasons:—
no one has taken the time and pains to examine a complete
('lückenlose') series of ovaries, and second, there have not been
any definite characteristics by which a young egg-cell might be
recognized. Concerning this second point these authors state
further:

Nun ist es von einem von uns (v. Winiwarter, '00) nachgewiesen
worden, dass das Ei der Säugetiere im Laufe der Wachstumsperiode
eine Reihe von Kernveränderungen durchmacht, welche so characteris-
tisch sind, dass sie mit Sicherheit erlauben, einen *jungen* Oocyten von
allen übrigen epithelialen Zellen des Ovariums zu unterscheiden. Von
diesem Prinzip ausgehend, hat sich schon damals einer von uns (v.
Winiwarter) dahin ausgesprochen, dass eine Neubildung von Eiern
nur dann als bewiesen gelten könne, wenn die als neugebildete Eier
angesehenen Elemente die characteristischen Kernmetamorphosen
der ersten Entwicklungsstadien des Ovariums erkennen lassen ("que
les preténdus ovules de nouvelle formation montreraient les méta-
morphoses nucléaires caractéristiques des premiers stades de développe-
ment de l'ovaire"). ('08 a, p. 613–614.)

It seems to me that this is rather an unwarranted assumption,
made by von Winiwarter himself in 1900 and repeated by von
Winiwarter and Sainmont in 1908. The account given by these
authors of the development of these egg-cells, is very carefully
worked out. The egg-cells, in their development, pass through
certain stages which have been very carefully characterized,
and then every one undergoes degeneration. It is not quite
clear just what justification there is for the dictum that any
cells of new formation, in order to be considered egg-cells, must
pass through the same stages in their development as the earlier
formed cells which degenerate and disappear. It would seem
that the ultimate fate of any cells of new formation should have
some bearing on the question of whether they are to be considered
definitive egg-cells. If these cells, formed after birth, can be
shown to develop into the definitive ova, if they can be traced
through all the stages up to eggs in mature Graafian follicles, it
would seem that the question of whether or not their earlier
development was like that of the cells formed in the embryo,
was a matter of slight importance.

To return to the two points raised by them concerning a new formation of egg-cells; —I think my series of ovaries is complete enough to satisfy any requirements. As for their second consideration, it seems to be rather an unwarranted assumption; the ultimate fate of the cells under discussion is what should decide their status, and not "the nuclear metamorphoses characteristic of the first stages of the development of the ovary." Perhaps their promised later chapter will throw some light on this question.

The problem of the origin of the definitive ova is one which has been of interest to many workers, but is not yet definitely settled. I shall take up this point only briefly, referring to the papers by Swift ('14 and '15) and Firket ('14) for a more detailed discussion.

The two more important views as to the origin of the definitive germ cells are: (1) that they develop by a process of differentiation from the mesothelial cells of the germinal epithelium covering the ovary; and (2) that they develop from 'primordial germ cells' or 'Ureier,' which themselves have had their origin elsewhere (entoderm) and have migrated to the epithelium of the genital ridge, there to become differentiated into the ova. The first is the older view, and, presented by Waldeyer in 1870, is today held probably by a majority of those who have considered the question. The second conception is comparatively recent and probably grew out of Hoffmann's work in 1893. He showed ('93) that the 'primordial germ cells' or 'Ureier' which Waldeyer and his school believed were differentiated from cells of the germinal epithelium, were, in birds, present in the embryo, long before the germinal epithelium or gonad had appeared, and that they were found far from the site of the future reproductive organ. They were found in entoderm, in splanchnic mesenchyme and between the two, and later migrated to the mesothelial covering of the gonad when it started its development. He believed that the definitive ova were the direct descendants of these 'primordial germ cells.'

'Primordial germ cells' have been described in a number of forms, including fishes, reptiles, birds, and mammals. For a more detailed discussion and bibliography reference is again

made to Swift ('14 and '15) and Firket ('14). To mention a few cases among the mammals, Jenkinson ('13) states that in the rabbit the 'primordial germ cells,' originating in the splanchnopleure of the yolk-stalk, migrate to the gonad and there develop into the definitive ova; he believes that the definitive germ cells of the mouse have a similar origin. He adds, however:—"This mode of origin of the germ cells does not, of course, preclude the formation of others from the cells of the sex-cords, that is from the germinal epithelium, and it is indeed quite possible that this occurs." Kirkham ('16) says that the primordial germ cells in the mouse give rise to the definitive ova, and Rubaschkin ('12) holds that they persist and form the definitive ova in the guinea pig.

It is evident, then, that this second view is becoming more generally accepted. There is, however, a fatal weakness in the evidence heretofore presented in its support. Those who hold this conception have, for the most part, been content to trace these 'primordial germ cells' in or to the germinal epithelium, and then assume that they proceed there to develop into the definitive ova. The case has been considered proven when these cells had been followed into the embryonic ovary. Very few have studied the further history of these cells. Swift (loc. cit.), who is very positive that the primordial germ cells form the definitive ova,[4] carried his investigations only as far as an embryo of fourteen days incubation, stating that d'Hollander ('04) and Sonnenbrodt ('08) describe the development of these cells into definitive ova. As a matter of fact, d'Hollander did not

[4] Swift states ('15, p. 450) that v. Berenberg-Gossler "has confirmed the findings of Swift in all the essential points." This is rather misleading, for the fact is that while von Berenberg-Gossler does confirm the actual facts—that large cells, 'primordial germ cells', are present in duck embryos of 24 to 32 somites, he does not accept Swift's interpretation of this. He states:—Alles in allem, bin ich der Ansicht, dass das ganze verhalten dieser zellen in hohem grade davor warnt, sie für keimbahnzellen zu halten, und dass ihre Genese überhaupt ihre Geschlechtszellennatur sehr zweifelhaft macht. . . . Als Hauptergebnis meiner bisherigen Untersuchungen sehe ich die Erkenntnis an, dass man von einer Keimbahn bei Sauropsiden nicht mehr reden kann." (Von Berenberg-Gossler, '14, p. 261–262. The emphasis is his.)

study material from chicks more than twenty days after hatching (v. p. 161), and his evidence on this point is therefore not conclusive. Sonnenbrodt studied material from chicks just hatched to hens several years old; he states that the egg-cells in the ovary at hatching are oocytes, and that they develop into the definitive ova. He apparently accepts d'Hollander's views as to the origin of these oocytes. Although neither of these works alone is conclusive on this point, together they serve to support Swift's view.

Nevertheless, it has not been conclusively demonstrated that these 'primordial germ cells' develop into the definitive ova. In fact, there is weighty evidence to show that they play no part in the formation of the latter. Firket ('14) states that Dustin, Kuchekewitsch, and Allen admit that these 'primordial germ cells' degenerate totally or partly in Amphibians and Sauropsids; Allen and Popoff admit a degeneration in the testis and Skrobansky in the ovary of mammals. Kirkham ('16) states that in the male mouse the 'primordial germ cells' all degenerate, while in the female they form the definitive ova. Von Winiwarter and Sainmont ('08) hold that these 'primordial germ cells' in the cat are temporarily hypertrophied cells and have nothing to do with the process of oogenesis.

Firket ('14) studied material from chick embryos from eighty-two hours incubation to hatching, and in addition, the ovaries from chicks just hatched up to young hens of six months, an age when the hens of most breeds are sexually mature and have begun to lay. He believes that his work proves the sexual or genital nature of these 'primordial germ cells,' or 'primary gonocytes,' as he prefers to call them. His studies show that the gonocytes of the medullary zone (formed by the first of the two embryonic proliferations from the germinal epithelium, as von Winiwarter described for the cat) develop into oocytes which pass through the first stages of the growth period and then degenerate. They have all disappeared in the chick fourteen days after hatching. The oocytes of the cortical zone (second embryonic proliferation) practically all degenerate, although he states that he can not be sure that they all do. There is a new formation of germ cells

in the cortical region, from cells derived from the germinal epithelium, and from these the definitive oocytes develop; but it is not improbable, at least, that a small number of the 'primordial germ cells' as well, are differentiated into definitive ova. One of his conclusions may be pertinent here:—

Il faut, donc, morphologiquement parlant, considérer les gonocytes primaires (primordial germ cells) des Vertébrés comme étant un rappel phylogénique des gonocytes définitifs des classes inférieurs, notament des Cyclostomes et des Acraniens. L'épuisement graduel, dans la série phylogénique des éléments de cette lignée a nécessité l'apparition, au cours de l'ontogènese, d'une seconde lignée de gonocytes, moins précoces. ('14, p. 330–331)

In the mouse, the evidence here presented shows that the definitive ova originate from cells of the germinal epithelium by a process of differentiation, and that this process takes place between birth and sexual maturity. The primitive germ cells also arise from the germinal epithelium, in the embryo. But the relations of the 'primordial germ cells' are not clear. Jenkinson ('13) and Kirkham ('16) both think that they form the definitive ova in the mouse. But Kirkham's paper is merely an abstract, unaccompanied by figures, and therefore can not be considered conclusive. Jenkinson devotes a few pages in his book to this question, but is hardly convincing.

It would seem that, in the mouse, there are three possible courses of development open for the 'primordial germ cells':— 1) they may persist and form the primitive germ cells or oocytes and so eventually degenerate; 2) they may, after reaching the germinal epithelium, develop into the definitive germ cells; or, 3), they may degenerate completely. The evidence presented, I think, shows that neither the first nor the second is the course followed. The objection mentioned above, that no one has traced these cells through to mature ova, also disposes of the second possibility. There is, indeed, the further possibility that the 'primordial germ cells,' after entering the germinal epithelium, lose their size and characteristics and become indistinguishable from the mesothelial cells. It would be extremely difficult to prove or disprove this, but the burden of the proof would, it seems to me, rest upon any one who sup-

ported such a view. The last course, degeneration, seems to be the most probable fate of these cells.

There are not many examples in the literature of a new formation of egg-cells after birth. Van Beneden ('80) described in the adult bat a formation of egg-cells from the germinal epithelium. From his description and figures, the process resembles the formation of oocytes in the mouse before sexual maturity; but, as he did not connect this process with that in the embryo, it is difficult to say whether it is strictly comparable with the condition in the mouse.

Lane-Claypon ('05), in a study of the ovary of the rabbit, concludes that from the germinal epithelium are formed definitive ova, follicle cells, and interstitial cells. These interstitial cells, from their origin, are potential egg-cells, and, under the proper stimulus, are capable of developing into ova. In this case, the proper stimulus is provided in some way by pregnancy, and a number of these cells become differentiated into oocytes. Apparently these conclusions have not been confirmed by other authors, and hence lose something of their force.

Von Winiwarter and Sainmont ('08) state that in the cat, at about the age of three and one-half or four months, a renewal of the activity of the germinal epithelium provides a new supply of germ cells which develop into the definitive ova, when all the egg-cells of the first and second proliferations have degenerated. In a note appearing about the same time ('08[a]), they state that these definitive ova come either entirely from this third proliferation, or partly from it and partly from undifferentiated cells left over from the second.[5]

[5] "Es tauchen nun jetzt in den Epithelhaufen und Strängen der Corticalis Kleine Gruppen von Zellen auf, deren Kerne im Staubförmigen oder deutobrochen Stadium sind. Diese Formen waren schon seit langer Zeit nicht mehr vorhanden, und da sie den ersten Stufen des Wachstums des Oocyten entsprechen, ist es augenscheinlich, dass sie mit einer Neubildung von Eiern zusammenhängen. . . Wir glauben bewiesen zu haben, dass in Säugetierovarium nicht nur sämtliche Markstränge, sondern auch alle Eier und Follikel der primitiven Corticalis dem Untergang anheimfallen. Die definitiven Eier entstammen entweder von undifferenzierten Zellen der zweiten Proliferation (Pflügersche Schläuche) oder von Zellen der dritten Wucherung oder invaginations épithélials. Es ist uns nicht möglich, wenigstens morphologisch, die Elemente der einen und anderen zu unterscheiden." (v. Winiwarter and Sainmont, '08 a, p. 616.)

Kingsbury ('13), while admitting that his material was too scant to permit a conclusive statement, inclined to the opinion that there was no evidence of a new formation of ova by a third proliferation from the germinal epithelium of the cat's ovary.

R. Van der Stricht ('11) apparently does not consider this question at all, as, in his work on the vitellogenesis of the cat's egg, he makes no mention of such a new formation of ova. But, as he accepts von Winiwarter's terminology and course of development for the egg-cells, and states, further, that oocytes with diplotene or dictyé nuclei are found in the adult ovary, it may be assumed that he derives the definitive ova from the second proliferation.

Rubaschkin ('12) states that he can corroborate the conclusions of von Winiwarter and Sainmont that the cells of the first and second proliferations in the cat degenerate. He says nothing, however, about a third proliferation, but one is led to infer that in his opinion one does occur. He further states that Waldeyer had described a proliferation of cells in the post partum development of the ovaries of several animals, but I think Rubaschkin has been misled. In the reference mentioned by him, Waldeyer ('70) says that he believes there is no new formation of egg-cells from the surface epithelium, but that any epithelial down-growths present in post partum ovaries of dogs and rabbits are left over from the embryonic proliferation. Moreover, he states:—

Auch bei Katzen, von welchen ich mehrere zur Zeit der Frühjahrs-brunst untersuchte, fand ich, wie gesagt, Nichts von einer derartigen Neubildung. Meine Untersuchungen sind so zahlreich, dass, wenn sie wirklich vorkommen sollte, wir eine sehr seltene Ausnahme und am allerwenigsten eine Regel vor uns hätten. ('70, p. 45).

Rubaschkin also says that he found a third proliferation of cells from the germinal epithelium of the ovary of the guinea pig which occurs before birth and which he considers the source of the definitive ova.

Firket ('14) describes in the embryo chick a third proliferation of cells from the germinal epithelium which he states forms the most if not all of the definitive ova. As mentioned above, however, he is not able to state definitely that all the mature ova

come from this source. In both these last cases however, it may be questioned whether such a formation of egg-cells before birth is strictly comparable to a proliferation of cells after birth and before sexual maturity.

Felix ('12) in describing the development of the ovary in man says that there is an early proliferation of cells from the germinal epithelium, forming the 'epithelial nucleus.' Later, a 'young cortical zone' becomes differentiated, whether from the outer part of the epithelial nucleus, or by a renewed activity on the part of the epithelium resulting in a proliferation of cells, he is unable to say with certainty, although inclining toward the former view. With the exception of this possible proliferation from the germinal epithelium, there is no addition of epithelial cells to the ovary. After the tunica albuginea is formed (in embryos of 180 mm. length) no cells can be added to the interior of the ovary. Apparently there is no possibility of a new formation of ova from the germinal epithelium.

From the foregoing, it is clear that a new formation of germ cells after birth is not of very general occurrence. It may be that, as von Winiwarter and Sainmont ('08 a) suggest, such a formation of ova has been overlooked because a careful study has not been made of a complete series of ovaries. Certainly, the study of a complete series of ovaries between birth and sexual maturity and into adult life, has shown that in the mouse there is such a proliferation of germ cells after birth.

It is possible that the criticism may be made that the egg-cells described above, in the germinal epithelium of postnatal ovaries, as developing primary oocytes, are, in reality, cells which have already passed through the earlier stages of development in the embryonic ovary, and are, perhaps in von Winiwarter's diplotene or dictyé stages. I have gone over this carefully and am confident that such is not the case. In the first place, these egg-cells differ in many particulars from the cells described as diplotene or dictyé, and are not to be confused with them. Secondly, these post natal oocytes are actually smaller than the primitive oocytes of the later stages (cf. figs. 21 and 22 with 28 to 31). Thirdly, intermediate forms can be observed between these cells

and ordinary cells of the germinal epithelium on the one hand, and between these cells and oocytes in mature Graafian follicles on the other. The evidence, then, presented by a study of a series of mouse ovaries between birth and sexual maturity shows rather conclusively that there is a new formation of germ cells after birth, and that the definitive ova come from these cells.

· Rubaschkin ('12) from his work on the guinea pig states that developing egg-cells may be distinguished from other cells in the ovary by a difference in the mitochondrial content. Mitochondria in the germ cells appear exclusively in the form of granules, while in other cells, including those of the germinal epithelium rods or threads are found. He thus distinguishes sharply between germ cells and epithelial cells, in accordance with his theory of the 'Keimbahn.' The granular form is the type found in the embryonic cells; as differentiation of the cells proceeds the mitochondria become transformed into rods and threads. The germ cells, accordingly, show their embryonic or undifferentiated condition by the granular type of their mitochondria.

Firket ('14) finds that, in the chick, the type of mitochondria is not constant in the germ cells. Swift ('14) states that the mitochondria in the 'primordial germ cells' of the chick are usually rods, although granules are frequently present also. This is directly opposed to the results of Tschaschin ('10), a student of Rubaschkin, who describes the mitochondria of the 'primordial germ cells' of the chick as exclusively granular.

In this connection the work of Lewis and Robertson ('16) is of interest. In tissue cultures of the testicular follicles of the grasshopper, Chorthippus curtipennis, the mitochondria were observed in the living germ cells in the course of their development. Granular in the primary spermatagonia, the mitochondria become granular threads in the secondary spermatogonia and assume a long thread-like appearance during mitosis. In the growth period of the primary spermatocytes, the mitochondria are again of the granular form. This would show that in this form the shape of the mitochondria is not constant in the germ cells during their development.

In the mouse, the mitochondria in the developing definitive oocytes are almost entirely of the granular type. The cells of the germinal epithelium have a small amount of cytoplasm in which are a few granular mitochondria (fig. 47, upper cell). In the course of the growth of one of these cells as a primary oocyte, the number of mitochondria increases, keeping pace with the development of the cell (fig. 47, lower cell.) The mitochondria are of the granular type until the cell is quite large, in a follicle of cuboidal cells, single-layered or even stratified, when rods and threads begin to appear (figs. 50, 51). Thus it is evident that the granular type of mitochondria prevails in the earlier stages of the developing definitive oocytes.

But in the mouse the mitochondria of the cells of the germinal epithelium are also of the granular form. And further, in the follicle cells surrounding the oocytes, the mitochondria, distributed chiefly in the part of the cells toward the oocyte, are granular, rod-like, or thread-like. In the cells of some follicles granules appear, and in the cells of others rods or threads are found; in the same follicle, some cells may have granules and others rods or threads, and it is not at all rare to find all kinds of mitochondria in the same cell (fig. 52). It is seen, then, that, in the mouse, while the granular type of mitochondria is predominent, perhaps, in the developing definitive oocytes, this is not a distinctive feature, for epithelial cells and follicle cells as well have a similar mitochondrial content.

This might be expected from the work of Lewis and Lewis ('15) on mitochondria in tissue cultures. They find that the mitochondria can be observed in the living cell, unstained, and that these mitochondria are not constant in form, but change their shape repeatedly. Rods or threads may be seen to break up into granules, and granules fuse to form larger granules. Accordingly, one would not expect to find the mitochondria of any one shape constant in or peculiar to any particular kind of cell.

Schaxel ('11) states that the shape of the mitochondria varies according to the method of fixation and staining:—that with the Benda technique, rods or threads predominate, while after

Altmann's or similar methods, granules are found. While I have not made a special study of this point, my results seem to bear out the conclusions of Schaxel; however, although mitochondria of one type or the other may predominate in preparations made by one or the other of these methods, both granules and rods or threads are found in both kinds of preparations. Lewis and Lewis think it quite probable that the mitochondria, 'malleable' or plastic as they appear to be, may have their shape affected by different methods of treatment.

Moreover, it is possible that the mitochondria represent the structural expression of the reducing substances concerned in cellular respiration (Kingsbury, '12). As Lewis and Lewis suggest, it is possible that they are continually being formed and as continually destroyed (oxidized) in the cytoplasm in the course of the metabolic activity of the cell. It is evident that a pronounced change in the metabolism of the egg-cell is correlated with its marked growth in size, from a small cell in the germinal epithelium to a mature ovum. Furthermore, the part played by the follicle cells in the growth of the oocyte must also affect the metabolic activity of those cells. It would seem reasonable, then, to conclude that it is only natural that the mitochondria should not be constant in shape in any one particular kind of cell, but should vary in different cells, or even in the same cell at different times.

In returning now to the question of the post partum proliferation of germ cells, the present discussion will take up only the work of von Winiwarter and Sainmont on the cat in connection with my results in the mouse. From the little these authors have said about this new formation of egg-cells, the process is limited to a brief period of time shortly before sexual maturity (three and one-half to four months). As a result of a multiplication of cells in the germinal epithelium, masses or strands of cells grow down into the tunica albuginea, retaining a connection with the epithelium. In these strands, certain of the cells appear with their nuclei in the 'deutobroque' stage. The germ cells of embryonic origin have largely degenerated by this time, so there is room in the ovary for these new egg-cells which, to-

gether with their follicle cells—which are apparently left over from the first proliferation (v, footnote 3, p. 281)—will make up the definitive cortex. Von Winiwarter and Sainmont do not say so in so many words, but, since they repeat (cf. p. 281 to 283) the assumption made by the senior author in 1900, that cells of a new formation must pass through the nuclear transformations seen in the germ cells of embryonic origin, the inference is that these cells of this post partum proliferation do possess such a nuclear history. The promised later chapter, dealing with this particularly, will doubtless clear up a number of the questions arising from their preliminary account.

In the mouse the period during which egg-cells are formed from the germinal epithelium is prolonged, from birth or shortly after, nearly to sexual maturity, instead of being limited to a small part of that time. The process is much more marked during the first half of this period, and becomes gradually slower until it stops shortly before sexual maturity. Correlated with this prolonging of the period during which germ cells are formed from the germinal epithelium is the entire absence of anything resembling 'cords' or tubular down-growths in the ovary after birth. As has been described above (p. 268 and 269), the germ cells arise singly and make their way individually out of the germinal epithelium into and through the tunica albuginea. It might be considered that the 'tubular' or 'cord-like' down-growths, such as those found in the cat, are here, shallowed out and retarded, reduced to single cells. It is entirely probable that this prolonging of the period, during which definitive oocytes arise from the germinal epithelium, and the 'down-growth' of individual cells, instead of groups of cells, are to be correlated with the small size of the ovary in the mouse. There is not enough room in the organ to contain all the definitive ova, as well as the degenerating primitive oocytes, and, as a result, the process of formation of the former is retarded and prolonged over a long period, and the germ cells arise singly instead of in groups. These definitive egg-cells are added outside the earlier formed primitive oocytes, and room for their growth is provided for by the degeneration

and resorbtion of these latter and their follicles, as well as by the growth of the whole ovary.

From this it is apparent that the potentiality of the germinal epithelium for germ-cell formation lasts for a relatively long time in the mouse. At birth, the cells of the germinal epithelium seem equally capable of developing into oocytes, follicle cells, or epithelial cells, and it is not evident just what the factors are which determine their eventual fate. As the ovary becomes more mature and the cells more differentiated, this potentiality of the cells of the germinal epithelium is lost and after sexual maturity no more egg-cells or follicle cells are derived from the epithelium.

The question of synizesis and synapsis[6] has been given marked attention during the last few years. Whether synizesis represents a real condition in the development of the germ cells (egg or spermatozoon) has been discussed quite thoroughly and will be taken up only briefly here. Reference is made to the works of von Winiwarter and Sainmont ('08), Duesberg ('08), Meves ('07), and others who discuss the question and the literature. Here it will be enough to say that Meves, Duesberg, McClung, and many others consider synizesis an artifact due to faulty fixation. The first two authors, however, admit that there is at a definite period in the development of the germ cells, a tendency, more or less marked in different forms, on the part of the chromatin to contract when brought into contact with the fixative. On the other hand, von Winiwarter and Sainmont, and probably a majority of those who have given the matter consideration, believe that synizesis is a normal stage in the development of the germ cells, of rather marked theoretical importance.

The existence of synapsis or a conjugation of chromatin threads is affirmed and denied; and those who believe it takes place are not agreed on the manner of its occurrence. Union side by side

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[6] There is some confusion in regard to the usage of these terms. Von Winiwarter and Sainmont use 'synapsis' to mean a contraction of the chromatin to one side of the nucleus. The better usage seems to be, however, to restrict this term to the conjugation of the chromatin threads or chromosomes, and to employ 'synizesis' to apply to the stage where the chromatin is contracted in the nucleus, as McClung suggested in 1905. Accordingly, the terms will be used here in this sense.

(parasynapsis) and end to end (telosynapsis) have been described in different forms; and in some of these same forms, union of any sort has been denied. For example, von Winiwarter and Sainmont describe a parallel or side by side conjugation of chromatin threads in the cat, and R. Van der Stricht ('11), while accepting the results of these authors on many points, states that he finds no evidence whatever for such an occurrence.

In the mouse the primitive germ cells, as has been described, pass through in the course of their development, a stage which may be termed synizesis. The contraction is more marked, perhaps, in ovaries whose preservation was not the best, but the condition is present, nevertheless, in well-fixed material. Probably, therefore, its occurrence here is not to be considered an artifact.

In the development of the definitive germ cells, however, which are formed after birth, there is not the slightest indication of synizesis. There is no period in the differentiation of these oocytes when there is the least appearance of a contraction of the chromatin (figs. 24 to 46). In the mouse, synapsis does not occur, either in the development of the primitive germ cells, or in the differentiation of the definitive oocytes. In the former, there is no evidence of a union, side by side or end to end, of the chromatin threads. These bands of chromatin exhibit no parallelism whatever until they begin to split lengthwise in what is termed the diplotene stage, rather late in development. In the definitive oocytes there are no definite, well-defined chromatin threads in the whole course of early development, from the cell in the germinal epithelium to the nearly mature oocyte in its Graafian follicle, and accordingly, there can be no question of a union of chromatin bands at all.

There can be no doubt about the facts in regard to the absence of synizesis and synapsis in the development of the definitive oocytes in the mouse. A careful search was made of the growing egg-cells in the germinal epithelium of ovaries from birth to sexual maturity for just these stages. At first it was thought that they would be encountered, and their absence was doubted. But further study showed convincingly that these stages were

not part of the developmental history of these definitive ova. This agrees with the results Duesberg ('08) reports for the spermatogenesis in the rat; he finds no indication of synapsis. I might say here that the early part of the development of the definitive oocytes in the mouse bears a noticeable resemblance to the growth period of the spermatocytes in the rat, according to Duesberg ('08). Compare his figures 4 to 14 with my figures 28 to 40.

It is evident, then, that there is no general agreement as to the facts of synizesis and synapsis, that is, whether there is or is not a union, side by side or end to end, of chromatin threads during or after a period when the chromatin is more or less contracted in the nucleus. Naturally, then, the interpretations placed on these phenomena do not agree. Three views may be mentioned and briefly discussed here.

The first is the one held, perhaps, by most workers at present. This is that, during synapsis in oocyte or spermatocyte, chromosomes of maternal and paternal origin unite side by side, and that there may occur an interchange of materials during the more or less complete fusion. In one of the maturation divisions following, maternal chromosomes are separated from paternal; thus, since whole chromosomes pass into daughter cells, a reduction in the number of chromosomes is brought about. This is the view of those who hold what have been termed 'ultimate particle' theories of development and inheritance, based on Weismann's theories and the hypothesis of the individuality of the chromosomes. These bodies are made up of elements or particles— 'factors' (which may be ultramicroscopic) —linearly arranged, and derived from each parent. These 'factors' are the 'determiners' of the characters ('unit characters') in the new individual. During synapsis, when maternal chromosomes conjugate with paternal, and later, when these chromosomes separate, there is a segregation and redistribution of these 'factors,' so the mature ovum or each spermatozoon has a set of 'factors' differing from those of oocyte or spermatocyte, the character of which has been determined in all probability by chance. The characters of the new individual are determined by the factors brought in by each germ cell in fertilization. Those who hold

this view have used the behavior of the chromosomes to explain certain modes of inheritance and have constructed complicated theories to account for the facts. It would seem, however, that they have confused the explanation of the phenomena with the teleological significance they have attached thereto.

Another school (Meves, Duesberg, et al.) avoids the difficulty of explanation or interpretation by denying the facts. For them, synizesis is merely a tendency on the part of the chromatin to contract, at a certain period in the development of the germ cells, and the contraction figure is due to imperfect fixation in a faulty technique. Synapsis does not exist; those who describe this condition have confused the seriation of stages, and the apparent conjugation is really a splitting and separation of chromatin threads, a precocious preparation for the following division (first maturation division).

A third view is that attributed to R. Hertwig by von Winiwarter and Sainmont ('08), by Kingsbury and Hirsch ('12) and by Levy ('15), although I have been unable to find the reference where he discusses just this point. This view is that synizesis and synapsis represent a suppressed or abortive mitosis.

According to this view, on the one hand, synizesis represents 'an attempt on the part of' the spermatogonia to divide again—which fails; while on the other hand, the reputed conjugation of chromosomes occurring at about this time is but the imperfect fission and subsequent fusion of daughter chromosomes of such abortive division (Kingsbury and Hirsch, '12).

In discussing the degeneration of secondary spermatogonia in Desmognathus, Kingsbury and Hirsch point out a resemblance between the degeneration stages and synizesis, and make the suggestion that synizesis may represent or be an expression of a "running out of the spermatogonial stock." It may be the checking or terminating of the period of multiplication. Synapsis is apparently absent in Desmognathus.

If synizesis and synapsis represent an abortive mitosis, it would be expected that somatic cells would show similar phenomena under the proper conditions. And, indeed, the conclusions of Marcus ('07) as reported by Popoff ('08) demonstrate this.

In the development of the thymus, Marcus found that the cells, after a period of mitosis, enter on a phase somewhat similar to the growth period of the germ cells, which leads through suppressed or abortive mitosis to degeneration. Marcus found, just before the abortive mitosis, cells in stages which he considered true synizesis. Popoff ('08), in a discussion of this, considers that this finding of synizesis in somatic cells puts an end to the special significance this stage has been given in the development of the germ cells. The significance of synapsis depends on, first, the hypothesis of the individuality of the chromosomes, and, second, on the occurrence of synizesis and synapsis exclusively in the germ cells. Popoff, rejecting the first and considering the second proved not to be true, denies any special significance to synizesis and synapsis.

Metz ('16) finds that in the Diptera, although nothing resembling synizesis is evident, there occurs in the somatic cells a pairing of chromosomes which is very similar to synapsis (conjugation of chromosomes). He states:—

The similarity between the figures in the somatic cells of flies and those in the germ cells of many animals (including flies) makes it seem very probable that essentially the same cause is operative in both cases. If this be true it would seem that in the development of a fly each cell-division is preceeded by an attempt at synapsis. Or, in other words, the tendency to undergo synapsis is so marked as to bring about a close approximation of homologous chromosomes during each cell generation. ('16, p. 255).

In the mouse, synizesis occurs only in the development of the primitive germ cells, all of which degenerate, as has been described above. It may be that, even in this early stage in the development of these cells, degeneration has set in, manifesting itself as yet, however, only in this manner. The splitting of the chromatin threads later (diplotene stage) may be a precocious longitudinal division in preparation for a mitosis which never occurs. This may be due (R. Hertwig, Kingsbury and Hirsch, et al.) to a derangement of the nucleo-cytoplasmic relationship, brought about by a setting in of degenerative processes. Certain of the forces governing this relationship may become inoperative and others abnormally strong so that the "attempt on the part

of the cell" to perform its usual functions results in such conditions as synizesis and, in some forms, of synapsis.

This would explain the occurrence of synapsis in the second proliferation of germ cells in the cat as described by von Winiwarter and Sainmont. In the third proliferation described by them, it is to be inferred that synizesis and synapsis occur, but the disturbance in the 'play of forces' governing the nucleo-cytoplasmic relationship is not great enough to bring about their suppression; the cells after a 'checking,' recover and go through the maturation process. In the case of the somatic cells described by Metz, it might be that the disturbance of this relationship is not great enough to interfere markedly with cell-devision. In the mouse, the 'disturbance in the play of forces' may be greater in the young (embryonic) ovary, perhaps because of its immature condition, and bring about the complete degeneration of the cells, while in the adult or sexually mature mouse this may not be marked enough to manifest itself at all.

The results of Wodsadelek ('16) on the spermatogenesis of the mule may be of interest here. He finds that the course of spermatogenesis is apparently normal up to the beginning of the growth period of the primary spermatocytes, at which time the germ cells begin to degenerate. The author states that there is no evidence of synizesis, but that there is a more or less marked attempt at synapsis or conjugation on the part of a varying number of chromosomes He says:

Cells of this nature, in which a great deal of fusion had apparently taken place, invariably show more or less pronounced indications of decay, and the question arises as to whether this unusual amount of fusion is due to the condition of decay or whether the degeneration sets in because of the unusual amount of fusion. It appears, however, that the great amount of fusion is caused by the existing degenerate condition of the cell in general, for invariably masses of chromatin material bearing no resemblance to normal chromosomes or threads are present in these cells. ('16, p. 20).

In these spermatocytes, the attempt at synapsis is apparently an indication of the degenerate condition of the cells. The more of the chromosomes which 'pair,' the more marked the degenera-

tion. It might not be going too far to conclude that in cases where synizesis and synapsis occur as undoubted facts, the cells are in a more or less marked condition of degeneration. In some cases, such as the primitive germ cells of the mouse, the cells of the second proliferation of the cat (von Winiwarter and Sainmont), etc., the degeneration is so pronounced that the cells never recover. In other cases, for example synizesis and synapsis, as described in the germ cells of an increasingly large number of forms, the degenerate condition is so slight that although it brings about synizesis and, in many instances, synapsis, the cells recover and proceed to maturation.

It was stated in the earlier part of this paper that the egg-cells of the first or embryonic proliferation in the mouse all degenerate and disappear. The evidence for this is both direct and circumstantial. In ovaries of mice, from about seventeen days post partum up to those sexually mature and adult, egg-cells in their follicles may be seen in various stages of degeneration and atresia. Usually the atretic follicles are large, and at first are located near the center (future medulla) of the ovary, but smaller degenerating follicles are in the primitive cortex as well. As stated above, some of these degenerating egg-cells undergo a degenerative fragmentation and may form a first polar body and second polar spindle, and may even break up into fragments, with or without nuclei, so that the whole process resembles parthenogenesis (Kingery, '14). This is evidence, of course, that a large number of these germ cells of embryonic origin degenerate and are resorbed. Furthermore, in ovaries from a few days after birth to sexual maturity there is a peripheral zone of egg-cells in primary follicles made up of a single layer of flattened cells. This zone, which may be from three to six or eight cells deep, is composed of definitive oocytes I, formed from the germinal epithelium after birth and located in and under the tunica albuginea. A study of ovaries between birth and sexual maturity shows that, in the mouse as in other forms, there is formed an over-supply of oocytes, and a vast number of these egg-cells must fail to reach maturity. The development and differentiation of the ovary has had a centrifugal direction all

along, and most probably this is adhered to in this instance. The primitive germ cells, centrally located, are the first to start on their course of degeneration, a process which later involves many of the definitive oocytes, probably beginning with those more deeply situated. This degeneration of definitive egg-cells, which in all likelihood sets in before sexual maturity, is continued through the whole sexual life of the individual, as is, of course, too well known to need emphasis. Since, then, a large number of definitive oocytes degenerate, and since these are situated more superficially than the primitive germ cells, of which an extremely large number certainly degenerate, it is not unreasonable to conclude that all these primitive oocytes degenerate and are resorbed, and that the definitive ova are all of a later, that is, post natal, origin from the germinal epithelium.

SUMMARY AND CONCLUSIONS

1. In the development of the ovary of the mouse there are two proliferations of cells from the germinal epithelium. The first, occurring before birth, gives rise to germ cells, the 'primitive germ cells,' all of which degenerate and are resorbed; the second, extending from birth or a few days after nearly to sexual maturity, forms the definitive ova.

2. The evidence here presented, while not conclusive, indicates the possibility that the rete ovarii is formed by in-growths from the mesonephros.

3. A periovarian capsule (bursa ovarica), which develops as a fold of the peritoneum, encloses the ovary more or less completely. The oviduct penetrates this capsule, which is a closed sac with no evident opening into the peritoneal cavity in about 50 per cent of the cases examined.

4. The follicle cells of the primitive oocytes arise from 'indifferent cells' which, also originating from the germinal epithelium, grow down into, or are left behind in, the ovary along with the germ cells. In the course of development, they come to surround the egg-cells, forming thus the primitive follicles.

5. The follicles of the definitive oocytes arise also from the germinal epithelium. While the egg-cell is developing in the

pithelium, the cells adjacent to it extend up around and down under it, enclosing it while it is still in the epithelium. Thus, when the oocyte is left behind, or under the epithelium in the tunica albuginea, it is surrounded by a primary follicle made up of a few flattened cells.

6. In the mouse the primitive germ cells have a course of development different from that of the definitive oocytes. The former, of the first or embryonic proliferation, undergo synizesis; then they pass through the stages pachytene, diplotene, dictyate or dictyé (of von Winiwarter) and are then overtaken by degeneration, which may be atrophy or a degenerative fragmentation.

The cells formed from the germinal epithelium after birth, the definitive oocytes, have a different history; their chromatin is in the form of an irregular network, clumps of chromatin at the intersections, with from two to five nucleoli in the meshes. With the growth of the oocyte, the chromatin becomes attenuated and stains more faintly until there is the appearance of irregular granules of chromatin connected by very slender chromatin strands and the remnants of the linin reticulum, and one large faintly staining nucleolus with frequently one or two others more deeply stained. This condition persists until the egg is in a mature Graafian follicle, ready for maturation.

7. The evidence from a study of the mouse ovary, while not, perhaps, conclusive on this point, tends to show that the 'primordial germ cells' ('Ureier') probably degenerate, playing no part in the development of the definitive ova.

Oocytes originating from cells of the germinal epithelium by a process of differentiation, the 'primitive germ cells,' develop to a certain point and then degenerate. None of these cells take part in the formation of the definitive ova.

8. The definitive germ cells develop by a process of differentiation from cells of the germinal epithelium. Stages can be observed, transitional between mesothelial cells on the one hand and primary oocytes in Graafian follicles on the other. This differentiation takes place after birth and before sexual maturity, constituting a 'new formation' of germ cells.

9. Mitochondria, which, although granular in the developing definitive oocytes, are also found as rods or threads in the germ cells and frequently appear in all forms in the follicle cells, are not to be considered characteristic of the germ cells. Certainly no one particular form is constant in the germ cells, a condition which is not surprising when one considers the probable nature of the mitochondria and the different methods for demonstrating them.

10. The new formation of germ cells from the germinal epithelium is prolonged and extends from birth or shortly afterwards to approximately sexual maturity. This prolonging of the potentiality of the germinal epithelium for germ cell production and the absence of 'cell-cords' are probably to be correlated with the small size of the ovary of the mouse.

11. Synizesis is found constantly in the development of the primitive oocytes; while in the differentiation of the definitive ova, neither synizesis nor synapsis occur. In the case of the primitive germ cells, which are fated to degenerate, the suggestion is repeated that synizesis may represent a stage in degeneration, wherein the normal relations of nucleus and cytoplasm, and the forces governing them are disturbed. The fact that similar stages have been found in somatic cells militates against the attributing of any special genetic significance to these conditions. '

12. The evidence shows that all the primitive oocytes (embryonic proliferation) develop to a certain extent and then degenerate. This is accomplished by the time the mouse is sexually mature.

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Figures 8 to 12 were drawn in outline with a projection lantern and the details filled in from the specimens. The other figures were all drawn with a camera lucida, at table level. Text figures 1 to 6 were made with the aid of a Zeiss apochromatic objective, 2 mm., 1.4 N. A., and compensating ocular × 8, at a magnification of 1875 diameters; the cuts, reduced to one third, are × 625. Figure 7 was drawn at a magnification of 133 and reduced to one third (× 44). The other figures, with the exception of 51, were made with a Zeiss apochromatic objective, 2 mm., 1.4 N. A., and compensating ocular × 12, at a magnification of 2667 diameters; reduced one fourth off, they are × 2000 in the plates. Figure 51 was drawn with compensating ocular × 6, and is half this magnification.

I wish to express my appreciation of the careful and painstaking work of Mr. R. S. Gutsell who made all my drawings for me.

ABBREVIATIONS

Cu H., Weigert's copper hematoxylin
Fl., Flemming's fluid
Herm., Hermann's fluid
I.H., Heidenhain's iron hematoxylin

P.A.F., Picro-aceto-formol (Bouin's)
p.p., Post-partum
Tri., Flemming's triple stain (safranin, gentian violet, and orange G.)

PLATE 1

EXPLANATION OF FIGURES

8 Transection of ovary of embryo 11 mm. long. The oviduct (Müllerian duct) is at the left in a fold which later forms the perioan capsule. × 67.

9 Transection of ovary of embryo 17 mm. × 67.

10 Transection of ovary of mouse between 9 and 19 hours after birth. The capsule completely surrounds the ovary. × 67.

11 Transection of ovary of mouse 10 days after birth. The capsule was left out of this drawing. × 67.

12 Transection of ovary of mouse 15 days post partum. The center is filled with large follicles containing primitive oocytes. × 33.

13 Transection of ovary of mouse 39 days old—practically sexually mature. The definitive cortex filled with definitive oocytes in their follicles is shown, and the medulla with its blood vessels, stroma, etc. × 33.
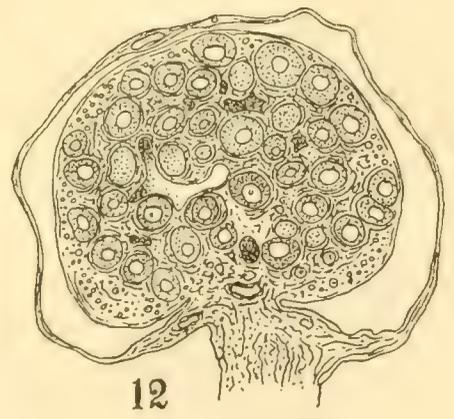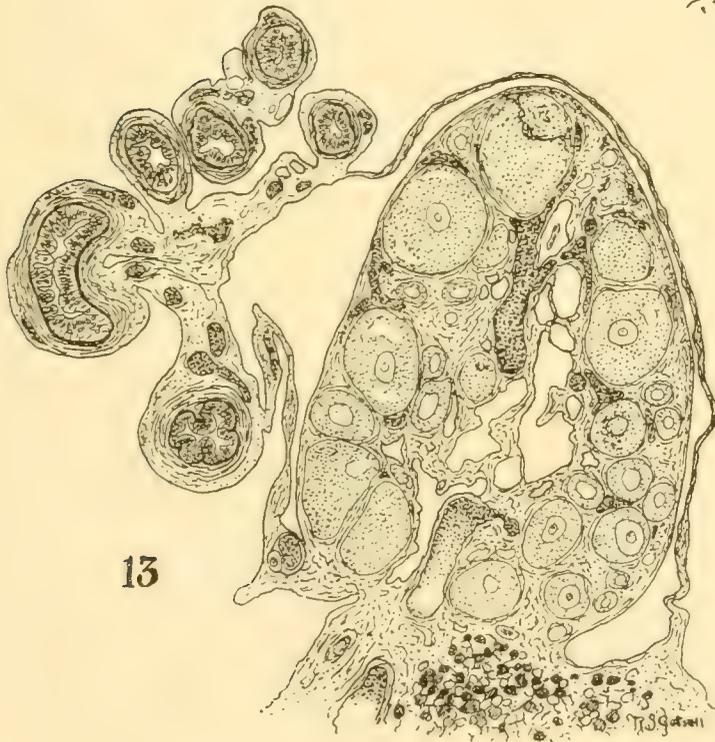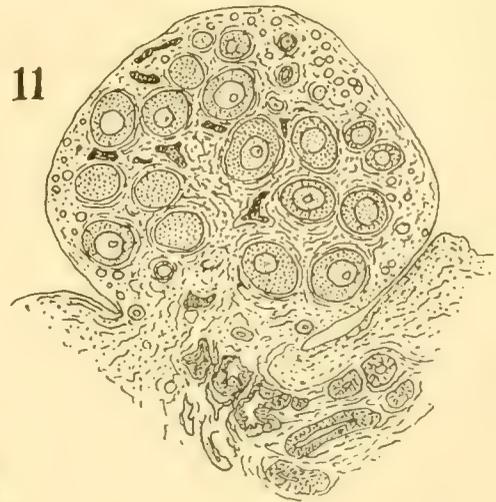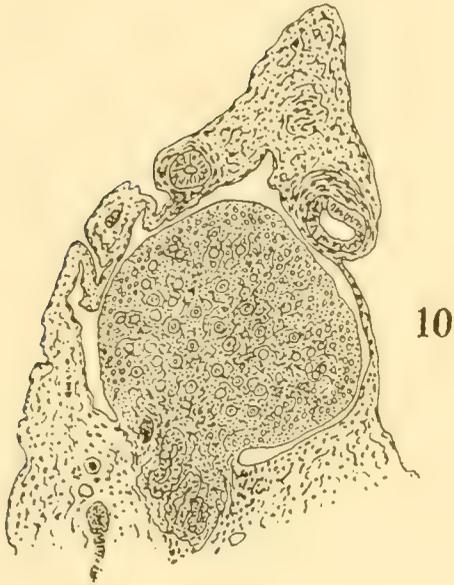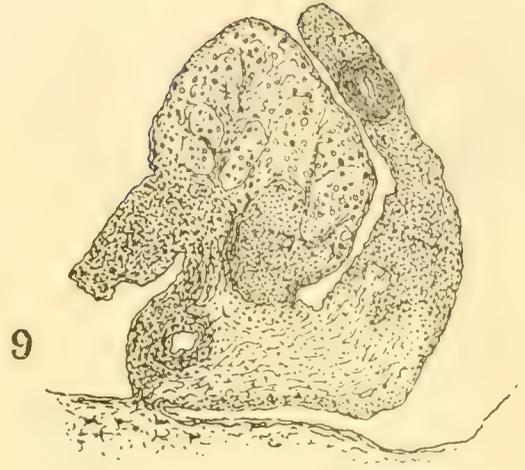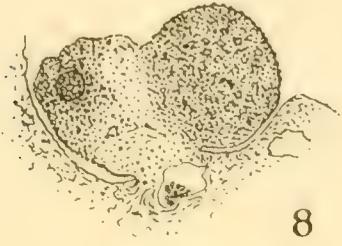
14 Cell of germinal epithelium, 'protobroque.' 16 mm. embryo. *Herm.,* *I.H.*

15 Primitive oocyte in the epithelium. Transition from protobroque to leptotene. Same ovary as 14.

16 Primitive oocyte in leptotene stage. Two nucleoli are visible. 14 mm. embryo. *P.A.F., I.H.*

17 Beginning of synizesis, primitive oocyte. 17 mm. embryo. Carnoy's *I.H.*

18 Synizesis, primitive oocyte. 16 mm. embryo. *Fl., Tri.*

19 Pachytene stage, primitive oocyte. 19 mm. embryo. *Herm., I.H.*

20 Diplotene stage, primitive oocyte. Embryo near term. *Herm., I.H.*

21 Primitive oocyte, transition from diplotene to dictyate (dictyé). Same ovary as figure 20.

22 Dictyate stage. Primitive oocyte in follicle of a single layer of flattened cells. The idiosome is visible. Mouse three days p.p. *Fl., Tri.*

23 Dictyate stage. The nucleus only of a primitive oocyte in a follicle of 4 or more layers of cells, with a cavity (antrum) just forming. Thirteen days p.p. *Fl. Tri.*

24 Cell of the germinal epithelium, resembling 'protobroque' stage. Twenty-eight days p.p. *Fl., Tri.*

25, 26, 27 Cells of the germinal epithelium in mitosis. Mouse eleven days p.p. *P.A.F., I.H.*

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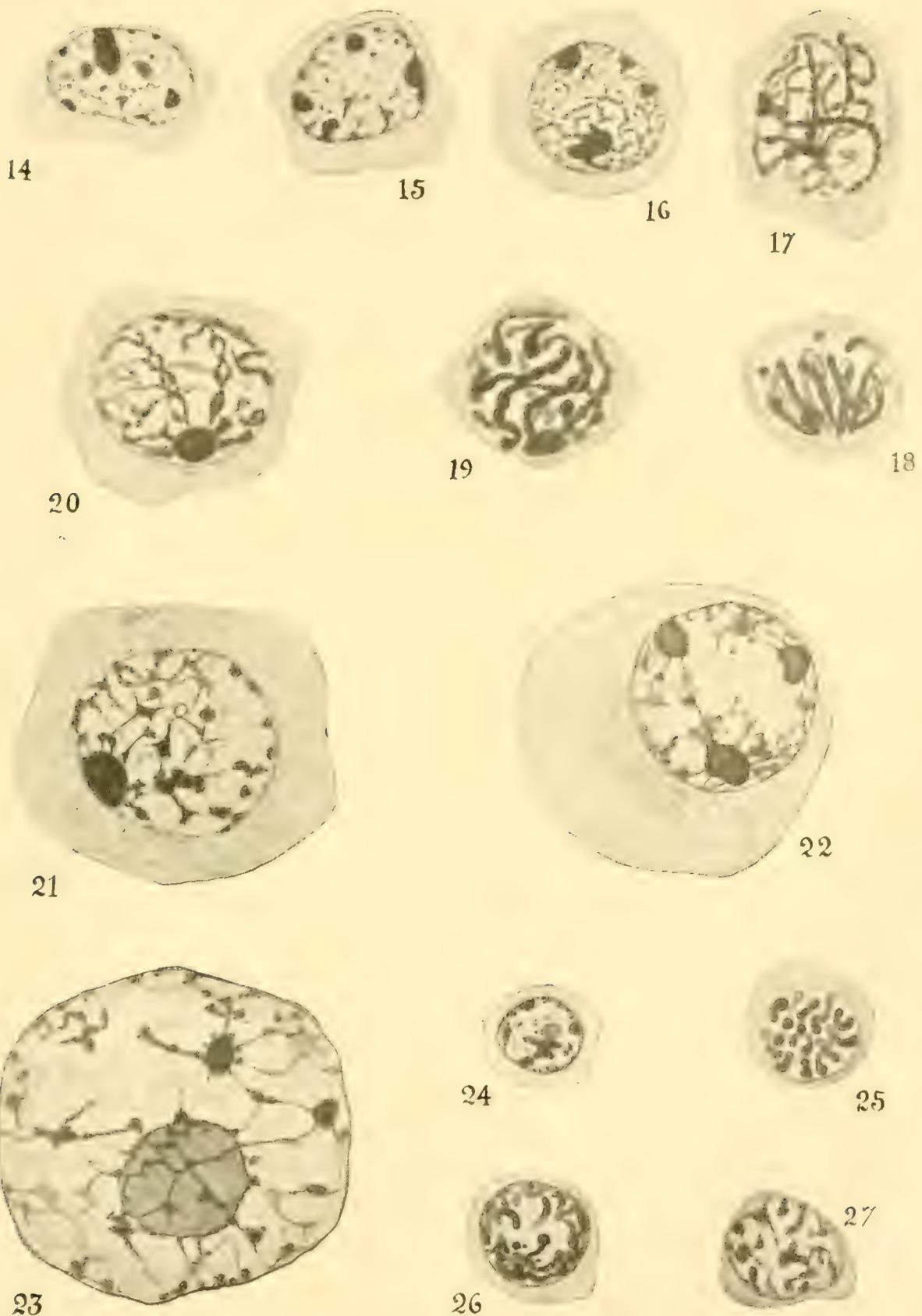
28 Three cells from the germinal epithelium (the free surface is up). 'd' is a definitive oocyte in stage 'a' ('protobroque'?), while the others are undifferentiated epithelial cells. Mouse eleven days p.p. *P.A.F.*, *I.H.*

29 Germinal epithelium. 'd' is a definitive oocyte in stage 'a'; 'e' and 'f' are oocytes in stage 'b'; 'g' is an ordinary epithelial cell, and 'h' is a cell of the tunica albuginea. Same ovary as figure 28.

30 to 33 Stage 'b'. 30 and 33 are from the same ovary as figure 28; 31 is from a mouse twelve days p.p., *Herm.*, *I.H.* and 32 is from a mouse twelve days p.p., *Fl.*, *Tri.*

34 Transition, 'b' to 'c'. Same ovary as figure 28.

35 Transition, 'b' to 'c'. The germinal epithelium has been torn away from the ovary, probably through shrinkage, carrying with it a developing oocyte. Same ovary as figure 28.

36 Definitive oocyte, stage 'c'. Early stage of follicle formation. The free surface of the epithelium is up. Mouse ten days p.p., *Fl.*, *I.H.*
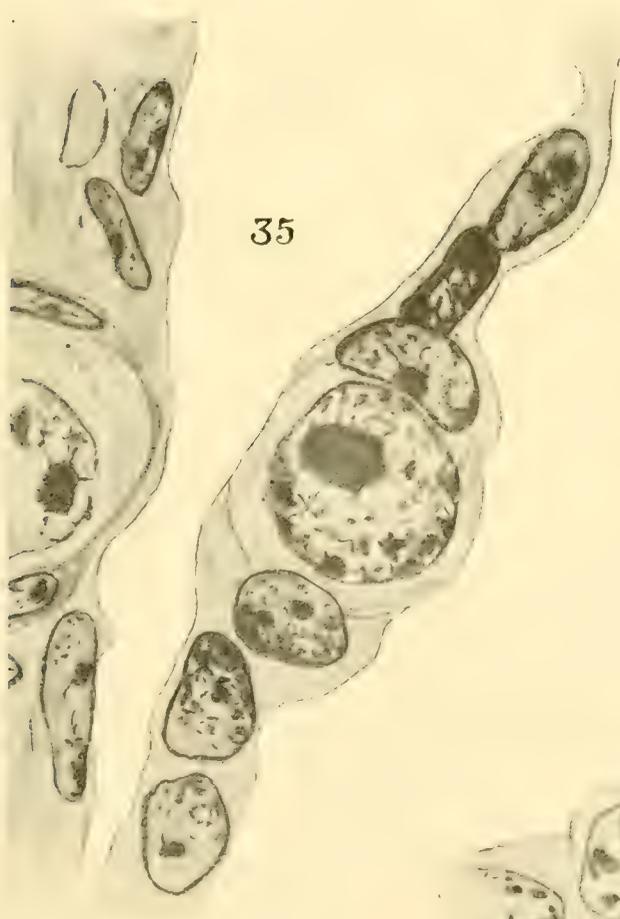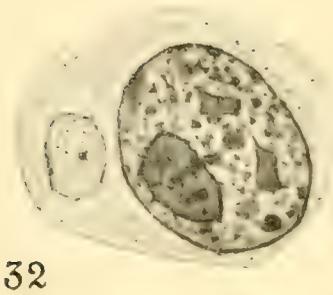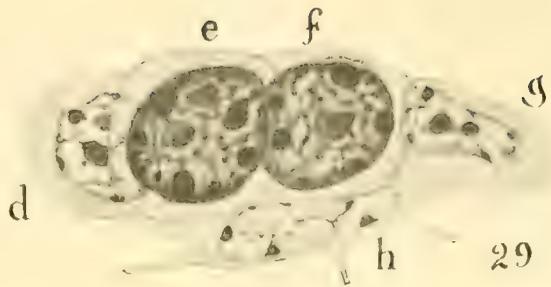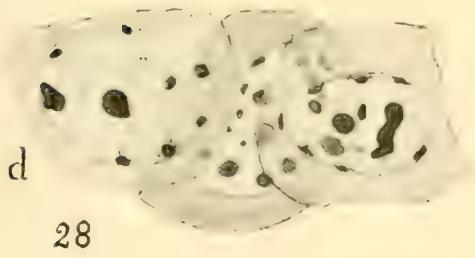
37   Stage 'c'.   This oocyte was surrounded by a follicle of flattened cells and was partly in the tunica albuginea beneath.   Same ovary as figure 28. (Eleven days p.p., *P.A.F.*, *I.H.*)

38   Stage 'c'.   Follicle formation.   The free surface of the epithelium is toward the left.   Same ovary as figure 37.

39   Stage 'c'.   This oocyte was in a follicle of flattened cells, under the epithelium and surrounded by the connective tissue cells of the tunica albuginea.   Same ovary as figure 37.

40   Stage 'c'.   Primary oocyte in follicle just under the germinal epithelium. Mouse twenty-eight days p.p. *Fl.*, *Tri.*

41   Oocyte in primary follicle of flattened cells.   Mouse sexually mature (ninety days p.p.).   Carnoy's, *I.H.*

42   Stage 'c'.   Oocyte in primary follicle of cuboidal cells.   Mouse sexually mature (sixty-seven days p.p.).   *Fl.*, *I.H.*

43   Stage 'c'.   Oocyte in follicle of tall cuboidal cells which are in two layers in places.   Same ovary as figure 42.

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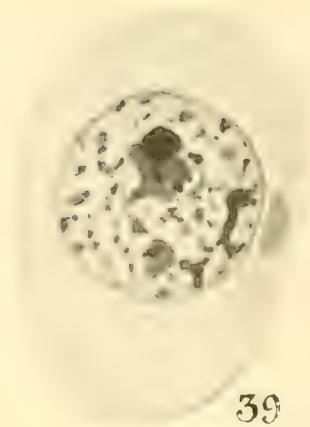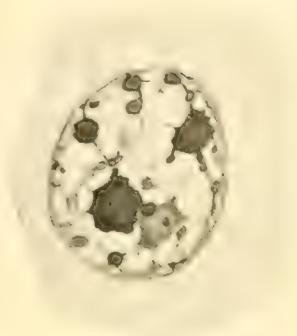
PLATE 5

44 Stage 'c'. Nucleus of oocyte in follicle of 2 to 3 layers of cuboidal cells. Mouse sexually mature (sixty-seven days p.p.). *Fl.*, *I.H.* (Same ovary as figure 42)

45 Stage 'c'. Nucleus of definitive oocyte in follicle of 3 to 5 layers of cells. A slight vacuolation in the follicle indicates the beginning of the formation of a cavity (antrum folliculi). Adult mouse, pregnant. *P.A.F.*, *I.H.*

46 Stage 'c'. Nucleus of definitive oocyte in mature Graafian follicle (this follicle is shown in text figure 7). Adult mouse, *Herm.*, *I.H.*

47 Two cells from the germinal epithelium of an ovary twenty-eight days p.p. The upper cell is an indifferent epithelial cell and the lower is a definitive oocyte in stage 'a'. A mitochondrial technique was used (Benda's fluid, *Cu H*).

48 Definitive oocyte in germinal epithelium, stage 'b'. To show mitochondria. Zenker's + 2 drops of acetic acid; potassium dichromate, fourteen days; *Cu H*. Mouse seventeen days p.p.

49 Oocyte in the germinal epithelium, with cells flattened around each end (figs. 28, 29). Mitochondria and idiosome shown. Same ovary as figure 48.

50 Oocyte in the germinal epithelium, about the same stage as figure 28 or 29. Mitochondria and idiosome are shown. The former are mostly granules, but there is an indication of the formation of granular threads. Mouse eleven days p.p. Same technique as ovary shown in figure 48.

51 Oocyte in follicle of 3 to 4 layers of cells, showing the uniform distribution of the mitochondria. The idiosome is also shown. Mouse twenty-six days old. Zenker's + 2 drops of acetic acid; potassium dichromate, three weeks; *Cu H*. × 1000.

52 Part of an oocyte and adjacent follicle cells. Note that the mitochondria in the egg-cell are all granular, while both granules and threads appear in the follicle cells. Same ovary as figure 47.

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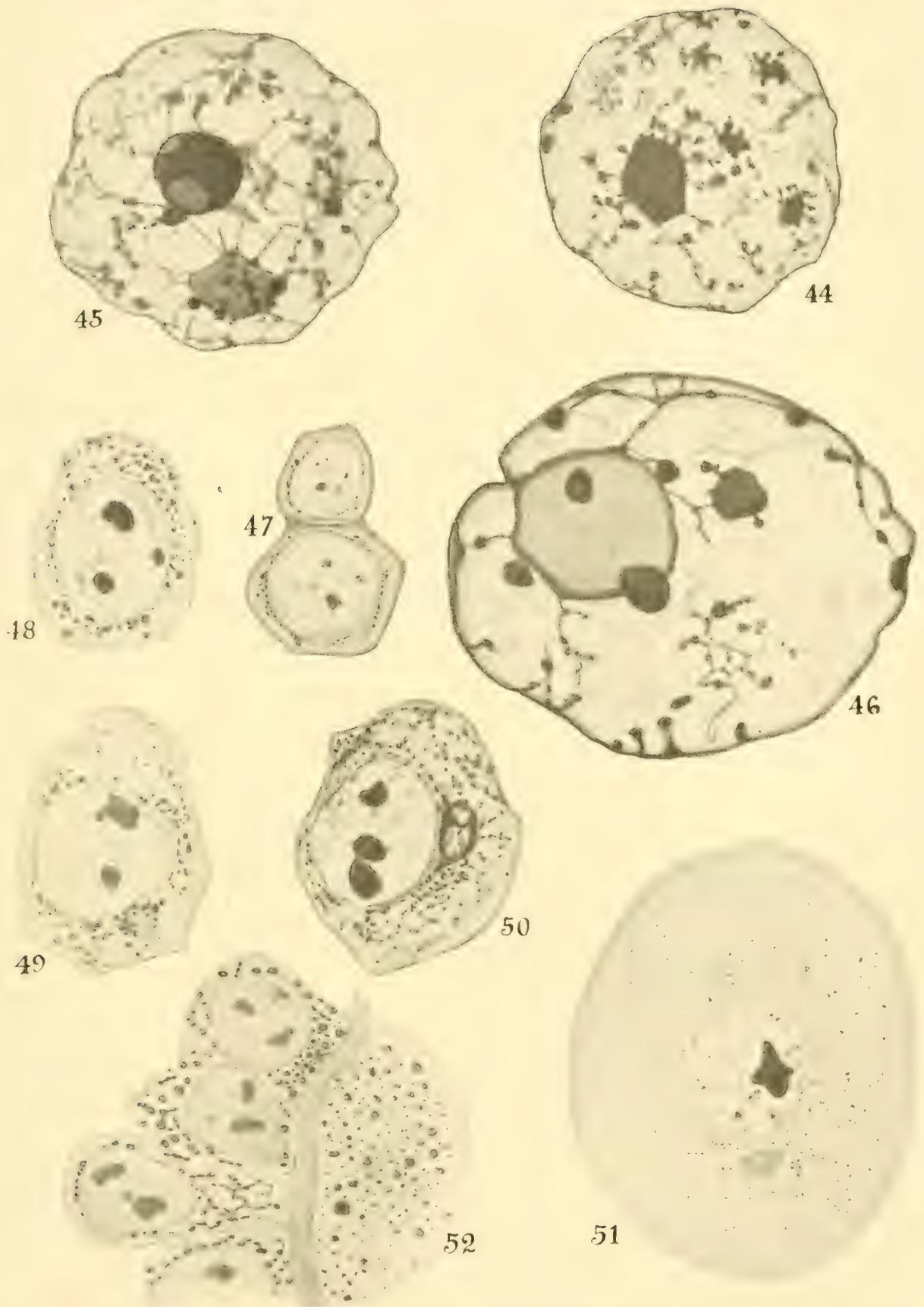
A STUDY OF THE CAUSES AND THE EXTENT OF VARIATIONS IN THE LARVAE OF ARBACIA PUNCTULATA

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ONE HUNDRED AND FIFTY-TWO FIGURES

CONTENTS

INTRODUCTION

The investigation presented in this paper was undertaken
for the purpose of determining the normal development of the
larvae of Arbacia punctulata under standard laboratory condi-
tions, the extent and causes of variation which they exhibit, and
the probable range of variation which they may undergo in
their natural environment.

Much of the information available as to normal development
during the period usually employed in experimental work must
be gathered from control cultures accompanying the various
lines of experimentation. These studies, however, most often
deal with some limited phase of growth, such as fertilization or
cleavage and hence offer but fragmentary accounts of normal
development. It has seemed desirable, therefore, to obtain
more detailed knowledge than is at present recorded as to de-
velopment under laboratory conditions.

To accomplish this, two lines of experimentation were pur-
sued: first, the observation of control cultures raised under
conditions as nearly constant as possible; and second, the modi-
fication of the medium by varying the individual factors which
constitute it. Through the first line of experimentation, it was
hoped, not only to obtain a standard type that might serve as a
basis for future experimental work, but also to determine the
extent and causes of such variations as occur under constant
conditions of the surrounding medium. Through the second line

of experimentation, it was hoped to obtain some data on the specific effects of the various factors in the environment and on the laws governing the reactions of the embryos to these fluctuating conditions. And finally, by determining the limits to which variations could be pushed in the developing embryos, it was hoped to ascertain the probable extent of variations in the larvae of Arbacia punctulata in their natural environment.

LITERATURE

Studies of limited phases of this problem have been made by previous investigators on Arbacia and on other American and European species, mainly Toxopneustes, Strongylocentrotus, Echinus and Spaerechinus.

Tennent ('10) made a study of the eggs and embryos of Toxopneustes variegatus with a view to obtaining more detailed knowledge than had been previously recorded, of the development under laboratory conditions. The variations observed by him "fall into three classes,—fluctuating variations, defects, multiplicities. The fluctuating variations were those of length and correlation," and were determined by the statistical method. The defects consisted in the absence of a skeletal rod or of an arm, or of a malformation of a part of the body; the multiplicities in the presence of more than the normal number of skeletal rods or more than the usual number of arms. The frequent appearance of some characteristic defect or multiplicity among the embryos of a series of cultures in which the eggs or the sperm were all derived from a single individual, offered an opportunity for the study of inheritable variations.

Vernon ('95 and '00) also applied statistical methods as being preeminently suited to a study of variations. In these two papers he attempted to determine not only the range of variations but also their causes: the effect, if any, that changes in environment may produce, and the period in the life of the embryo at which it is most susceptible to modifications in environmental conditions. He established "that Echinoderm larvae vary from the average in respect of size of their various

parts according to the law of frequency error, in the same man-
ner as Galton found to be the case for man . . . ." He
arrived at the conclusion "that the larvae show a considerable
variation in size apart from any influence caused by conditions
of environment. These variations Weismann considers to be
due to actual differences of the germ plasm."

Moreover, he demonstrated that changes in the surrounding
medium increase considerably the extent of variability, and
so, he argued, offer a wider opportunity for the operation of
natural selection. Having determined as accurately as possible
the normal size and variability, he investigated the modifica-
tions consequent upon seasonal changes and such alterations
of the medium as might occur in nature; as, for instance, changes
of concentration of the sea-water, changes of temperature,
changes of amount of oxygen and of carbon dioxide, and changes
produced by addition of uric acid and of urea. As Vernon's
object was primarily to determine the extent of ultimate vari-
ation produced by the different experimental conditions to which
he subjected the ova and embryos, his measurements were taken
at the eighth day of development, the time when the larva of
Strongylocentrotus lividus normally attains its maximum size.
This offered no opportunity for the observation of any gradual
adjustment of the organism to its changed environment or any
progressive lessening of its power of resistance, as could be
detected by measurements at frequent intervals.

One of the earliest investigations of the effects of changes
of the constitution of sea-water upon normal development of
the sea-urchin was made by Pouchet and Cambry ('89). Since
calcium is the element most concerned in formation of the skele-
ton, they concluded that decrease in the relative amount of this
constituent of sea-water must produce some characteristic effect
upon growth; that this effect would not be incurred by the
skeleton alone, because it is an integral part of the tissues of the
animal; and hence that modifications of those tissues from which
it is built must also be involved. They failed in an attempt to
raise larvae in an artificial Ca-free medium, but succeeded in
doing so in sea-water in which as much as nine-tenths of the

calcium was replaced by sodium. They found that the latter medium not only produced lack of skeleton, but also diminished rate of growth, decreased size, and lowered vitality, so that a much smaller percentage reached the gastrula stage than when normal sea-water was employed.

Herbst ('93), investigating the effects of changed composition of the sea-water upon development, concluded that the modifications produced in this manner are brought about as the result, not of chemical but of physical changes, especially of osmotic pressure. He states that the effect of like amounts of different salts decreases with increasing molecular weights of the latter; but this law holds only for the equi-basic salts of one and the same metal. To the salts of different metals, tissues are permeable in varying degrees; that is, the epithelium of the Echinoid larva is almost negligibly permeable to lithium, but somewhat readily to potassium and sodium. He believed "dass die Seeigel larven den Salzen des umgebenden Mediums gegenüber ein gewisses Auswahlvermögen geltend machen and nur solche Stoffe aus dem Meerwasser aufnehmen, welche für ihre Entwicklung notwendig sind."

In a later paper ('95), however, he altered his views in regard to the cause of modifications produced by lithium salts and concluded that the effects could be of a chemical as well as of a physical nature;—"dass die Zellen der Echinidenlarven die Salze des betreffenden Metallen aufnehmen . . . . und zurückhalten."

Still later ('95), he carries further his studies of the effects of changes of the medium, employing various salts of lithium and sodium. The results of these experiments show, he says, "dass man mit sämmtlichen Lithiumsalzen den normalen Entwickelungsgang in derselben Weise würde abändern können, wenn die Säure, an welche das Metall gebunden ist, nicht schädlich auf die Eier resp. Larven wirken würde. An dem Zustand der Zellen jener Blastulae, welche in einer Mischung von Lithium aceticum gestorben sind, sieht man, dass dieselben durch Essigsäure abgetödtet worden sind. Es scheint hiernacht, als würde das organische Lithium-salze innerhalb der Larvenzellen

in freie Säure und Metall gespalten und letzteres vielleicht an das lebende Protoplasma gebunden, während die Säure selbst frei bleibe." The action of the salt will then be specifically that of the metal in those instances where the acid itself is not harmful or where some other substance is present which neutralizes the effect of its anions.

In his succeeding series of articles he deals with three main problems: ('97) what inorganic substances are indispensible in the medium for the normal development of the Sea-urchin; ('01) what rôle does each of these substances perform; and finally ('03), are the chemical processes which accompany embryonic development immovably fixed, or does the developing organism possess the power to regulate its chemical composition, even in a varying medium so that the normal end product will be obtained.

In these investigations he confirms the conclusions arrived at previously, that each substance in the medium produces its specific effect and that the various elements normally present in the sea-water may, in only a few instances and to a limited degree, be replaced by closely allied substances. Throughout his discussions he emphasizes repeatedly the fact that the effects vary in intensity on the individuals derived from the eggs and sperms of different parentage and even on those of the same parentage. Moreover, he shows that eggs obtained in different years and seasons in some instances respond differently to the same treatment. Temperature also plays an important rôle in producing modifications.

Fischel ('09), on account of the important rôle played by the chlorides of potassium, magnesium and calcium in regulating the oxidation processes in artificial parthenogenesis of sea-urchin eggs, investigated the action of these salts upon normally fertilized eggs in order to determine their specific effect in parthenogenesis, and to obtain more detailed knowledge of fertilization itself. He replaced part of the sea-water with iso- or hyper-tonic solutions of the above mentioned salts and concluded that the injuries produced resulted from chemical modifications of the protoplasm. Longer exposure to these altered conditions or briefer subjection to stronger solutions, involved

also fundamental alteration in physical characters,—mainly increased osmotic pressure in the blastocoele, resulting in inflation and death. Fischel believed that these substances, which played so important a part in regulating oxidations in artificial parthenogenesis, were intrinsically harmful, but were controlled by the processes set into action by membrane-formation. He observed, as had the previous investigators, that, while the limits of endurance for the different Echinoids employed were narrow and about the same for all, yet the response to environment was not absolutely identical for all the species nor for all the individuals of the same species.

Hyper- and hypo-tonic solutions were employed by Vernon ('95), who diluted and concentrated the natural sea-water to the limits of endurance, ranging from about 850 cc. diluted to a liter on the one hand, to 1150 cc. concentrated to a liter on the other. He found that the maximum growth was attained in a solution containing 50 cc. of distilled water per liter and resulted in a 15.6 per cent increase in size.

The variations in early development produced by changes of temperature have been the subject of numerous investigations. One of the earliest observations recorded on this subject was that of Herbst ('95). While he did not report any direct effect of temperature on the developing embryos, he believed that the markedly different response to experimental conditions shown by the eggs of two successive years, was due to the difference in temperature of the sea-water of the regions from which the eggs and sperms were obtained.

The effects of temperature and season on development were made the subject of a detailed investigation by Vernon ('95). He concluded that the larvae of Strongylocentrotus lividus "reach their maximum body length when impregnation is performed at about 17°.5 to 21°.5, whilst at temperatures above or below these limits, the larvae become smaller, and that too in greater proportion the more the temperature varies from the favorable limits." Subjection to the altered temperature for one minute during fertilization proved quite as effective as

longer treatment in producing reduction in size, while briefer periods gave proportionally less marked results.

In his later paper ('00) his earlier observations were confirmed, and additional ones on later stages showed that "this degree of reaction diminished in more or less regular proportion from the time of impregnation onwards." So marked were his results that he believed future research would reveal the justification for assuming this to be "a general law of variation" not only for temperature but also for other experimental conditions.

He found that season affected growth in Strongylocentrotus, a result which he ascribed to differences in maturity of the eggs and sperms. During the period in which his experimental work was done, namely, from the beginning of April until the first of October, two maxima were obtained, one early in May and one in September, with a minimum about the middle of August. Impregnations made at this time resulted in 24.9 per cent decrease in size below those made on the ninth day of May. Observations on Spaerechinus supported, in the main, those upon Strongylocentrotus, although in general the former were less modified by changes in the environment.

Driesch ('93), in his Entwicklungsmechanische Studien, gives a brief table showing the more rapid development of the Spaerechinus embryos at increased temperature 31°, as compared with a control culture at 19°. A difference, already apparent at the two-cell stage, gradually increased during the succeeding period until, at the time when the control had begun to show a 'prismform' structure, the others were typical plutei with long arms.

Peter ('05) showed that the cells of Echinus and Spaerechinus, under an increase of 10° in temperature, divided two and one-half times as often,—an acceleration comparable to that of chemical reactions at greater temperature.

Marcus ('06) experimented with Strongylocentrotus lividus and found a decided increase in rate of development in his 'Warmekultur' (22°) over that of the 'Zimmerkultur' (17° to 19°), and a still greater increase above the 'kaltekultur' (9°). In the first the primary mesenchyme cells were approximately

seventy-two in number while in the second they were about forty-five and in the last, twenty-five. But the less numerous underwent a compensating increase in diameter, so that cell size was in inverse ratio to cell number.

Godlewski ('08) confirmed the conclusions of Marcus for Echinus, that "die erhöhte Temperatur, die grössere Konzentration der Salze im Seewasser, der höhere Alkalinitätsgrad des Umgebenden Mediums begünstigen die Häufigkeit der Zellteilungen während der Furchung," so that the embryos which have developed under these conditions contain a greater number of cells than those which have developed in lower temperature or in more dilute sea-water or in a lower degree of alkalinity. But, since during the following period no considerable increase of absolute mass of the nuclear substance of the embryo occurs, and since through the divisions of this cleavage period the nuclear substance is distributed to a greater number of cells, it results that the nuclei of those blastulae which have developed in lower temperature or in lower salt- or hydroxylconcentration, are smaller than those which have been raised under the reverse condition. Consequently the absolute amount of nuclear substance seems to be independent of external factors.

Loeb ('08) determined the temperature-coefficient of the eggs of Strongylocentrotus purpuratus for the time between fertilization and the first cleavage division, and found that it varied from 3.91 for the interval between 3° and 13°, to 1.74 for the interval between 12° and 22°, with an average of 2.86 for each ten degrees of temperature. Peter's average ('05) was 2.15. Loeb's coefficient for the time from the beginning of the first to the beginning of the second division, ranged from 3.33 for the interval between 5° and 15°, to 1.91 for 10° to 20°, with an average of 2.56. He argued that since the temperature-coefficient for the 'Lebensdauer' of the fertilized and unfertilized egg was two for each degree of temperature, "die chemischen Vorgänge welche die Lebensdauer dieser Organismen bedingen, nicht identisch sein können mit den Vorgängen, welche der Entwickelung zugrunde liegen."

Herbst ('06), in his Vererbungsstudien II, as a preliminary to a study of the effects of changes of temperature in hybridized forms, gives a brief summary of the effects of similar changes on the larvae from straight fertilized eggs of Strongylocentrotus, Echinus and Spaerechinus. In Strongylocentrotus, a tendency toward increase in body-length, with arm-length approximately unchanged, gave a slight increase in total size. With Echinus, body-length became less whereas arm-length showed only a slight increase, so that the resulting individuals were relatively smaller. In Spaerechinus, the body-length was also less, but owing to a much greater increase in length of arm-rods the developed plutei were of considerably greater size. In all, he found with increased temperature a tendency toward increased complexity of skeleton, as shown by the number of rods and accessory processes, accompanied in Strongylocentrotus by the beginnings of lattice-formation.

Peter (08) also subjected the eggs of Spaerechinus granularis and Echinus microtuberculatus to such modifications of the medium as were conducive to more rapid growth, such as increased temperature and heightened alkalinity, and found a greater variability, as measured by number of primary mesenchyme cells, correlated with accelerated development. He employed three grades of temperature, a 'Kaltekultur' at about 13° to 16°, a 'Warmekultur' about 23° to 26°, and a 'Zimmerkultur' between the two. In the warmer cultures variability was increased and was accompanied generally but not always by an increase in the mean number of skeleton-producing cells. A similar effect was produced by increasing the alkalinity, whereas acidity resulted in changes similar to those occasioned by lower temperatures. In none of these experiments, however, was rate of growth, he argued, decreased below that probably occurring under natural conditions since even in his 'Kaltekultur' the temperature was somewhat above that of the open sea in the region from which his specimens were taken; and in the acid solutions, although rate of development was decreased, it was not less than that of his 'Kaltekultur.'

Hence he argued that, since variability was decreased with decreased rate of development, the extreme variants occurring in the cultures subjected to this lowered temperature would not lie beyond that of those found in the open sea. But in his more rapidly developing cultures the variability would be increased beyond these limits; consequently the extreme variants in these solutions would lie in either direction beyond the limits of his normal growth-curve,—a fact which he believes of significance in the establishment of species. He discusses the relative variability of individuals of the same parentage as compared with that of the offspring of different parentage, and finds that of the latter wider and more intense.

Loeb ('98) reported the accelerating effect of alkalies and the retarding effect of acids upon the early embryonic and larval development of Arbacia. He ascribed the beneficial action of alkalies to an acceleration and the harmful effect of acids to an inhibition of oxidation processes. This effect was scarcely discernible during the first few hours of development, but by the second and sometimes the third day the larvae subjected to the alkaline solution were noticeably in advance of the controls, while the development of those in the acid sea-water was retarded or completely inhibited.

Herbst ('03), working with a medium of artificial sea-water containing magnesium sulphate and the chlorides of sodium, potassium and calcium, found that a slight degree of alkalinity, which might not pass above or below a certain point, was essential to development. The optimum concentration varied in the different genera, and, upon approaching the limits of endurance, the individual differences in the offspring of the same parents were made more pronounced. In contrast to the results of Loeb with Arbacia, Herbst found that for Spaerechinus and Echinus the sea-water possessed the optimum concentration of hydroxyl and that addition of alkali caused a retarding of development similar to that which occurred when too little was present.

Moore, Roaf and Whitely ('05) emphasized the 'steadying action' of the sea-water upon variations in the concentration

of the hydroxyl and the hydrogen ions on account of the mixed phosphates and carbonates which it contains, whereby addition of acid or alkali brings about a readjustment of the equilibrium instead of a corresponding wide swing in the concentration of the hydrogen and hydroxyl ions. They found that a disturbance of the equilibrium toward the acid side was much more harmful than toward the alkaline, since addition of alkali or of alkaline salts such as di-sodic phosphate in small amounts produced a beneficial effect upon growth, whereas acids or acid-salts were from the start inhibitory in their action. The concentration of the hydrogen and the hydroxyl ions seemed to be the determining factor in development, while the action of the cation entered as a specific factor only in case the hydrogen and hydroxyl concentrations were low, as with the phosphates of the alkalies.

Loeb ('06) placed newly fertilized eggs of Strongylocentrotus lividus in neutral van't Hoff solution and found that none developed beyond the four- to eight-cell stage. Addition of 0.1 cc. N/100 KOH to 50 cc. of the solution allowed a few eggs to reach the blastula stage; addition of 0.2 cc. allowed 60 per cent to reach the gastrula stage and 0.4 and 0.8 cc. N/100 KOH to 50 cc. permitted all to develop into larvae. With greater concentrations of the hydroxyl, the eggs were more or less injured, until in 0.8 cc. N/100 KOH none could segment. He did not, however ('13), believe the rate of development affected, as it was not noticeably raised by the addition of 0.5 cc. or 0.7 cc. N/10 NaOH to a neutral solution of M/2 (NaCl + KCl + CaCl$_2$). The increased rate of oxidations in the presence of excessive amounts (more than 0.8 cc. N/10 NaOH to 50 cc. of the neutral solution) "suffices to suppress the development of the egg" and hence "cannot be utilized for any conclusions upon the normal oxidations in the sea-urchin egg."

METHODS

The experimental work for this paper was done at Woods Hole, Massachusetts, during the summers of 1914 and 1915. In the first year, the experiments were carried on during the latter part of July, all of August and early September, and in the

second summer extended from the twelfth of June until the first of August.

Upon being brought into the laboratory, the sea-urchins were placed in clean glass aquaria with running water, and used as soon as possible, generally within twenty-four hours after being received. Usually the experiments were begun at once. Each sea-urchin was washed, first in fresh water, then in sterilized sea-water, cut horizontally through the test, and inverted over a Syracuse watch-glass. If the eggs were ripe, they were extruded at once through the genital pores, and only those individuals which responded immediately were used. Eggs and sperms were examined under the microscope, and the spermatozoa employed only when they were very active, and the eggs only when nearly one hundred per cent were mature.

The solutions to be used in the experiment were previously prepared and placed in separate beakers, while a similar series of solutions were kept in readiness in a set of watch-glasses. Into each of the former a portion of the eggs was dropped, and into the latter sufficient of the sperm to give a slightly milky suspension. About two drops of each sperm-suspension was then added to its corresponding portion of eggs, and the mixture quickly stirred and allowed to settle. By having everything in readiness and by using a fresh pipette for each solution, the entire operation could be concluded within about five minutes; consequently the eggs and sperm were subjected to the action of the changed medium only a few seconds before being brought together. This was highly desirable, in order to eliminate as far as possible any specific effect of the altered medium upon the unfertilized sex-products.

As soon as the eggs had settled, the supernatant liquid was siphoned off, fresh solution added and the process repeated once every fifteen minutes during the first hour. The eggs, which by this time had usually reached the two-cell stage, were poured into finger-bowls and allowed to stand until they swam at the surface of the water, when they were transferred with as little of the liquid as possible to finger-bowls containing fresh solutions. In the cultures in which the blastulae did

not swim but remained on or near the bottom of the dish the liquid was siphoned off from above as thoroughly as possible, and the blastulae poured into the fresh solutions. Transfers were made at the close of each twenty-four hours of development, so that during the five days of the experiment nine changes were made: four during the first hour, one after about six hours, and after twenty-four, forty-eight, seventy-two and ninety-six hours respectively.

All the sea-water employed was obtained from the harbor at a sufficient distance from shore to insure freedom from shore-contamination, conveyed in glass vessels and filtered before using. During the experiments the finger-bowls were kept covered with glass plates to prevent evaporation and kept standing in the aquarium surrounded by running sea-water. From time to time the temperature of this water was compared with that of the open sea at high tide and about three feet below the surface, and at no time did it show a greater difference than one-half of one degree. The greatest possible cleanliness was observed throughout, every dish and instrument being sterilized each time it was used.

Rate of growth was determined by measurement of the skeleton, as this seemed to afford the most nearly constant character and the one most capable of accurate determination. A micrometer eye-piece, Zeiss no. 4 ocular and objective D were employed. The measurements in this paper are in these micrometer units, each one of which is equivalent to 0.036 mm. Four measurements were made upon each pluteus; the first, from the posterior end of the right body-rod to the end of the right anal arm (fig. a, *A. R.*); the second, from the end of the left body-rod to the end of the left anal arm; the third, from the posterior end of the right body-rod to the end of the right oral arm (text fig. a, *O. R.*); and the fourth, from the end of the left body-rod to the end of the corresponding oral arm. Each measurement thus included an arm and a body length, excepting in the young forms where the skeletons were still represented by the tri-radiate spicules. In this case the lina a–a′ (fig. b) would correspond to the first two measurements given

above, and a–a'' to the last two. In whatever position the pluteus lies some foreshortening must necessarily occur. Vernon ('95) selected measurements taken from the side as most nearly accurate; but on account of a divergence laterally of the arms at the oral end of the pluteus, some error must necessarily occur under this mode of treatment. Moreover, by this method, measurements could be taken only on one side of the animal. Since one of the purposes of the present investigation was to determine any irregularities in the skeleton of each individual, measurements from both sides of the body were desirable. By selecting only those specimens which lay in one definite position,—that is, flat against the slide with anal surface directed upwards, the slight error due to foreshortening was reduced to a minimum.

Figs. a and b

EXPERIMENTAL

The summer egg-production of Arbacia at Woods Hole during 1915 was divided into two periods. The periods of sexual maturity were of longer duration for the males than for the females, as the former developed somewhat in advance of the latter and remained active later. In 1914, an early period began in the latter part of May while the following summer the first mature females were not obtained until about the sixth of June. In 1915, about a week after the first ripe specimens were obtained, practically all were mature. During the next two weeks nearly every female opened possessed ovaries filled with ripe eggs and the males extruded exceedingly active sperm. A gradual decline in the percentage of mature individuals then occurred until the middle of July when scarcely one could be obtained.

A week of almost complete rest followed, succeeded by a period
of sexual maturity, which also showed an early maximum
and a gradual decline in the proportion of ripe individuals.
By the middle of September all the females possessed pale and
exhausted ovaries, while the males were to a large extent active.
The spermatozoa, however, were less vigorous than those obtained early and became inactive in a much shorter time after
extrusion.

Plot 1 gives the general growth curves of the control cultures
taken during the entire period of experimentation. Hours
after fertilization are plotted as absissa, size as ordinate. A
represents the average of four control cultures run during
August and the two weeks of September 1914; B, 24 during the
early part of the following summer; and C, nine during the
latter half of July, 1915. A and C were taken during corresponding periods of egg-production and were arranged thus for
the purpose of comparing the general growth curves of the
two years. D represents the measurements of all the control
cultures for the entire period. E and F are lines drawn through
the extreme variants in the control averages.

Averages of the control cultures of the various periods

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
A..............	4.64	45.34	59.62	68.66	74.21
B..............	19.31	60.77	76.23	80.90	81.54
C..............	8.79	58.81	74.68	78.19	80.60
D..............	15.31	58.25	73.62	78.68	80.34

Before taking up a more detailed consideration of the separate cultures, some general observations may be drawn from
these figures. D, the average of 3600 individuals raised during
widely varying seasons of two successive summers, may be
taken as our standard growth-curve. When the embryo was
24 hours old, the skeleton measured 15.31 and extended nearly
through the body. Figure 1 represents a normal skeleton of
approximately this size. When the normal individual was 48
hours old, the four arms were well developed and the skeleton

had elongated to 58.25, an increase of 42.94. This represents the period of most rapid growth.

During the next 24 hours a retarding in rate of skeletal development took place, and at the end of 72 hours the embryo measured 73.62, an increase of 15.37 or about one-third of that obtained the previous day. During the next 24 hours rate of growth diminished still more, and, at the close of this period, the larva had reached 78.68, an increase of only 5.06, or 6.8 per cent.

At the age of 120 hours the larva had attained its maximum average length, 80.34, which is only 2.1 per cent in excess of
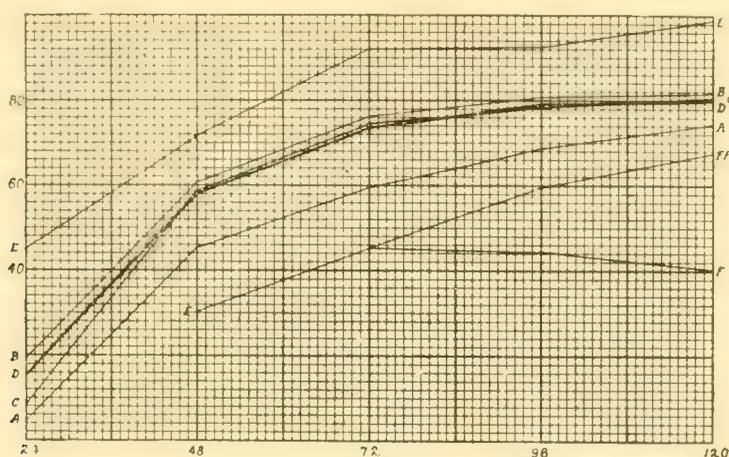
Plot 1

that 24 hours previous. At this time, however, a wide variation prevailed among the larvae of different cultures. Some were just attaining their greatest length while others had already passed this point and had begun to undergo reduction. As resorption of the skeleton took place very rapidly, individuals of this age were found with anal arms almost completely lacking and oral arms scarcely more prominent (fig. 24).

It was evident, not only from observation of the activity of the sperms and of the percentage of mature females, but also from a comparison of the growth-curves of these periods, that the individuals raised from eggs of the early period were much more vigorous. Their development during the first 24 hours was more rapid, and they attained a much greater size. Those obtained during the second period of 1914 were much less

vigorous than those of the early period of 1915. They were
smaller, developed more slowly, and, in the experimental solu-
tions, showed a greater divergence from the normal type. The
same may be said, though to a less degree, of those raised during
the corresponding period of the following summer. The first
few hours of slow development were followed by an interval of
more rapid growth, and at the age of 48 hours they were of
about normal size. Ultimately, however, in all the cultures there
occurred a nearly complete readjustment, and at the age of 120
hours, the averages of the several periods, in contrast to their
variabilities, showed the least deviation from type.

The first series of experiments was undertaken for the purpose
of determining the effects of dilution and concentration of normal
sea-water on the developing embryo.
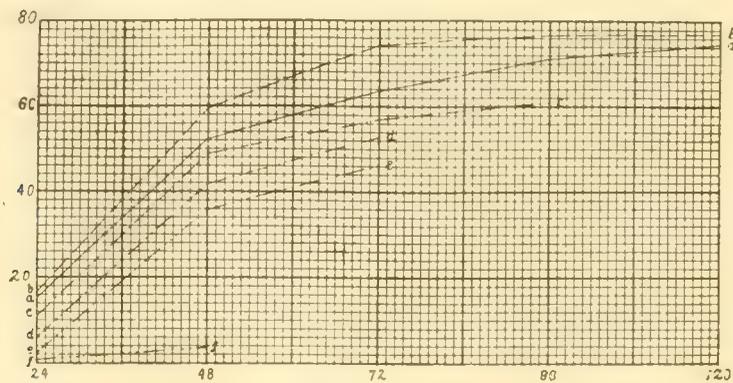
Plot 2

In Experiment 1, the following solutions were employed:

Experiment 1. Plot 2. August 10, 1914

a..............................Sea-water (control)
b..............................94 cc. sea-water + 6 cc. distilled water
c..............................88 cc. sea-water + 12 cc. distilled water
d..............................82 cc. sea-water + 18 cc. distilled water
e..............................76 cc. sea-water + 24 cc. distilled water
f..............................70 cc. sea-water + 30 cc. distilled water
g..............................64 cc. sea-water + 36 cc. distilled water

Tables 1 to 6 give the standard deviation, mean length,
and coefficient of variation for one hundred individuals, four
measurements each, and Plot 2, the growth-curves for the anal
arms. Figures 1 to 22 illustrate one typical individual from
each of the solutions on the various days of the experiment.

TABLE 1

Constants, Experiment 1. Solution a (control)

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
24 hours	Right anal...........	15.50 ± 0.32	4.76 ± 0.22	30.77 ± 1.60
	Left anal...........	15.50 ± 0.32	4.78 ± 0.22	30.89 ± 1.60
	Right oral...........	15.50 ± 0.32	4.76 ± 0.22	30.77 ± 1.60
	Left oral...........	15.50 ± 0.32	4.78 ± 0.22	30.89 ± 1.60
48 hours	Right onal...........	52.28 ± 0.33	4.93 ± 0.23	9.44 ± 0.45
	Left anal...........	52.00 ± 0.34	5.08 ± 0.24	9.78 ± 0.47
	Right oral...........	40.49 ± 0.28	4.17 ± 0.19	10.32 ± 0.49
	Left oral...........	40.58 ± 0.27	4.12 ± 0.19	10.17 ± 0.49
72 hours	Right anal...........	63.78 ± 0.28	4.21 ± 0.20	6.60 ± 0.31
	Left anal...........	63.66 ± 0.28	4.17 ± 0.19	6.56 ± 0.31
	Right oral...........	45.54 ± 0.28	4.27 ± 0.20	9.39 ± 0.45
	Left oral...........	45.58 ± 0.28	4.21 ± 0.20	9.24 ± 0.44
96 hours	Right anal...........	71.25 ± 0.28	4.27 ± 0.20	5.99 ± 0.28
	Left anal...........	71.07 ± 0.29	4.38 ± 0.20	6.16 ± 0.29
	Right oral...........	56.36 ± 0.23	3.43 ± 0.16	6.09 ± 0.29
	Left oral...........	56.47 ± 0.22	3.34 ± 0.15	5.91 ± 0.28
120 hours	Right anal...........	74.72 ± 0.30	4.46 ± 0.21	5.97 ± 0.28
	Left anal...........	74.78 ± 0.29	4.42 ± 0.21	5.92 ± 0.28
	Right oral...........	66.03 ± 0.18	2.71 ± 0.12	4.10 ± 0.19
	Left oral..	66.04 ± 0.18	2.76 ± 0.13	4.18 ± 0.19

A comparison of the growth-curve of the control individuals of this experiment with that of A, Plot 1, shows a much more rapid development in this culture during the early stages than was that of the general average for that period. Compared with the total average of the controls (D), they were of about the same length at the close of the first 24 hours; they then developed more slowly, and, through a proportionately greater growth the fourth day, attained a size 71.25, only slightly less than that of the general average, 78.68.

It may be noted that the specimens in culture *b* were larger throughout the entire period than were those of culture *a;* those of *c, d* and *e* were smaller. In *f,* the skeletons were present at 24 hours only as minute centers which at the close of the next day had not differentiated beyond the tri-radiate

TABLE 2

Constants, Experiment 1.　Solution b

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
24 hours	Right anal	16.51 ± 0.35	5.19 ± 0.24	31.47 ± 1.64
	Left anal	16.59 ± 0.34	5.17 ± 0.24	31.21 ± 1.62
	Right oral	16.51 ± 0.35	5.19 ± 0.24	31.47 ± 1.64
	Left oral	16.59 ± 0.34	5.17 ± 0.24	31.21 ± 1.62
48 hours	Right anal	59.76 ± 0.33	5.00 ± 0.23	8.37 ± 0.40
	Left anal	59.81 ± 0.34	5.06 ± 0.24	8.46 ± 0.40
	Right oral	44.56 ± 0.26	3.86 ± 0.18	8.67 ± 0.41
	Left oral	44.60 ± 0.26	3.90 ± 0.18	8.74 ± 0.42
72 hours	Right anal	74.21 ± 0.29	4.40 ± 0.20	5.93 ± 0.28
	Left anal	74.04 ± 0.29	4.39 ± 0.20	5.93 ± 0.28
	Right oral	52.60 ± 0.26	3.89 ± 0.18	7.41 ± 0.35
	Left oral	52.65 ± 0.26	3.97 ± 0.18	7.54 ± 0.36
96 hours	Right anal	76.85 ± 0.28	4.29 ± 0.20	5.58 ± 0.26
	Left anal	76.85 ± 0.29	4.37 ± 0.20	5.59 ± 0.27
	Right oral	61.81 ± 0.23	3.44 ± 0.16	5.57 ± 0.26
	Left oral	61.73 ± 0.22	3.39 ± 0.16	5.50 ± 0.26
120 hours	Right anal	77.00 ± 0.27	4.13 ± 0.19	5.36 ± 0.25
	Left anal	76.89 ± 0.27	4.09 ± 0.19	5.32 ± 0.25
	Right oral	67.69 ± 0.20	3.08 ± 0.14	4.55 ± 0.21
	Left oral	67.65 ± 0.20	3.08 ± 0.14	4.56 ± 0.21

stage.　In solution *g*, cleavage occurred, though slowly and irregularly, and resulted in somewhat abnormal looking blastulae; but at an age of 24 hours almost all of them had died, while none of those remaining showed evidences of forming skeletons.

The most characteristic modification produced in the skeleton was reduction of the typical structures. This may be seen especially well in figures 18, 19 and 20, representing *a*, *b* and *c* at the age of 96 hours. In *b*, the spines were less prominent, the dorsal body-branches were smaller and the ventral body-branches less complex. In figure 20, all these modifications are more pronounced. In the more dilute solutions a retarded fusion of the transverse rods and of the posterior ends of the body-rods generally occurred, as may be observed in *c* at the age of 96 hours (fig. 20).

TABLE 3

Constants, Experiment 1. Solution c

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
24 hours	Right anal..........	11.05 ± 0.21	3.25 ± 0.15	29.47 ± 1.52
	Left anal.......,...	11.06 ± 0.22	3.27 ± 0.15	29.62 ± 1.53
	Right oral..........	11.05 ± 0.21	3.25 ± 0.15	29.47 ± 1.52
	Left oral...........	11.06 ± 0.22	3.27 ± 0.15	29.62 ± 1.53
48 hours	Right anal.........	48.86 ± 0.33	4.91 ± 0.23	10.05 ± 0.48
	Left anal..........	48.77 ± 0.32	4.87 ± 0.23	9.99 ± 0.48
	Right oral..........	38.08 ± 0.24	3.65 ± 0.17	9.60 ± 0.46
	Left oral..........	38.08 ± 0.24	3.67 ± 0.17	9.64 ± 0.46
72 hours	Right anal..........	57.18 ± 0.26	3.98 ± 0.19	6.97 ± 0.33
	Left anal..........	57.18 ± 0.27	4.07 ± 0.19	7.13 ± 0.34
	Right oral..........	43.74 ± 0.24	3.61 ± 0.17	8.25 ± 0.39
	Left oral.......:....	43.62 ± 0.24	3.64 ± 0.17	8.36 ± 0.40
96 hours	Right anal..........	60.93 ± 0.25	3.76 ± 0.17	6.17 ± 0.29
	Left anal..........	60.86 ± 0.25	3.83 ± 0.18	6.30 ± 0.30
	Right oral..........	49.09 ± 0.24	3.57 ± 0.17	7.28 ± 0.34
	Left oral..........	49.86 ± 0.23	3.50 ± 0.16	7.02 ± 0.33
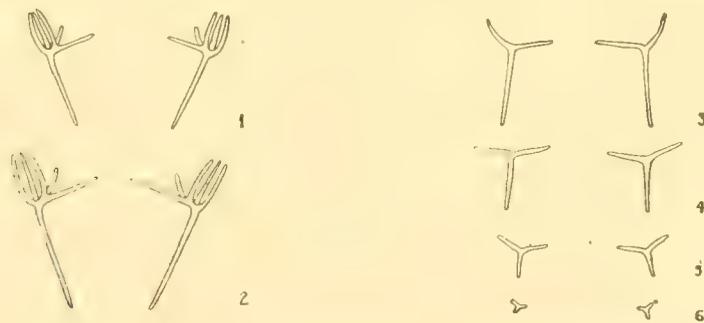
Figs. 1 to 22 Experiment 1. Sea-water diluted with distilled water.
Fig. 1   1a, 24 hrs. Control.
Fig. 2   1b, 24 hrs. Size increased above the control.
Fig. 3   1c, figure 4 1d, figure 5 1e, figure 6 1f, 24 hrs. Gradual inhibition in size, otherwise normal.

The next experiment in this series was made the following summer to determine more closely the limits within which increase of size could be obtained by dilution of the sea-water, and also the effects which increased concentration would produce upon the developing embryo. Moreover, previous ex-

periments performed this second season had shown that the
larvae were more viable and it was hoped to maintain those in
the dilute solutions alive longer in order to determine the results
at the period of their maximum size.
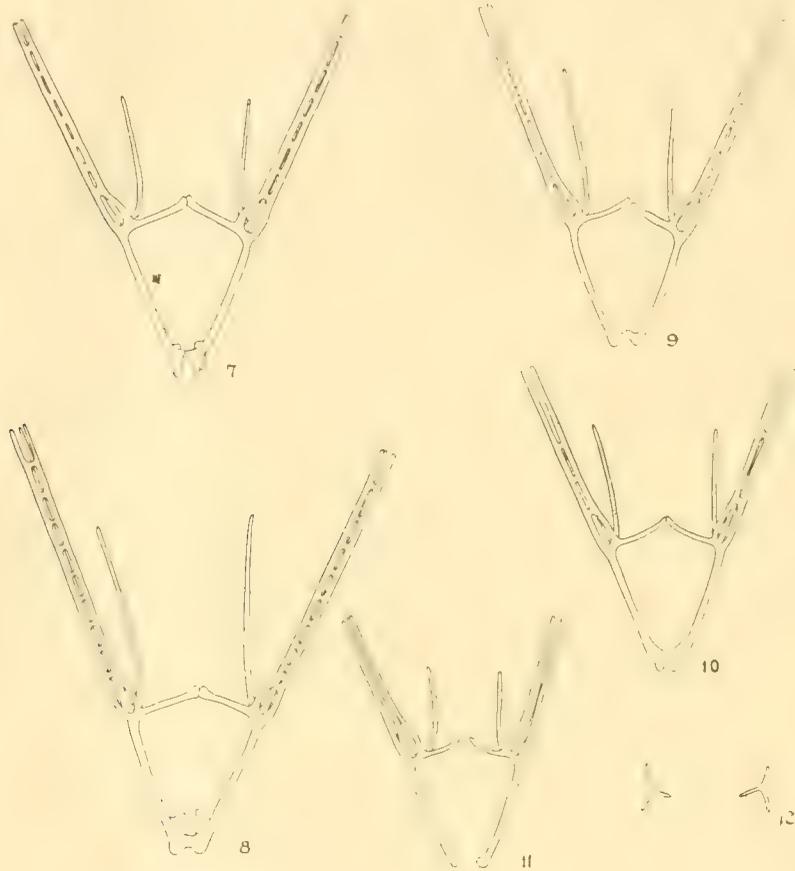
Fig. 7 1*a*, 48 hrs. Control.
Fig. 8 1*b*, 48 hrs. Larger than control.
Fig. 9 1*c*, 48 hrs. Of about the same size as control.
Fig. 10 1*d*, 48 hrs. Somewhat smaller than control and less highly developed.
Fig. 11 1*e*, 48 hrs. Smaller and in less advanced stage of development.
Fig. 12 1*f*, 48 hrs. Has not passed beyond tri-radiate stage.

The following solutions were employed: (S. W. is used in
the following tables to designate normal sea-water. C. S. W.
indicates normal sea-water concentrated to one-half its volume
by continuous heating at 70°C.).
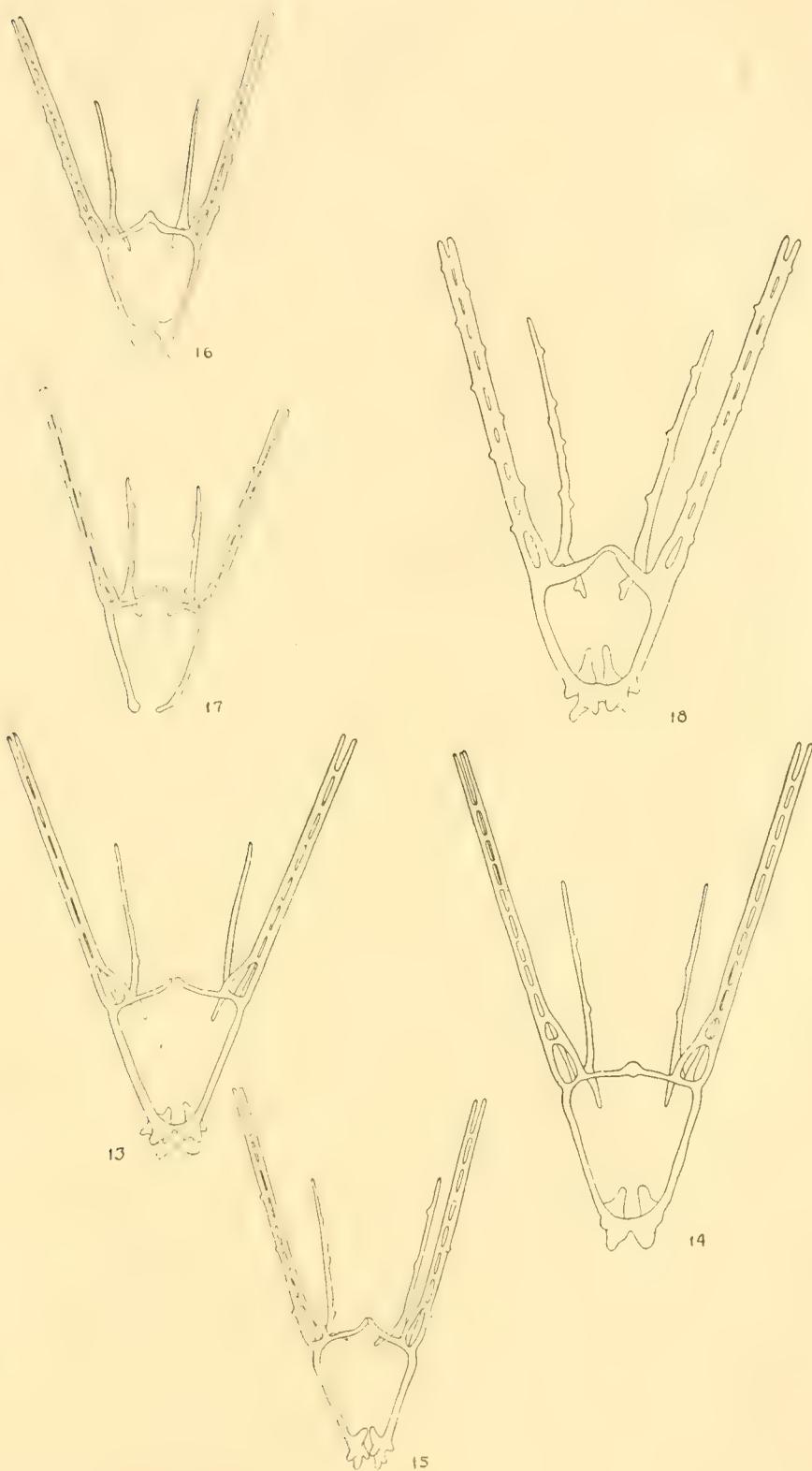
Fig. 13   1a, 72 hrs.   Control.
Fig. 14   1b, 72 hrs.   Larger than control, otherwise normal.
Fig. 15   1c, figure 16 1d, figure 17 1e, 72 hrs.   Gradual inhibition as to size.
Fusion retarded.
Fig. 18   1a, 96 hrs.   Control.

TABLE 4

Constants, Experiment 1.   Solution d

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
24 hours	Right anal..........	5.99 ± 0.16	2.43 ± 0.11	40.58 ± 2.23
	Left anal...........	5.90 ± 0.16	2.40 ± 0.11	40.76 ± 2.19
	Right oral..........	5.99 ± 0.16	2.43 ± 0.11	40.58 ± 2.23
	Left oral...........	5.90 ± 0.16	2.40 ± 0.11	40.58 ± 2.19
48 hours	Right anal..........	41.67 ± 0.32	4.79 ± 0.22	11.50 ± 0.55
	Left anal...........	42.04 ± 0.31	4.68 ± 0.22	11.14 ± 0.53
	Right oral..........	33.59 ± 0.22	3.26 ± 0.15	9.73 ± 0.46
	Left oral...........	33.55 ± 0.22	3.36 ± 0.16	10.03 ± 0.48
72 hours	Right anal..........	52.87 ± 0.27	4.04 ± 0.19	7.64 ± 0.36
	Left anal...........	53.05 ± 0.27	4.14 ± 0.19	7.81 ± 0.37
	Right oral..........	40.91 ± 0.21	3.17 ± 0.15	7.76 ± 0.37
	Left oral...........	40.84 ± 0.21	3.23 ± 0.15	7.90 ± 0.37
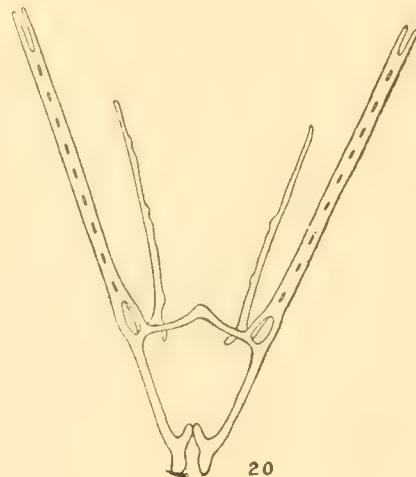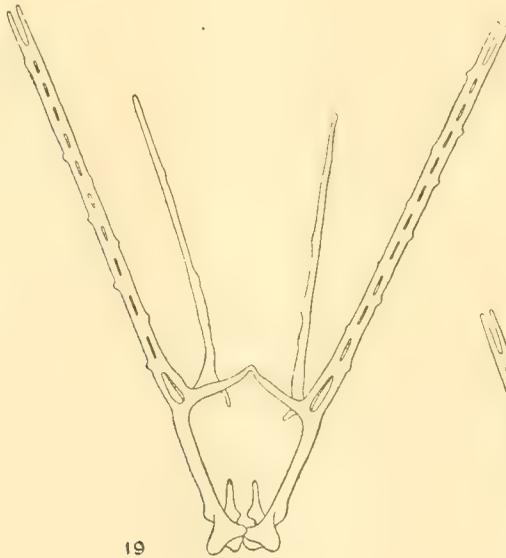
Fig. 19   1*b*, 96 hrs.   Larger than control.

Fig. 20   1*c*, 96 hrs.   Smaller than control.   Body-rods still unfused at the posterior ends.

TABLE 5

Constants, Experiment 1. Solution c

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
24 hours	Right anal	2.35 ± 0.13	1.98 ± 0.09	84.54 ± 6.28
	Left anal	2.35 ± 0.13	1.98 ± 0.09	84.54 ± 6.28
	Right oral	2.51 ± 0.13	2.04 ± 0.09	81.35 ± 5.91
	Left oral	2.51 ± 0.13	2.04 ± 0.09	81.35 ± 5.91
48 hours	Right anal	35.98 ± 0.30	4.55 ± 0.21	12.65 ± 0.61
	Left anal	36.03 ± 0.31	4.60 ± 0.21	12.79 ± 0.61
	Right oral	29.29 ± 0.25	3.71 ± 0.17	12.69 ± 0.61
	Left oral	26.36 ± 0.24	3.65 ± 0.17	12.43 ± 0.60
72 hours	Right anal	46.37 ± 0.21	3.23 ± 0.15	6.98 ± 0.33
	Left anal	46.38 ± 0.21	3.19 ± 0.15	6.89 ± 0.33
	Right oral	35.91 ± 0.18	2.77 ± 0.13	7.71 ± 0.37
	Left oral	33.95 ± 0.18	2.77 ± 0.13	7.73 ± 0.37
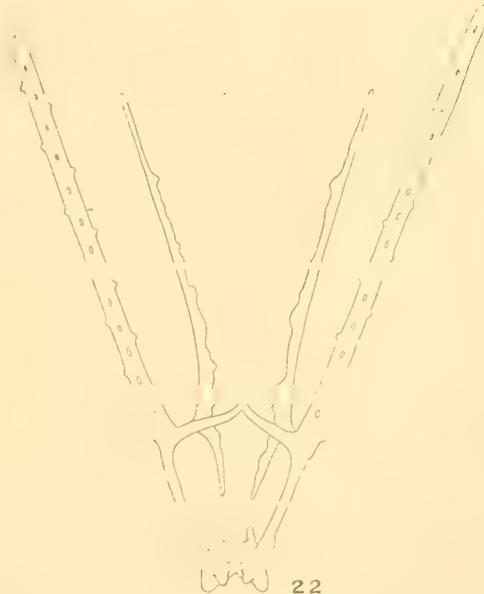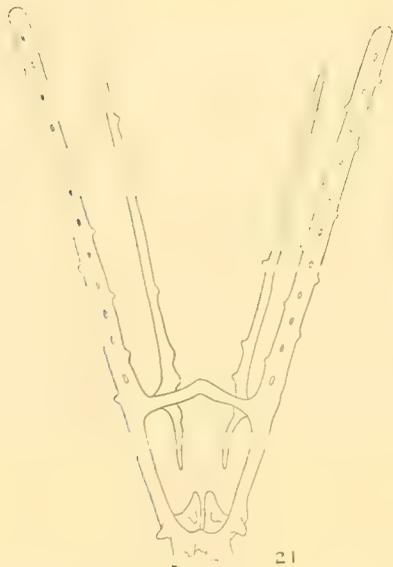
Fig. 21 1a, 120 hrs. Control.

Fig. 22 1b, 120 hrs. Specimen normal in all respects except size, which is increased above that of control.

TABLE 6

Constants, Experiment 1.   Solution f

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
24 hours	Right anal.........	0.82 ± 0.07	1.14 ± 0.05	139.45 ± 14.70
	Left anal.........	0.83 ± 0.07	1.12 ± 0.05	135.35 ± 13.94
	Right oral.........	0.82 ± 0.07	1.14 ± 0.05	139.45 ± 14.70
	Left oral...........	0.83 ± 0.07	1.12 ± 0.05	135.35 ± 13.94
48 hours	Right anal.........	3.86 ± 0.23	3.51 ± 0.16	91.09 ± 7.08
	Left anal...........	3.86 ± 0.23	3.51 ± 0.16	91.09 ± 7.08
	Right oral,.........	4.24 ± 0.24	3.61 ± 0.17	85.37 ± 6.38
	Left oral...........	4.24 ± 0.24	3.61 ± 0.17	85.37 ± 6.38

Experiment 2.   Plots 3 and 4.   June 20, 1915

h............................... 65 cc. S. W. + 35 cc. distilled water
g............................... 70 cc. S. W. + 30 cc. distilled water
f............................... 75 cc. S. W. + 25 cc. distilled water
e............................... 80 cc. S. W. + 20 cc. distilled water
d............................... 85 cc. S. W. + 15 cc. distilled water
c............................... 90 cc. S. W. + 10 cc. distilled water
b............................... 95 cc. S. W. + 5 cc. distilled water
a...............................100 cc. S. W.
b+............................... 95 cc. S. W. + 5 cc. C. S. W.
c+............................... 90 cc. S. W. + 10 cc. C. S. W.
d+............................... 85 cc. S. W. + 15 cc. C. S. W.
e+............................... 80 cc. S. W. + 20 cc. C. S. W.
f+............................... 75 cc. S. W. + 25 cc. C. S. W.

The following measurements were obtained:

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS	144 HOURS	168 HOURS
h...........							
g...........	16.62	32.10	44.78	47.85			
f...........	27.43	63.14	76.13	81.47	82.14		
e...........	32.21	68.82	79.89	83.83	85.81	86.13	84.46
d...........	33.92	71.88	83.03	86.42	88.71	83.21	72.47
c...........	34.87	74.91	87.20	90.03	85.93	75.34	58.31
b...........	35.90	73.15	85.09	88.74	79.11	62.11	44.39
a...........	36.82	70.14	81.95	85.66	71.77	44.63	37.91
b+.........	32.07	66.78	78.10	80.73	68.34	49.27	42.84
c+.........	25.13	62.15	73.91	73.81	85.11	53.48	48.13
d+.........	14.51	53.97	65.95	66.87	61.73	57.36	54.26
e+.........	3.20	43.96	56.09	56.29			
f+.........							

The control specimens, *a*, underwent a rapid early growth which resulted in a size at the age of 24 hours more than twice that of the average. The maximum point on their growth-curve occurred at an age of 96 hours, followed by a steady diminution in size. At the close of 144 hours the arms were shortened to a small fraction of their previous length (fig. 23), and on the following day practically nothing remained of the
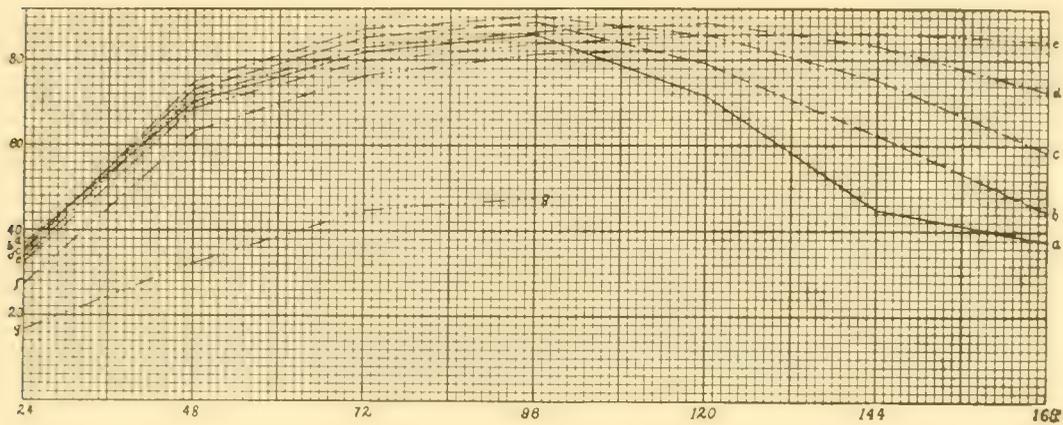
Plot 3
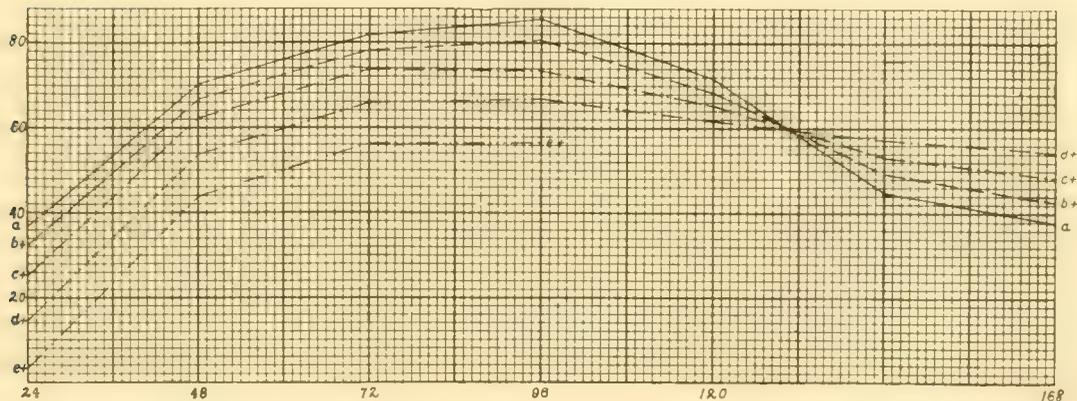
Plot 4

skeleton except the body-rods with greatly reduced ventral branches and dorso-ventral connectives (fig. 25).

In comparing the growth-curves of the specimens in the various solutions, we find that a primary inhibition of growth occurs in the diluted sea-water, increasing in intensity in proportion to the dilution of the medium. A secondary readjustment occurs, however; consequently the specimens in *b*,

c, d and e attain a greater size than those in a, with c largest, b and d somewhat smaller and e about equal to a. The highest points in the growth-curves of the larvae in b and c are reached at 96 hours, coincident in time with that of a. Those in d do not reach their maximum until 120 hours and in e until 144

Figs. 23 to 26 Experiment 2. Sea-water diluted with distilled water.

Fig. 23 2a, 120 hrs. Resorption of the skeleton has commenced. Anal arms are affected more noticeably than oral.

Fig. 24 2a, 168 hrs. Resorption more advanced. Anal arms almost disappeared. Oral arms reduced.

Fig. 25 2a, 168 hrs. Drawn from left side. Resorption still more advanced. Anal and oral arms almost entirely disappeared.

Fig. 26 2d, 96 hrs. Slight irregularities in the skeleton, and retarded fusing of the lateral connective and body-rods.

hours, indicating ultimate retarding of development. Moreover, in these more dilute solutions, the secondary readjustment fails to occur and the specimens in g attain an ultimate size only about one-half as great as those in a. Those in h underwent cleavage but failed to form skeletons.

In the solutions of greater concentration than that of the normal sea-water (Plot 4), there occurred a proportionately greater reduction in size,—an effect which must be looked upon in the light of an inhibiting of growth rather than of a
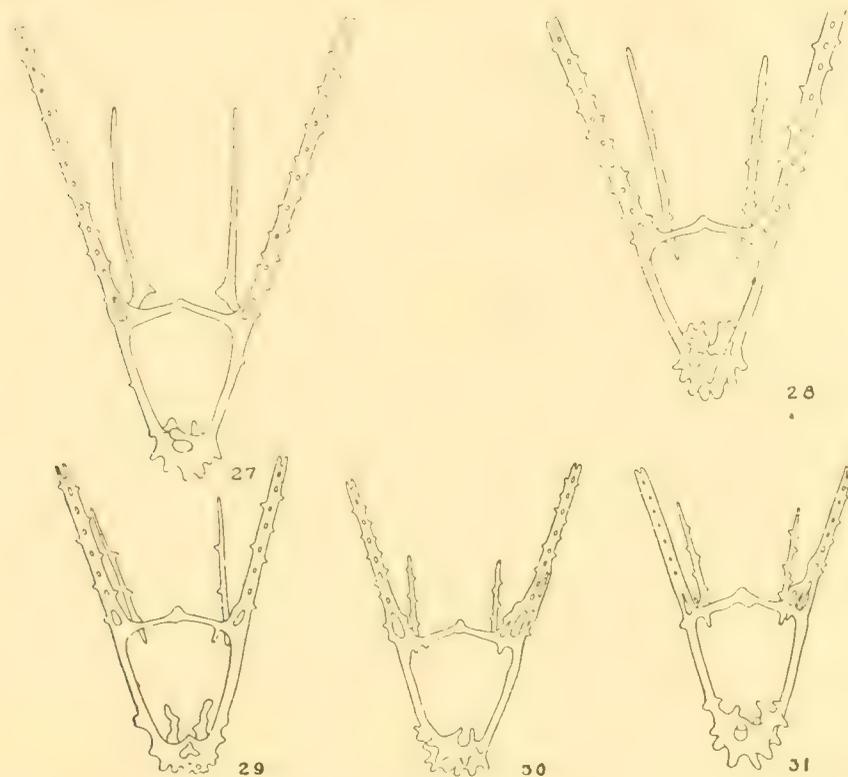
Figs. 27 to 31 Experiment 2. Normal sea-water concentrated.

Fig. 27 2a, figure 28 2b +, figure 29 2c +, figure 30 2d +, figure 31 2e +, 96 hrs. Prominent processes on the posterior parts of the body-rods and the ventral body-branches. Fusing of the lateral connectives with heavy deposit of calcareous material. Irregularity of structure of skeleton.

Fig. 32 Experiment 3. Sea-water gradually concentrated by evaporation.

Fig. 32 3d, 96 hrs. Slender rods as in dilute solutions, but processes more prominent; irregularity of form as in concentrated sea water.

retarding of development, since all reach their maxima at an age of 96 hours. An exception must be made in the case of $c+$, but its diminution is exceedingly slight and the maximum may lie close to the 96 hour point. The decrease in size in a as indicated by its growth-curve is shared to a less extent by $b +$, and still less by $c +$ and $d +$; $e +$ reached its maximum at the

age of 96 hours, but for the last 24 hours growth had practically ceased. During the entire period, however, this culture was well within the range of the control culture, F, plot 1. In $f+$, the eggs underwent cleavage, but did not develop further.

The modifications in the skeleton in these diluted solutions were of the same type as in the preceding experiment,—a reduction in the size and number of processes and a delayed fusion of the lateral connectives and of the body-rods (fig. 26). This tendency toward reduction in skeletal structures seemed to have no correlation with increase or decrease of size, since it was displayed by those larger as well as by those smaller than the control.

Conversely, in the solutions of greater concentrations a tendency toward increased complexity of structure appears, accompanied by the reduction in size described above. Figures 27 to 31 illustrate one individual from the control and one from each of the more concentrated solutions after 96 hours. Figure 26 is taken from d, 15 per cent dilution, on the same day. This increased complexity of structure is shown by the greater prominence of the spines as well as by the greater size and number of the processes on the posterior part of the body-rods. In contrast to the delayed fusion of the individual elements in the diluted solutions, those in the concentrated ones show a tendency, not only toward early fusion, but also toward excessive depositing of calcareous material at the points of contact of the transverse rods.

As the limits of endurance are approached in the more diluted and concentrated solutions, a tendency toward irregularity of structure appears. This is illustrated somewhat in figure 26, where an accessory spine on the right side of the larva near the base of the arm-rods, interrupts the otherwise highly symmetrical condition. It is shown more clearly in the following drawings (figs. 27 to 31), where each increased concentration produces a higher degree of irregularity.

The third experiment in this first series was undertaken for the purpose of observing whether the organism would undergo

less modification if subjected gradually to the changed conditions, and of determining what regulations, if any, would occur in the process of adjustment.

Solutions similar to those used in the preceding experiment were prepared and were let stand in the open air with a control kept closely covered to prevent evaporation. A second control was allowed to evaporate along with the other experimental solutions. The temperature of the sea-water at the beginning of the experiment was 17.25°C. The volume and specific gravity of each solution were taken every day, and at the close of the experiment the estimated specific gravity was checked against the determined.

Five dishes of each of the various solutions were set out together at the beginning of the experiment, and all allowed to evaporate. Each day one of these was utilized to supply the corresponding culture with fresh medium.

Volume of the solutions

CONC.	0 HOURS	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
per cent						
70	200	191.5	184.5	179.0	169.0	160.0
75	200	191.7	184.6	179.1	169.1	160.2
80	200	191.75	184.75	179.1	169.2	160.3
85	200	191.0	184.0	178.7	168.6	159.5
90	200	191.0	184.1	178.75	168.7	159.6
95	200	191.4	184.0	178.6	168.7	159.6
100	200	191.0	184.2	178.9	168.9	159.6
105	200	191.3	184.4	179.1	169.0	160.0
110	200	191.0	184.0	178.5	168.6	159.5
115	200	191.7	184.6	179.1	169.1	160.1
120	200	191.5	184.5	179.0	169.0	160.0
Average........	200	191.35	184.33	178.89	168.9	159.85

Specific gravity (estimated) of the solutions

	0 HOURS	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
g..............	1.01561	1.01631	1.01694	1.01736	1.01848	1.01953
f..............	1.01673	1.01748	1.01846	1.01860	1.01980	1.02092
e..............	1.01784	1.01864	1.01936	1.01984	1.02112	1.02232
d..............	1.01896	1.01981	1.02058	1.02108	1.02244	1.02371
c..............	1.02007	1.02097	1.02178	1.02232	1.02376	1.02510
b..............	1.02119	1.02214	1.02299	1.02356	1.02508	1.02650
aa.............	1.02230	1.02330	1.02420	1.02480	1.02640	1.02790
b+.............	1.02341	1.02447	1.02541	1.02604	1.02772	1.02929
c+.............	1.02453	1.02563	1.02662	1.02728	1.02904	1.03069
d+.............	1.02565	1.02680	1.02782	1.02817	1.03036	1.03208
e+.............	1.02676	1.02797	1.02883	1.02976	1.03168	1.03348

Measurements of larvae. Experiment 3. Plot 5 (diluted sea-water). Plot 6 (concentrated sea-water). June 17, 1915

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
g..............	17.34	39.91	60.10	77.91	91.47
f..............	23.25	48.92	69.97	84.24	94.32
e..............	28.21	56.18	79.81	88.17	95.19
d..............	32.74	68.30	86.10	92.28	95.86
c..............	34.26	69.41	84.77	90.13	92.71
b..............	36.07	66.21	79.69	84.05	84.17
aa.............	37.14	62.39	72.11	74.17	74.75
a..............	38.10	67.83	82.13	88.78	93.25
b+.............	32.12	54.79	64.15	66.76	66.82
c+.............	25.44	46.68	55.32	57.90	58.15
d+.............	13.21	38.84	46.06	47.87	48.09
e+.............	1.03	27.26	33.24	34.02	34.21

In comparing the growth-curve of the control for this experiment with that of our normal, we find a much more rapid early development resulting in a size more than twice that of the average. Constant and gradual growth was maintained during the four following days, so that each point on the growth-curve was considerably in excess of the normal (D, plot 1), and at the close of the experiment the specimens from this control culture measured 93.25, or 12.91 more than the general average 80.34.

The primary inhibiting effect of the various degrees of dilution may be seen by a comparison of the points of their growth-curves (Plot 5) at the 24 hour period. These points fall from 38.10, the size of the control, through 37.14, the average of culture *aa*, started in normal sea-water and allowed to evaporate for 24 hours, and terminate with 17.34 (*g*), started in 30
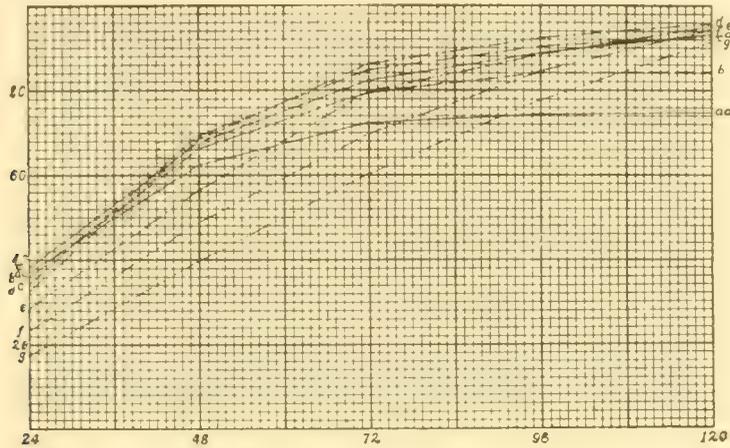
Plot 5
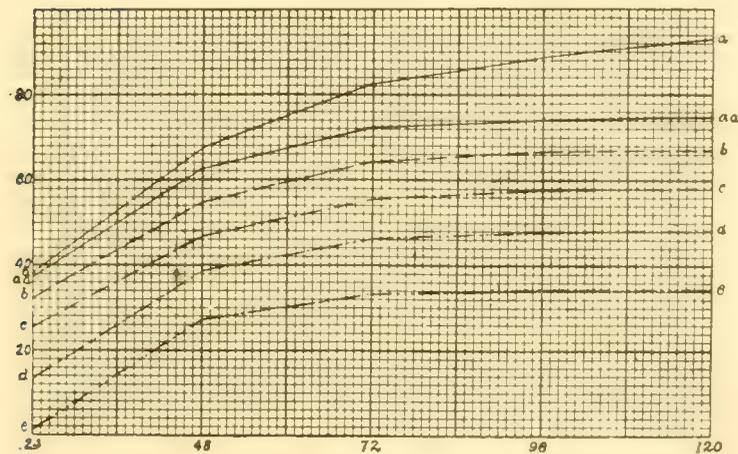
Plot 6

per cent dilution but now evaporated to 95.7 per cent of its original volume.

In the solutions of greater concentration than that of normal sea-water each culture showed a corresponding decrease in size, until in *e* + the skeletons were represented merely by minute tri-radiate spicules of very nearly the same size as

those in the corresponding culture of Experiment 2. Meanwhile, however, the solution had become concentrated by evaporation to a specific gravity of 1.02797, a concentration slightly greater than that of solution $f +$, Experiment 2, specific gravity 1.02788, in which development had not passed beyond the blastula stage.

At the end of 24 hours, all the cultures in the experiment were in the same order, as regards size, as those in Experiment 2 had been at the same age, and showed about the same inhibition of growth, although they were now in sea-water of approximately 4.3 per cent greater concentration.

The specimens in aa averaged slightly less than those in a, and, since these two cultures were started in normal sea-water, this decreased rate of growth cannot be ascribed to any primary inhibition due to sudden changes of osmotic pressure, but must have been due to the gradually increasing concentration. This effect was still more evident on the following days. Inhibition became more pronounced as the solution became more concentrated, until, at the end of 72 hours, a specific gravity of 1.02790 was attained. This was slightly in excess of that of solution h, Experiment 2, in which the eggs failed to develop beyond late cleavage.

In the cultures with diluted sea-water the early effects were similar to those in the corresponding solution of Experiment 2; but, as concentration gradually took place, secondary effects were produced. On the one hand, as those solutions of moderate dilution became evaporated to a density greater than that which Experiment 2 showed to be the optimum for growth, the effects specific to concentration replaced those peculiar to dilution; and the secondary characteristics were more evident the earlier the time of their appearance. On the other hand, in those cultures which were initially diluted more highly than was advantageous for growth, such as e, f and g, there was produced an early inhibition of growth. But as the medium became less injurious, secondary readjustment occurred and growth became more rapid as the concentration approached the ideal range.

In 72 hours, solution *c* had become concentrated to about the same specific gravity as that of *a*. We have then, at this stage two solutions of almost identical concentration, one of which has been maintained constantly in this condition and the other of which has reached it through a process of gradual evaporation; yet in the latter, the size, notwithstanding an early inhibition, has increased materially above the normal. As the concentration increases beyond that of sea-water, rate of growth decreases, and at the age of 120 hours the plutei are 0.5 per cent smaller than the control. We have therefore, at this point, two solutions of different concentration,—the one, that of normal sea-water with a specific gravity of 1.0223, and the other, that of a concentrated solution of a specific gravity of 1.0251, an increase of 12 per cent, in which the larvae, by widely different courses of treatment, have reached the same end. The ultimate effect could, of course, only be speculated upon, but at this point two facts are evident;—first, that we have in two similar solutions individuals of the same parentage, reaching entirely different conditions through unlike treatment; and second, we have the same individuals, through further dissimilar treatment, converging to the same point.

In all these cultures of Experiment 3 the specimens were the offspring of the same parents, but were subjected to such widely different conditions that there was produced an increasingly great primary inhibition of growth in the solutions from *a* to *g*. Those in which this early inhibition was least marked, i.e., those of the least dilution, are exposed at the close of the experiment to the most unfavorable conditions. To what, then, is this early inhibition due? To what extent may early inhibition be overcome by later favorable conditions? In how far may favorable conditions during the first hours of cleavage so benefit the organism that later adverse circumstances fail to produce modification of normal growth? And is an increased size at any one period necessarily indicative of or coincident with acceleration of development?

The primary inhibition could be ascribed to either of two causes,—first, shock attendant upon sudden change of medium

from normal sea-water to one of changed concentration; or second, an influence directly injurious to growth during the earliest stages of development in those solutions which later act most favorably, such as a 10 or 15 per cent dilution. Solution *aa* is one from which any element of shock must have been eliminated. At the close of 24 hours it has a specific gravity of 1.0233, or 4.4 per cent greater than the control. If early inhibition during the first 24 hours were the results of unfavorable conditions alone, we should expect the inhibition in growth in each solution to increase gradually as the concentration becomes greater, but instead we obtain the following results:

Percentages of decrease in size compared with the control

HOURS	aa	b+	c+	d+	e+
24	2.5	15.7	33.2	65.3	97.2
48	8.0	19.2	31.1	42.7	59.8
72	12.2	21.8	32.6	43.9	59.5
96	16.4	24.7	34.7	46.0	61.6
120	19.8	28.3	37.6	48.4	64.3

In the *aa* solution, the inhibition is slight the first day, and increases in intensity as the concentration gradually becomes greater. In solution *b* +, the inhibition at the end of 24 hours is only slightly less than that at the end of 48 hours. In *c* +, the inhibition is greater at the close of 24 hours than at the close of 48, after which it increases in intensity. The same is true of solutions *d* + and *e* + to an even more marked degree, and in the latter the recovery is not complete until the third day.

From these facts we may conclude that there is an early and separate inhibition factor due solely to sudden changes of medium and that subsequent inhibition or acceleration of growth is a secondary factor which may operate independently. In view of this, we may be justified in interpreting the early inhibition produced in the solutions of high dilution, which later cause acceleration in growth rate, as one due to the sudden change of physical conditions and not to a retarding effect

produced directly upon the developing embryo during the periods of blastula and gastrula formation.

Vernon ('95), working upon Strongylocentrotus, concluded that subjection for one minute to a temperature above or below that of the control was quite as effective in producing an ultimate reduction in size as exposure for 1 hour. In his experiments with sea-water of increased and decreased densities ('00), he found that a brief subjection to diluted sea-water (for example, from the time of fertilization until 6 or 12 hours after) produced a decrease in growth rate, whereas continued exposure (1 to 144 hours) brought about an increase, and he suggested that primary reduction in size was due to shock attendant upon transfer from dilute to normal sea-water. No such effects, he believed, resulted from transfer from normal sea-water to dilute; for ova used in the same experiment, which were kept for 5 and for 24 hours respectively in normal sea-water and then transferred to dilute sea-water for the remainder of the period, resulted in a 2.6 and 2.8 per cent increase of size. But it seems probable that there may have been a 'shock' quite as great in the latter instance as in the former. In the first experiment, however, when he subjected the larvae for the briefer periods to the dilute sea-water, they were removed from the more favorable medium before the effects of the primary inhibition could be completely overcome. But when they were left for a longer time (such as, 144 or 192 hours) in this medium, the effects of the more dilute solution had sufficient time in which to compensate for both transfers and an ultimate increase in size was induced. The same explanation could be offered for the second experiment, when the larvae were kept for the first few hours in normal sea-water and then transferred to a more dilute medium. The ultimate effect of this later long subjection to more favorable conditions was, as we should expect, a growth more than sufficient to compensate for the shock produced by short exposure to the more concentrated medium. Had this subjection been of longer duration and treatment with dilute medium briefer, he might have obtained larvae of normal or even of reduced size.

Herbst ('06), investigating the effect of increased temperature, found an increase in the number of roots of the multiple arm-rods and in the number with latticed structure. If they were removed from the warmer medium before the time of mesenchyme formation, the simple rods characteristic of Strongylocentrotus prevailed. If they were removed at the gastrula stage, multiple-formation was inhibited to a greater degree than when they were transferred at the pluteus stage.

This leads directly to the consideration of our second question: "To what extent may early inhibition be overcome by later favorable conditions?" In Experiment 3, cultures f and g, we have examples of early treatment with a medium so adverse that, under continuous subjection, life could scarcely be maintained (compare with f and g, Experiment 2), followed by a later more favorable medium within the range of optimum concentration. At the close of the experiment the larvae approximate so closely the control that they may scarcely be termed subnormal. As far as size is concerned, we have a complete compensation for early inhibition; but the ultimate growth attained is not equal to that which would have occurred had the larvae been subjected during the entire period to a concentration equal to that of these solutions at the close of the experiment, nor so slight as that which would have been produced by continued subjection to solutions of the original concentrations. This tends to confirm Vernon's conclusion that "the range of the harmful effect of an adverse condition is always greater than the succeeding influence of a favorable one. The larvae never attain the maximum size to which they can develop, nor the minimum at which they can maintain life."

Our third question, "In how far may favorable conditions during the first few hours of cleavage so benefit the organism that the later adverse circumstances fail to produce ultimate modification of growth," is more difficult to answer, since, as the data given above show, any sudden change at the time of impregnation has a deleterious effect upon the organism. If the transfer be to a medium intrinsically beneficial, and the subjection be of sufficiently long duration, the injury may be only temporary.

In solution c, Experiment 3, we have a medium that, if used continuously, would produce maximum growth; but in this experiment it has become, at the close of the fifth day, concentrated to a strength which if employed for the entire period, would have caused a decided decrease in size. At the close of the experiment the larvae are slightly smaller than the control. Although this decrease is almost negligible, it is evident that early exposure has not been so beneficial to the organism as to produce an ultimate increase in size. This becomes the more significant when we consider that the latter period is of shorter duration,—two days as compared with three days of more favorable conditions. In solution d, also, at first the dilution is greater than is most advantageous for development; at the close of 24 hours, evaporation has brought the solution to within the range of the optimum, yet the one day of unfavorable medium reduces the percentage of excess of size to a figure considerably closer to the normal. It seems conclusive, then, that later conditions of an adverse nature, although briefly employed, may undo the effects of an early beneficial medium; but this later force has not been able to injure the organism to such an extent that it approaches on either side the limits of growth.

One further question raised by Experiment 2 remains to be considered. Is a size greater than the normal indicative of more advanced development, or is it really beneficial to the organism? Resorption of the skeleton at this period of the life history of Arbacia is an abnormal process due to inanition of the larvae raised under these experimental conditions, and hence would probably be incurred first by those which have been most vigorous and have developed most rapidly. If rate of resorption of the skeleton be taken then, as an index of development, it follows from the facts shown in Plot 3 that the individuals of those cultures which have produced increase of size above the control, are not really in a more advanced stage of development. Thus, although the specimens of d and e attain a greater maximum than those of a, they reach it later, indicating an inhibition of development rather than an acceleration. In other words, we cannot conclude that *any* condition which

may be productive of a size greater than the normal is necessarily the optimum for development.

Just as increased growth is not necessarily correlated with accelerated development, so decreased size need not be associated with abnormality of structure. In the solutions of Experiment 3 in which a size above the normal is attained, there are discernible some effects of previous adverse conditions. An example of this is given in figure 32, which represents a specimen from solution *d*, Experiment 3, 96 hours. Although this individual,—one of the extreme variants in the solution, attained a size of 103, and its measurements taken on the two sides would indicate a perfect symmetry, slight irregularities, such as the presence of an accessory rod at the base of the right arm and a bowing of the left body-rod suggest some abnormalities of development. This skeleton, moreover, lacks the distinctive marks of those associated with growth in dilute solutions, that is, general simplicity of structure. The spines are about as numerous as under normal conditions, the ventral body-branches are possibly even more highly-developed and the point of fusion of the transverse rods is somewhat prominent. All these suggest some influence of the concentrated condition of the solution and suggest that a change of medium, even when the larva is approaching its maximum size, may produce some effect upon the skeleton.

Vernon ('95) gives a graph (fig. 5, loc. cit.) of the effect of salinity on growth of Strongylocentrotus. He found "that the larvae reached their maximum growth in a solution containing 50 cub. centims. of distilled water per liter, where they are 15.6 per cent larger than those developed in water of normal condition. With greater dilution than this they steadily decrease in size again, till in a solution containing 150 cub. centims. of distilled water per liter, they are on an average 4.3 per cent smaller than the normal. . . . . When the sol. contains only 25 cub. centims. of distilled water per liter, the larvae are 9.5 per cent larger than the normal, and when only 12.5 cub. centims., 5 per cent larger."

Vernon's measurements correspond to body-length alone, but since he also determined the arm-lengths and found them practically unaffected by alterations of medium, the general shape of his growth-curve, if not his actual percentages, may be used for comparisons.

Vernon found in Strongylocentrotus that maximum growth occurred at 950 cc. sea-water diluted to one liter; with Arbacia it lies at about 900. In concentrations of less density than this, the general growth-curve falls off somewhat more rapidly in Strongylocentrotus than in Arbacia. In the former at about 875 cc. sea-water to the liter the growth rate is approximately equal to that in normal, while the same is true with Arbacia at about 825. Vernon was unable to secure development in water of greater dilution than 150 cc. distilled water to the liter, whereas Arbacia under similar treatment reaches a size above the normal. Upon further dilution of the sea-water, Arbacia larvae show a steady decrease in size until 750 cc. per liter is reached, when, as the limit of endurance is approached, a sudden drop occurs. A somewhat similar curve is produced in sea-water of greater concentration, though with a less abrupt fall. Vernon, however, figures a very slight decline, so that as a concentration of 1150 cc. to the liter is approached, a point beyond which growth is impossible, the decrease in size is exceedingly slight.

In view of the great variation in growth produced by slight modifications in the concentration, Vernon suggests that the variation known to exist in the concentration of sea-water must have some influence upon ths size of the larvae. Garrey ('15) gives a table of the freezing point depressions of ocean water in various localities, ranging from $\triangle = 1.093$ (Dakin) at Kiel harbor, to $\triangle = 2.29$ (Bottazi) at Naples. At Woods Hole the concentration varies considerably in different localities and under different conditions such as tides and rain-falls, as shown by Garrey in an earlier paper ('04). Here he also gives a table of freezing point depressions of water from various localities in that vicinity which shows variations in $\triangle$ ranging form $\triangle = 1.78$ in the laboratory tap water after one extremely heavy rain, to

$\triangle$ = 1.84, his maximum value, obtained from water of the Basin
of the United States Fisheries Laboratory. Garrey gives his
average freezing point depression at Woods Hole at $\triangle$ = 1.81,
which he says is equivalent to a specific gravity of 1.02426. His
variation in concentration, then from $\triangle$ = 1.78 to $\triangle$ = 1.85
would range from 1.02385 to 1.02479 at 31.5°C. Taking 1.02426
therefore as the normal concentration of the sea-water in that
region, we would obtain a range of variation of about 5 per
cent in Arbacia larvae between these two extremes.

The second series of experiments dealt with the effects of
acidity and alkalinity, and for this purpose the following solutions
were employed:

Experiment 4, 5...............................Sea-water + CH_3COOH
Experiment 6, 7, 13...........................Sea-water + $NaOH$
Experiment 8, 9, 10...........................Sea-water + $NaHCO_3$
Experiment 11, 12.............................Sea-water + Na_2CO_3

The Na_2CO_3, being technically a salt, grades over into the
next series of experiments, but its alkaline reaction, due to
the fact that it is the salt of a strong base with a weak acid,
relates it more closely in its physiological effects to the other
experiments of this series.

Two experiments were performed with the acid solutions.
The first was begun September 8, 1914. This was the last
experiment of that season, and mature females were exceed-
ingly difficult to obtain. Moreover, the death-rate, even in
the control cultures, was so high that the eggs did not lend
themselves well to experimental conditions. The data obtained
are given, however, as a basis for comparison with similar
experiments under more favorable circumstances.

The solutions employed were:

Experiment 4. Plot 7. September 8, 1914

a...........................Sea-water
b...........................99.75 cc. S. W. + 0.25 cc. N/10 Acetic acid
c...........................99.50 cc. S. W. + 0.50 cc. N/10 Acetic acid
d...........................99.25 cc. S. W. + 0.75 cc. N/10 Acetic acid
e...........................99.0 cc. S. W. + 1.0 cc. N/10 Acetic acid

The corresponding measurements were:

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a...............	7.73	56.88	66.33	71.57	76.77
b...............		12.83	32.09	43.85	50.61
c...............		2.36	18.71	34.86	
d...............		0.61	8.85	29.34	

On June 15 of the succeeding year the second experiment was begun and solutions of the following composition employed:

Experiment 5. Plot 8. June 15, 1915

a.......................Sea-water
b......................99.75 cc. S. W. + 0.25 cc. N/10 Acetic acid
c......................99.50 cc. S. W. + 0.50 cc. N/10 Acetic acid
d......................99.25 cc. S. W. + 0.75 cc. N/10 Acetic acid
e......................99.0 cc. S. W. + 1.00 cc. N/10 Acetic acid
f......................98.75 cc. S. W. + 1.25 cc. N/10 Acetic acid
g......................98.50 cc. S. W. + 1.50 cc. N/10 Acetic acid

The corresponding measurements were:

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a...............	5.92	56.27	74.13	88.73	98.95
b...............	5.74	53.29	71.88	83.93	93.20
c...............	5.34	49.86	68.84	80.12	86.14
d...............	4.67	44.71	63.79	70.30	73.32
e...............	3.62	37.28	55.21	59.17	
f...............	2.20	23.92	43.02		

The growth curve for the control in Experiment 4 indicates a size somewhat larger than that of the general average of the controls for that season. Compared with the control of Experiment 5, it measures somewhat more at the ages of 24 and 48 hours; then undergoes an inhibition of growth, resulting in a size at the age of 120 hours but little larger than the average of 5a at the age of 72.

Only three of the cultures that were treated with acids in Experiment 5 formed skeletons; in 5e the eggs segmented and finally reached an early gastrula stage, but were abnormal and did not swim at the surface. In b, c and d there was an extremely rapid development following a slow growth during the first few

hours. The unusually great increase of average size must
have been due to the large death-rate which gradually elimi-
nated all but the most nearly normal by the 72 hour period.
By this time all were less active and, in solutions *c* and *d*, were
disintegrating.

In the corresponding experiments of the following year the
controls were far below the general average at the age of 24
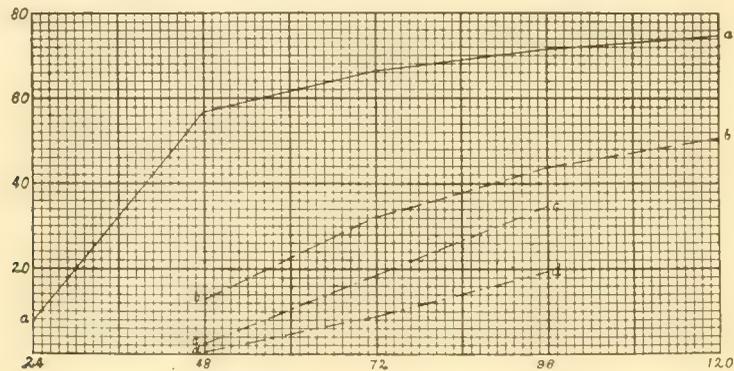
Plot 7
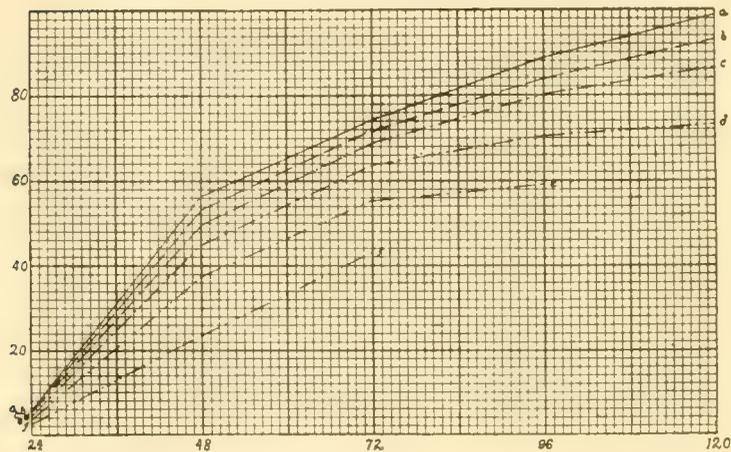
Plot 8

hours, but they then grew so rapidly that on the last two days
they were the extreme high variants on the growth-curve,
Plot 1 E.

The skeletons undergo characteristic modifications. All the
parts are more slender than in the normal, as shown in figure 41,
drawn from an individual of solution *b* at the 72 hour stage.
A comparison with figure 40, representing one of the control at
the same age, demonstrates the lighter structure of all the parts.

In very weak solutions, however, this does not involve a reduction in the number or size of the spine-like processes of the arms; but in higher concentration of acid the latter effect also is produced (fig. 42, 5c). Correlated with the disappearance of these spines, there is a retarded fusion of the separate rods in
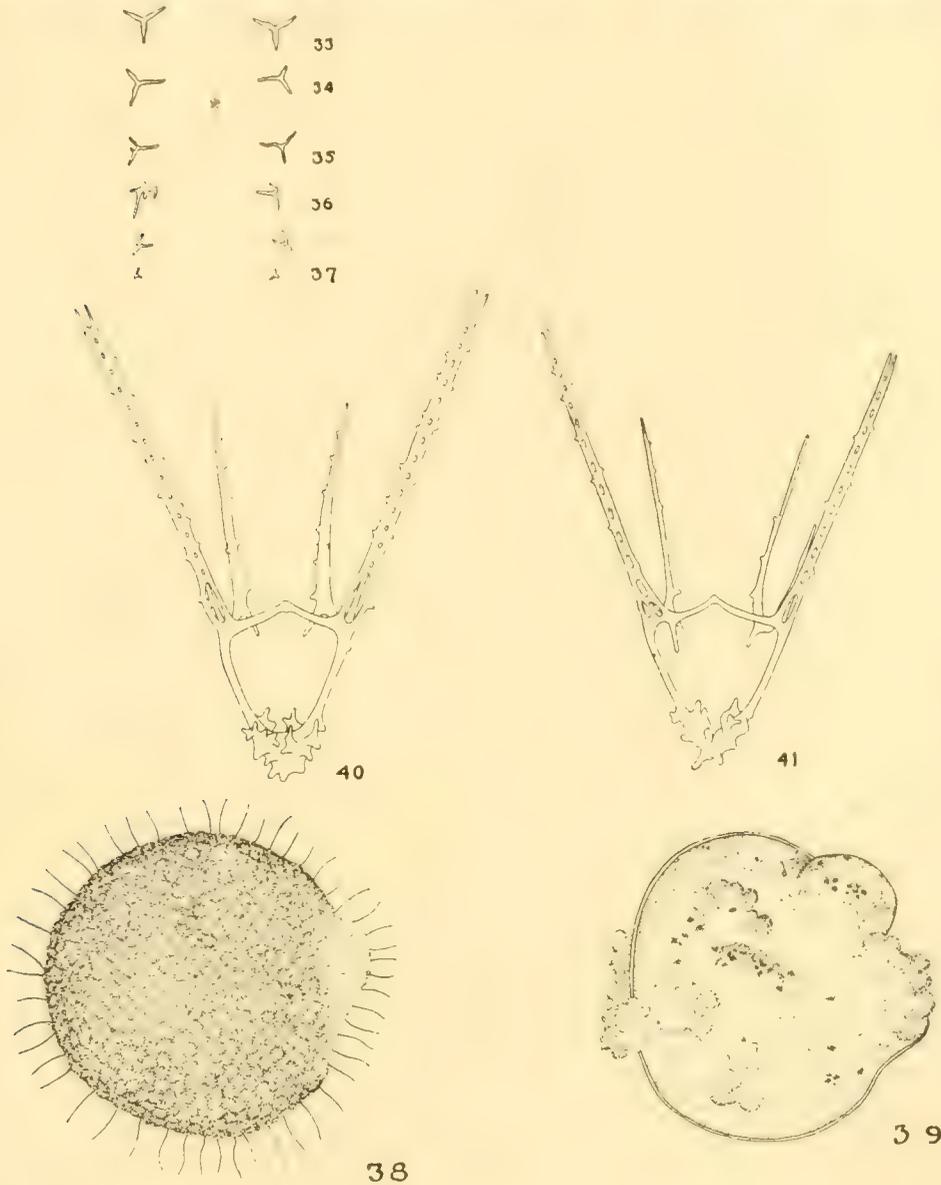
Figs. 33 to 48.   Experiment 5.   HCl.

Fig. 33   5a, 24 hrs.   Control.

Fig. 34   5b, figure 35 5c, figure 36 5d, figure 37 5e, 24 hrs.   Diminution of size and irregularity of structure.

Fig. 38   5f, 24 hrs.   Blastula stage, somewhat irregular.

Fig. 39   5g, 24 hrs.   Egg cytolyzed.   Pigment collected in irregular clumps.

Fig. 40   5a, 72 hrs.   Control.

Fig. 41   5b, 72 hrs.   All the parts more slender.

the anal arms (figs. 43 to 46, solutions *d, e* and *f*). Figure 45 from 5*e*, 72 hours, represents a typical specimen, nearly symmetrical, with arm- and body-rods forming a widely spread V. There is no evidence of spines and the number of cross-bars in the arms is greatly reduced. The ventral branches are lacking.

In contrast with the gradual disappearance of spines and cross-bars, there is a tendency toward formation of accessory
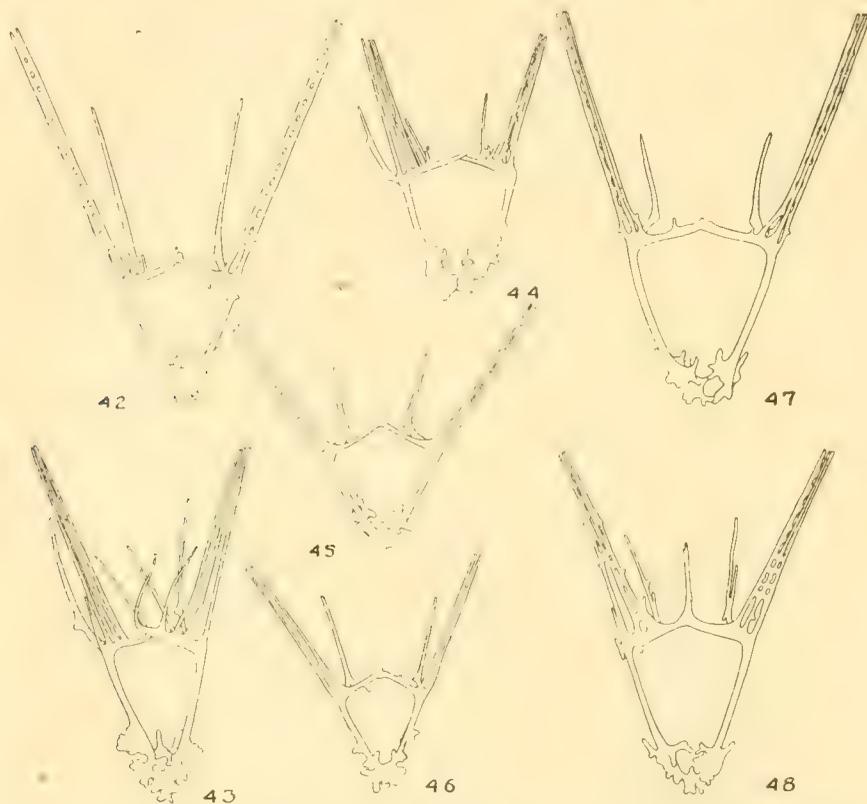
Fig. 42   5*c*, 72 hrs.   Reduction in size.   All the parts more slender.   Spines lacking from the arm-rods.

Fig. 43   5*d*, 72 hrs.   Slender and more reduced in size.   Arm-rods unfused, with accessory rods across the lateral connectives.   Posterior body-rods prominent.

Fig. 44   5*e*, 72 hrs.   Same characters, more pronounced.

Fig. 45   5*e*, 72 hrs.   Body symmetrical, slender.   Arm-rods slightly fused, spreading.

Fig. 46   5*f*, 72 hrs.   Same characters, still more marked.   Greater reduction in size.

Fig. 47   5*d*, 96 hrs.   Same characters, not so pronounced as in the stronger solutions.   Four rods in each arm.

Fig. 48   5*e*, 96 hrs.   Irregularity of structure.   Accessory rods on oral arms and at point of fusion of lateral connectives.

rods in the arms. Figure 43, V*d*, is a typical specimen. Four
unfused rods are present on each side, arising at intervals along
the transverse rods and the ends of the latter have bent ante-
riorly and elongated into additional ones. In figure 47, V*d*, 96
hours, four rods are present in each anal arm, and the promi-
nent openings in the lattice-work indicate incomplete fusion.

TABLE 7

Constants, Experiment 6. Solution a (Control)

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
36 hours	Right anal..........	34.65 ± 0.19	2.89 ± 0.13	8.36 ± 0.40
	Left anal...........	34.60 ± 0.19	2.91 ± 0.13	8.43 ± 0.40
	Right oral..........	22.11 ± 0.10	1.56 ± 0.07	7.06 ± 0.33
	Left oral...........	22.15 ± 0.10	1.55 ± 0.07	7.03 ± 0.33
48 hours	Right anal..........	46.48 ± 0.19	2.84 ± 0.13	6.11 ± 0.29
	Left anal...........	46.48 ± 0.19	2.84 ± 0.13	6.11 ± 0.29
	Right oral..........	26.65 ± 0.14	2.21 ± 0.10	8.31 ± 0.39
	Left oral...........	26.60 ± 0.15	2.24 ± 0.10	8.42 ± 0.40
72 hours	Right anal..........	63.67 ± 0.19	2.82 ± 0.13	4.43 ± 0.21
	Left anal...........	63.67 ± 0.18	2.74 ± 0.13	4.31 ± 0.20
	Right oral..........	37.29 ± 0.15	2.29 ± 0.10	6.16 ± 0.29
	Left oral...........	37.32 ± 0.16	2.41 ± 0.11	6.47 ± 0.31
96 hours	Right anal..........	69.47 ± 0.19	2.87 ± 0.13	4.13 ± 0.19
	Left anal...........	69.58 ± 0.19	2.96 ± 0.14	4.25 ± 0.20
	Right oral..........	44.79 ± 0.19	2.90 ± 0.13	6.48 ± 0.31
	Left oral...........	44.64 ± 0.19	2.87 ± 0.13	6.45 ± 0.30
120 hours	Right anal..........	74.59 ± 0.23	3.54 ± 0.16	4.75 ± 0.22
	Left anal...........	73.71 ± 0.23	3.41 ± 0.16	4.63 ± 0.22
	Right oral..........	48.71 ± 0.23	3.49 ± 0.16	7.18 ± 0.34
	Left oral...........	48.70 ± 0.23	3.45 ± 0.16	7.09 ± 0.34

Although all the typical parts of the skeleton are present, the
specimen is marked by a slight lack of symmetry. In many
individuals, this irregularity is much more evident, especially in
regard to the cross-bars in the anal arms (fig. 48 5*e*, 96 hours).
Here even the oral arms have shared in multiple rod-formation
and, at the point of fusion of the transverse rods, an additional
rod is formed.

TABLE 8

Constants, Experiment 6. Solution b

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
24 hours	Right anal	1.48 ± 0.08	1.32 ± 0.06	89.19 ± 6.84
	Left anal	1.48 ± 0.08	1.32 ± 0.06	89.19 ± 6.84
	Right oral	1.26 ± 0.07	1.18 ± 0.05	93.65 ± 7.41
	Left oral	1.26 ± 0.07	1.18 ± 0.05	93.65 ± 7.41
36 hours	Right anal	35.01 ± 0.23	3.42 ± 0.16	9.79 ± 0.47
	Left anal	34.98 ± 0.22	3.36 ± 0.16	9.61 ± 0.46
	Right oral	21.32 ± 0.17	2.56 ± 0.12	12.04 ± 0.57
	Left oral	21.35 ± 0.17	2.55 ± 0.12	11.94 ± 0.57
48 hours	Right anal	41.97 ± 0.22	3.26 ± 0.15	7.78 ± 0.37
	Left anal	41.95 ± 0.21	3.23 ± 0.15	7.71 ± 0.37
	Right oral	28.51 ± 0.17	2.54 ± 0.12	8.92 ± 0.42
	Left oral	28.47 ± 0.16	2.45 ± 0.11	8.62 ± 0.41
72 hours	Right anal	45.55 ± 0.27	4.15 ± 0.19	9.11 ± 0.43
	Left anal	45.56 ± 0.27	4.09 ± 0.19	8.99 ± 0.43
	Right oral	34.94 ± 0.19	2.93 ± 0.14	8.41 ± 0.40
	Left oral	34.98 ± 0.18	2.78 ± 0.13	7.97 ± 0.38
96 hours	Right anal	49.78 ± 0.27	4.10 ± 0.19	8.23 ± 0.39
	Left anal	49.76 ± 0.27	4.13 ± 0.19	8.31 ± 0.39
	Right oral	38.59 ± 0.24	3.61 ± 0.17	9.35 ± 0.45
	Left oral	38.59 ± 0.22	3.40 ± 0.16	8.83 ± 0.42
120 hours	Right anal	51.33 ± 0.30	4.51 ± 0.21	8.79 ± 0.42
	Left anal	51.32 ± 0.30	4.58 ± 0.21	8.93 ± 0.42
	Right oral	42.18 ± 0.20	3.08 ± 0.14	7.30 ± 0.34
	Left oral	42.16 ± 0.20	3.09 ± 0.14	7.33 ± 0.35

In Experiment 6 alkali was added to the sea-water in the following concentrations:

Experiment 6. Plot 9. September 5, 1914

a................................Sea-water
b................................99.33 cc. S. W. + 0.66 cc. M/10 NaOH
c................................98.66 cc. S. W. + 1.33 cc. M/10 NaOH
d................................98.0 cc. S. W. + 2.0 cc. M/10 NaOH

The growth-curve for the control culture resembles rather closely that of the general average for this period, except that

TABLE 9

Constants, Experiment 6. Solution c

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
24 hours	Right anal..........	3.21 ± 0.12	1.87 ± 0.08	58.49 ± 3.62
	Left anal...........	3.21 ± 0.12	1.87 ± 0.08	58.49 ± 3.62
	Right oral..........	3.37 ± 0.12	1.87 ± 0.08	56.09 ± 3.41
	Left oral...........	3.37 ± 0.12	1.87 ± 0.08	56.09 ± 3.41
36 hours	Right anal..........	35.15 ± 0.27	4.06 ± 0.19	11.57 ± 0.55
	Left anal...........	35.10 ± 0.26	3.97 ± 0.18	11.33 ± 0.54
	Right oral..........	23.98 ± 0.22	3.36 ± 0.16	14.03 ± 0.68
	Left oral...........	23.85 ± 0.22	3.34 ± 0.15	14.01 ± 0.68
48 hours	Right anal..........	40.63 ± 0.28	4.16 ± 0.19	10.25 ± 0.49
	Left anal...........	40.63 ± 0.28	4.16 ± 0.19	10.25 ± 0.49
	Right oral..........	29.15 ± 0.25	3.85 ± 0.18	13.20 ± 0.64
	Left oral...........	29.06 ± 0.25	3.79 ± 0.18	13.08 ± 0.63
72 hours	Right anal..........	43.21 ± 0.29	4.32 ± 0.20	9.99 ± 0.48
	Left anal...........	43.11 ± 0.25	4.22 ± 0.20	9.79 ± 0.47
	Right oral..........	33.32 ± 0.23	3.55 ± 0.16	10.67 ± 0.51
	Left oral...........	33.13 ± 0.22	3.33 ± 0.15	10.06 ± 0.48
96 hours	Right anal..........	45.13 ± 0.30	4.53 ± 0.21	10.05 ± 0.48
	Left anal...........	45.01 ± 0.30	4.56 ± 0.21	10.14 ± 0.48
	Right oral..........	32.78 ± 0.25	3.78 ± 0.18	11.55 ± 0.54
	Left oral...........	32.96 ± 0.25	3.80 ± 0.18	11.53 ± 0.55
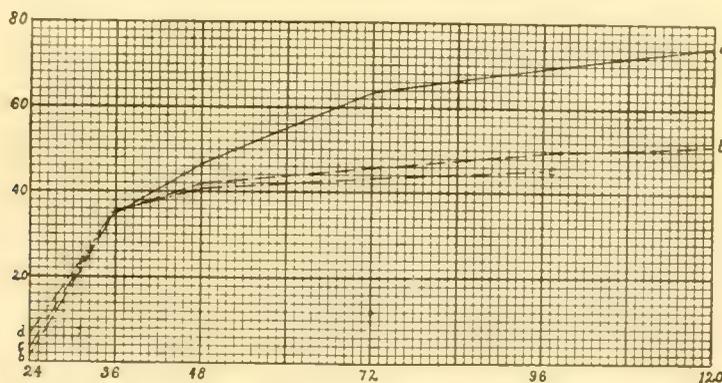
Plot 9

at 24 hours the measurements of the general average indicate the presence of small tri-radiate spicules, whereas in this solution no skeletal structures have appeared. In solution *b* (fig.

TABLE 10

Constants, Experiment 6.   Solution d

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
24 hours	Right anal..........	6.20 ± 0.27	4.00 ± 0.19	64.67 ± 4.18
	Left anal..........	6.20 ± 0.27	4.00 ± 0.19	64.67 ± 4.18
	Right oral.........	6.04 ± 0.27	4.07 ± 0.19	67.41 ± 4.41
	Left oral..........	6.04 ± 0.27	4.07 ± 0.19	67.41 ± 4.41
36 hours	Right anal.........	35.23 ± 0.28	4.29 ± 0.20	12.20 ± 0.56
	Left anal..........	35.42 ± 0.28	4.26 ± 0.20	12.05 ± 0.58
	Right oral.....	28.05 ± 0.23	3.41 ± 0.16	12.17 ± 0.58
	Left oral	28.03 ± 0.22	3.41 ± 0.16	12.14 ± 0.58
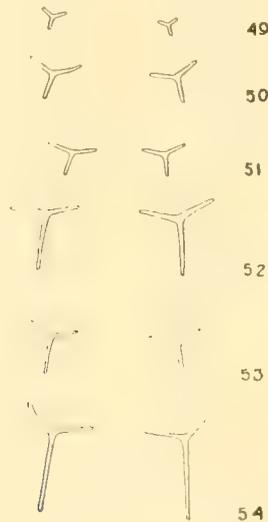
Figs. 49 to 70.   Experiment 6.   NaOH.

Fig. 49   6a, 24 hrs.   Control.

Fig. 50   6b, figures 51 and 52 6c, figures 53 and 54 6d, 24 hrs.   Increased size in the solutions of greater alkalinity.

50), however, most of the specimens are more advanced and possess skeletons ranging from minute rounded centers which can be detected only by their bright shiny appearance, to distinct tri-radiate spicules.   In solution c (figs. 51 and 52) and d (figs. 53 and 54), the tri-radiate character of the spicules is evident in nearly all, while in many of d the posterior rays have begun to differentiate into body-rods.

At the age of 36 hours, the individuals of culture a possessed skeletons of approximately the same size as those of the other

cultures; all the averages were now so nearly equal that no conclusive evidence could be drawn as to the relative heights of the various growth-curves at this particular period (figs. 55 to 60). At the age of 48 hours, however, a was decidedly larger than b and c (figs. 61 to 63) and an increased difference was evident
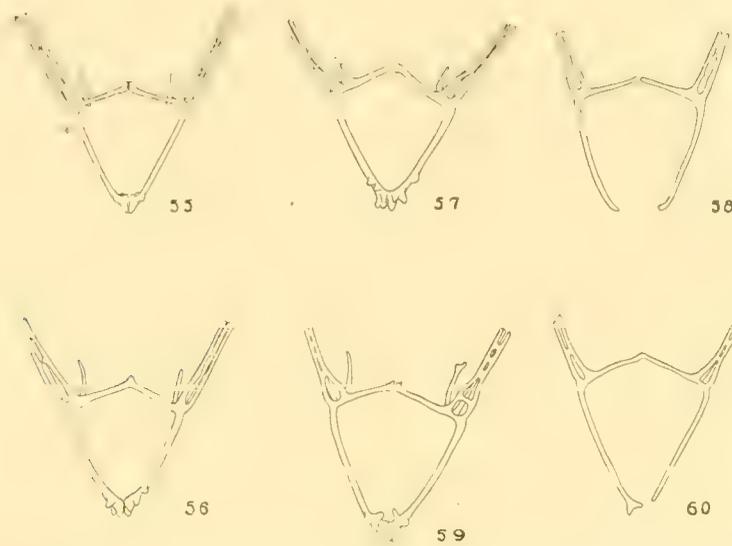
Fig. 55 $6a$, 36 hrs. Control. Figure 56 $6b$, figures 57 and 58 $6c$, figures 59 and 60 $6d$, 36 hrs. All of approximately the size. Variation increased and asymmetry gradually acquired in the stronger solutions.
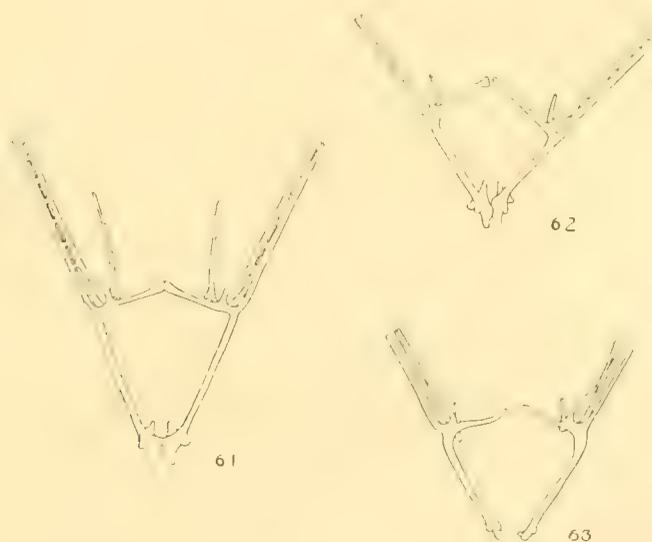
Fig. 61 $6a$, 48 hrs. Control.

Fig. 62 $6b$, figure 63 $6c$, 48 hrs. Greater inhibition of growth in the alkaline solutions. Retarded fusion of the various elements. Irregularity.

at 72 (figs. 64 to 66) and 96 (figs. 67 to 69); c did not develop past the 96 hour stage.

Although all the specimens were of approximately the same size at the age of 36 hours, an inhibition of development was
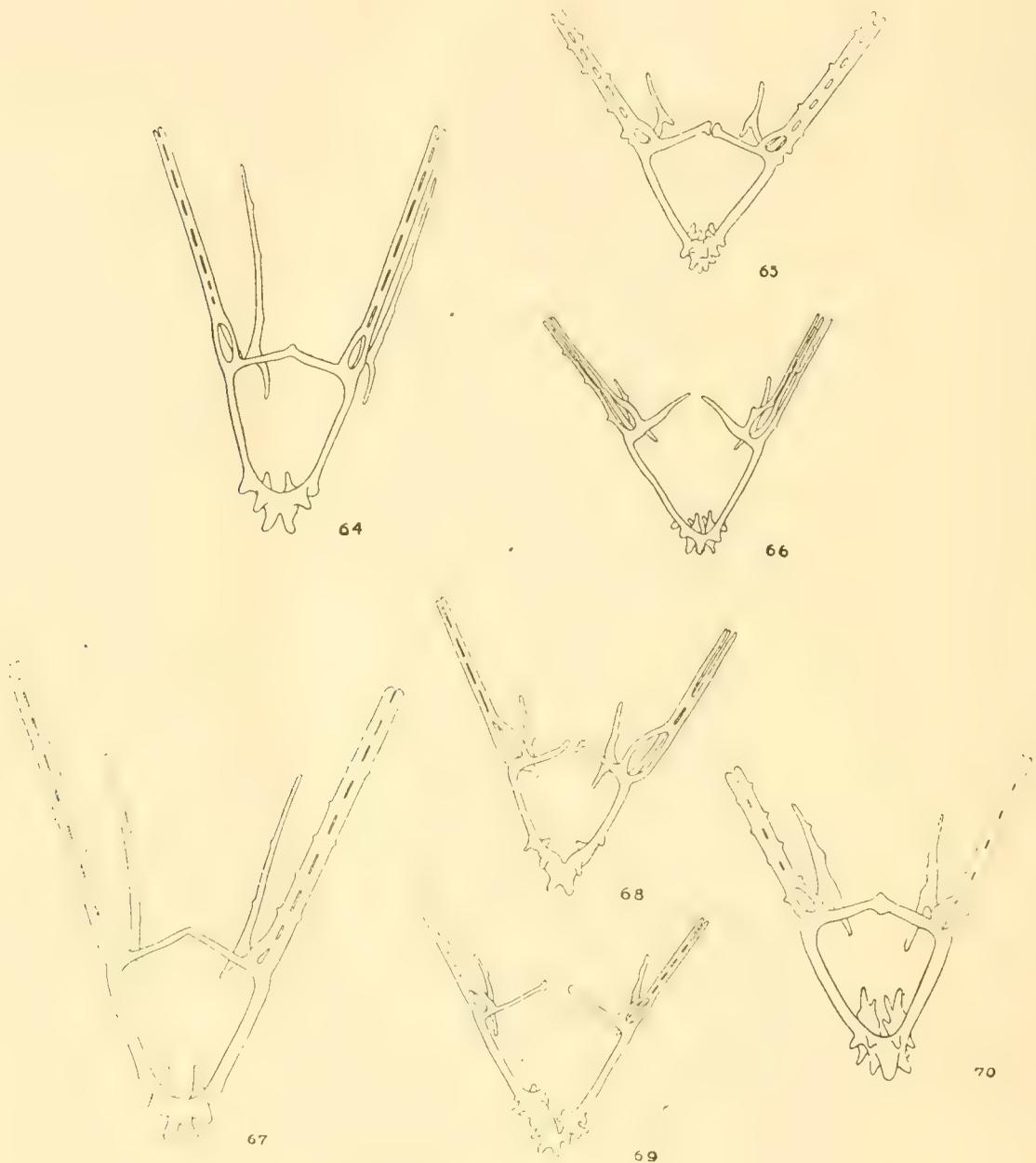
Fig. 64   6a, 72 hrs.   Control.

Fig. 65   6b, figure 66 6c, 72 hrs.; figure 67 6a, Control; figure 68 6b, figure 69 6c, 96 hrs.; figure 70 6b, 120 hrs.   Same characters, becoming more pronounced at the later periods and in the stronger solutions.

evident in the stronger solutions. Figure 55 represents a typical individual of the control cultures, and practically all the specimens were in a similar stage of development. The same may be said of solution *b* (fig. 56). But in solution *c*, figures 57 and 58 represent two of the extreme variants and it may be seen that, although of approximately the same size, both larvae are in a less advanced stage than either of the above. This is true also of those in *d* (figs. 59 and 60).

In the stronger solutions, moreover, there enters the element of irregularity of structure, apparent only in the minuter details. In the specimen represented by figure 62 (*b*, 48 hours), for instance, fusion of the transverse rods would probably not have occurred in the mid-line. In figure 63 (*c*, 48 hours), one body-rod is longer and heavier than the other, and the individual elements in the left anal arm are very slightly distorted. In figure 68 (*b*, 96 hours), the transverse rods are unequally developed and that on the right side seems to have undergone a secondary elongation in order to meet the other.

This experiment was repeated a number of times and a slight variation was found in the exact time at which the control culture outgrew those in the alkaline solution. In one instance the specimens in solutions *b* were still of larger size at 48 hours, but by 72 hours, the only apparent difference was the greater degree of symmetry of those of the control. In this set of solutions, too, there was less difference in size between the specimens of the control and those of the alkaline solutions at the late stages,—a condition which seemed to prevail in all the experiments of the early part of the following year, when the specimens in general were more viable, and those in the experimental solutions seemed able to live under more extreme conditions. On the other hand, in experiments when the controls themselves were less vigorous, the difference in size between them and the specimens in the treated solutions appeared more exaggerated in later stages, following an earlier appearance of the typical irregularities. For instance, figures 71, 72, 73 and 74 (Experiment 7) are drawn from the 24 hour stage of specimens from solutions corresponding to *a*, *b*, *c* and *d* of Experiment 6.

At this time, a possessed small tri-radiate spicules and later formed skeletons which were normal in all but size, measuring only 3.7, 41.56, 53.22, 62.37 and 64.24 on the various days. The alkaline solutions showed at 24 hours a much greater excess of size over the controls than did the corresponding culture of Experiment 6, but the skeletons were irregular and in later stages became far more abnormal.

At this time the specimens in culture a were apparently in a healthy condition, swimming about and their mouths and alimentary tracts undergoing periodic contractions. This also was true of those in solutions b and c, and slightly less marked in d and e. In f the specimens were still swimming at the surface, but were less active, and in g there was a much higher death-rate and many of the specimens were swimming lower in the dish.

The third experiment in Series II was made with $NaHCO_3$.

Experiment 8. Plot 10. July 10, 1914

a................................Sea-water
b................................224 cc. S. W. + 1 cc. 0.45M $NaHCO_3$
c................................223 cc. S. W. + 2 cc. 0.45M $NaHCO_3$
d................................222 cc. S. W. + 3 cc. 0.45M $NaHCO_3$
e................................221 cc. S. W. + 4 cc. 0.45M $NaHCO_3$
f................................220 cc. S. W. + 5 cc. 0.45M $NaHCO_3$
g................................219 cc. S. W. + 6 cc. 0.45M $NaHCO_3$

The corresponding growth measurements were:

	18 HOURS	24 HOURS	48 HOURS	72 HOURS	84 HOURS	96 HOURS	120 HOURS
a	14.10	44.83	70.07	92.01	49.73	44.37	40.31
b	20.23	46.81	70.84	90.92	68.13	61.91	56.24
c	25.19	48.20	72.13	89.95	75.24	69.10	62.11
d	29.92	50.92	71.46	88.88	79.14	73.92	66.45
e	34.87	49.85	69.72	87.10	81.97	76.65	71.04
f	32.01	47.20	68.21	84.89	85.83	79.85	74.92
g	28.14	44.13	66.10	81.17	84.01	81.97	78.15

The control growth-curve resembles that of Experiment 2 in that it reaches an early maximum which is followed by resorption of the skeleton. Here, the period of greatest growth occurs still earlier, before the 72 hour stage, after which a

rapid decrease in size takes place. As concerns rate of growth, the specimens in the experimental solutions behave similarly to those in the sea-water to which NaOH had been added. Solutions *b*, *c*, *d* and *e* produced during the first 18 hours an increasingly beneficial effect and *g* surpassed *a* by a smaller per-

Figs. 71 to 74.   Experiment 7.
Fig. 71   7*a*, 24 hrs.   Control.
Fig. 72   7*b*, figure 73 7*c*, figure 74 7*d*, 24 hrs.   Solutions same as in the previous experiments.   In this series the characters were more pronounced and the irregularities very prominent at 24 hrs.
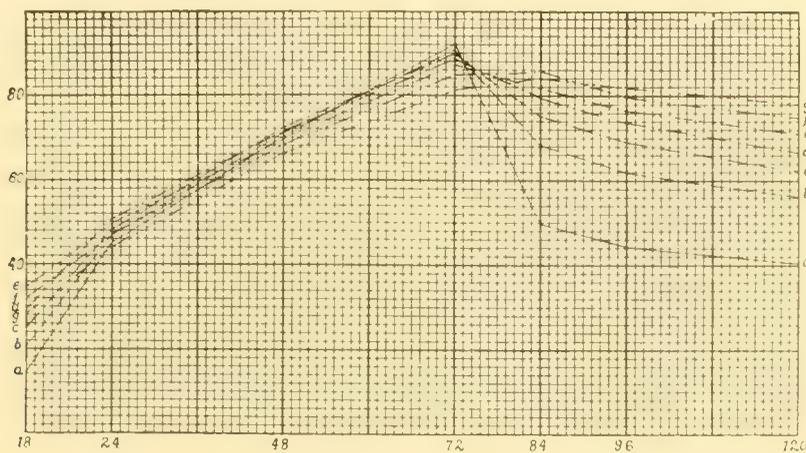
Plot 10

centage, indicating that the degree of alkalinity most conducive to growth at this period has been exceeded.

At 18 hours, then, the concentration of solution *e* was optimum for growth; at 24 hours, that of *d*; at 48 hours, that of *c*; and at 72, that of *a*, as is indicated by their positions on the growth-curves on the various days.   Hence, although an increased alkalinity produced an accelerated initial development,

there was a gradual shifting toward the bottom, of the growth-curves of those in higher concentrations of the hydroxyl, with the specimens of the control ultimately the largest.

The growth-curves of f and g reach their maxima 24 hours later than the others. In solutions of less alkalinity, skeletal resorption was more rapid and at the close of 120 hours the positions of all the cultures were the reverse of that indicated at the age of 72 hours.

Another experiment was made with $NaHCO_3$ added in greater proportions in order to determine the effects of still higher concentrations of the acid salt. For this purpose the following solutions were employed:

Experiment 9.   Plot 11.   July 15, 1915

```
a...............................Sea-water
b.............................98.5 cc. S. W. + 1.5 cc. 0.45M NaHCO₃
c.............................97.0 cc. S. W. + 3.0 cc. 0.45M NaHCO₃
d.............................95.5 cc. S. W. + 4.5 cc. 0.45M NaHCO₃
e.............................94.0 cc. S. W. + 6.0 cc. 0.45M NaHCO₃
f.............................92.5 cc. S. W. + 7.5.cc. 0.45M NaHCO₃
g.............................91.0 cc. S. W. + 9.0 cc. 0.45M NaHCO₃
```

The corresponding measurements were:

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a	28.84	74.05	85.91	92.13	95.08
b	34.15	76.09	86.12	90.81	92.74
c	30.21	68.91	78.18	82.14	83.69
d	25.94	62.87	70.25	72.94	73.98
e	21.30	57.89	64.19	65.97	66.15
f	12.11	50.24	56.13	57.89	58.02
g	2.07	41.32	45.77	47.15	

In this experiment also, there was shown the specific effect of alkalinity upon length of skeleton. Solution b, which was approximately equivalent to $8e$, produced a similar modification of the rate of growth. In all the others, the concentrations of the hydroxyl were greater than in any of Experiment 8; c, however, caused an acceleration during the first day; in

all the rest the specimens were smaller throughout the entire period.

In Experiment 10 still greater percentages of NaHCO₃ were employed in order to obtain the limit of endurance at early stages. In Experiment 9 an inhibition of growth was plainly discernible in the stronger solutions, even during the early periods, but judging from the active movements of the specimens during the first 48 hours, it was evident that much stronger solutions could be employed.
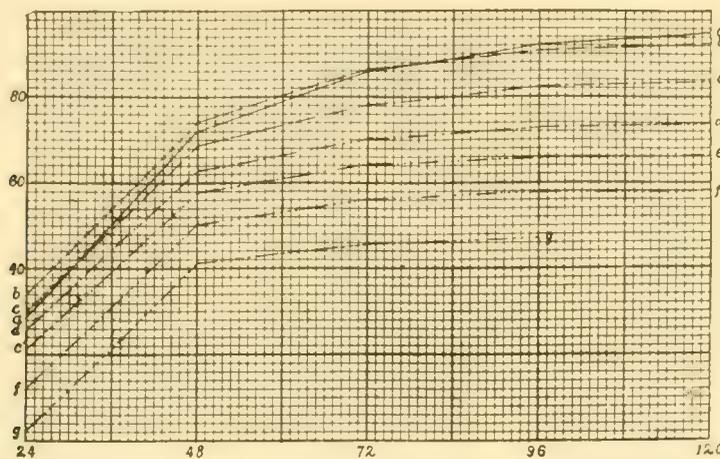
Plot 11

Experiment 10. July 15, 1915

a.................................Sea-water
b.................................90 cc. S. W. + 10 cc. 0.45M NaHCO₃
c.................................80 cc. S. W. + 20 cc. 0.45M NaHCO₃
d.................................70 cc. S. W. + 30 cc. 0.45M NaHCO₃
e.................................70 cc. S. W. + 40 cc. 0.45M NaHCO₃
f.................................50 cc. S. W. + 50 cc. 0.45M NaHCO₃

Figures 75 to 80 show specimens from the various solutions at the age of 24 hours. In *a* (fig. 75) the three branches of the tri-radiate spicule have elongated into body-rod, transverse rod and dorso-ventral connective, and the rods of the anal arms are beginning to form. In *b* (fig. 76) gastrulation has just taken place, producing individuals which appear abnormal only in lack of skeletons. This culture, however, contained on

the following day a small percentage of individuals with skele-
tons represented by small heavy tri-radiate spicules The
specimens in solution c (fig. 77) were irregular and abnormal
looking and were moving about slowly on the bottom of the
dish. Gastrulation had begun, but had proceeded only a little
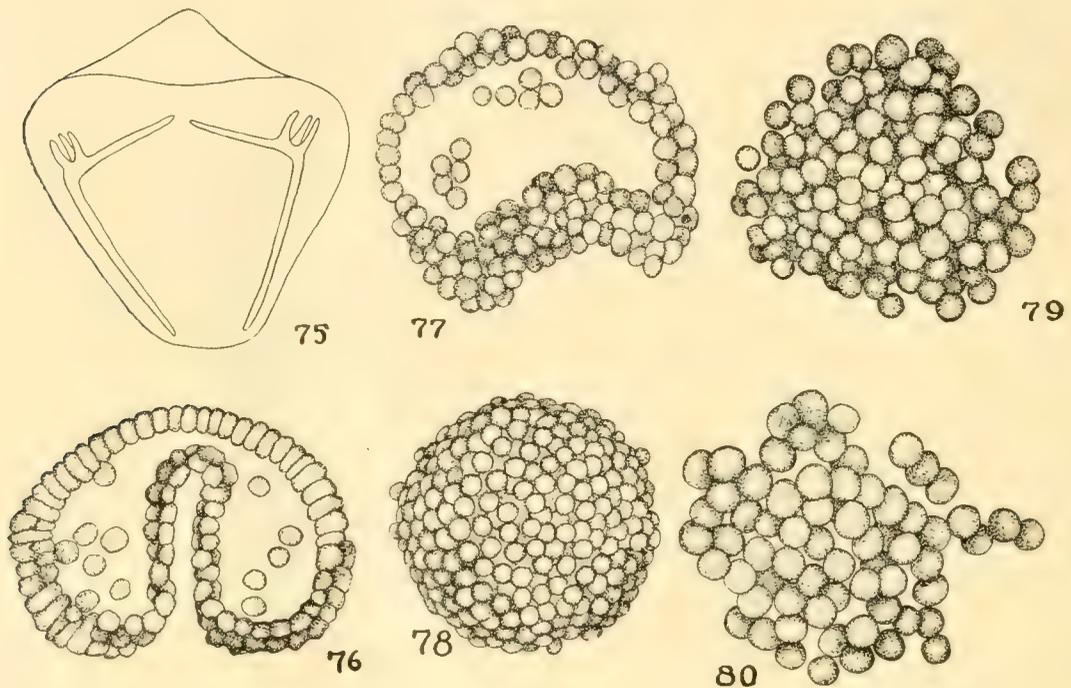
Figs. 75 to 80.   Experiment 10. $NaHCO_3$.
Fig. 75   10a, 24 hrs.   Control.
Fig. 76   10b, 24 hrs.   Gastrulation. No skeletons. (Optical section.)
Fig. 77   10c, 24 hrs.   Gastrulation just commenced. No further develop-
ment. (Optical section.)
Fig. 78   10d, 24 hrs.   Blastula.
Fig. 79   10e, 24 hrs.   Morula-like mass of cells.
Fig. 80   10f, 24 hrs.   Morula-like mass of cells in less advanced stage of
development.

way and the walls presented a rough and uneven appearance.
In the other three solutions (figs. 78 to 80) all were dead; d
(fig. 78) after reaching the blastula stage, and e and f, a late
cleavage.   In e (fig. 79), the smaller cells indicate a slightly
more advanced stage than in f (fig. 80), but both have become
irregular, morula-like masses.

The effects upon the skeletons caused by subjection to the carbonate solutions were very different, in all respects except size, from those produced by the increased hydroxyl concentration. In the latter there was no appreciable tendency toward a thickening of the skeletal structures nor toward a reduction of parts, but in the carbonate solutions these were the most prominent and characteristic changes. Figure 81 represents one of the control specimens (Experiment 9), 48 hours old and figure 82, one from c at the same age. All the structures are heavier, especially the rods of the oral arms and the body-
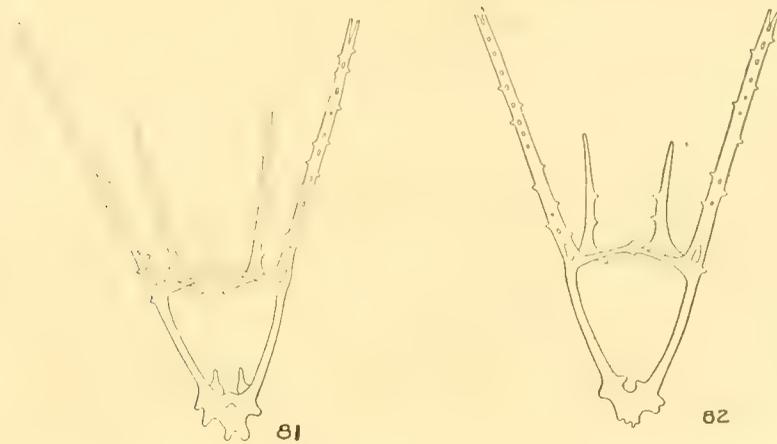
Figs. 81 to 87   Experiment 9 NaHCO₃.
Fig. 81   9a, 48 hrs.   Control.
Fig. 82   9c, 48 hrs.   Structures heavier, especially oral arms and body-rods.

rods. There has been a precocious closing of the interspaces in the anal arms and the spines are reduced in number. A fusion of the skeletal elements often occurs early, accompanied by decided thickenings at the points of contact. In other instances, even at a late period, the individual elements remain distinct. The ventral body-branches are frequently present as thick, fused, club-shaped masses giving little or no indication of separate processes (fig. 83, f, 72 hours). At other times, especially in the stronger solutions, no ventral body-branches are present (fig. 84, g, 48 hours). Figures 85, 86, and 87 are taken from solution f at the age of 72 hours, and depict the typical modifications. Figure 85 represents one nearly resembling the normal

and is the most usual type. It is very much simplified in out-
line, thickened in every portion of the skeleton, with only slight
traces remaining of the lattice-formation in the anal arms, and
with a reduction in the number of spines and processes of the
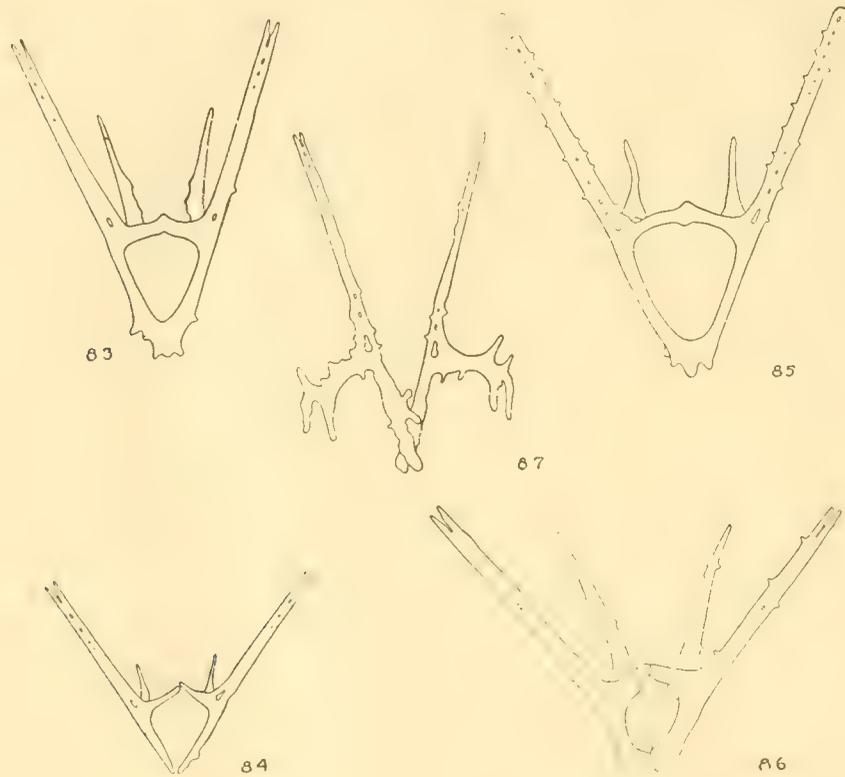
Fig. 83 9*f*, 48 hrs. All the principal parts heavier. Precocious fusion of
the arms. Lack of processes. Reduction in size.

Fig. 84 9*g*, 48 hrs. Same chracaters except that here the lateral connec-
tives and the body-rods have not fused. Specimen very symmetrical.

Fig. 85 9*f*, 72 hrs. Usual type. Skeleton thickened, simplified in structure.
Slight traces of lattice-formation. Reduction in the number of spines and
processes. Highly symmetrical.

Fig. 86 9*f*, 72 hrs. Skeleton heavier than in figure 85, the two halves
unfused.

Fig. 87 9*f*, 72 hrs. Two portions of the skeleton unfused. Dorso-ventral
connectives (?) turned laterally, with oral arms (?) turned posteriorwards.

body-rods, yet it shows no indication of lack of symmetry. The
tendency toward irregularity of structure under the various
other experimental conditions described above is entirely lack-
ing here, and a comparison of the measurements taken of the
two sides of the body in these cultures shows quite as little

tendency toward variations in this respect as is shown in the control.

In the specimen illustrated by figure 87, the main part of the additional calcareous deposit occurred on the dorso-ventral connectives which were turned laterally instead of dorsally. The oral arms may be said to be directed posteriorly, or to be lacking entirely, and the dorsal body-branches to be very highly developed. A few individuals of this type were found, not only in this experiment in the stronger solutions, but also in similar cultures with individuals from different parents.

Experiment 11 deals with the effects of Na_2CO_3, which was employed because upon hydrolysis it gives hydroxyl ion concentration greater than that given by $NaHCO_3$. The following solutions were used:

Experiment 11. Plot 12. July 9, 1915

a..Sea-water
b.....................................99.8 cc. S. W. + 0.2 cc. 0.45M Na_2CO_3
c.....................................99.6 cc. S. W. + 0.4 cc. 0.45M Na_2CO_3
d.....................................99.4 cc. S. W. + 0.6 cc. 0.45M Na_2CO_3
e.....................................99.2 cc. S. W. + 0.8 cc. 0.45M Na_2CO_3
f.....................................99.0 cc. S. W. + 1.0 cc. 0.45M Na_2CO_3
g.....................................98.8 cc. S. W. + 1.2 cc. 0.45M Na_2CO_3

The following measurements were obtained:

	24 HOURS	36 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a	24.40	55.72	68.16	84.31	92.26	96.48
b	27.82	60.55	71.94	85.97	91.90	94.37
c	36.72	62.68	72.13	83.89	88.03	89.78
d	32.14	56.13	64.17	74.76	77.08	78.01
e	20.30	38.32	45.12	54.73	55.91	56.22
f	5.63	15.69	21.34	28.14		
g		0.06	2.85			

The specimens of the control culture of this experiment greatly exceed in size those of the general average for the corresponding season. Here again, the effect of the increased alkalinity is shown in the growth-curves produced by the various solutions. The specimens in *b*, *c* and *d* are in advance of those in *a* at the ages of 24 and 36 hours, but *b* drops behind after

48 hours and c after 72. By the 96 hour period, all are smaller than those in the control.

Frary and Nietz ('15) give a table of the hydrolysis of sodium carbonate in solutions at 25°C., according to which 0.005M Na_2CO_3 furnishes concentration of hydroxyl ions equivalent to a 0.00081M NaOH solution. By interpolating in the table, the addition of 0.8 cc. 0.45M Na_2CO_3 (11e) to 100 cc. water would furnish approximately the same concentration of hydroxyl ions as addition of 0.66 cc. M/10 NaOH (6b). These, being in distilled water, would not have the same values as when added to sea-water, but it seems significant that in the
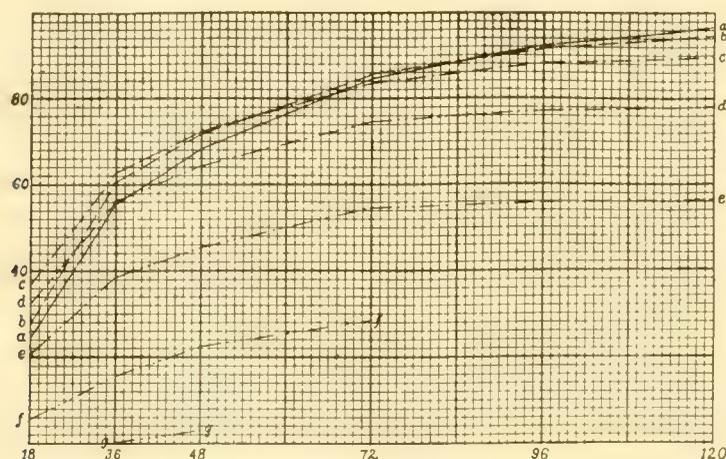
Plot 12

two experiments, these two solutions produce strikingly similar modifications of the growth-curves.

The modifications found in the Na_2CO_3 solutions were of very much the same type as in those of the $NaHCO_3$, except that the changes were not apparent so early. In these solutions the lattice-formation was very evident, even more so than in the control cultures, and persisted until a later stage. Figures 88, 89 and 90 represent specimens from a, c and d at 48 hours; figures 91 and 92, specimens from solution e; figures 93 and 94, two from solutions f; figure 95, one from g. In comparison with those of the same age in Experiment 9, it may be seen that the skeleton is heavier and the lattice-formation persists later. For instance, the specimens represented in figure 91

(11*e*) and in figure 86 (9*f*) are of approximately the same size and both are 48 hours old. In figure 91, the arms are somewhat heavier, but the individual rods comprising them have not fused so greatly and the spines, though reduced in
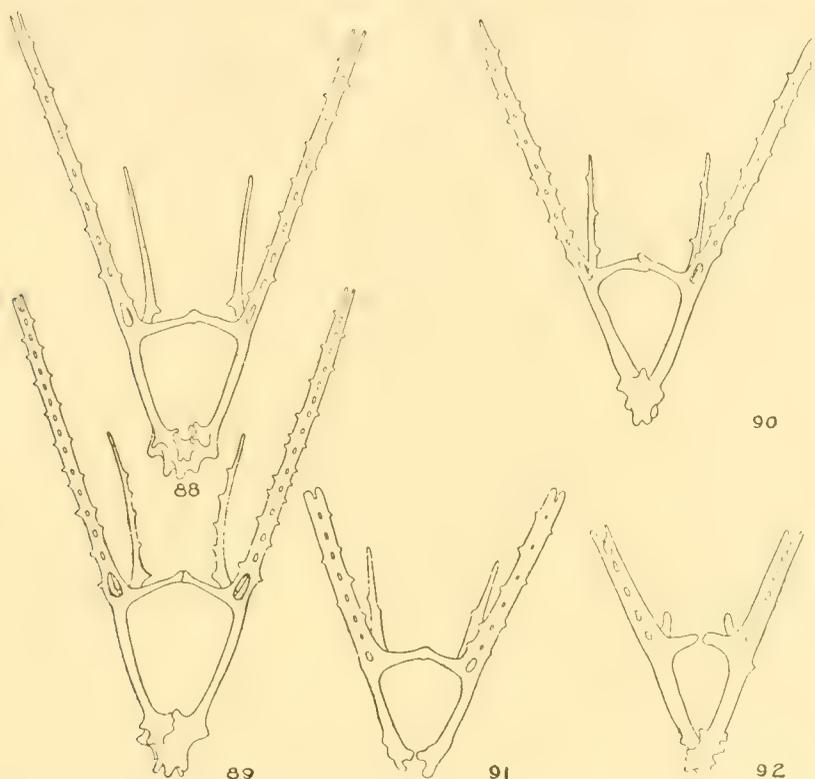
Figs. 88 and 89.   Experiment 11. Na_2CO_3.

Fig. 88   11*a*, 48 hrs.   Control.

Fig. 89   11*c*, 48 hrs.   All the structures slightly heavier, but in a less advanced stage of fusion than the control, indicating influence of increased Na content.

Fig. 90   11*d*, 48 hrs.   Same as above, but influence of Na less pronounced. Resembles more closely those in the $NaHCO_3$ solutions.   Symmetry.

Figs. 91 and 92   11*e*, figures 93 and 94 11*f*, figure 95 11*g*, 48 hrs.   Increasing inhibition of growth.   Gradual loss of evidence of Na influence and acquirement of CO_3 characters; such as, heavy symmetrical skeletons, with highly fused arm-rods.

number, are still present.   In other specimens of the same solution (fig. 92) spines are lacking.   Figures 93 and 94 represent two of the extreme variants of solution *f*.   In 92, spines have disappeared, but the rods have not fused to a greater extent than in the control culture (fig. 88).   Figures 96 and 97 are

from solutions e and f at 72 hours and 98 from e at 96 hours.
The same features characterize these specimens except that
here fusion of the individual rods in the arms has been almost
complete.

All these figures are characterized by a high degree of sym-
metry. This quality is evident, even in those cultures where
skeletons have been reduced to the extent indicated in figure
95. In the presence of an excess of carbonate, then, in contrast

Fig. 96   11e, figure 97 11f, 72 hrs.; figure 98 11e, 96 hrs.   Complete disap-
pearance of Na characters and appearance of CO_3.

to the other experimentally altered media, asymmetry is not
correlated with inhibition of growth.

The most pronounced effect of addition of acids or alkalies
to the medium in which the Arbacia eggs are developing is the
decrease or increase in rate of growth. As shown in the ac-
companying experiments, acid invariably produces a retarding
and inhibiting effect; alkalies, in small amounts, cause an ac-
celeration of growth in early stages, resulting in increased size.
In later stages, however, these same cultures show a retarda-
tion of the rate of development so that ultimately they lag

behind the control. In solutions more highly alkaline, the excessively precocious development results in irregularity and asymmetry and finally in complete inhibition.

Loeb ('98) in an early paper called attention to this effect of acids and alkalies upon the rate of development. He stated, however, that alkalinity produced a scarcely noticeable effect in the first few hours, and a gradually increasing one on the second and third days. "So gewinnt man den Eindruck," he says, "als ob die Hydroxylionen des Alkali eine langsame aber stetige Zunahme in der Geschwindigkeit der Entwicklung bedingen." But after three or more days, disintegration of the larvae set in and he could no longer determine any difference between those in alkaline solutions and those in sea-water. He suggested that by this time the alkali was largely neutralized by the acids produced in the process of growth.

Later, in his "Artificial Parthenogenesis and Fertilization" he takes an entirely different position. He says that "he has since vainly attempted to show that the rate of development of the sea-urchin egg can be increased with the increase of the concentration of hydroxylions in the sea-water. This leads him to believe that these eggs develop best in a solution in which the concentration of hydroxylions equals that of the sea-water; and that while it is possible to delay their development by a lowering of this concentration, no acceleration can be produced if the C_{OH} in the sea-water is raised."

That some effect upon rate of development is produced in the earliest stages, was demonstrated by the following experiment in which solutions of the same constitution as in Experiment 11 were employed. Each culture was divided into two portions and at one hour after insemination one of each was killed quickly by the introduction of a few drops of fixing fluid, and a count taken of the number in the one- and two-cell stages. After 18 hours, the percentage of those possessing skeletons was taken in the other portions.

Experiment 12.   July 9, 1915

```
a............................Sea-water
b............................99.8 cc. S. W. + 0.2 cc. 0.45M Na₂CO₃
c............................99.6 cc. S. W. + 0.4 cc. 0.45M Na₂CO₃
d............................99.4 cc. S. W. + 0.6 cc. 0.45M Na₂CO₃
e............................99.2 cc. S. W. + 0.8 cc. 0.45M Na₂CO₃
f............................99.0 cc. S. W. + 1.0 cc. 0.45M Na₂CO₃
g............................98.8 cc. S. W. + 1.2 cc. 0.45M Na₂CO₃
```

The following data were obtained:

Time after insemination

	1 HOUR PER CENT IN 1-CELL	1 HOUR PER CENT IN 2-CELL	18 HOURS PER CENT WITH SKELETONS	18 HOURS SIZE OF SKELETONS
a.............	14.0	86.0	53.2	0–2
b.............	8.0	92.0	82.1	0–4
c.............	4.7	95.3	100.0	2–4
d.............	3.3	96.7	84.6	0–3
e.............	13.5	86.2	58.3	0–2
f.............	18.8	81.2	0.0	
g.............	23.9	76.1	0.0	

A somewhat similar experiment was performed with NaOH and the effects at a still earlier stage noted.

Experiment 13.   July 29, 1915

```
a............................Sea-water
b............................99.33 cc. S. W. + 0.66 cc. N/10 NaOH
c............................98.66 cc. S. W. + 1.33 cc. N/10 NaOH
d............................98.0  cc. S. W. + 2.00 cc. N/10 NaOH
```

The following data were obtained:

Time after insemination

	2.5 MINUTES. PER CENT WITH FERT. MEMBR.	1 HOUR, 20 MINUTES. PER CENT IN 2 CELL	1 HOUR, 20 MINUTES. PER CENT IN 4-CELL	1 HOUR, 20 MINUTES. PER CENT IN 8-CELL
a.............	42.6	4.1	95.9	
b.............	63.7	0.8	99.2	
c.............	81.5		97.4	2.6
d.............	27.1	98.8	1.2	

These experiments indicate that the effect of alkalinity upon rate of development is immediate, and decreases in intensity during later stages. This later retardation of growth may be a secondary effect produced as a result of the embryo's having exceeded its optimum rate. In other words, there may be a point beyond which rapid development ceases to be beneficial to the organism and hence precocious growth may be conducive to later inhibition. This fact is shown more clearly in the case of the very alkaline solutions where overrapid development leads at once to irregularity of structure and inhibition of growth.

The specific effect of the hydroxyl may be said, then, to be an acceleration of the rate of growth in the early stages, succeeded by inhibition. In very weak solutions both these effects are slight, but the greater the alkalinity, the more rapid the acceleration and the more marked the succeeding asymmetry and inhibition. The effect, of course, is not exactly proportional to the amount of alkali added, since, in the higher concentrations, some magnesium and calcium hydroxides must precipitate out.

In the experiments given above (Experiment 6), the limit of endurance was reached when 2 cc. N/10 NaOH were added to 98 cc. sea-water, but in an experiment of the following year, the limit was found to be 3 cc. N/10 NaOH to 97 cc. sea-water. The concentration productive of maximum rate of growth at each stage was found to be about the same, the only material difference in the reaction of the embryos to the changed environment being a greater degree of resistance, characterized by a more retarded setting in of the typical abnormalities.

In the carbonate solutions we also have acceleration of development by the alkalinity produced as a result of the hydrolysis of $NaHCO_3$ and Na_2CO_3, both salts of a strong base with a weak acid. In the bicarbonate solutions, as we should expect, acceleration is slighter and in stronger solutions is overshadowed by the specific effect of the carbonate. In the sodium carbonate, especially in the weaker solutions, a third element enters, the specific effect of the sodium, a fact which may be

explained by the presence of a greater proportion of the sodium in the molecule added.

The specific effect of the carbonate must be considered to increase the bulk of the skeleton, combined with a strong tendency toward regularity and symmetry. This latter quality is so pronounced that it inhibits completely the opposite tendency produced by alkalinity, that is, irregularity and asymmetry. In the stronger carbonate solutions, even in the earliest stages, the retarding and inhibiting effect of the carbonates suppresses completely any tendency for increased rate of development that would otherwise occur.

In the preceding set of experiments, the one in which Na_2CO_3 was employed gave some indication of the specific effect of the sodium; but since this is an alkaline salt, the modifications produced by its cation were complicated or completely masked by the greater effects of the anion and of the increasing alkalinity.

In the following series, neutral salts were employed, NaCl, in a series of iso- and hyper-tonic solutions.

Experiment 14. Plot 13. July 16, 1915

```
a..........................................Sea-water
b.......................................90 cc. S. W. + 10 cc. 0.52M NaCl
c.......................................80 cc. S. W. + 20 cc. 0.52M NaCl
d.......................................70 cc. S. W. + 30 cc. 0.52M NaCl
e.......................................60 cc. S. W. + 40 cc. 0.52M NaCl
f.......................................50 cc. S. W. + 50 cc. 0.52M NaCl
g.......................................40 cc. S. W. + 60 cc. 0.52M NaCl
```

The corresponding measurements were:

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a.............	22.83	69.91	84.10	88.10	89.13
b.............	25.74	72.94	88.17	92.01	93.31
c.............	20.91	68.24	85.23	89.90	91.65
d.............	16.27	61.70	81.82	87.61	89.94
e.............	9.90	54.19	78.43	84.73	
f.............	3.21	41.97	68.34		
g.............		26.62	28.73		

The individuals in this experiment were considerably larger than those of the general average for the corresponding period of that summer. The specimens in solution *b* exceeded in size those in *a* during the entire period; those in *c* and *d*, although smaller at the age of 24 hours, grew more rapidly and when 120 hours old, were larger than those of the control. Those in *e* underwent an initial inhibition, but at the age of 96 hours they attained almost as great a size as those in *a;* but failed to live through the following day.

If the production of the specific effects of the carbonate is due to the fact that molecular $MgCO_3$ and $CaCO_3$ are pushed
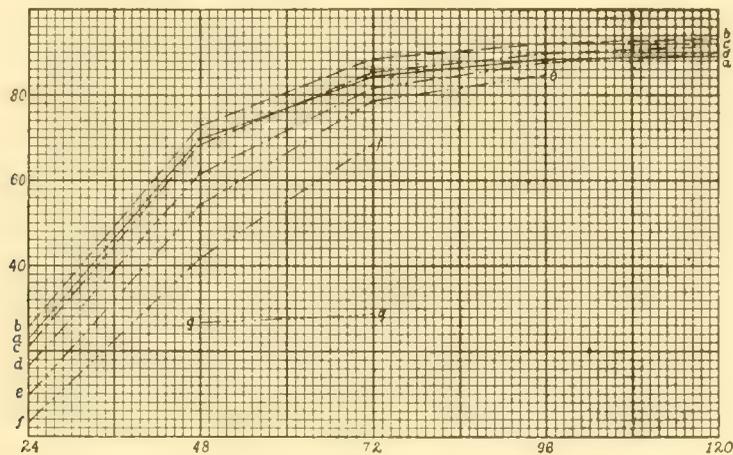
Plot 13

nearer to the point of saturation and rendered more available for the use of the embryo, then dilution of sea-water with NaCl should produce modifications consistent with a less saturated condition; and this is what actually occurs. The skeletons are more slender than in the normal individual, and are characterized by conspicuous perforations in the lattice-work of the arms, and thorn-like spines and processes of the body-rods.

Since the alkalinity of the sea-water is reduced by dilution with neutral NaCl solution, we might expect a reduction in size; but, on the contrary, an increase above the normal occurs— in Experiment 14 even at 30 per cent dilution. But it is significant that in solutions *c* and *d*, in which the specimens ultimately attained a greater maximum growth than did those in *a*, an inhibition was produced during the first day, the time

when alkalinity is most essential for growth. The increased growth might be accounted for by the fact that in the solutions in which sea-water is diluted with iso-tonic NaCl, a reduction of the salts of Mg and Ca, two elements antagonistic to Na, occurs, and the stimulating effect of the Na might be expressed in the increased size. This is the most striking modification produced by the NaCl solutions.
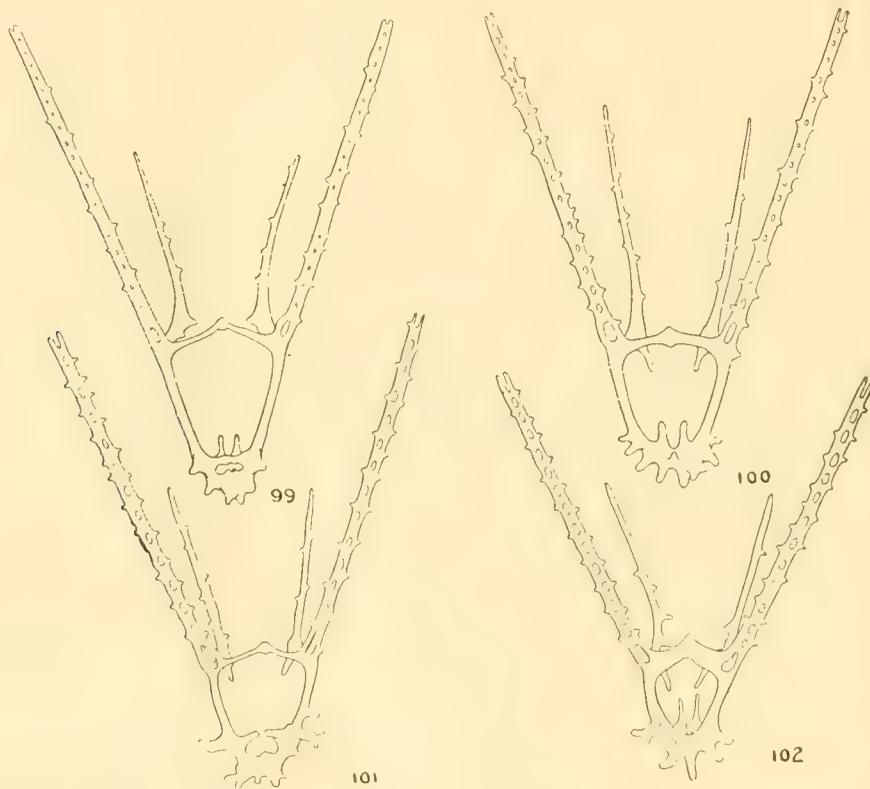
Figs. 99 to 102 Experiment 14. NaCl, 0.52M.
Fig. 99 14a, 72 hrs. Control.
Fig. 100 14c, figure 101 14e, figure 102 14f, 72 hrs. Characterized by reduced body-size and proportionately longer arms. The processes while not reduced in number are longer and more thorn-like. The lattice-work in the arms is more open, although the connecting branches are about as numerous as in the normal.

Another effect of increased NaCl content is the decreased length of the body-rods. Figures 99 to 102 illustrate all these modifications. Figure 99 represents the control at 72 hours; figure 100, one from culture c; figure 101, from e and 102 from f. In the solutions of gradually increased NaCl content, we may observe the longer and more spiked character of the typical proc-

esses, the greater prominence of the ventral and dorsal branches, the less highly fused condition of the arm-rods, and the reduced size of the body-rods. Were this last named characteristic not correlated with a proportionate increase of arm-length, we might ascribe it merely to the dilution of some necessary constituents of the sea-water, but since total size is not reduced, we must conclude that it is a specific effect of the Na.

In these solutions, then we have four specific effects:

a) A condition as to thickness of skeleton, diametrically opposed to that which occurs in the carbonate solutions, and expressed in slender, perforated skeletons with conspicuous processes.

b) A growth exceeding that of the normal in the solutions of slighter dilution, caused possibly by decrease in the proportion of Mg and Ca. This excessive growth occurs during the later periods.

c) A tendency toward inhibition of growth during the first day of the experiment, the time when decrease of alkalinity produces its most marked effect.

d) Decreased length of body-rods.

Experiment 15 was made with solutions of 0.56M NaCl. Three solutions were employed, a control, *a; b*, of 10 cc. 0.56M NaCl to 90 cc. sea-water; and *c*, of 20 cc. 0.56M NaCl to 80 cc. sea-water.

The corresponding measurements were:

Experiment 15. Plot 14. July 21, 1915

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a	4.27	56.82	73.13	83.12	86.01
b	7.53	55.76	72.19	82.09	86.96
c	3.25	52.84	71.37	81.80	83.77

In Experiment 16, NaCl was used in solutions of still higher concentrations:—

Experiment 16. Plot 15. July 21, 1915

a . . . . Sea-water
b . . . . 90 cc. S. W. + 10 cc. 0.60M NaCl
c . . . . 80 cc. S. W. + 20 cc. 0.60M NaCl
d . . . . 70 cc. S. W. + 30 cc. 0.60M NaCl
e . . . . 60 cc. S. W. + 40 cc. 0.60M NaCl
f . . . . 50 cc. S. W. + 50 cc. 0.60M NaCl
g . . . . 40 cc. S. W. + 60 cc. 0.60M NaCl
h . . . . 30 cc. S. W. + 70 cc. 0.60M NaCl
i . . . . 20 cc. S. W. + 80 cc. 0.60M NaCl
j . . . . 10 cc. S. W. + 90 cc. 0.60M NaCl
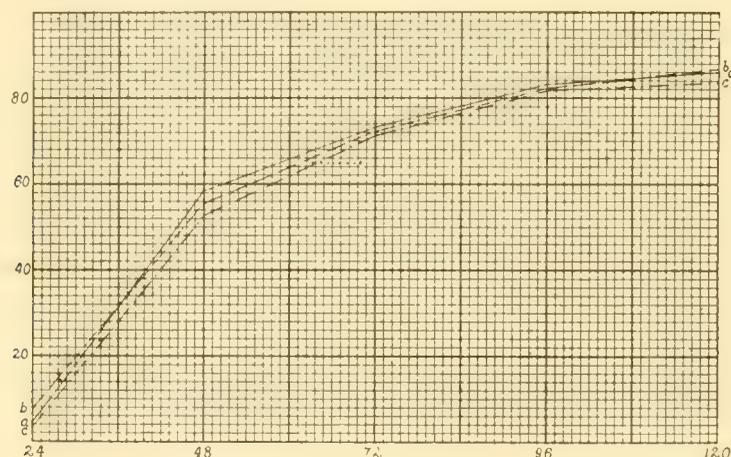
Plot 14

The corresponding measurements were:

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a	2.93	67.01	79.23	54.67	48.72
b	2.12	63.92	75.75	46.13	41.34
c	1.39	59.83	71.46	37.81	36.18
d	0.74	53.77	64.28	36.23	34.21
e	0.30	46.62	53.93	35.19	33.88
f	0.03	38.86	43.76	39.78	
g		30.24	31.42		
h		19.94	20.02		
i					
j					

The growth-curve for the control of Experiment 15 agrees very closely during the first three days with that of the gen-

eral average for the second period of the summer of 1915 (Plot 1 C) but during the following two days it increased more rapidly and exceeded the total average for the first part of the summer (Plot 1 B). In Experiment 16, the control showed the retarded growth characteristic of most of the experiments made later in the season. At the age of 24 hours, it was only 2.93 or 19.1 per cent of that of the total average of all the experiments (Plot 1 D) and 33.3 per cent of that of the general average for the later period of that season (Plot 1 C). Growth was very rapid the following two days, and at the age of 72 hours it reached the highest point in its curve. This maximum is considerably lower than that in any of the other experiments
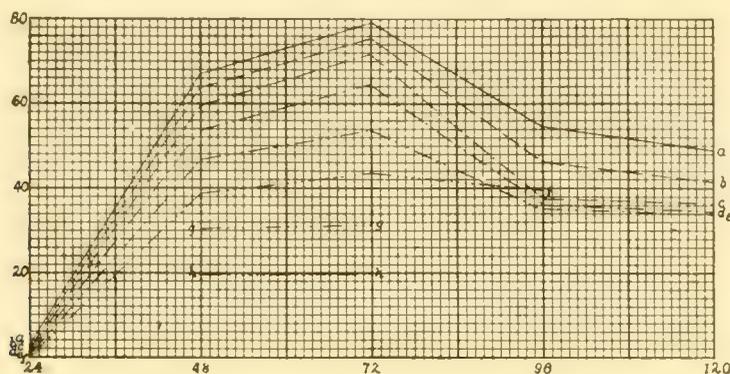
Plot 15

in which it has been attained precociously, and again demonstrates that rate of development is not necessarily correlated with size.

Figures 103 and 104 represent cultures *g* and *h* at 72 hours and show, together with the reduction in size obtained in hypertonic solutions, the typical abnormalities characteristic of NaCl solutions: comparatively long and slender oral arms, and short body-rods which at the posterior region are perforated by many openings. In cultures *i* and *j*, the eggs underwent cleavage but the larvae did not form skeletons.

The following measurements were taken from a similar set of cultures in which development was more gradual; the maximum was not reached until the 120th hour.

Experiment 17. Plot 16. July 24, 1915

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a...............	6.23	58.31	73.17	79.17	82.14
b...............	4.39	55.44	70.14	76.11	78.76
c...............	2.67	52.92	67.27	72.75	75.33
d...............	1.34	50.13	63.78	69.19	71.42
e...............	0.28	47.28	60.59	65.80	66.23
f...............		43.96	48.83	50.20	
g...............		33.08	34.05		
h...............		12.82			
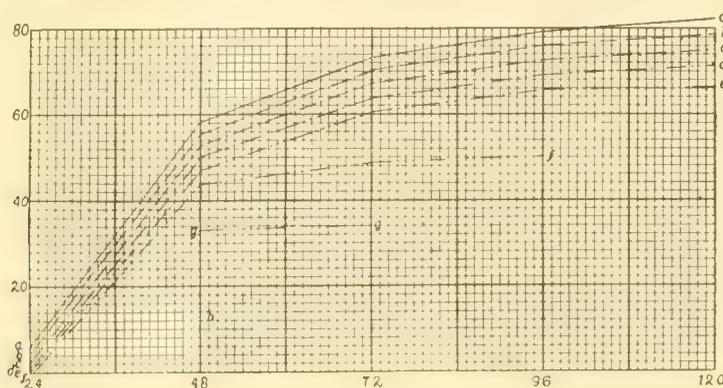
Plot 16

The control developed rather slowly at first, but ultimately reached a size slightly greater than that of the general average. In the experimental solutions, decrease of size was very slight and the various cultures maintained very nearly the same proportions throughout. In *e*, 40 cc. 0.60M NaCl to 60 cc. seawater, the solution of greatest NaCl percentage in which the specimens survived 120 hours, the average size was 80.6 per cent of that of the control. Except *h*, all the cultures in which the specimens formed skeletons lived the same length of time as did those in the previous experiment.

In the experiments in which the solutions of NaCl employed were of higher concentrations, an important variation in the medium was produced,—an increase of osmotic pressure. In solution 15*b*, the physical effect of the hypertonic solution was about sufficient to neutralize the physiological effect of the sodium, but in solutions of increased density, such as, 15*c*, 16*b*, 17*b*, etcetera, the inhibiting effect of the hypertonic solution outweighed the stimulating effect of the sodium.

Fischel ('09) used solutions of N/2 NaCl with sea-water in various proportions in his experiments with Echinus micro-tuberculatus, Strongylocentrotus lividus and Arbacia pustulosa. Only in the case of the first named did he obtain an acceleration in the rate of development; in the other two the opposite effect was produced. When he subjected the eggs of Arbacia, one half hour after fertilization, for the brief period of 30 minutes to a mixture of 87.5 parts sea-water to 12.25 parts 0.50M NaCl, they developed much more slowly than did the control. The inhibition was not especially apparent during cleavage, but appeared after blastula formation. He figures two which had been treated in this manner and at 3 days 20 hours and 4 days 18 hours respectively, they had not passed beyond a late gastrula stage. If this reaction of Arbacia pustulosa is typical, either some fundamental difference must exist between punctulata and the species employed by Fischel, or profound disturbances were set up when the embryos were transferred from the experimental solution back to sea-water. The slight change of osmotic pressure involved seems hardly adequate to account for the extensive injury incurred. Rather it seems necessary to conclude that the two species respond in a fundamentally different way to similar experimental conditions.

In order to ascertain the effects of NaCl in hypertonic solution without dilution of the other constituents of sea-water, the salt was added directly to the sea-water in the following amounts:—

Experiments 18 and 19

a..Sea-water
b..100 cc. S. W. + 0.255g. NaCl
c..100 cc. S. W. + 0.375g. NaCl
d..100 cc. S. W. + 0.50 g. NaCl

From these two experiments with similar solutions, the following results were obtained:

Experiment 18. Plot 17. September 9, 1914. Tables 11 to 14

	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a...............	40.57	58.90	71.62	77.46
b...............	28.46	46.15	56.60	63.39
c...............	11.60	28.03	39.25	45.77
d...............	0.67	7.68	17.88	27.61

Experiment 19.   Plot 18.   September 9, 1914

	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a	30.22	45.46	59.43	67.55
b	2.70	27.42	40.26	45.85
c	.	11.80	27.77	31.45
d			7.91	
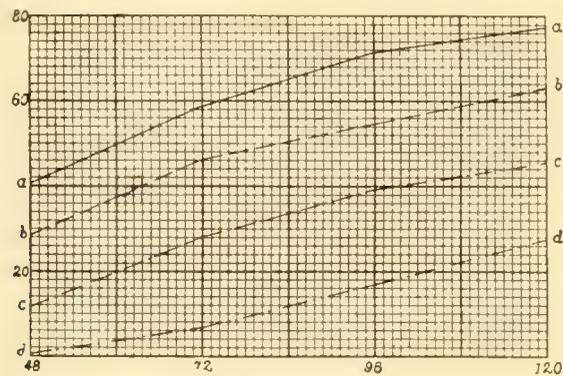
Plot 17
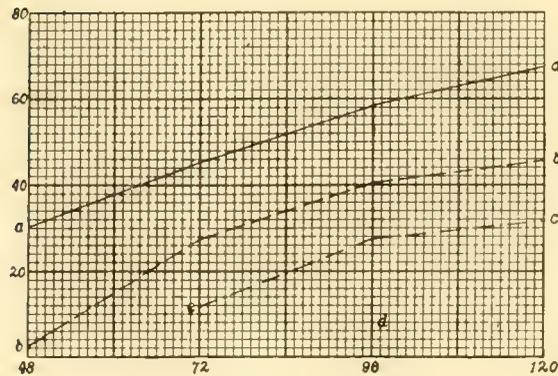
Plot 18

These two experiments were performed very late in the summer of 1914, and, as may be seen, even the controls developed so slowly that they did not possess skeletons at the age of 24 hours. In Experiment 18, however, rate of growth was fairly rapid the following day and at the age of 48 hours the specimens were 10.5 per cent smaller than the average for that period.

In these cultures we find some of the modifications typical of hypertonic solutions and some of those specific to NaCl. Figures 105 to 116 constitute a series of drawings from the various cultures of Experiment 18. In solutions of pure sea-water

concentrated by evaporation there is, as we have seen, a tendency toward increase in the number of spines and other accessory processes. This also is typical for NaCl solutions, in which these processes assume slender, spike-like forms. Under both variations of the medium, asymmetry appears in the stronger solutions. The main point of difference between the effects of these two changes of the medium is that in the pure, concentrated sea-water, fusion of the arm-rods occurs rather

TABLE 11

Constants, Experiment 18. Solution a (control)

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
48 hours	Right anal..........	40.57 ± 0.20	2.96 ± 0.14	7.31 ± 0.35
	Left anal...........	40.51 ± 0.19	2.93 ± 0.13	7.24 ± 0.34
	Right oral..........	30.71 ± 0.09	1.40 ± 0.06	4.56 ± 0.21
	Left oral...........	30.74 ± 0.09	1.40 ± 0.06	4.56 ± 0.21
72 hours	Right anal..........	58.90 ± 0.20	3.09 ± 0.14	5.25 ± 0.25
	Left anal...........	58.90 ± 0.20	3.08 ± 0.14	5.23 ± 0.25
	Right oral..........	41.89 ± 0.18	2.71 ± 0.12	6.49 ± 0.30
	Left oral...........	41.90 ± 0.18	2.70 ± 0.12	6.46 ± 0.30
96 hours	Right anal..........	71.62 ± 0.25	3.76 ± 0.17	5.25 ± 0.25
	Left anal	71.45 ± 0.26	3.86 ± 0.18	5.40 ± 0.25
	Right oral..........	47.10 ± 0.18	2.75 ± 0.13	5.84 ± 0.27
	Left oral...........	47.15 ± 0.18	2.77 ± 0.13	5.88 ± 0.28
120 hours	Right anal..........	77.46 ± 0.29	4.36 ± 0.20	5.64 ± 0.26
	Left anal...........	77.40 ± 0.28	4.16 ± 0.19	5.38 ± 0.25
	Right oral..........	51.67 ± 0.20	3.06 ± 0.14	5.93 ± 0.28
	Left oral...........	51.63 ± 0.21	3.12 ± 0.14	6.05 ± 0.29

early, whereas in the NaCl solutions, a retardation of the process is evident.

The specimens in the cultures made hypertonic by the addition of NaCl show the modifications common to the two methods discussed above,—that is, prominent processes and slight irregularities of structure. In respect to fusion of the arm-rods, they agree more closely with the specimens in the solutions in which sea-water was diluted with iso-tonic NaCl, in that a retardation of this process takes place. It is evident, then, that

TABLE 12

Constants, Experiment 18. Solution b

		MEAN	STANDARD DEVIATION	COEFFICIENT OF CORRELATION
48 hours	Right anal	28.46 ± 0.23	3.46 ± 0.16	12.15 ± 0.58
	Left anal	28.46 ± 0.23	3.46 ± 0.16	12.15 ± 0.58
	Right oral	28.58 ± 0.23	3.52 ± 0.16	12.34 ± 0.59
	Left oral	28.58 ± 0.23	3.52 ± 0.16	12.34 ± 0.59
72 hours	Right anal	46.15 ± 0.25	3.80 ± 0.18	8.25 ± 0.39
	Left anal	46.03 ± 0.25	3.74 ± 0.17	8.13 ± 0.38
	Right oral	35.47 ± 0.17	2.55 ± 0.12	7.20 ± 0.34
	Left oral	35.08 ± 0.16	2.51 ± 0.12	7.17 ± 0.34
96 hours	Right anal	56.60 ± 0.29	4.34 ± 0.20	7.68 ± 0.36
	Left anal	56.64 ± 0.29	4.37 ± 0.20	7.71 ± 0.37
	Right oral	38.23 ± 0.20	2.97 ± 0.14	7.79 ± 0.37
	Left oral	38.21 ± 0.20	2.97 ± 0.14	7.79 ± 0.37
120 hours	Right anal	63.39 ± 0.35	5.30 ± 0.25	8.37 ± 0.40
	Left anal	63.42 ± 0.35	5.29 ± 0.25	8.35 ± 0.40
	Right oral	39.34 ± 0.21	3.26 ± 0.15	8.28 ± 0.39
	Left oral	39.34 ± 0.23	3.45 ± 0.16	8.78 ± 0.42

TABLE 13.

Constants, Experiment 18. Solution c

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
48 hours	Right anal	11.60 ± 0.24	3.66 ± 0.17	31.55 ± 1.64
	Left anal	11.60 ± 0.24	3.66 ± 0.17	31.55 ± 1.64
	Right oral	11.96 ± 0.23	3.45 ± 0.16	28.86 ± 1.48
	Left oral	11.96 ± 0.23	3.45 ± 0.16	28.86 ± 1.48
72 hours	Right anal	28.03 ± 0.28	4.17 ± 0.19	14.90 ± 0.72
	Left anal	27.89 ± 0.26	3.97 ± 0.18	14.25 ± 0.69
	Right oral	20.23 ± 0.14	2.13 ± 0.10	7.05 ± 0.33
	Left oral	20.32 ± 0.15	2.24 ± 0.10	7.40 ± 0.35
96 hours	Right anal	39.25 ± 0.36	5.37 ± 0.25	13.69 ± 0.66
	Left anal	39.37 ± 0.36	5.40 ± 0.25	13.72 ± 0.66
	Right oral	29.44 ± 0.23	3.47 ± 0.16	11.79 ± 0.57
	Left oral	31.50 ± 0.18	2.78 ± 0.13	8.83 ± 0.42
120 hours	Right anal	45.77 ± 0.42	6.34 ± 0.30	13.86 ± 0.66
	Left anal	45.83 ± 0.42	6.23 ± 0.29	13.60 ± 0.66
	Right oral	33.10 ± 0.22	3.30 ± 0.15	9.99 ± 0.53
	Left oral	33.02 ± 0.30	4.50 ± 0.21	13.63 ± 0.66

TABLE 14

Constants, Experiment 18. Solution d

		MEAN	STANDARD DEVIATION	COEFFICIENT OF CORRELATION
48 hours	Right anal	0.67 ± 0.08	1.29 ± 0.06	193.45 ± 26.78
	Left anal	0.76 ± 0.09	1.42 ± 0.06	187.11 ± 25.24
	Right oral	0.67 ± 0.08	1.29 ± 0.06	193.45 ± 26.78
	Left oral	6.76 ± 0.09	1.42 ± 0.06	187.11 ± 25.24
72 hours	Right anal	7.68 ± 0.35	5.24 ± 0.25	68.33 ± 4.32
	Left anal	7.74 ± 0.33	3.91 ± 0.23	63.51 ± 4.02
	Right oral	7.68 ± 0.35	5.24 ± 0.25	68.33 ± 4.32
	Left oral	7.74 ± 0.33	3.91 ± 0.23	63.51 ± 4.02
96 hours	Right anal	17.88 ± 0.45	6.80 ± 0.32	38.05 ± 2.06
	Left anal	17.88 ± 0.45	6.80 ± 0.32	38.05 ± 2.06
	Right oral	17.88 ± 0.44	6.66 ± 0.31	37.28 ± 2.01
	Left oral	17.88 ± 0.44	6.68 ± 0.31	37.28 ± 2.01
120 hours	Right anal	27.61 ± 0.42	6.33 ± 0.30	22.94 ± 1.15
	Left anal	27.85 ± 0.45	6.74 ± 0.32	24.20 ± 1.22
	Right oral	18.38 ± 0.35	5.25 ± 0.25	28.56 ± 1.44
	Left oral	18.28 ± 0.38	5.67 ± 0.27	31.05 ± 1.11

TABLE 15

Constants, Experiment 19. Solution a (control)

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
48 hours	Right anal	30.22 ± 0.15	2.35 ± 0.11	7.78 ± 0.37
	Left anal	30.22 ± 0.15	2.35 ± 0.11	7.78 ± 0.37
	Right oral	30.20 ± 0.15	2.33 ± 0.11	7.72 ± 0.37
	Left oral	30.20 ± 0.15	2.33 ± 0.11	7.72 ± 0.37
72 hours	Right anal	45.46 ± 0.17	2.53 ± 0.12	5.56 ± 0.26
	Left anal	45.43 ± 0.16	2.50 ± 0.11	5.51 ± 0.26
	Right oral	32.03 ± 0.10	1.60 ± 0.07	5.00 ± 0.23
	Left oral	32.03 ± 0.10	1.59 ± 0.07	4.97 ± 0.23
96 hours	Right anal	59.43 ± 0.20	3.04 ± 0.14	5.11 ± 0.24
	Left anal	59.47 ± 0.21	3.11 ± 0.14	5.23 ± 0.24
	Right oral	41.35 ± 0.19	2.93 ± 0.13	7.09 ± 0.34
	Left oral	41.41 ± 0.19	2.95 ± 0.14	7.13 ± 0.34
120 hours	Right anal	67.55 ± 0.21	3.16 ± 0.15	4.69 ± 0.22
	Left anal	67.45 ± 0.21	3.16 ± 0.15	4.68 ± 0.22
	Right oral	45.38 ± 0.19	2.89 ± 0.13	6.38 ± 0.30
	Left oral	45.41 ± 0.19	2.81 ± 0.13	6.20 ± 0.29

TABLE 16

Constants, Experiment 19.　Solution b

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
48 hours	Right anal............	2.70 ± 0.11	1.70 ± 0.08	62.96 ± 4.02
	Left anal............	2.70 ± 0.11	1.70 ± 0.08	62.96 ± 4.02
	Right oral............	2.83 ± 0.11	1.69 ± 0.08	59.76 ± 3.73
	Left oral............	2.83 ± 0.11	1.69 ± 0.08	59.76 ± 3.73
72 hours	Right anal............	27.42 ± 0.24	3.68 ± 0.17	13.42 ± 0.65
	Left anal............	27.42 ± 0.24	3.68 ± 0.17	13.42 ± 0.65
	Right oral............	23.26 ± 0.08	1.27 ± 0.06	5.49 ± 0.26
	Left oral............	23.21 ± 0.09	1.33 ± 0.06	5.75 ± 0.27
96 hours	Right anal............	40.26 ± 0.27	4.02 ± 0.19	10.00 ± 0.48
	Left anal............	40.32 ± 0.26	3.92 ± 0.18	9.74 ± 0.46
	Right oral............	35.00 ± 0.16	2.51 ± 0.12	7.19 ± 0.33
	Left oral............	35.06 ± 0.17	2.56 ± 0.12	7.32 ± 0.34
120 hours	Right anal............	45.85 ± 0.25	3.74 ± 0.17	8.17 ± 0.38
	Left anal............	45.78 ± 0.25	3.75 ± 0.17	8.19 ± 0.39
	Right oral............	38.53 ± 0.23	3.43 ± 0.16	8.91 ± 0.42
	Left oral............	38.61 ± 0.23	3.41 ± 0.16	8.84 ± 0.42

TABLE 17

Constants, Experiment 19.　Solution c

		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
72 hours	Right anal............	11.80 ± 0.51	7.62 ± 0.36	64.64 ± 4.17
	Left anal............	11.80 ± 0.51	7.62 ± 0.36	64.64 ± 4.17
	Right oral............	11.84 ± 0.50	7.46 ± 0.35	63.07 ± 4.03
	Left oral............	11.84 ± 0.50	7.46 ± 0.35	63.07 ± 4.03
96 hours	Right anal............	27.77 ± 0.29	4.30 ± 0.20	15.49 ± 0.75
	Left anal............	27.77 ± 0.29	4.30 ± 0.20	15.49 ± 0.75
	Right oral............	27.89 ± 0.29	4.37 ± 0.20	15.67 ± 0.76
	Left oral............	27.89 ± 0.29	4.37 ± 0.20	15.67 ± 0.76
120 hours	Right anal............	31.45 ± 0.27	4.08 ± 0.19	12.97 ± 0.62
	Left anal............	31.42 ± 0.27	4.15 ± 0.19	13.20 ± 0.63
	Right oral............	28.21 ± 0.17	2.58 ± 0.12	9.16 ± 0.44
	Left oral............	28.22 ± 0.16	2.48 ± 0.11	8.81 ± 0.42

TABLE 18

Constants, Experiment 19. Solution d

		MEAN	STANDARD DEVIATION	COEFFICIENT OF CORRELATION
96 hours	Right anal............	7.91 ± 0.54	8.10 ± 0.38	102.50 ± 8.60
	Left anal............	7.91 ± 0.54	8.10 ± 0.38	102.50 ± 8.60
	Right oral........:...	9.19 ± 0.57	8.57 ± 0.40	93.24 ± 7.35
	Left oral............	9.19 ± 0.57	8.57 ± 0.40	93.24 ± 7.35

although NaCl is present in highest quantities in sea-water, the effects of concentration by evaporation are not merely specific for NaCl but are complicated by the presence of the other constituents, which are increased in the same proportion.

Figure 105 is a drawing of a specimen from the control at 48 hours and figure 106, of a specimen from solution *b*. In the latter there occurs a fusion of the posterior body-rods with developing processes, in contrast to the simple unfused condition of the corresponding elements in *a*. The arm-rods, on the contrary, are still separate, and fusion is only slightly more advanced in 108, which represents a specimen from the same culture 72 hours old. In figure 109, *c* 72 hours, the characteristics depicted are the same but are more pronounced. Figures 111 and 112 are taken from *b* and *c* at the age of 96 hours. In the latter, normal fusion of the arm-rods has taken place but the long spine-like processes are conspicuous. Figures 114 and 115 represent the same cultures at the age of 120 hours and in most respects are characteristic. In solution *d* (fig. 116), inhibition has been so great that a true pluteus form is not attained by the embryo and only the rudiments of arm-processes are acquired. This is in marked contrast to the results of experiments in which isotonic NaCl solutions are substituted for portions of the sea-water, as in the latter case, body-size is greatly decreased in proportion to arm-length.

The hydroxyl ion, the carbonates and the sodium, are all studied in detail by Herbst ('03), together with the other constituents of sea-water, in an effort to determine the exact rôle of each in development. In investigating this problem, he employed artificial sea-water from which the element under con-

sideration was omitted and noted the resulting abnormalities. Whatever processes were modified or inhibited he assumed to be directly affected by the lack of that substance.

Sodium and hydroxyl he looked upon as general conditions of development, since their omission from the medium resulted in complete inhibition of all the life processes. By general condition he does not mean that the substance is necessarily indispensible for every separate process, but for some one or more fundamental process operating at each period of development. He contrasts the rôle of calcium to that of sodium in its effect on the cohesion of the cells and ascribes the injurious action of the Ca-free medium to the "Auflockerung des Zellen-verbandes," which he assumes to be caused directly by the sodium. It may be that this lack of cohesion of the cells is concerned with the increased size of the larvae in the NaCl solutions.

Herbst assigns an important rôle to the carbonates in skeleton-formation, since in the alkaline CO_2-free medium only small, rudimentary tri-radiate spicules are laid down. Later on, however, the same larvae may begin to form arms and become fairly normal. He suggests that the secondary regulation may

Figs. 103 and 104   Experiment 16.   NaCl, 0.60 M.

Fig. 103   16g, 72 hrs.   Greatly reduced in size, but proportions maintained about as in the normal.   Many perforations in the posterior fused portion of the body-rods.   Anal arms unfused except for one cross-branch.   Processes all very long and prominent.   Oral arms elongated.

Fig. 104   16h, 72 hrs.   Still further reduction in size, about 1/5 the size of the control.   All the typical modifications for NaCl solutions accentuated.

Figs. 105 and 106   Experiment 19.   Sea-water + NaCl.

Fig. 105   19a, 48 hrs.   Control.

Fig. 106   19b, figure 107 19c, 48 hrs.; figure 108 19b, figure 109 19c, figure 110 19d, 72 hrs.; figure 111 19b, figure 112 19c, figure 113 19d, 96 hrs.; figure 114 19b figure 115 19c, figure 116 19d. 120 hrs.   In the stronger solutions increasingly great reduction in size, and early fusion of the body-rods, followed by develop-

ment of numerous heavy processes in the posterior region. Fusion of the arm-rods inhibited. Irregularity of structure. In d, figures 113 and 116, a typical pluteus-like form is not attained. This is in marked contrast to the solutions in which isotonic NaCl is substituted for portions of the sea-water. In the latter, arm-length is increased in proportion to body-length.

be due to the formation of H_2CO_3 from the CO_2 of the air, since $H_2CO_3 + 2KOH \rightleftarrows K_2CO_3 + 2H_2O$.

Herbst considers hydroxyl also an indispensable constituent of the medium for sea-urchin development and it is significant that the processes which he finds especially affected are those occurring early in development,—for example, fertilization and the formation of the fertilization membrane. In his artificial medium the optimum concentration varied in the different forms employed. For Echinus it lay deeper than for Spaerechinus and the spermatozoa could endure a lower percentage than the ova. Cleavage was accelerated up to a certain degree of alkalinity; size and symmetry of the larva were also affected, and were decreased both above and below a certain degree of the hydroxyl.

Herbst undertook to determine whether acids were evolved by the embryos in the process of development and found by the employment of various indicators that no strong acid was given off. He performed a series of experiments in aerating his OH-free water with

a), air containing CO_2,

b), CO_2 free air,

c), CO_2 and NH_3 free air,

and concluded that the beneficial effect of the hydroxyl ions is to neutralize the carbonic acid which is present in the water in greater or less amounts, and which may be increased through the metabolism of the animals.

In the experiments recorded in this paper, however, the solutions in which the eggs were developing were changed so often during the first few hours after fertilization, that no opportunity was given for a decided accumulation of the products of metabolism. Hence the neutralization of acids could not be urged as an explanation of the fact that addition of NaOH caused increase of rate of development. Even did this occur, still higher concentration of hydroxyl would not produce increasingly greater acceleration.

The experiments of this series indicate, then, that:—

a) Addition of acids produces an inhibitory effect upon growth.

b) Increase of alkalinity above the normal causes acceleration, accompanied by irregularity of form, and finally, inhibition. When this increase of alkalinity is slight, the resulting injury is small, but with greater concentrations of the OH ion the effect is correspondingly more pronounced. In very strong solutions, varying from two to three cc. of N 10 NaOH per 100 cc. of sea-water, cleavage is irregular, and abnormal blastulae are formed which fail to undergo gastrulation.

c) Addition of carbonates produces a double effect:—

1) Increase of OH concentration, by which the typical effect of increased alkalinity upon rate of growth is brought about.

2) Increased bulk of skeleton, due possibly to the fact that the Mg and Ca carbonates are nearer to the point of saturation in the modified medium.

The following experiment was made for the purpose of determining the effects upon development of carbon dioxide, the product of metabolism present in greatest proportions in the sea-water. To what extent it could exert any influence upon the young sea-urchins in their natural environment must be largely a matter of conjecture, but some effect is to be inferred from the fact that very minute amounts of acid produce marked inhibition in development and decrease of size.

A saturated solution of CO_2 in sea-water was prepared and, for the various cultures, portions of this were diluted with sea-water as tabulated below. The solutions were changed the usual number of times until the blastulae began to swim, at about six hours after fertilization, then three times daily. Each time, they were prepared immediately before using, so as to maintain as nearly as possible a constant amount of CO_2 in the medium. The following concentrations were employed:

Experiment 20.   Plot 19.   July 2, 1915

a.....................Sea-water
b.....................99.5 cc. S. W. + 0.5 cc. S. W. saturated with CO_2
c.....................99.0 cc. S. W. + 1.0 cc. S. W. saturated with CO_2
d.....................98.5 cc. S. W. + 1.5 cc. S. W. saturated with CO_2
e.....................98.0 cc. S. W. + 2.0 cc. S. W. saturated with CO_2
f.....................97.5 cc. S. W. + 2.5 cc. S. W. saturated with CO_2
g.....................97.0 cc. S. W. + 3.0 cc. S. W. saturated with CO_2

The corresponding measurements were:

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a...............	6.01	64.23	77.47	81.04	83.14
b...............	5.24	61.13	73.20	76.15	77.47
c...............	4.52	57.85	68.77	72.21	73.19
d...............	4.09	54.88	65.86	67.89	68.97
e...............	3.71	54.91	62.69	64.91	65.37
f...............	3.41	49.72	59.90	61.88	62.03
g...............	3.20	47.80	57.13	58.79	

The specimens in the control culture for the above experiment developed slowly at first, being much smaller than the general average, but on the following day they increased more rapidly and at the close of the experiment very slightly surpassed the general average in size.
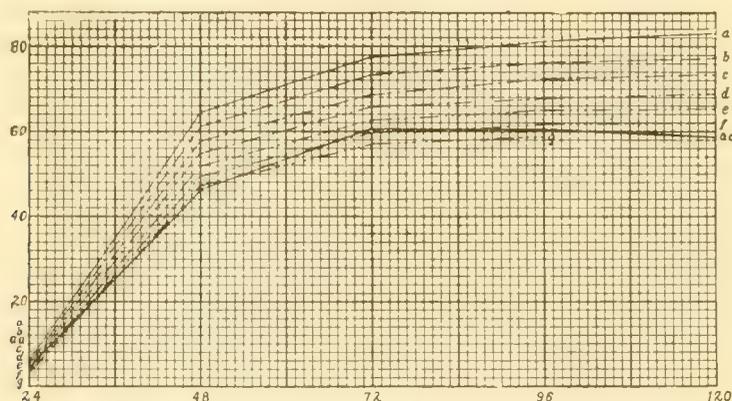
Plot 19

The curves constructed from the averages of the specimens in the experimental solutions are remarkable for their regularity, each indicating a slightly greater inhibition of growth than does that of the preceding one. Even the specimens in culture g, which lived only 96 hours, possessed a fairly high and regular growth curve, which was not at any time outside of the range of the control cultures, Plot 1, F.

Figures 117 to 142 are drawn from the various cultures on succeeding days. Figures 117 to 121 are from specimens 24 hours old and illustrate the most marked characteristics produced by subjection to CO_2,—the appearance of radial symmetry.

Figure 117 is from the control, 118 from solution d, 119 from e, 120 and 121 from f. In the control (fig. 117) all the specimens examined were strictly bilateral and the same was true in b. In c a very few, not more than one or two in each hundred measured had three spicules present at the age of 24 hours, but in solution d (fig. 118) this phenomenon was rather more common and appeared in about ten per cent of the specimens. In solution e (fig. 119), four spicules were developed in a still larger percentage, while some possessed three and the remainder, two. In solution f, four seemed to be the predominating number among those in which radial symmetry was produced (fig. 120), while many possessed three and a few, five (fig. 121). In solutions of increasing CO_2 content, there was very little inhibition of growth, as may be seen from the figures, and in each specimen all the spicules were apparently equal and normally shaped.

Figures 122, 123 and 124 are from solutions a, b and c respectively at the age of 48 hours. The skeletons are approximately of the same size, but those in the CO_2 cultures are heavier and slightly less regular. This change is still more marked in solution d (fig. 125) and in addition a small accessory spicule is present in the right side. These accessory spicules were much less frequent in the specimens 48 hours old than in those 24, and in many cases were still smaller than at the early period, indicating that resorption was taking place. Figure 126 illustrates another specimen from the same culture. Its skeleton is somewhat more regular and is not so heavy as that represented in figure 125, but the accessory spicule which lies at the left side has enlarged and possesses the main parts characteristic of normal skeletons: body-rod, dorso-ventral connective, anal and oral arm-rods. In the right side, compensatory structures have developed in the form of accessory rods on the lateral connectives. In the side in which the accessory skeleton is present, the arm-rod is more slender, and instead of the usual three, is composed of two single rods in a less advanced stage of fusion.

Figures 127 and 128 are from solution e. The specimen represented in 127 was smaller but the skeleton was heavier and more irregular. In 128, four spicules are present but although irregu-

lar and abnormal, they are of approximately equal size. Figures
129, 130 and 131 are from culture f. Figure 129 represents a
specimen below the average size, but it is regular and about like
that of a normal individual 36 hours old. In figure 130 the actual
length of the skeleton is very little greater but the form more
nearly approaches that of a true pluteus. It is still more irregu-
lar than any of the preceding and has a small accessory spicule
in the right side. In figure 131, skeletal structures are present
but while they are large and massive, they have not passed be-
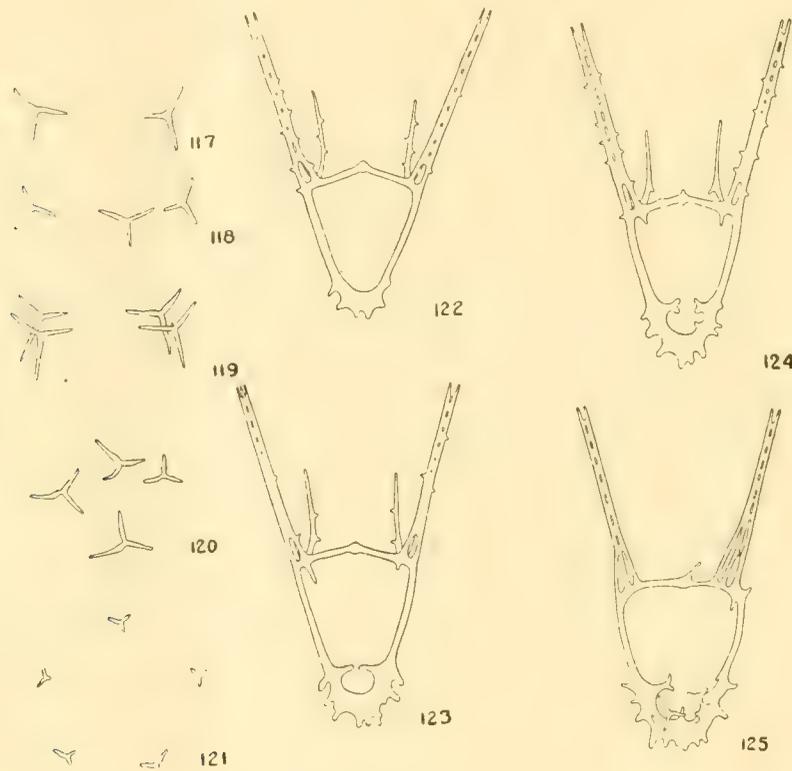
Figs. 117 to 142. Experiment 20. CO_2.
Fig. 117   20a, 24 hrs.   Control.
Fig. 118   20d, 24 hrs.   Three skeletal centers of about normal size.
Fig. 119   20e, 24 hrs.   Four centers. Size approximately normal.
Fig. 120   20f, 24 hrs.   Four centers.
Fig. 121   20f, 24 hrs.   Five centers. Reduction in size.
Fig. 122   20a. 48 hrs.   Control.
Fig. 123   20b, 48 hrs.   Skeleton somewhat heavier and less regular. About
the same size as control.
Fig. 124   20c, 48 hrs.   Same characters more pronounced.
Fig. 125   20d, 48 hrs.   Specimen less symmetrical. Accessory spicule present
on the right side.

yond the tri-radiate stage. Figures 132, 133 and 134 are from culture *g*. They are still smaller than those of solution *f*, but in the main show the same characteristics. Figure 132 is com-
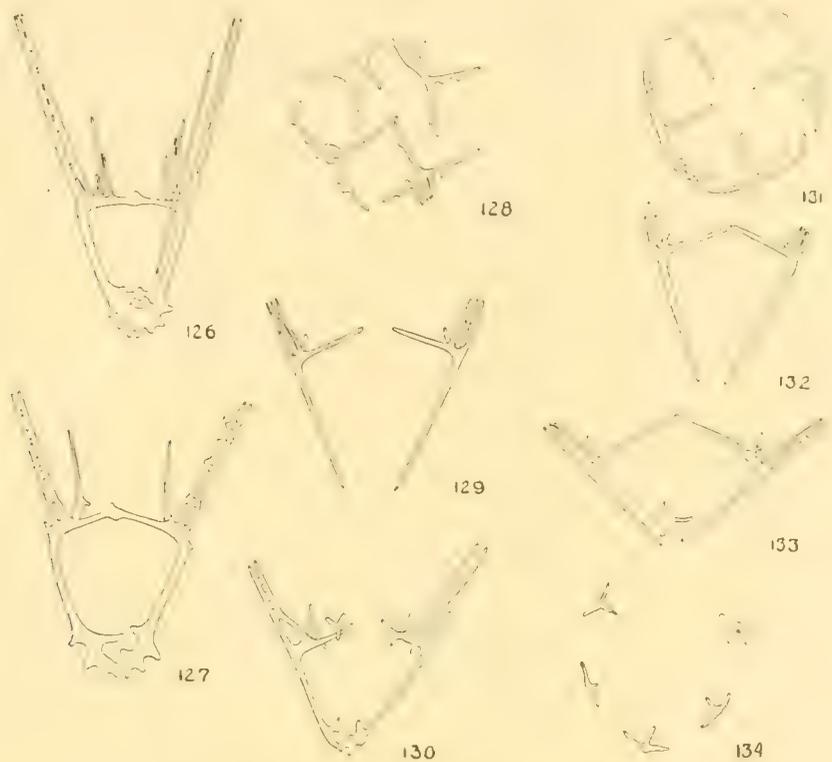
Fig. 126 20*d*, 48 hrs. Skeleton somewhat more regular and less heavy than the preceding, but an accessory skeleton on the left side has enlarged and developed most of the parts typical of a normal skeleton.

Fig. 127 20*e*, 48 hrs. Skeleton heavier, reduced in size, irregular. No accessory spicule.

Fig. 128 20*e*, 48 hrs. Four centers present, all of about equal size and highly abnormal.

Fig. 129 20*f*, 48 hrs. Inhibition of growth very evident. Skeleton about like that of a normal individual of 36 hrs. age.

Fig. 130 20*f*, 48 hrs. Skeleton of about the same size as that of figure 129, but more nearly of a true pluteus form. Irregular, and possessing accessory rods. Accessory spicule present on the right side.

Fig. 131 20*f*, 48 hrs. Four abnormal skeletal centers.

Fig. 132 20*g*, 48 hrs. Inhibition of growth still more pronounced than in figure 129. About like a normal individual of 18 hrs.

Fig. 133 20*g*, 48 hrs. Specimen about the same size as in figure 132, but with true pluteus form. Slightly irregular and with arm-rods unfused. Accessory spicule present.

Fig. 134 20*g*, 48 hrs. Five spicules present, which are irregular and still in the tri-radiate stage.

parable to 129, but indicates a slightly less advanced condition.
.Figure 133, like 130, is more irregular and possesses an acces-
sory spicule. In 134, five spicules are present, but are small
and even at this stage show decided irregularity of form.
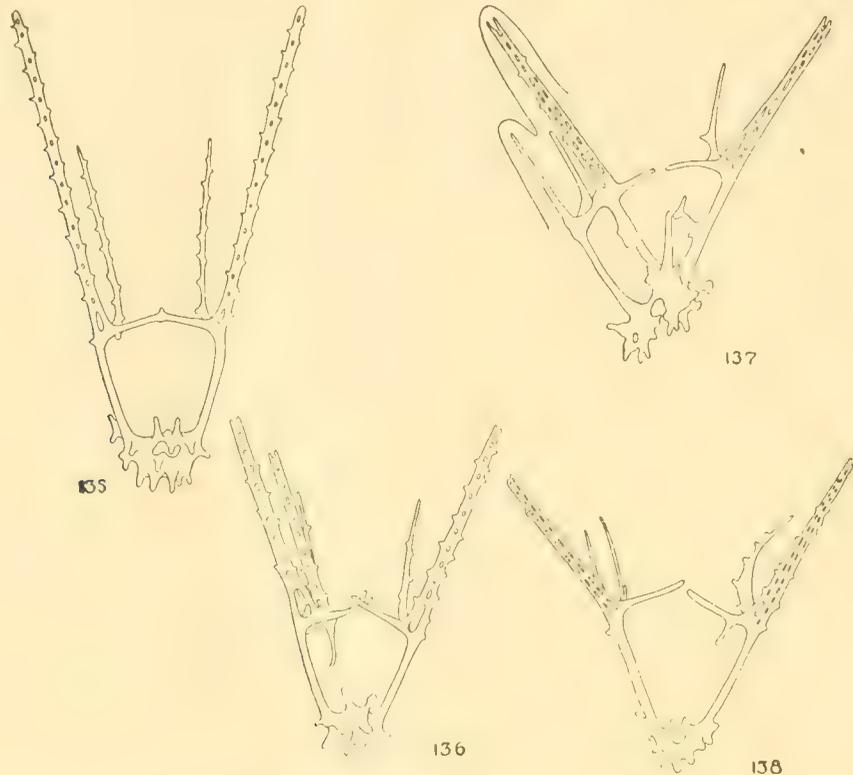
Fig. 135   20*a*, 72 hrs.   Control.
Fig. 136   20*e*, 72 hrs.   Skeleton smaller than control, but fairly normal.
Large accessory skeleton on the right, fused to the primary at the base of the
arm-rods.
Fig. 137   20*f*, 72 hrs.   Asymmetrical.   Several rods in each arm, with spines
missing from the right.   Accessory skeleton on the right side, with all the typi-
cal parts.   The anal rod of the secondary skeleton forms an accessory arm; and
the oral rod of the secondary functions instead of the oral rod of the primary,
which is missing on this side.
Fig. 138   20*f*, 72 hrs.   Slight irregularities.   Spines missing from the right
side.   Left oral arm is exceedingly heavy, while that on the right is of very
light structure and possesses an accessory rod.

Figure 135 represents a larva from the control 72 hours old.
In figure 136, solution *e*, the primary skeleton is smaller than
the normal, but otherwise is fairly regular.   In the right side
there is a large secondary skeletal structure, consisting of arm-
rods fused to about the same extent as those in the primary, short

body rod, and transverse rod. It is connected with and apparently fused to the primary skeleton at the base of the arm-rods. Figures 137 and 138 are from *f*. Although the latter at first glance would seem to be rather symmetrical, closer inspection reveals that in many details the two sides are unlike. In the right,
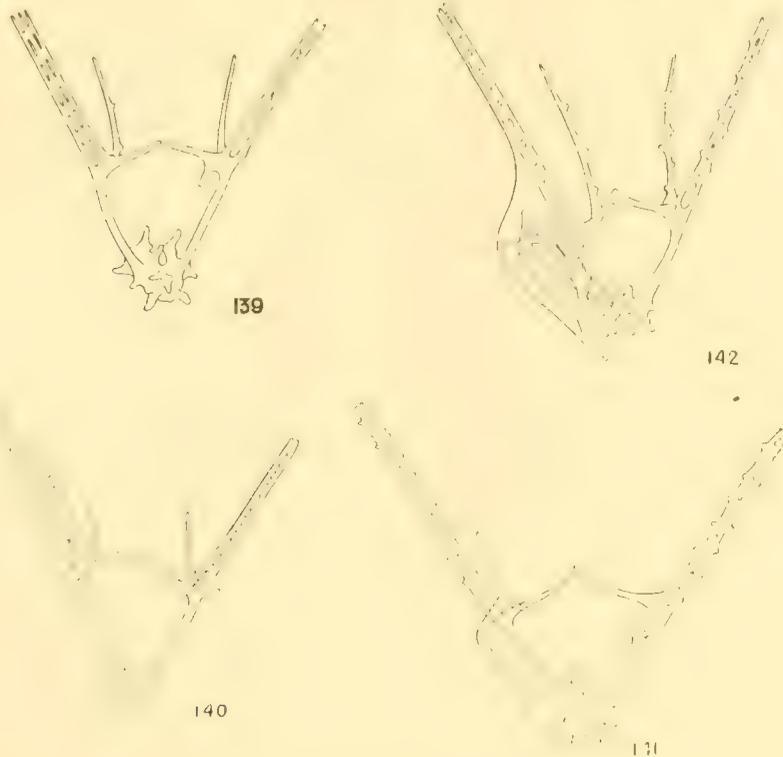

Fig. 139 20*g*, 72 hrs. General form of the pluteus highly symmetrical, but right side heavier than the left and four well fused rods present in the corresponding anal arm. Left anal arm possesses only two rods which are just beginning to unite in two places.

Fig. 140 20*g*, 72 hrs. In this also, the right side of the skeleton is much heavier than the left, and possesses an accessory skeleton. Right oral arm is lacking in the primary structure, and its place is supplied by that of the secondary. This oral arm is small and gives the pluteus an abnormal form.

Fig. 141 20*f*, 96 hrs. Primary skeleton highly symmetrical. Accessory spicule on the left side and secondary skeleton on the right, having a well developed body-rod with ventral branches. The other portions cannot be positively identified.

Fig. 142 20*g*, 96 hrs. Primary skeleton less regular. Secondary skeleton on right side, consisting of two body-rods with ventral branches, and dorsoventral connectives (?) with dorsal body-branches. The secondary skeleton probably originated from two centers both of which subsequently shifted to the right side.

spines are missing from the arm-rods, while in the left they are
abundant and are heavy and irregular. In this side the oral
arm is abnormally massive, whereas the corresponding struc-
ture in the opposite side is slender and has an accessory rod.
In figure 137 a secondary skeleton is present in the right side.
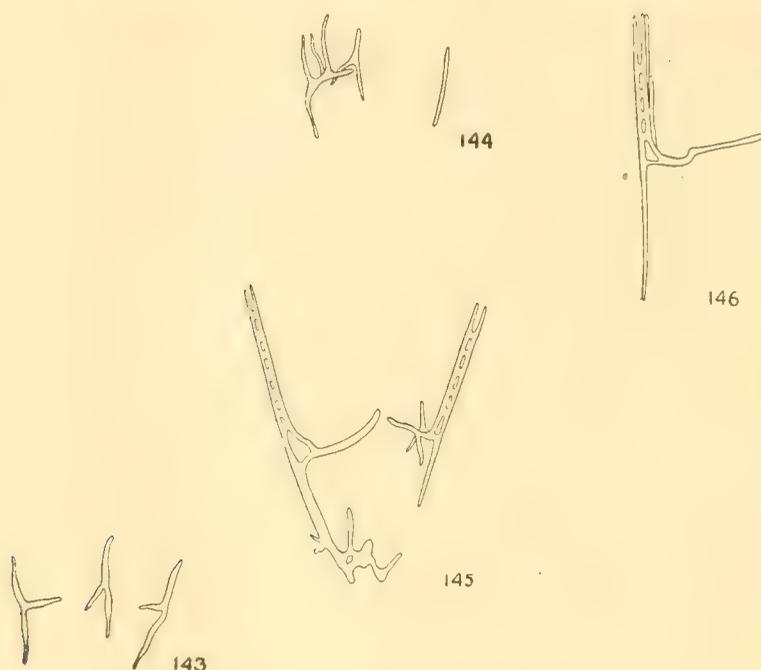
Figs. 143 to 146 Experiment 20. Stale sea-water.
Fig. 143 20*aa*, 24 hrs. Three spicules present, radially arranged, but irregular
in form.
Fig. 144 20*aa*, 24 hrs. Three centers again present, but extremely irregular.
One spicule is small and abnormal, but has some of the typical parts,—body-rod,
arm-rods and lateral connective. One is still tri-radiate, while the third is in
the form of a straight rod.
Fig. 145 20*aa*, 48 hrs. Skeleton on right side somewhat irregular, but pos-
sessing all the normal parts excepting dorso-ventral connective and oral arm-
rod. On the left side these are present but the body-rod is incomplete. That
of the right side has bent forward and is developing a compensatory structure.
Fig. 146 20*aa*, 48 hrs. Only one skeletal structure present which lacks dorso-
ventral connective and anal arm.

It possesses arm-rods, dorso-ventral connective and body-rod
with ventral branches by which it is fused to the primary skele-
ton. It is also joined by what apparently corresponds to the
lateral connective. Its anal rod is not so closely applied to
that of the primary skeleton, as are those in the specimens pre-
viously considered, and here the pluteus has developed an ac-

cessory arm, as indicated by the outline in the drawing. The oral arm-rod is lacking from the primary skeleton, and the corresponding structure of the secondary functions in its stead; but since the point of origin of this secondary oral arm-rod is lower in the body of the pluteus than that of the primary of the opposite side, the animal, in spite of a rather high degree of symmetry characterizing the primary skeleton, is irregular and abnormal looking.

Figures 139 and 140 are from specimens of solution g, and are also 72 hours old. In the latter the accessory skeleton is present, and though smaller than the primary has nearly all the characteristic parts,—body-rod, anal arm-rod composed of two fused single rods, greatly reduced oral arm-rod and dorso-ventral connective. The primary and secondary skeletons are joined by the ventral branches of their body-rods. In this animal, the oral arm-rod, though much smaller than normal and united with the anal arm-rods by an exceedingly short dorso-ventral connective, likewise functions instead of the corresponding structure of the primary. Contrary to the usual compensatory regulation, the skeleton in the side possessing the accessory structure, is more massive than in the side where these parts are missing. In 139 also, one side of the skeleton is much heavier than the other, and the anal arms possess three well fused rods in contrast to two unfused ones in the opposite side.

Figure 142 is from the same culture after 96 hours of development. Here the accessory parts are more complicated and harder to identify. Two body-rods with corresponding ventral branches are evidently present, and the parts which are bent over and fused to the right body-rod of the primary skeleton are possibly dorso-ventral connectives with dorsal body-branches. Rudiments of anal arm-rods are present. This secondary skeleton may have originated from a single center, as in some cases it is known that one side of a skeleton may cross over and supply the missing parts on the opposite side; but this generally extends only to some one definite element and does not involve a duplication of all the structures. Hence it seems probable that this skeleton must have arisen from

two centers, which have grown very symmetrically as regards each other.

Figure 141 represents a specimen from culture f at an age of 96 hours. Here two secondary skeletons are present, that in the right having developed most of the characteristic parts while that in the left side has not grown beyond the tri-radiate stage.

This production of radial symmetry is the most characteristic change caused by the employment of carbon dioxide. Herbst obtained it in Spaerechinus and Echinus by reducing the amount of potassium or sulphate in the medium. In the latter case, the number of skeletal centers sometimes reached seven. With the addition of lithium halides he obtained a similar effect, which resulted in the appearance of three, four or five centers.

Fischel obtained radial symmetry by the employment of hypertonic KCl solutions, by which also the number might be increased to five. It seems difficult to reconcile the fact that absence of potassium and hypertonic solutions with an excess of potassium could produce the same result. Herbst suggests that the reduced larval size in K-free sea-water may be due to a failure on the part of the tissues to imbibe water in sufficient quantities. If this in any way affects the symmetry, we should expect the same results to be obtained with hypertonic solutions. This, however, does not occur in Fischel's other experiments nor is any evidence of it to be observed in the experiments with hypertonic solutions described above.

The appearance of an uneven number of skeletal centers cannot be due to mere doubling of that of one side. Herbst says, "Ist nur ein Dreistrahler vorhanden, so kann man wegen der unregelmäsigen Lage nicht sagen, ob dies der rechte oder linke ist, und ebensowenig kann man bei Vorhandensein von dreien behaupten, dass der linke oder der rechte verdoppelt sei." He describes the gradual appearance of bilateral symmetry thus; "Man kann aber trotzdem als Regeln bezeichnen, dass zwei von den Dreistrahlern, deren Lage mehr an die Norm erinnert, die anderen in der Entwickelung überholen so dass schliesslich

doch noch bilaterale Larven von annähernd Plutcusform aus den ursprünglich mehr radiär aussehenden Larven hervorgehen."

This is what occurs in Arbacia. During the first 24 hours, all the skeletal centers develop equally (figs. 118 to 121) and in very strong solutions the radial symmetry is maintained until later periods (figs. 128, 131 and 134, e, f and g, 48 hours). Either these larvae fail to develop further, or else, as Herbst describes, two spicules which most closely approximate the normal in position, elongate and give the animal a bilateral form. The other skeletal centers may remain without undergoing any change for some time, (fig. 133 g, 48 hours), but subsequently a shifting occurs, so that the centers come to lie upon one side or other of the larva. This is especially true when the third spicule ultimately develops into a secondary skeleton (fig. 126 d, 48 hours, etc.), and generally it is fused to one side of the primary skeleton (figs. 137 f, 72 hours and 140 g, 72 hours). In figure 141 f, 96 hours, an accessory skeleton has remained at each side, but in 142 g, 96 hours, both have shifted to one side and fused with the primary skeleton. In no instance did radial symmetry obtain in so late a stage as is shown by Herbst ('03), in his figure 13. When a third arm appears, as in figure 137 f, 72 hours, it is more or less closely applied to one of the primary.

Addition of CO_2 to the sea-water does not cause any tendency toward the appearance of those characteristics specific to the carbonate solutions. Since addition of CO_2 produces increase of CO_3 and HCO_3 ions, the absence of any tendency toward the carbonate effect when CO_2 is added, gives further evidence for the conclusion reached previously, that those modifications are caused by the molecular calcium and magnesium carbonates, rather than by the CO_3 or HCO_3 ions.

In another culture run parallel to this experiment, the fertilization was made in normal sea-water and the medium left unchanged for the following five days, in order to determine whether the modifications were similar to those of the carbon dioxide solutions. It was not to be expected that the growth-curve would be like those of the others since in this instance the carbon dioxide would appear in the medium as a gradual ac-

cumulation. The growth-curves indicate a considerable inhibition during the first 24 hours, the period when the presence in the medium of such substances as inhibit or accelerate growth produces the greatest effect. The measurements for the succeeding days (aa) were 24 hours, 4.28; 48 hours, 56.18; 72 hours, 60.38; 96 hours, 60.47; and 120 hours, 58.47.

In this solution a tendency toward radial arrangement of the skeletal structures was evidenced in the early stages, followed by irregularity in the later. Figures 143 and 144 represent specimens at the 24 hour stage, and figure 145, at the end of 48 hours. Some, as in figure 146, have skeletons in one side only, while others appear normal except for decreased size. The animals had far from a healthy appearance, their movements were less regular, and many had sunk to the bottom of the dish. At the end of 120 hours a large number were dying.

These modifications were not typical altogether of carbon dioxide solutions, although the tendency toward radial symmetry is one of its characteristic effects. The abnormalities caused by a medium which remains unchanged for several days, is not then, due to the accumulating carbon dioxide alone, but probably to the presence of other products of metabolism as well. The dishes were shallow, and while they were closely covered to prevent evaporation, sufficient air-space was left above the surface of the water to allow plenty of oxygen for the developing embryos. Vernon, by a gas determination, estimated that the amount of oxygen in the water actually increased and the amount of carbon dioxide in his cultures during the eight days in which his experiments were run. Yet when such water was used as a medium for a second culture, a decrease in size of 7.9 per cent and 7.3 per cent resulted.

Another factor which might be exceedingly potent in determining the qualities and rate of development of the larvae would be the degree to which the characters of the parents are inheritable, and the extent to which these are modified in different fertilizations. Since it was impossible to keep the sea-urchins for two generations, an experiment was performed similar to that done by Tennent ('10). He selected five fe-

males and divided the eggs of each into five portions. The sperm from five males was treated in the same way, and from each portion of sperm, one division of eggs from each female was fertilized. This resulted in 25 cultures which he observed for three days and noted the variations in the different cultures.

In the present experiment, since it was desired to take the usual number of measurements in each culture every day, only four males (I, II, III and IV), and four females (A, B, C and D) were selected. In this way 16 cultures were procured and observed for the usual five days.

The following measurements were obtained:

Experiment 21.   June 27, 1915

Plot 20

A

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
I............	6.91	48.82	64.80	75.15	81.29
II............	4.10	41.93	60.77	73.88	79.09
III............	2.14	37.78	55.68	68.78	77.90
IV	11.65	52.84	67.91	78.64	75.22

Plot 21

B

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
I............	23.16	63.88	79.91	86.20	90.01
II............	18.64	56.92	76.15	82.73	89.17
III............	16.29	52.74	70.59	79.87	83.90
IV............	24.18	67.94	82.63	89.95	81.78

Plot 22

C

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
I............	26.10	67.76	81.78	80.62	76.11
II............	19.97	61.83	77.10	78.13	73.26
III............	21.97	56.69	72.57	75.44	70.15
IV............	28.27	71.91	84.64	83.14	68.32

Plot 23

D

|	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
I............	15.78	62.80	75.79	84.21	90.09
II............	12.82	55.74	71.63	80.57	86.85
III............	8.67	51.66	66.54	78.33	83.91
IV............	18.15	66.79	79.10	85.14	81.87

Some conclusions of interest may be deduced from these figures. First, it may be noted that after 24 hours, the cultures from each separate female show a slight degree of variation as to size, and center about a mean which is characteristic for each. For instance, with A, the mean is 6.2: with B, 20.56; C, 24.04 and D 13.85. Some influence of definite characters
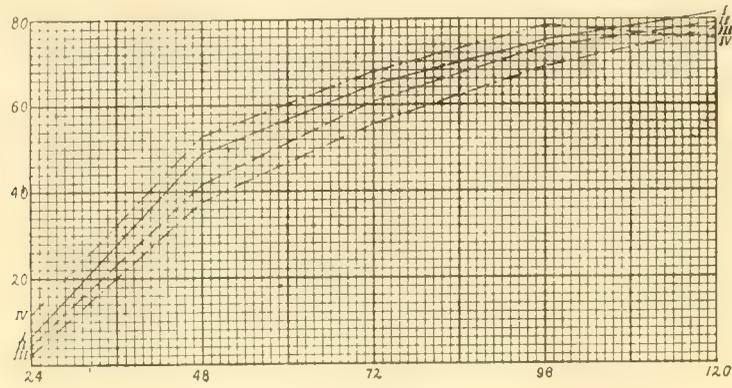

Plot 20
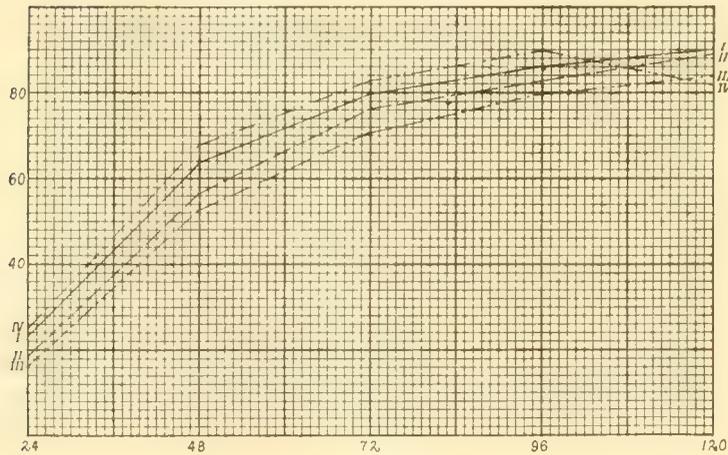
Plot 21
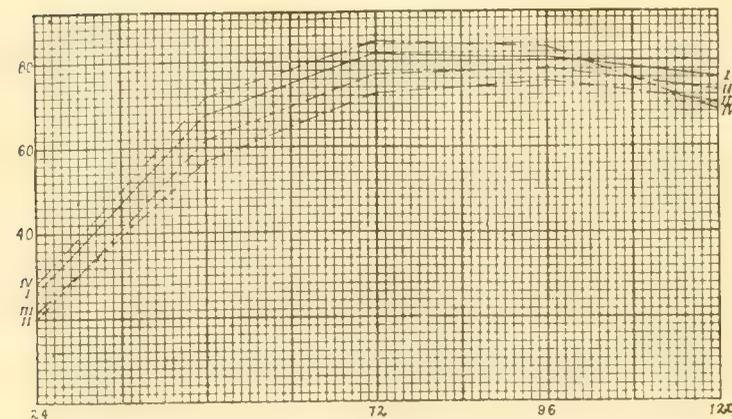
Plot 22
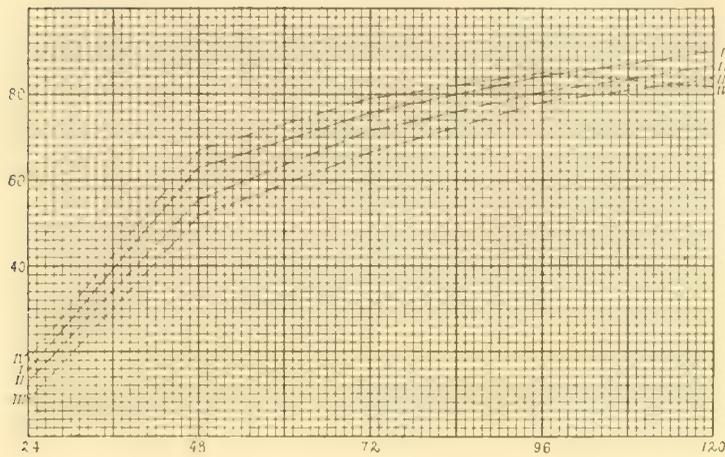
Plot 23

of the sperm is, however, also evident, since in all the sets, the order of the cultures as to size is identical, IV, I, II, III, except in C where III and II are reversed. This may be ascribed to experimental error in handling or measuring since no other explanation can be offered. At the close of the experiment, however, the order of all the sets is the same, being I, II, III, IV.

In all combinations except C (Plot 23), cultures I, II and III
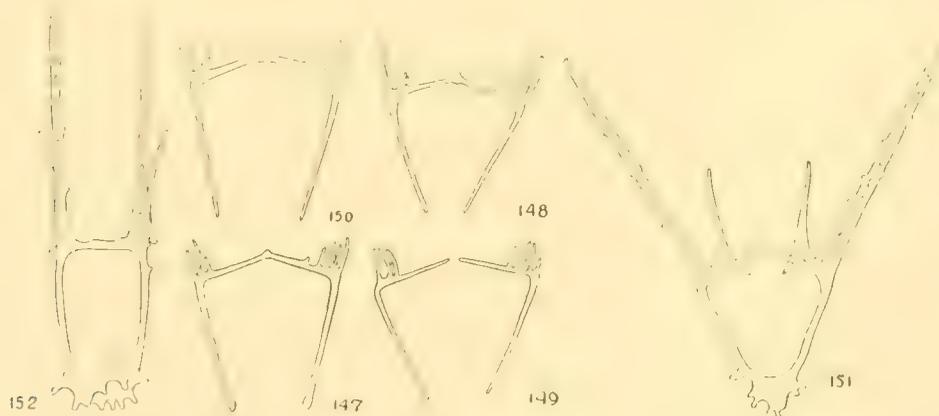
Figs. 146 to 152   Experiment 21.   Eggs from different females fertilized with sperm from different males.

Fig. 147   21 AI, figure 148 21 AII, figure 149 21 AIII, figure 150 21 AIV, 24 hrs. Specimens showing the tendency for appearance of four rods in place of three on the left side of a large number of larvae of all the cultures from eggs of Female A.

Fig. 151   21 BIII, 24 hrs.   Specimen with unusually widely spread arms. Present in a large proportion of all the cultures fertilized with sperm from Male III.

Fig. 152   21 BIII, 24 hrs.   Specimen with anal arms and body-rods parallel. This type appeared in about 1 per cent of all cultures from Male III.

grew slowly and continuously for the five days. On the other hand all of IV and all of C reached a maximum either at the 72 or the 96 hour period, indicating that this quality of rapid development must be contributed by all of the eggs and all of the sperm of those two individuals. The combined effects of these two forces is greater than that of either one alone, since C IV reaches a lower point on the curve than any other culture 120 hours old.

Some characteristic modifications in the skeletons were also observable. For instance, all the cultures of A contained a few individuals having four rods in one of the anal arms. This is not an unusual variation in Arbacia plutei and is found in many of the cultures. In this set, however, it occurred in greater proportions than ordinarily happens, and usually on the left side. Figures 147 to 150 are from AI, AII, AIII and AIV respectively. In each one four rods are present in this position and in AI there is the rudiment of a fifth which might ultimately have fused with the rest.

In all the solutions of III, a large number of plutei had the arms spread much more widely than is usual (fig. 151, BIII, 48 hours). In a count of 100 specimens from each solution taken after 48 hours, AIII possessed 79; BIII, 24; CIII, 81 and DIII, 97 with widely spread arms. In each of these solutions there were also a few of the type represented in figure 152 (BIII, 48 hours). The arms and body-rods were about the same length as in the normal but were almost parallel and formed right angles with the narrow transverse connectives and the broad plate-like posterior portion of the body-rods. The oral arms were spread much as in the normal specimens and hence projected at the sides beyond the anal. Individuals of this type averaged about one per cent of each of these cultures. There were none like them to be found in any others of the series, but in an entirely different solution fertilized with sperm from the same male, a still greater percentage of skeletons of this type was present.

In all the previous experiments, care was taken to use the germinal products as quickly as possible after extrusion, as it was thought that staleness might have some effect upon size or vigor of the larvae. To ascertain in how far this might be true, the eggs and sperm from two individuals were kept in separate vessels and from portions of these, fertilizations were made at the following times:

Experiment 22. July 22, 1915

		24 HOURS	48 HOURS	72 HOURS
a	8.45 a.m. (Immediately after extrusion)	27.4	68.7	84.3
b	10.44 a.m.	27.1	67.4	85.1
c	11.45 a.m.	27.8	69.1	83.9
d	1.00 p.m.	27.0	68.5	83.8
e	4.00 p.m.	26.9	68.2	84.2
f	7.15 p.m.	26.7	67.9	84.7

It may be seen from these figures that keeping the germinal products for 10 hours and 30 minutes effected no reduction of size. Moreover, these larvae seemed quite as vigorous as those fertilized immediately after extrusion.

In the experiment above, the eggs and sperm were kept in Syracuse watch glasses and covered with very little sea-water. Since the fact that they were thus in a highly concentrated condition might explain the lack of effect of long standing upon the vigor of the resulting individuals, another experiment was performed, and some of the eggs and sperm placed in separate vessels and covered with a considerable volume of water. Other portions were kept in a concentrated condition as in the previous experiment. All the eggs were mature and the sperm was very active. The following fertilizations were made: (C ♀ indicates eggs from the concentrated stock; D ♀, eggs from the dilute. C ♂ designates concentrated and D ♂[1] dilute sperm-suspension.)

Experiment 23

	9 a.m. July 23	*per cent fertilization*
a	Control	100.0

9 p.m. July 23

b	C ♀ × C♂	100.0
c	D ♀ × D♂	56.1

9 a.m. July 24

d	C ♀ × C ♂	100.0
e	D ♀ × C ♂	3.0
f	C ♀ × D ♂	0.0
g	D ♀ × D ♂	0.0
h	D ♀ × freshly extruded sperm	3.4
i	Freshly extruded eggs × D ♂	1.5
j	Freshly extruded eggs (used in *i*) × freshly extruded sperm (used in *h*)	99.7

The measurements from the various cultures were:

	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS
a	5.73	55.64	74.39	82.95	86.74
b	5.84	54.37	71.82	83.13	87.25
c	5.46	53.65	72.77	80.76	83.30
d	5.65	54.79	73.90	83.27	86.87
e		49.62	68.51		
f					
g					
h		56.34	77.23	85.54	
i					
j	6.33	52.87	74.96	84.68	88.36

Both controls, *a* and *j*, gave regular growth-curves which agreed very closely with the average for the period in which the experiments were made. As may be seen from the above measurements, when the egg supply and the sperm-suspensions were kept concentrated so that the action of the sea-water was reduced to a minimum, no abnormal effect was produced by delaying fertilization twelve or even twenty-four hours (*b* and *d*). But when the sperm was diluted so that all were in contact with the sea-water, a reduction in size of 3.9 per cent was produced (*c*).

After standing 24 hours in the dilute condition, the sperm was more injured than the eggs; for the latter when inseminated with concentrated sperm resulted in a 3 per cent mem-

brane-formation (e), while the eggs from the concentrated supply, fertilized with diluted sperm-suspension gave entirely negative results (f). The sperms, although slightly activated, were unable to penetrate even into freshly extruded eggs (i). In solution e, the small percentage that developed were of low vigor, were smaller than the control, and lived only three days. The eggs from the diluted stock fertilized with fresh sperm (h) also showed lowered vitality, so that before 120 hours all were dead.

In another series of experiments done in the same way, the sperm was so active at the end of 24 hours and the percentage of fertilizations was so high, that the germinal products were kept for several days and the experiments repeated. The following results were obtained:

Experiment 24. July 26, 1915. Percentage forming membranes

	24 HOURS	48 HOURS	72 HOURS	96 HOURS
C ♀ × C ♂................	100.0	100.0	100.0	97.0
D ♀ × C ♂................	100.0	97.3	87.5	47.3
C ♀ × D ♂................	98.6	94.2	80.6	25.7
D ♀ × D ♂................	99.7	91.7	74.4	10.0

Owing to the lack of time, the measurements of these larvae were not taken, but the cultures were kept for several days and the vigor of the resulting individuals observed. No reduction in size in the later fertilization was observable, but as in the previous experiment, this decrease would probably be so small that it could be determined only by actual measurement.

All from the 24 and 48 hour fertilizations were apparently as vigorous as the control which was started immediately after extrusion. After 72 hours, however, a lessened vitality was observable, but only when the sperm had been diluted. In these cultures the death-rate was so much higher that comparatively few attained the pluteus stage. After 96 hours, a lessened vigor was apparent in all the solutions. Normal plutei were formed in a and b, but in c the cleavage was irregular, and few normal individuals resulted.

Fisher ('03), also working on Arbacia at Woods Hole, performed somewhat similar experiments using diluted sperm-suspensions at various intervals to fertilize freshly extruded eggs. In some instances the last successful fertilization he was able to obtain was 24 hours after the sperm was removed from the male. In one case, the sperm was still able to fertilize the eggs after 133 hours, 30 minutes, and Fisher thought possibly it might have been active longer had he been able to continue the experiment. He found that "whenever fertilization occurs the fertilized egg develops to the adult stage . . . . never was a segmented egg found that did not develop into a pluteus." In this respect, his results disagree with those obtained above, where a slight effect on the size and a still greater effect upon the vigor of the resulting individuals were obtained.

Goldfarb ('15), made a similar study with Toxopneustes variegatus, and in most respects the results of this investigation agree with his. He found that "increasingly stale eggs of a given female, though fertilized by freshly prepared sperm suspensions, at each trial gave a decreasing number of fertilizations, and that this decrease was approximately the same for all females. . . . . The rate of cleavage is progressively retarded. The number that cleave irregularly is increased The number of atypic larvae is correspondingly increased." But his results disagree with those in this study, in the experiments with fresh eggs × stale sperm and stale eggs × fresh sperm. In both cases development was normal. Possibly the germinal products employed by Goldfarb had not been kept so long as those in the experiment above, or else Toxopneustes sperm and eggs may differ in viability from those of Arbacia.

DISCUSSION

We have seen that the control cultures in the previous experiments exhibited wide variation. As the plot containing the curves of the general averages shows, the condition of the larvae 24 hours old varies from no skeleton to one of 44.83, which is slightly larger than that of the control of Experiment 18 (sea-water + NaCl), 72 hours old. The widest variation is found in

larvae aged 120 hours, due to the fact that some have reached their maximum and resorption of skeleton has commenced. The lowest point attained at this time by any control still undergoing increase of size is 67.55, Experiment 19, Plot 1, FF. The wide variation exhibited by the control cultures may be ascribed partly to the fact that although they were raised under conditions as nearly standard as possible, some fluctuations in the environment could not be avoided.

No attempt was made during the course of the experiments to regulate the density of the sea-water,—a factor which the experiments on dilution and concentration show has a profound influence upon growth and development, and, under the conditions usually existing in the Woods Hole region, may produce a variation of about five per cent.

Fluctuations in the temperature were reduced to a minimum by keeping the finger-bowls in which the sea-urchins were raised surrounded by running sea-water. But this did not eliminate all variations in temperature since a gradual rise occurred during the course of the summer. On June 12, the date of the first determination for 1915, the sea-water registered 16.5°C. On June 20 it was 17.5°C., on June 27, 18.5°, on July 5, 19.5°, on July 16, 20.5° and on July 22, 21.5°. The highest point, 21.6°, was recorded on July 23. During the following week the temperature fell somewhat, and on July 28, the date of the last observation, was 20.8°.

Unfortunately, owing to lack of time, no experiments dealing with temperature as a separate factor could be carried out; hence no estimation can be made of the range of variation produced by these changes; but it was observed that the individuals in the cultures kept surrounded by running sea-water were more vigorous than were those exposed to the fluctuations ordinarily occurring in the laboratory.

Moreover, during the summers of 1914 and 1915, there was a wide difference in the size and vigor of the larvae raised at different seasons, confirming the conclusions of previous investigators that there is a seasonal variation. During the early period they grew rapidly and reached a high maximum

which might be attained at the 120 hour period or later, or might be passed as early as the 72. During the latter portion of the summer, growth was much slower, especially the first day, and continued steadily for the 120 hours that the larvae were under observation. The ultimate size, however, was not so great as during the early period. The averages of the maximum sizes during the two seasons were 86.23 and 82.25 respectively. But these distinctive characteristics were not absolute with the various periods,—for instance, in the second part of the summer (Period C), one of the seven averages reached its maximum at the 72 hour stage, and in all the periods there was considerable variation as to size of skeleton at the 24.

Usually those that underwent the most rapid growth during the first day were the ones that reached an early maximum. We have a typical instance of such an early maximum with high initial rate of growth in Experiment 21, CVIV, one of the cultures in which the eggs from different females were fertilized with sperm from different males. But this again does not hold absolutely, as in BVI, another culture of the same experiment, the measurements on the first day were exceptionally high, yet growth was continuous for the entire time.

Since, then, modifications due to environmental factors which could not be controlled under the conditions of these experiments, and modifications referable to different seasons, were insufficient to explain the wide variation that occurred in the larvae of the control cultures, other causes must be sought. Among the factors involved, as the above experiments show, are: variations in the degree of alkalinity; variations in the proportions of the different constituents of sea-water, which may be associated with changes in density; and the presence of the products of metabolism.

No definite data for the comparison of the alkalinity of the sea-water seem available. Determinations have been made in various regions, but owing to a difference in terminology and in the indicators employed, definite comparisons cannot be drawn. Loeb states that the concentration of the free OH ions in the sea-water at Pacific Grove "seems to lie between 10^{-6}

and 10^{-5} N, while that of Woods Hole is somewhat higher and may reach 10^{-5} N." Moore, Roaf and Whitely give that of Port Erin Bay, using the same indicator, phenol-phthalein, as 0.00023N, or 2.3×10^{-4} N.

The degree of alkalinity necessary for development, moreover, depends upon the character of the salts present. Herbst found that the most favorable alkalinity of his artificial sea-water was greater than that of the natural sea-water, yet addition of hydroxyl to the latter caused no acceleration of growth. He offered as a suggestion that possibly the concentration of hydroxyl could be less in the presence of such salts as the phosphates and carbonates of the sea-water, or else the phenol-phthalein is altered in its reaction in the presence of some salts found in the sea-water but not in his artificial solution. These facts indicate that the alkalinity of the sea-water is not a constant factor, and that, even under the same degree of hydroxyl concentration, the effects vary with varying concentrations of salts. That the latter are not always present in different waters in constant amounts has been adequately determined. Mayer ('14) states that a 0.60M NaCl solution is isotonic with the sea-water at Tortugas. Garrey ('04) gives 0.52M as equivalent to that at Woods Hole, 0.54M for Pacific Grove and 0.58M for Beaufort.

In the above experiments I have attempted to show some of the modifications produced by these factors operating separately. To determine the effects of all possible combinations and of gradual subjection would require much additional investigation. But since it is impossible to alter one constituent of the sea-water without involving other changes, some evidence of the combined effects of several independent factors has been produced; for instance, in the employment of 0.60M NaCl solutions dilution of the other constituents occurs coincident with increase of osmotic pressure; in the carbonate solutions decrease in the $CaCO_3-$ and $MgCO_3-$ content occurs at the same time as increase of alkalinity, and, in the gradual concentration of the sea-water, a condition of increased density replaces one of highly decreased concentration.

In how far may these combined factors modify normal development? In many of the experimental solutions in which conditions are so adverse that life is barely maintained, the measurements come well within the range of size of the controls. In the first experiment with concentrated medium (2), the measurements of the specimens subjected to 40 per cent concentration (e +), which lived only 96 hours, are greater than those indicated by 1F (Plot 1) for the corresponding days, and drops beneath those of 1FF only at the 96 hour stage. The same is true of the specimens in the solution of 30 per cent dilution, which lived only 96 hours, except that at the age of 72 hours they measured slightly more than those in 1F.

These two solutions represent the limits to which concentration and dilution could be pushed, and yet the size of the larva was not reduced below that of the extreme variants of the control cultures. In the second experiment with $NaHCO_3$ (9), the same conditions may be observed. In the culture with 91 cc. sea-water + 9 cc. 0.45 $NaHCO_3$, the larvae were in a dying condition at the close of the fourth day. At the ages of 24, 48 and 72 hours they were well within the range of the controls, and at the age of 96 hours were within 1F but below 1FF. Likewise in the experiment with carbon dioxide (20g), the same conditions occur. Not until 96 hours, after 24 hours of almost complete inhibition of growth, does the average measurement drop below that of 1FF.

It is significant that these three experiments were performed during the early period (B),—a fact which may account for the high resistance of the individuals to the foreign solutions. But in other experiments made during the same period, in which the controls seemed equally vigorous, life was maintained at a much lower level. For instance, in the experiment with gradual concentration of the sea-water (3), the specimens in the solution of 120 cc. sea-water concentrated to 100 (e +), the larvae lived 120 hours and seemed fairly vigorous at the close; but except at the 24 hour period, when skeletons were present merely as spicules scarcely tri-radiate, the measurements were never within the range of the controls. This leads to our

first general conclusion, that equally adverse conditions do not produce equally great reduction in size, and agrees with the results of the experiments with increased hydroxyl concentration, where the injury inflicted may be ascribed to over-rapid growth.

In Experiment 2, we have two experiments made with the same control, one of dilution and one of concentration of the sea-water. The specimens in the solution of 30 per cent dilution (g) and those in the solution of 120 cc. sea-water concentrated to 100 (e +), lived only 96 hours, and hence we may look upon them as having undergone practically the same extent of injury, yet a comparison of their growth-curves reveals the widely different effects of the two modes of treatment. We may conclude, therefore, that each modification of the medium produces its specific effect upon growth.

In those instances in which two constituents of the medium are changed, the effects of one may completely mask the effects of the other. Thus increased alkalinity tends to produce irregularity of structure and inhibition of growth, while the carbonates, although alkaline in reaction, are associated with a high degree of symmetry. Moreover, in cultures in which seawater was diluted with isotonic NaCl solution, dilution of the other constituents with increase of Na, produced a size above the normal. But when the NaCl solutions were hypertonic, the physical effects of increased osmotic pressure neutralized the physiological effects of the Na, and a growth-curve scarcely different from the normal was produced. As the solutions became still more hypertonic, all stimulating effect of the Na was lost, and a decreased size resulted.

In other instances, when both factors tend to produce the same result, the combined effect may be greater than either one alone. In the solutions of high degree of dilution, those in the first experiment, such as f (30 per cent dilution) possessed a much lower vitality than did those in the solution of the same dilution in the second (g).

This leads to our next general conclusion, that where the control is below the average size, the experimental solutions exert a more unfavorable influence. In the two experiments in

which NaCl was added to the sea-water (18 and 19) the control of the first was one characteristic of that period, and the measurements on the various days are not unlike those of the general average for A. In the second, all the values for the control are lower and correspond to Curve FF (Plot 1). A comparison of the figures obtained for the experimental solutions shows that each measurement in the second experiment is less than the corresponding one in the first, and the specimens were less vigorous.

Even when the experimental solution tends to produce increase of size, the injury is more intense when the control possesses a low average. For example, in the NaOH solutions, (6d) 2 cc. N/10 NaOH to 98 cc. sea-water represented the highest concentration of OH that could be endured by the larvae, but the following summer, when the animals were very vigorous, in one instance (0a) 3 cc. N/10 NaOH to 97 cc. sea-water was employed. It follows, then, that although abnormal conditions are not always productive of reduced size, a size below the normal is usually indicative of reduced vigor.

What, then, is normal development? We cannot say that the specimens of greatest size are necessarily normal, since some changes of the medium, such as dilution of the sea-water or concentration of the hydroxyl, produce increased growth but lead to secondary inhibition. Nor can we say that those are normal which have developed under standard conditions of the medium, since sea-water is exceedingly complex and variable. Moreover, we have seen that other factors, such as the season at which the eggs are produced and the staleness of the sexual products modify development, and that the eggs of different parents do not respond in the same way to similar treatment, nor do all the eggs of the same parent react identically.

Normal development we can regard only as the modal development, and the mode must vary according to the place and the time; that is to say, the environment. If some sudden change in the composition of the medium were to occur, the largest class to respond in a specific way would be regarded as normal; but a few extreme variants might perhaps be best fitted to the new

conditions. A fluctuating variation might therefore be beneficial to the individual and a mutation to the species also, even though it might be termed an abnormality.

What, then, may the range of variation in the Arbacia larvae be? No attempt could be made to answer this for mutations; but for such variations as are inherited and for such as are produced by modification of the medium, some idea may be drawn

EXPERIMENT	COMPOSITION OF MEDIUM	SOLUTION	PERCENTAGE OF INCREASE ABOVE CONTROL				
			24 hours	48 hours	72 hours	96 hours	120 hours
1	Dilution of the sea-water	b	6.5	14.3	16.3	7.8	3.0
2	Dilution and concentration of sea-water	b		4.2	3.8	3.5	
		c		5.7	6.4	5.1	
		d		2.4	1.3	0.2	
3	Gradual concentration of sea-water	c		2.3	3.2	1.6	
		d		0.7	4.7	3.8	2.7
		e					2.0
		f					1.1
6	Increased alkalinity	b	0.48/0	1.0			
		c	3.21/0	1.4			
		d	6.20/0	1.6			
8	Addition of NaHCO$_3$	b	4.4	1.0			
		c	7.5	2.0			
		d	13.5	1.8			
		e	9.8				
		f	5.2				
9	Addition of NaHCO$_3$	b	1.8	2.7	0.2		
		c	4.7				
11	Addition of Na$_2$CO$_3$	b	10.4	5.5	1.9		
		c	50.4	8.7			
		d	31.7				
14	Addition of 0.52M NaCl	b	12.7	4.3	4.8	4.4	4.6
		c			1.3	2.0	2.8
		d					0.9
15	Addition of 0.56M NaCl	b					0.9

from the above data. The following table gives the increase in size in percentages above the average of the control in each of the foregoing experiments. (Values are not given where decreased size has been produced nor after the control has passed the highest point on its growth-curve.)

This table gives some idea of the degree to which size may be increased in Arbacia larvae under experimental conditions. The percentage may be comparatively large when the embryos are 24 hours old, but becomes gradually less until finally when the larva are 120 hours old, it is limited to 4.6 (Experiment 14). This represents approximately the maximum increase in size, since if the factors which produce this excessive growth are applied in greater concentrations, an inhibition rather than a further increase occurs. But these figures do not stand for absolute values, since the controls, themselves, represent varying quantities. Would the effect be greater if the values of the control were low? The first experiment with dilutions (1) seems to indicate that it would, in the early stages; but at the close of the experiment increase of size is not relatively greater than in the other experiments.

Decrease in size may be so great under experimental conditions, that some cultures never approach the range of the control,—for example, the specimens in the second experiment in which NaCl is added to the sea-water, do not acquire skeletons until 96 hours old. These forms resemble the 'Dauer-blastulae' of Driesch, but have archenterons fully developed or short and bent over so that the mouth opens close to the blastopore. Strictly speaking, however, they are not Dauer-blastulae for if subjected to a more favorable medium they may resume development, but may not, as we have seen, "attain the maximum size to which they can develop." On the other hand, the larvae may endure adverse conditions of greater intensity if subjected gradually to the medium than if subjected suddenly, as shown by the experiment with gradually concentrated sea-water (3).

The excessively low values which may be produced in the early stages, indicate so great an injury that life cannot long

be maintained. The following percentages represent the maximum inhibition in each experiment when the control has reached its greatest size.

EXPERI-MENT	COMPOSITION	SOLU-TION	PERIOD	PER CENT
			hours	
2	Concentration and dilution......................	g	96	44.1
		e+	96	34.2
3	Gradual concentration.........................	g	96	1.8
		e+	96	63.3
4	CH₃COOH...	b	120	21.0
5	CH₃COOH...	d	120	25.8
8	NaHCO₃..	g	72	11.7
9	NaHCO₃..	f	120	38.8
11	Na₂CO₃...	e	120	41.7
16	NaCl 0.60M......................................	h	72	74.7
17	NaCl 0.60M......................................	e	120	19.2
18	NaCl (hypertonic)...............................	d	120	64.3
19	NaCl (hypertonic)...............................	c	120	53.4
20	CO₂..	aa	120	29.6
21	Eggs × Sperm of different parentage.............	c	120	3.9

These tables show the greater plasticity of Arbacia larvae in respect to decrease in size than in respect to increase. In practically all of these solutions, while the larvae had departed widely from the normal in the characters distinctive for the various substances employed, they seemed vigorous and capable of continued development. To what extent they could resist new adverse conditions that might be superimposed, or to what extent they could survive these same conditions at a later period has not been determined in these experiments; but these figures indicate to some extent the range of variation which may possibly occur in Arbacia larvae during early stages in their natural environment.

SUMMARY

In conclusion, the experiments recorded above tend to show that:

1. The factors producing variations in the growth and development of the larvae of Arbacia punctulata are of two types,— non-environmental or intrinsic and environmental or extrinsic.

2. Among the factors of a non-environmental nature are,—

a. Different reaction of the offspring of the same parents to similar environmental conditions.

b. Different reaction of the offspring of different parents to identical environmental conditions.

c. Different reaction of eggs produced at different seasons to similar environmental conditions.

d. Staleness of the sexual products.

3. Among the factors of an environmental nature are,—

a. Modifications in the concentration of the sea-water.

b. Modifications in the alkalinity of the sea-water.

c. Modifications in the salt-content of the sea-water.

d. Accumulations of the products of metabolism in the surrounding medium.

4. Each modification of an environmental nature produces its specific effect upon growth.

If two or more factors in the medium are varied, in case the modifications are of an antagonistic nature, the effects of one may completely mask the effects of the other; in case both factors tend to produce the same result, the combined effect may be greater than that of either one alone.

5. Sudden change of medium produces an inhibition of growth.

If the change be gradual, and from one of less to one of more favorable medium, a complete compensation for early inhibition may occur, but the ultimate growth is not equal to that which would have occurred had the larvae been subjected during the entire period to the more favorable environment.

If the transfer be gradual and to a less favorable medium, the injury would not be so great as if the larvae had been subjected continuously to the adverse conditions.

6. Size and degree of development are not correlated.

Increased size is not correlated with increased vigor.

Decreased size is correlated with reduced vigor.

Individuals subnormal as to size, when subjected to an unfavorable environment, undergo more extreme modifications than do normal specimens when subjected to similar conditions.

7. The larvae of Arbacia punctulata show greater plasticity in respect to decrease in size than in respect to increase. In such modifications of the medium as occurred in the foregoing experiments, the range of variation, extended, at the time of maximum size, from a 5.1 per cent increase above the size of the control, to a 63.3 per cent decrease.

This range of variation probably corresponds approximately to that of the larvae of Arbacia in their natural environment.

The subject of this paper was suggested by Dr. D. H. Tennent and the work has been carried out under his direction. The experimental part was done at the Marine Biological Laboratory at Woods Hole, Massachusetts, during the summers of 1914 and 1915. I am very glad to express my gratitude to Dr. Tennent for his constant help and guidance in this research during the two years of its progress.

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THE HISTORY OF THE EYE MUSCLES

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TWENTY FIGURES

The muscles which move the eye-ball are a specialized group whose functional importance is quite disproportionate to their size. They are muscles of ancient origin, presumably antedating by millions of years muscles such as those which move the eye-lids. As a result of the investigations of two generations of morphologists we are now in a position to sketch in general outline their probable phylogenesis. The present paper raises the problems—What has been the past history of the eye muscles? What changes have they undergone in their transformation of the fish into the mammal? How many myotomes enter into their formation? Are they, like the tongue and appendicular muscles, exotic in origin and derivatives of the post-otic lateral trunk muscles? To what do they owe their present isolation? Can their history be traced back into stages before eyes made their appearance?

Comparative anatomy has thrown very little light upon the history of the eye muscles. Like the eyes with which they are so intimately associated, they appear in the lowest vertebrates in essentially the same form as in man. Indeed their number and their nerve relations are the same in man as in the dogfish. Of the entire group of eye muscles only the superior oblique shows a functional change in the course of phylogeny. The direction of its pull is altered as the result of the development of the trochlear tendon. Comparative anatomy also reveals some aberrations in the innervation of the eye muscles and such curious modifications as in Astroscopus where some of the eye muscles are transformed in to electroplaxes with some striking changes in their innervation. Moreover, in reptiles and some

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mammals a retractor bulbi (oculi) makes its appearance as a derivative of the external rectus muscle (Johnson, '13; Fraser, '15), but in the great majority of vertebrates the number and the nerve relations of the eye-muscles remain identical and unchanged. Nature seems to have pursued with regard to them the policy of 'letting well-enough alone.' Their 'evolutionary potential' appears to be approximately zero.

Were we therefore dependent upon comparative anatomical evidence alone for our conclusions concerning the history of the eye-muscles, we should be obliged to consider them as an isolated and peculiar group, the pre-craniote history of which is unknown. While we should not feel forced to assert that they arose, Minerva-like, full-formed, nevertheless it would remain a matter of uncertainty or of speculation whether they were exotic or endogenous, whether visceral or somatic, in their origin. Comparative embryology, however, appears to justify the assertion that the eye-muscles are a remnant of the lateral trunk muscles which, in the ancestors of vertebrates, extended in an unbroken series throughout the entire length of the body. Of the parietal muscles anterior to the ear they alone have persisted, through their functional relations to the eye-ball. Their isolation is associated with the growth and enlargement of the otic capsules and of the cranial skeleton.

The details of this story have been slowly gathered. First, Balfour ('78) discovered the extension of the body cavity into the head region of Elasmobranch embryos, thereby demonstrating the fundamental similarity of head and trunk regions in Vertebrates. He also showed that the mesoderm of the head undergoes a segmentation independent of that of the visceral arches, resulting in the formation of the so-called head-cavities. Later, Marshall ('81) proved that the eye-muscles arise from the walls of these head-cavities. He asserted that four of the eye-muscles (those innervated by the oculomotor nerve) develop from the first head-cavity, the superior oblique muscle from the second and the external rectus muscle from the third. Subsequent investigation has repeatedly confirmed these results for all classes of vertebrates –Cyclostomes (Koltzoff '01), Elas-

mobranchs (VanWijhe '82, Miss Platt, '91, Lamb '02), Reptiles (Corning '00, Filatoff '07, Johnson '13), Mammals (Miss Fraser '15).

One point, however, in the ontogenesis of the eye-muscles from the head-cavities still remains in dispute. While most investi-
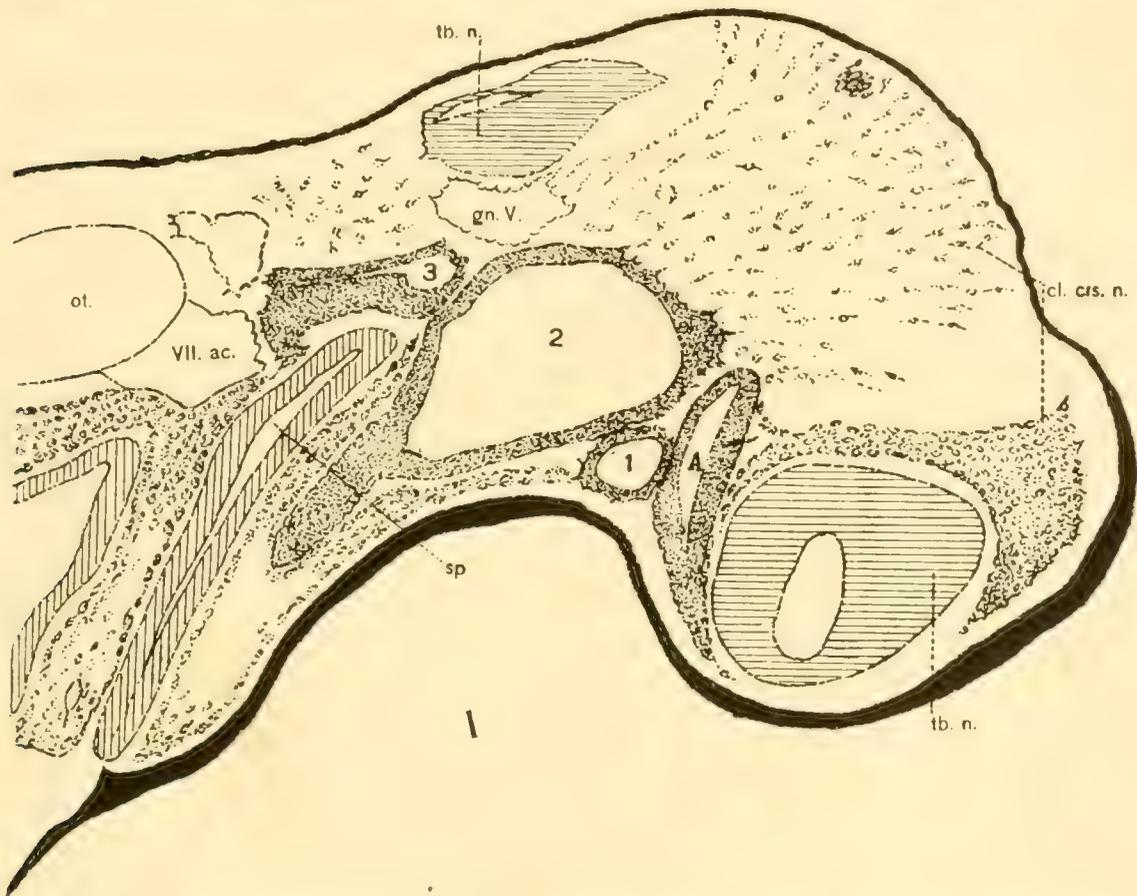

Fig. 1 A camera drawing of a parasagittal section of a 7 mm. Squalus embryo (Series IFF–2–2–16) showing the head cavities. The anterior cavity, not seen by Marshall but later discoverd by VanWijhe, is also shown. It soon degenerates while the other cavities differentiate into the eye muscles. *A*, anterior head cavity; *1, 2, 3*, first, second and third head cavities; *VII.ac*, acustico-facialis nerve anlage; *cl.crs.n*, neural crest cells; *gn.V*, trigeminal ganglion; *ot*, otic capsule; *sp*, spiracular pouch; *tb.n*, neural tube.

gators agree with Marshall in deriving the external rectus muscle from the third (hyoid) cavity, Dohrn ('04) and the writer ('09) have asserted that the second (mandibular) cavity also participates in its formation. Neither, however, published any figures or other evidence to support his assertion. The first, question, therefore, to which we may well turn our attention

is, How many head-cavities participate in the formation of the external rectus muscle?

Miss Platt ('91) was the first to observe that in Elasmobranch embryos a muscle which she calls 'muscle E' arises in such intimate connection with the external rectus muscle that for some time she believed that the mandibular cavity "took part with the third in the formation of the external rectus muscle." Subsequent observation has confirmed this observation of Miss Platt. There is disagreement only concerning the supposition that the mandibular component ('muscle E') degenerates. Does this 'muscle E'—the mandibular component of the embryonic external rectus muscle—degenerate?

In answering this question Miss Platt compared two stages ('91, figs. 5 and 6, pl. 5) which correspond quite closely with text figures 6 and 8 of this paper. She says (p. 86):

This section (sect. 5) also shows the peculiar relation existing between the external rectus muscle, now forming in the third head cavity, and the mandibular muscle, mus. E. If this cross section be compared with a similar section through an older embryo, represented in sec. 6, it will be seen that were the cells of the mandibular muscle (mus. E.) to fuse with those of the third head cavity (ext. rec.) at the stage represented by sec. 5, the resulting muscle would closely resemble in shape the external rectus of sec. 6. I am convinced, however, that such a fusion does not take place, for the limiting wall of the third head cavity can be traced until the muscle here formed comes to occupy the entire place once occupied (sec. 5) by the cells of the two muscles (ext. rec. and mus. E.). The cells of the mandibular muscle (mus. E.) gradually yield their place to those of the third head cavity and are ultimately lost in the general mesoderm. Thus a muscle, the rudiment of which appeared in the walls of the mandibular cavity prior to the origin of any of the eye muscles, completely disappears, although in the embryo of 22 mm. it is still relatively large as compared with the eye muscles.

In the description of a 27 mm. embryo of Squalus she goes on to say (p. 87): "The cells of the rudimentary muscle (mus. E.), so closely related to the external rectus, are now indistinguishable from the surrounding mesoderm, and the superior oblique muscle represents all that is left of the walls of the dorsal part of the mandibular cavity." Of the histological changes manifested by degenerating muscle cells Miss Platt presents no evidence whatever.

Of Miss Platt's 'muscle E' Lamb ('02, p. 195) says:

This latter muscle has now (in a 19 mm. embryo of Squalus) reached its maximum development. The anterior end curves not only outward but upward as well, so that the direction of the muscle is approximately dorso-ventral. From now on this muscle undergoes degeneration at as a 26 mm. stage scarcely a trace of it remains.

Proof of its degeneration, however, Lamb does not give. That Lamb is confused regarding the fate of the muscle is shown by the fact that in his figure 9, p. 185, 'muscle E,' which is correctly so labelled in the drawing, is incorrectly described in the paper as "proliferated from the hyoid somite," while it is named "the external rectus muscle!" Such contradictions are unfortunate in a paper which is otherwise a valuable contribution to the literature.

Johnson ('13, p. 161) apparently assumes the degeneration of the so-called muscle E (which occurs in Reptile embryos in the same relations as in Elasmobranchs) without taking the trouble to inquire into the evidence. He agrees with Miss Platt and Lamb that it degenerates and, like them, fails to prove the assertion. Miss Fraser ('15), whose paper is the latest dealing with the problem of the ontogenesis of the eye-muscles, is more cautious in her statements. She is willing to admit the possibility (p. 341) that in marsupials the second myotome "may contribute towards the formation of the m. externus rectus as in some fishes (Dohrn '04, Neal '09, '14)," but she adds "we have no direct evidence of this in Trichosurus."

Dohrn ('06) was the first to assert the persistence of the mandibular component of the external rectus muscle. He states in a foot-note (p. 243):

bei Scyllium kann man sich mit der grössten Sicherheit davon überzeugen, dass von einem Zugrundegenen des mandibularen Antheils des Rectus externus keine Rede ist, da man Schritt für Schritt die stärkere Verdickung der Masse und die Bildung der einzelnen Fasern innerhalb diesser Masse constatiren kann, während gleichzeitig die aus der III Kopfhöhle stammenden Muskelfasern immer näher an die der Mandibularhöhle aufrücken und zugleich von hinten her sparsamer werden, bis schliesslich die ganze Muskelmasse keinen Unterschied mehr darbietet.
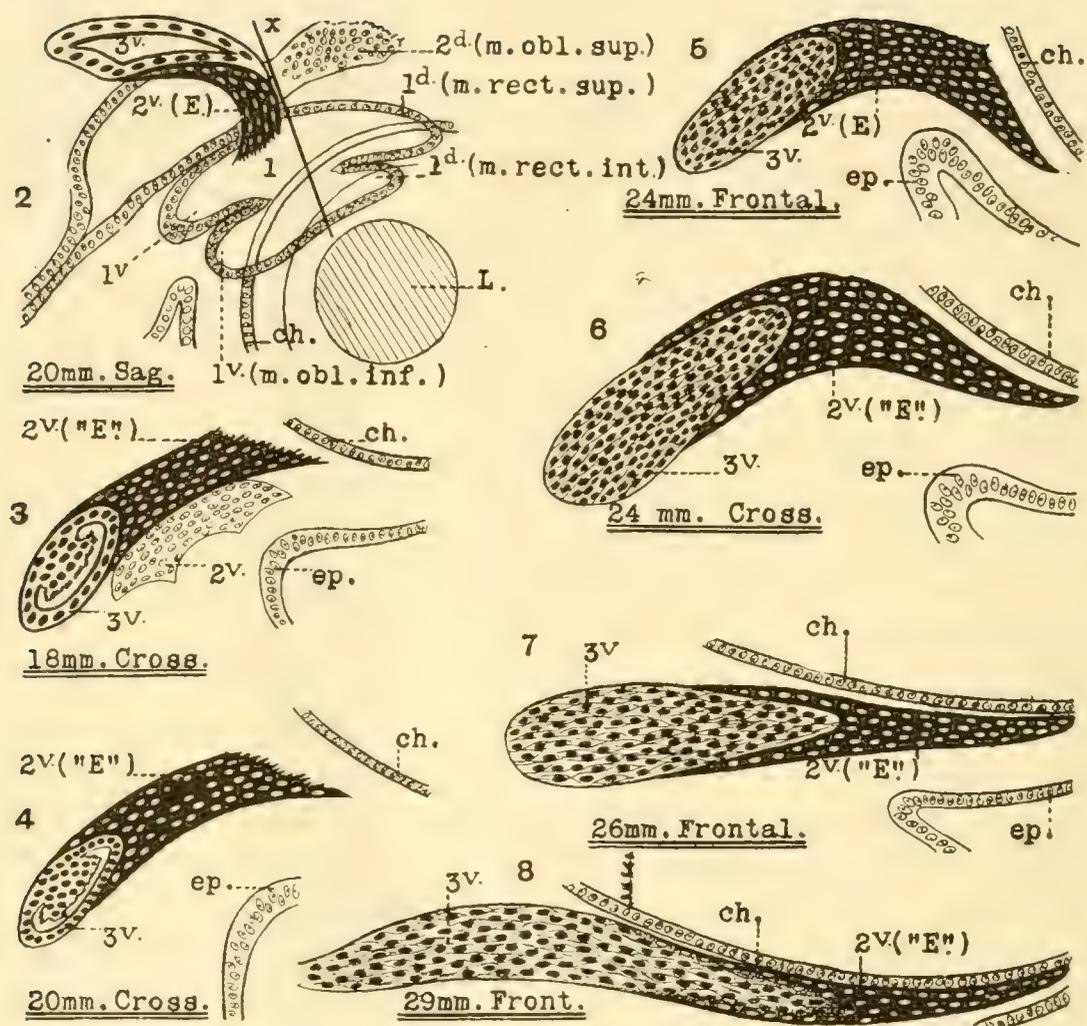
Figs. 2 to 8 Semidiagrammatic camera drawings of certain stages in the onto-genesis of the external rectus muscle as seen in Squalus embryos of 18 mm. to 29 mm. length. The mandibular component of the external rectus muscle (Miss Platt's muscle E) is shown in black with unshaded nuclei, while the hyoid component is drawn unshaded with black nuclei. Head-cavities 1, 2, and 3 are shown in topographic relation to the eye-ball only in figure 2. The anlage of the external rectus alone is shown in the remaining figures (3 to 8).

Fig. 2 The double-bimeric-origin of the external rectus muscle. The two components are in intimate contact from this stage on. The figure is drawn from parasagittal sections of a 20 mm. Squalus embryo. The division of the head cavities to form the six eye muscles has already begun.

Fig. 3 The anlage of the external rectus muscle in a cross section of an 18 mm. embryo of Squalus. The mandibular component (Mus. E.) appears as a differentia-tion of the mandibular cavity, the lateral wall of which has already begun to dis-integrate into mesenchyme. The multiplication of embryonic muscle cells in the median wall of the third head cavity has begun to obliterate the lumen of that cavity. In a 20 mm. embryo the conditions remain essentially unchanged (fig. 4).

The evidence which has convinced the writer of the persistence of the mandibular component of the external rectus muscle is summarized in text figures 2 to 8 of this paper. In earlier papers ('09, '14) the fact of its persistence was asserted, in agreement with Dohrn ('04), but no evidence was presented. The facts are as follows: In Squalus embryos of eighteen to twenty-four millimeters the anlage of the external rectus muscle shows two easily distinguishable elements, one (anterior) derived from the myotome of the mandibular cavity and which is recognizable as Miss Platt's 'muscle E' the other (posterior) formed from the myotome of the hyoid cavity (*mytm. 3*) The two elements differ, not only in their staining properties, but the distinction between the two may also be made out, as Miss Platt has stated, through the presence of a limiting membrane bounding the myotome of the third (hyoid) head-cavity. As a result, however, of the gradual disappearance of this membrane as development goes on it becomes increasingly difficult to distinguish the two elements. The difficulty is further increased because of the forward growth of myotome 3 (the hyoid element of the external rectus anlage), which comes eventually to lie above the mandibular element (mus. E.) Consequently, in embryos of 28 to 30 mm. it is possible to distinguish the two elements clearly only in cross sections of the muscle anlage. In still later stages the bounding membrane disappears altogether. A slight difference however in the direction of the long axes of the muscle fibers of the two elements makes it possible, even after the disappearance of

Fig. 5   The external rectus muscle as it appears in a 24 mm. embryo in frontal section. The muscle has thickened and elongated and the lumen of the third has disappeared. Cross sections of embryos at this stage show the muscle anlage much as in frontal sections (fig. 6).

Figs. 7 and 8   The external rectus is cut lengthwise in frontal (horizontal) sections of 26 mm. and 29 mm. Squalus embryos. In these stages it becomes increasingly difficult to distinguish the two components of the muscle, especially in frontal sections. The persistence of the mandibular component, however, is undeniable. *1d, 2d,* dorsal moieties of the first and second head cavities; *1v, 2v* ventral moieties of the same; *3v,* the third (hyoid) cavity; *E,* mandibular component (mus. E) of the external rectus muscle; *ch.* chorioid layer of the optic vesicle; *ep.* epidermis; *L,* lens; *x,* dividing line between dorsal and ventral moieties of the head-cavities.

the limiting membrane, to distinguish the two in cross sections. We see therefore that what disappears is not the mandibular element but the limiting membrane bounding the hyoid element, making it increasingly difficult and finally impossible to distinguish the two. Of the disintegration or degeneration of the muscle cells of 'muscle E' there is not the slightest evidence. On the contrary, in the stages during which degeneration has been said to occur, the embryonic muscle cells of both elements of the external rectus muscle undergo similar progressive dif-
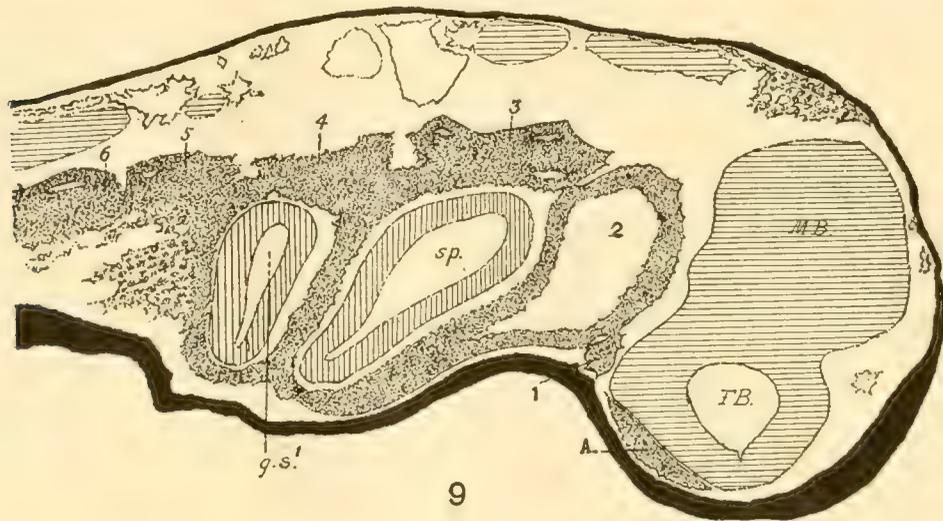
Fig. 9 A camera drawing of a parasagittal section of a 5 mm. Squalus embryo (Series ICC -1–2–1) showing VanWijhe's somites 1 to 6. They are seen to be dorsal segments of the mesoderm and their segmentation to be independent of the visceral arches. *A*, the anterior cavity; *1, 2, 3, 4, 5, 6*, VanWijhe's somites 1 to 6; *F.B.*, forebrain vesicle; *g.s.'*, first gill-pouch; *M.B.* midbrain vesicle; *sp.* spiracular pouch.

ferentiation as elongated spindle-shaped muscle fibers. In both, myofibrillae are visible in Squalus embryos of forty-five millimeters and transverse striae in embryos of one hundred millimeters.

Concerning the ontogenesis of the other eye-muscles there is no difference of opinion. All later observers, including the writer ('98), have confirmed the results of Marshall ('81). It is quite unnecessary, therefore, in this paper to repeat the description of what is so well known. In the light of the evidence presented in this paper, however, it seems necessary to revise

the well-known text-book formula for the ontogenesis of the
eye-muscles as follows: From the first or pre-mandibular head-
cavity arise the muscles innervated by the oculomotor nerve,
namely the recti superior, anterior and inferior, and the inferior
oblique; from the second or mandibular myotome are differen-
tiated the superior oblique muscle and the lateral portion of
the external rectus; from the third head cavity develops the
median portion of the external rectus muscle. A comparison
of the old and new formula may be made in tabular form:

The old formula for the ontogenesis of the eye-muscles

SOMITE	MUSCLES DERIVED	NERVE	NUMBER
Myotome 1.................	Rectus superior	Oculomotor	III
	Rectus internus	Oculomotor	III
	Rectus inferior	Oculomotor	III
	Obliquus inferior	Oculomotor	III
Mytome 2........:.............	Obliquus superior	Trochlearis	IV
Myotome 3....................	Rectus externus	Abducens	VI

Revised formula for the ontogenesis of the eye-muscles

Myotome 1d.................	Rectus superior	Oculomotor	III
	Rectus internus	Oculomotor	III
Myotome 1v.................	Rectus inferior	Oculomotor	III
	Obliquus inferior	Oculomotor	III
Myotome 2d....................	Obliquus superior	Trochlearis	IV
Myotome 2v.................	Rectus externus	Abducens	VI
Myotome 3v.................	Rectus externus		

But, however interesting and however important from the
embryological point of view such an account of the ontogenesis
of the eye-muscles in Squalus may be, it tells us little that is
morphologically significant. The questions still remain un-
answered—What is the morphology of the 'head cavities?'
What has been their past history! To the answer to these im-
portant questions we may now turn our attention.

The true morphology of the head cavities and the first reli-
able clue to the phylogenesis of the eye-muscles was revealed
by VanWihje ('82) who showed that the head cavities are mem-

bers of a series of mesodermic segments (somites) which, in Elasmobranch embryos, extend without interruption throughout head and trunk. He was thus able to demonstrate in craniote embryos an acraniote stage and to strengthen the conviction of morphologists that, in the ancestors of vertebrates, head and trunk were undifferentiated just as they are in Amphioxus today. The repeated confirmation of the presence of VanWihje's mesodermic segments in vertebrate embryos of widely divergent groups such as Cyclostomes (Koltzoff '01), Elasmobranchs (VanWijhe '82, Hoffman '95, Neal '96, Sewertzoff '98, Johnston '09, Braus '99), and Amphibia (Miss Platt '97) and the demonstration that they are serially homologous with those of the trunk has finally established the long-contested fact that the eye muscles are members of the series of lateral trunk myotomes.

The proof of this conclusion is complete. All of the objections formerly raised against the homology of pre-otic and post-otic mesodermic somites (VanWijhe's) have been adequately answered, and a brief summary shows how convincing is the evidence in favor of their serial homology with those of the trunk:—VanWijhe's somites are continuous with those of the trunk; beginning with the neck region, their differentiation is progressive; they differentiate into myotome and sclerotome, the former coming from the median wall as in the case of trunk somites; like trunk myotomes they are innervated by somatic motor nerve fibers; their segmentation is quite independent of that of the visceral arches; and they are dorsal in relation to the notochord and dorsal aorta.

Their ontogenesis in Elasmobranchs shows that the differention of the three anterior myotomes into the six eye muscles involves, primarily, the splitting of the myotomes into dorsal and ventral moieties. The process is exactly similar to the splitting of the post-otic myotomes of Petromyzon dorsal and ventral to the ear, and the facts suggest that in the ancestors of craniotes the lateral segmental muscles of the head region, in the course of phylogeny, became divided into dorsal and ventral elements as the result of a longitudinal splitting along the series

of lateral line sense organs.   Possibly, as many morphologists have thought, the lens and the otic capsule were at once time members of the series of lateral line sense organs.   The dorsal and ventral moieties of the first myotome subdivide again, thus forming the four muscles innervated by the oculomotor nerve, The dorsal element of the second myotome forms the superior oblique muscle, while the ventral unites with the third myotome
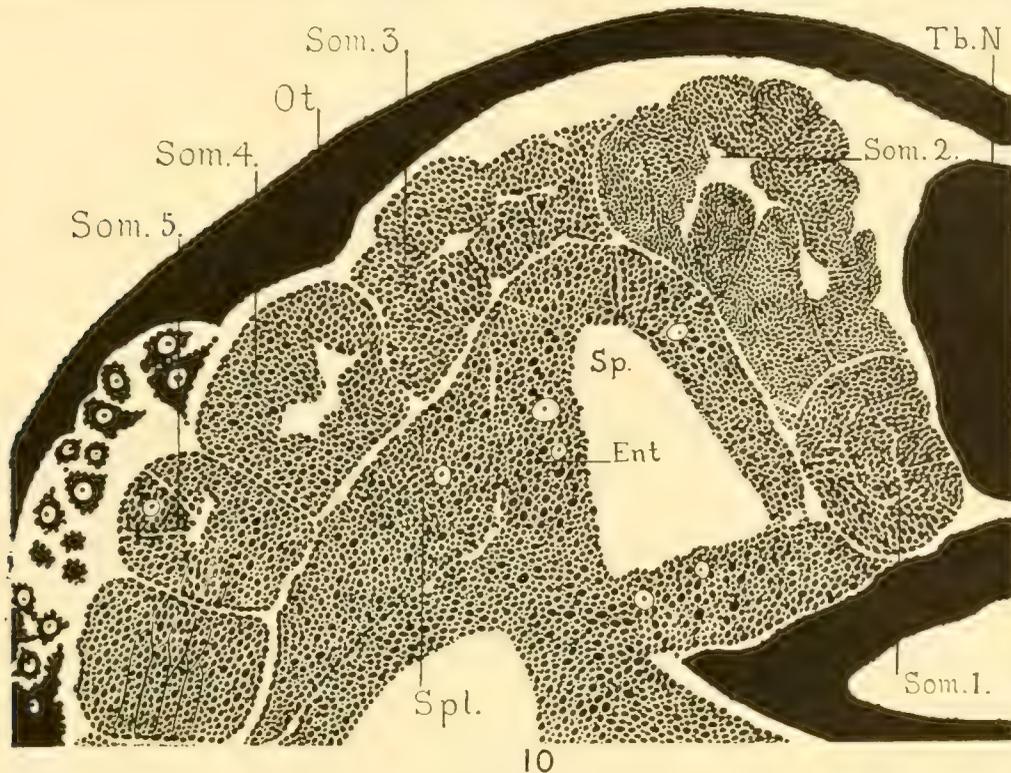
Fig. 10   A camera drawing of a parasagittal section of an 8-day (Naples) Petromyzon embryo, showing the mesodermic segmentation.   The auditory placode lies just posterior to the third somite.   Except for the absence of the anterior cavity, the mesodermic segmentation is comparable with that of Squalus fig. 8).   *Ent*, entoderm; *Ot*, auditory placode; *Som. 1*, *Som. 2*, *Som. 3*, *Som. 4* *Som. 5*, *Som. 6*, mesodermic somites 1 to 6; *Sp.* spiracular pouch; *Spl.* splanchnic mesoderm; *Tb.N.* neural tube (forebrain).

to form the external rectus muscle.   While, in Petromyzon, the following myotomes split above and below the ear and persist in the adult, in elasmobranchs the myotomes of the fourth, fifth and sixth disappear in ontogeny, leaving an hiatus between the eye muscles and those of the trunk and bringing about their characteristic isolation as a group of muscles in the adult (figs. 17 to 20).

The morphological and phylogenetic significance of these facts is plain. Instead of being relatively young muscles which have not arisen directly from the segmental muscles (as asserted by Ziegler '08), and instead of being post-otic muscles which have secondarily migrated into the preotic region (as suggested by McMurrich '12), the eye muscles are seen to be persistent portions of the pre-otic lateral trunk musculature which owe their persistence to a functional connection with the eye-ball. Their separation from the lateral trunk muscles and their isolation in the adult vertebrate are correlated with the great enlargement of the ear-capsule. Their presence in the pre-otic region therefore strongly suggests that in the ancestors of vertebrates the myotomic segmentation extended unbroken throughout the entire length of the body. Amphioxus is just such a form.

That the ancestor of vertebrates was Amphioxus-like is a very generally accepted conclusion of morphologists. In this connection the mesodermic segmentation discovered by Koltzoff ('02) in Cyclostomes (Petromyzon) is especially significant and important, since in this animal, according to Koltzoff, the segmentation of the cephalic mesoderm is primarily total and complete as in Amphioxus embryos and, moreover, the anterior mesodermic segments develop, as in Amphioxus, as dorsolateral diverticula of the entoderm. Furthermore, the lumen of each diverticulum is connected with the enteron just as in Amphioxus larvae. If Koltzoff's observations be correct, Cyclostomes stand in this, as in other respects, intermediate between Amphioxus and Elasmobranchs. Koltzoff's observations, however, have not been confirmed.

The importance of this evidence in its bearing on the past history of the vertebrate head has led me to examine sections of Petromyzon embryos in those early stages before hatching in which Koltzoff finds the mesodermic segmentation most clearly expressed. In at least two series of sections of eight-day (Naples) Petromyzon embryos the evidence presented seems to bear out Koltzoff's contention that the mesodermic segmentation in Cyclostomes is comparable with that of Elasmobranchs. While

the 'anterior' cavities are wanting (just as in some genera of Elasmobranchs), the preotic mesoderm shows a series of divisions, the relations of which to adjacent organs are comparable with those of VanWihje's first, second and third somites. These are shown in text figures 10 and 11. If Koltzoff be correct in asserting that the eye-muscles of Petromyzon are differentiated from the walls of the first three mesodermic segments, their comparability with the first three somites of VanWihje is indisputable.
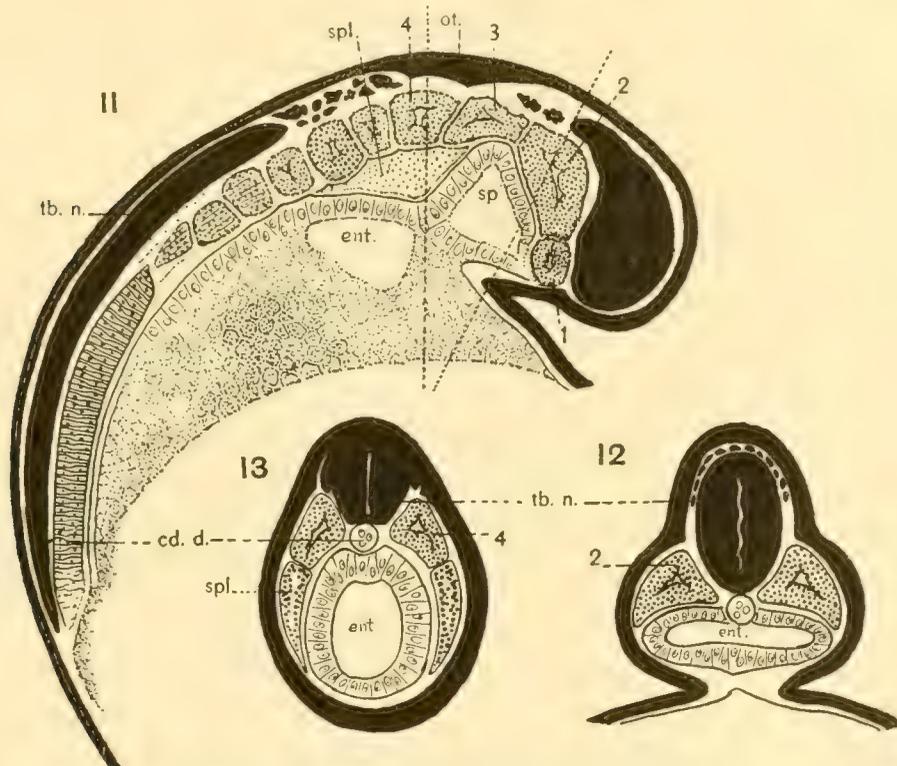
Figs. 11, 12, 13 Semidiagrammatic camera drawings of sections of Petromyzon embryos, showing the mesodermic segmentation in the region of the head. Figure 11 is from a parasagittal section of an 8-day embryo (Harv. embryol. coll. A, sect. 35–36). The topographic relations (and their later development according to Koltzoff '02) of the first three somites show them to be homologous with the first three somites in elasmobranchs.

Fig. 12 Cross section of an 8-day Petromyzon embryo through the region of somite 2 (fig. 11). The similarity of the somite to the mesodermic pouches of Amphioxus is obvious.

Fig. 13 is a cross section of a slightly older Petromyzon embryo cut in the region of the fourth somite, showing the differentiation of somatic and splanchnic mesoderm. 1, 2, 3, 4, Somites 1–4; cd.d. notochord; ent, enteron; ot, auditory placode; sp. spiracular pouch; spl, splanchnic mesoderm; tb.n. neural tube.

I regret that my own observations are not sufficiently extended to enable me to confirm Koltzoff's statement, but I know of no reason for doubting his conclusions. If they are correct, Petronyzon resembles an Acraniote in having all of its myotomes persist in the adult. In all other Craniotes at least some myotomes in the ear region degenerate in embryonic stages. Koltzoff's discovery consequently now puts us in a position to compare a larval Craniote with a larval Amphioxus and thus to carry the history of the eye-muscle back to an Amphioxus stage, that is, to a stage before eyes were differentiated. Here, however, we are confronted with the difficulty of exact homology between the myotomes of Amphioxus and those of Craniotes.

The absence in Amphioxus of eyes, ears and serial brain vesicles deprives the morphologist of the accustomed fixed points of comparison. The considerable divergence of opinion regarding the metameric homologies of Amphioxus is, therefore, not surprising. The most reasonable conjecture appears to the writer to be the assumption that the first permanent myotome (Van-Wijhe's 1st) of Craniotes is exactly homologous with the first permanent myotome of Amphioxus. This supposition is strengthened by the relations to the 'anterior cavities.' For, in both Amphioxus and Craniote embryos, are found mesodermic masses or paired cavities anterior to the first permanent myotomes. These are the 'anterior entodermic diverticula' of Amphioxus and the 'anterior head-cavities' of Craniotes (Elasmobranchs and Ganoids). The exact homology of these mesodermic cavities is based, not only on their relation to the first permanent myotomes, but also on the important circumstance that in Ganoids (Amia) the anterior head-cavities open to the exterior by an external opening, just as do the anterior entodermic diverticula of Amphioxus, in which the left cavity opens to form the pre-oral pit. In Amia

Figs. 14, 15, 16 Diagrams of acraniote stages of cyclostome and elasmobranch embryos in comparison with a larval Amphioxus. All three show an homologous mesodermic segmentation. *1, 2, 3, 4,* etc., somites 1, 2, 3, 4, etc; *a,* anterior cavities; *c.s.g.,* club-shaped gland; *end,* endostyle; *g.s.°,* first (transient) gill-pouch; *g.s.'* first (permanent) gill-pouch; *hyp.* hypophysis; *L,* lens; *M,* mouth; *N,* nasal pit; *n'ch,* notochord; *n'p,* neuropore; *ot,* otic capsule; *sp.* spiracle; *th'r,* thyreoid.
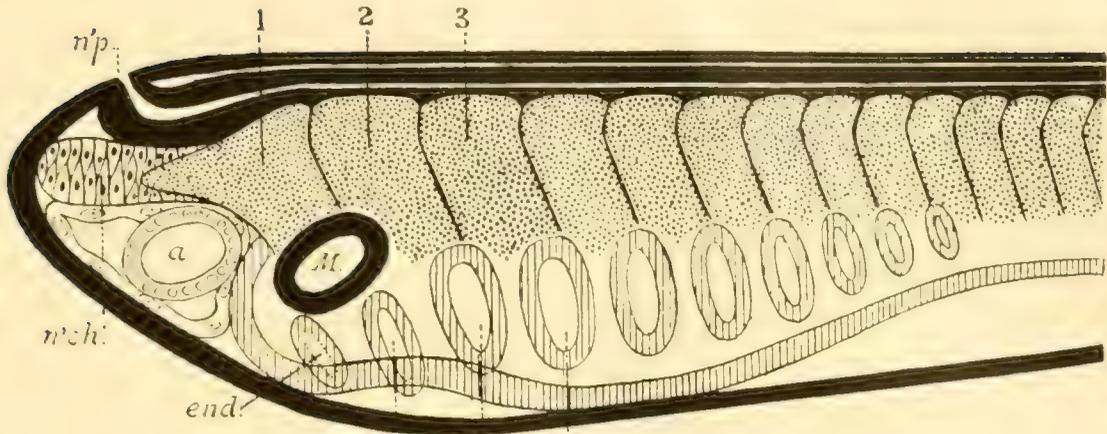
Larval Amphioxus.

14
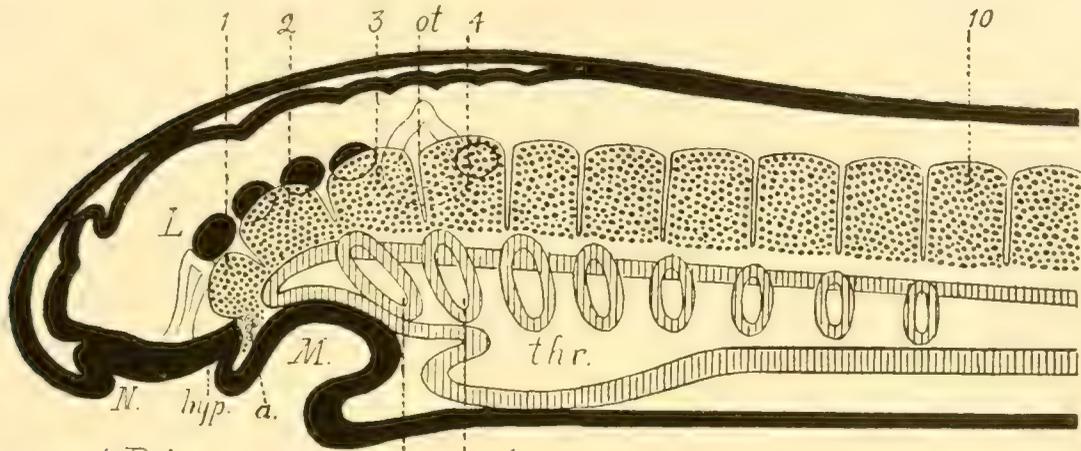
Larval Petromyzon.

15
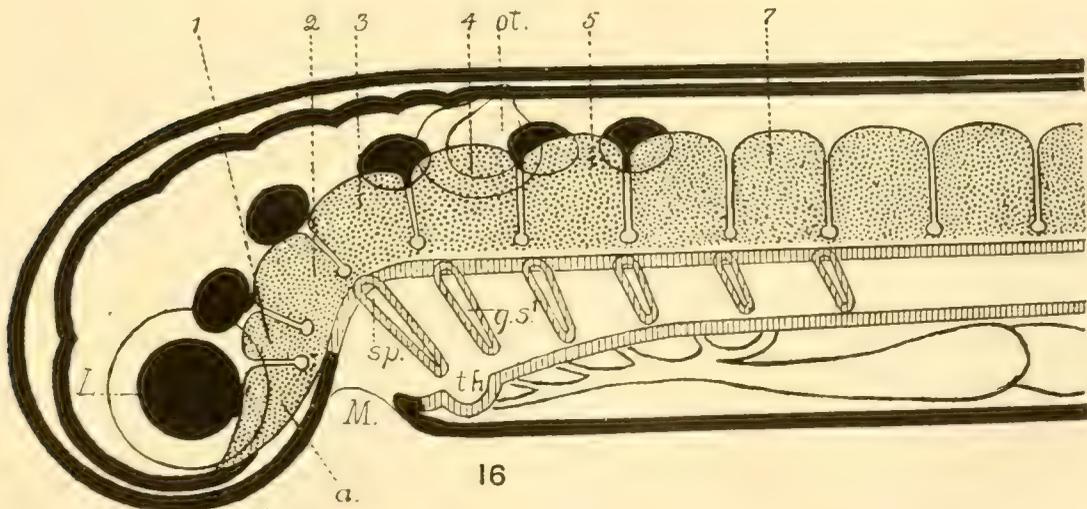
Squalus Embryo.

16

(Reigard '02) they open to form the suckers of the Ganoid larva. Similarly in Balanoglossus, it will be remembered, the anterior (proboscis) cavities open to the exterior by the proboscis-pore, which is sometimes right and sometimes left in its position. ·Since these cavities ·are the most anterior in the chordate body, since they lie immediately anterior to the first permanent myotomes and since they open—or at least one of them opens—to the exterior in Amphioxus and a Craniote (Amia), and since they are peculiar in this respect, their exact homology seems not unreasonable and strengthens the assumption of the homology of the first permanent myotomes in Craniotes and Acraniotes.

Assuming, therefore, on the basis of this evidence, the exact homology of the latter, the history of the eye-muscles may be seen to be the history of the transformation of the first three myotomes of an Amphioxus-like ancestor into the definitive six eye-muscles of man. This history may be very briefly summarized: Primarily, as in Amphioxus, the three anterior myotomes were members of an unbroken series of segmented muscles extending throughout the entire length of the body. When lateral line organs and enlarged cranial ganglia associated with them made their appearance, the anterior myotomes became split lengthwise into dorsal and ventral moieties. Further separation and displacement followed the enlargement of the optic and otic vesicles. In this way eventually two sets of muscles, one dorsal and one ventral, were brought in close proximity to the enlarging optic vesicles with which they finally became functionally associated (figs. 17 and 18).

How these two sets of muscles became gradually transformed into the eye-muscles is revealed by their ontogenesis in Elasmobranchs. Each of the divisions (dorsal and ventral) of the first myotome divides again, thus forming the four muscles innervated by the oculomotor nerve. The second myotome undergoes no further subdivision. Its dorsal moiety becomes the superior oblique muscle and is innervated by the trochlearis nerve. Its ventral portion, however, unites with the third myotome to form the external rectus muscle and becomes innervated by the abducens nerve. The dorsal moiety of the third myotome does

not differentiate muscle fibers. Like the myotomes of somites four, five and six, it has disappeared phylogenetically, leaving the eye muscles as an isolated group unconnected with the post-otic myotomes (figs. 19 and 20).

During the phylogenetic transformation of the eye muscles noteworthy changes occur in their nerve connections. Since,

Larval Amphioxus.

17

Larval Petromyzon.
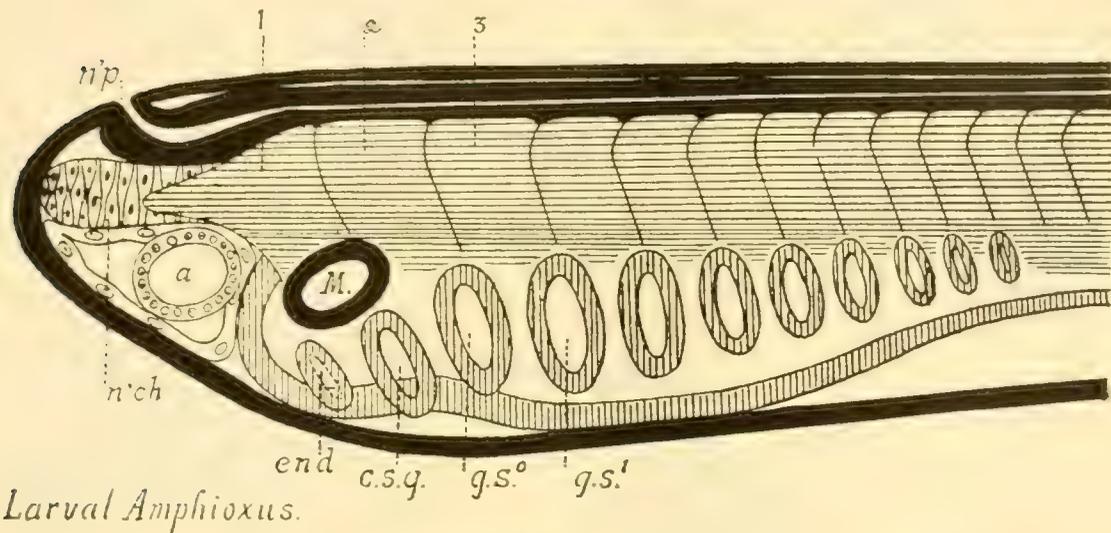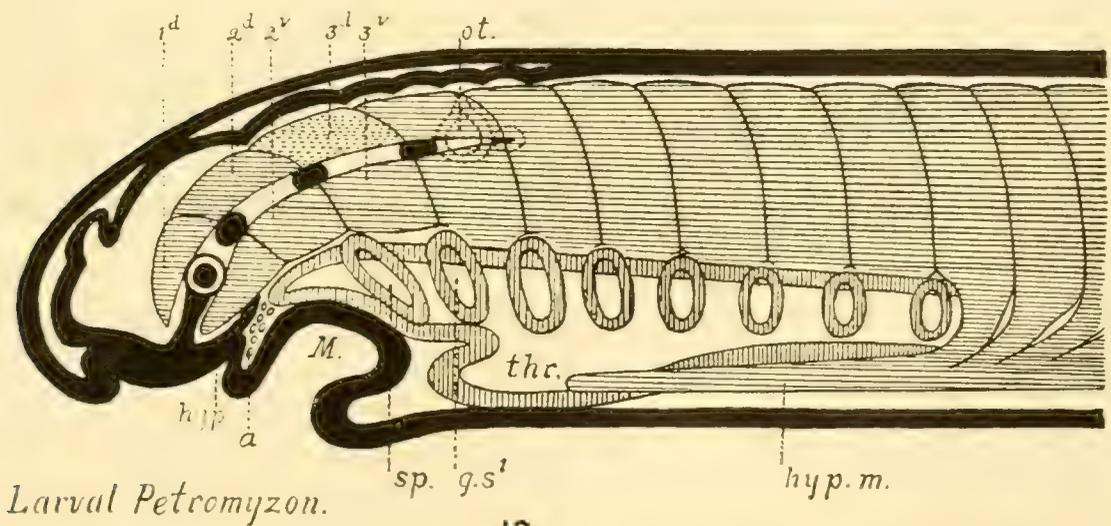
18

Figs. 17, 18 Pre-gnathostome stages in the history of the eye muscles. Diagrams based upon the myotomic relations in Amphioxus and Petromyzon embryos. All of the myotomes persist in Petromyzon (Koltzoff) as in Amphioxus, but they split into dorsal and ventral moieties in the case of the five anterior ones. Abbreviations the same as in figures 14 to 16.
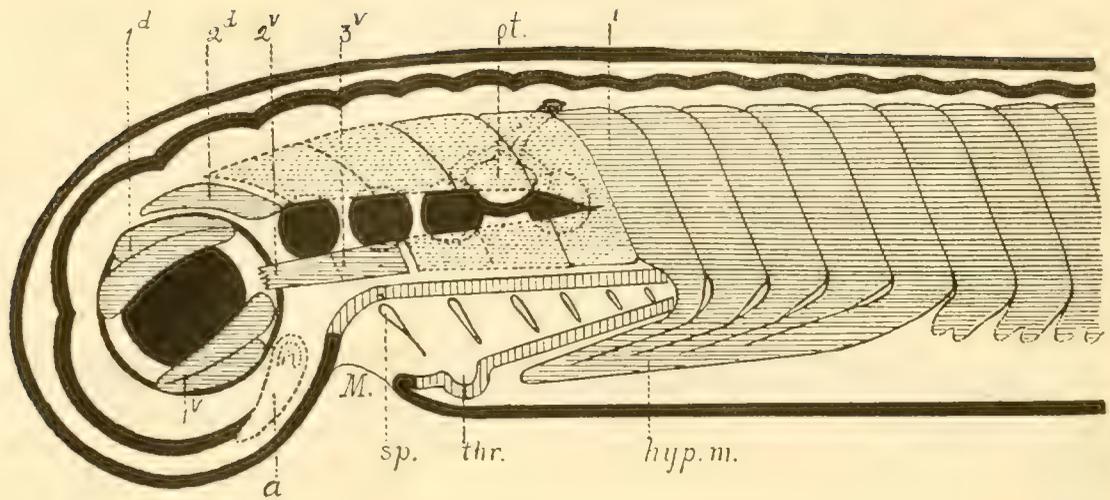
Larval Squalus.   19
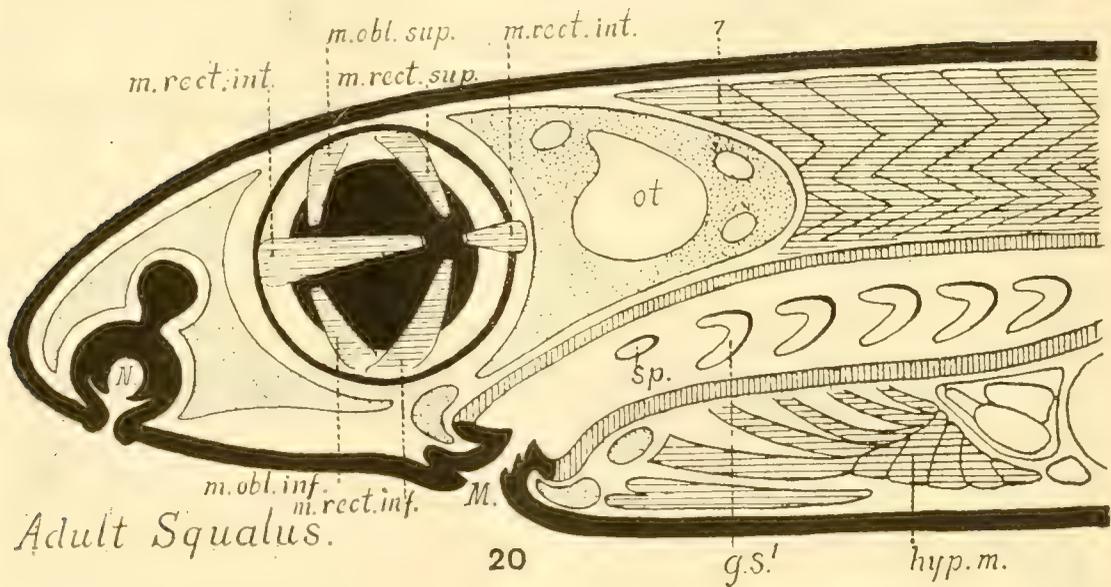
Adult Squalus.   20

Figs. 19, 20 Diagrams showing two later stages in the phylogenesis of the eye muscles, based upon their ontogenesis in elasmobranchs. The myotomes which degenerate in ontogeny are indicated by dotted lines. In figure 19 the anlagen of the six eye muscles are already differentiated. Their relation to the dorsal and ventral moieties of the myotomes is indicated. The adult relations of the muscles are seen in figure 20. They remain essentially the same in man. *1d,* dorsal moiety of the first myotome; *1v,* ventral moiety of the first myotome; *2d, 2v,* dorsal and ventral moieties of the second myotomes; *3v,* ventral moiety of the third myotome; *a,* anterior cavities; *7,* seventh myotome; *g.s.¹,* first gill-slit; *hyp.m,* hypoglossus musculature; *M,* mouth; *N,* nasal pit; *ot,* otic capsule *sp,* spiracle; *th'r.,* thyreoid.

however, these have been discussed at considerable length in earlier papers by the writer ('09, '12, '14), and since no facts are presented which are irreconcilable with the conclusions stated above, it seems unnecessary to do more than refer to them here. While the first myotome retains throughout phylogenesis its primitive nerve relations to the oculomotor nerve, the somatic motor nerve of the second myotome (the trochlearis) acquires a dorsal chiasma and retains connection only with the dorsal moiety of the muscle. The ventral portion, uniting with the third myotome, becomes innervated by the abducens nerve, the somatic motor nerve of a more posterior metamere. The conclusion that such modified metameric nerve relations may have occurred through a process of nerve substitution or piracy is in harmony with what we now know of the method of nerve histogenesis (Harrison '11) and of the primary independence of nerve and muscle (Parker '10). Consequently the modified metameric nerve relations of the eye muscles present no serious objections to the phylogenetic conclusions reached in this paper.

Objection to the foregoing description of the phylogenesis of the eye muscles may be raised on the ground of the uncertainty that Amphioxus represents a form ancestral to vertebrates. To some this will seem a serious objection. But that Amphioxus embodies more completely than any other existing animal the general characteristics of the chordate type from which the Vertebrates have sprung, is an opinion held by the great majority of vertebrate morphologists. Familiarity with embryonic and larval stages of Amphioxus, Cyclostomes, and Elasmobranchs greatly strengthens the conviction that this opinion is sound. The evidence presented in this paper is in full accordance with the belief.

An attempt to carry the history of the eye muscles back into pre-chordate stages leads eventually to the problem of the origin of the segmental musculature—in other words to the problem of the origin of the mesoderm. The logic of the previous discussion would lead to the conclusion that originally the eye muscles were metameric diverticula of the invertebrate intestine. Further than this we could scarcely proceed.

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CELL INCONSTANCY IN HYDATINA SENTA[1]

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The highly determinate development of certain invertebrates has long been known. The number as well as the fate of the early embryonic cells has been established in numerous cases, and is exceedingly regular. The number of cells in the embryo may be the same in all individuals, even though the number in the adults of the same species is variable. It is only in comparatively recent years, however, that this constancy of number of cells has been discovered in adult animals of certain species in which the cells are numerous. Martini ('09 a, '09 b, '09 c, '12) has claimed such constancy in nematodes, rotifers, and tunicates and Van Cleave ('14) in Eorhynchus. The latter author cites a number of other cases.

The extension of constancy of cell number to adult animals seems to me to make new demands upon the explanations offered to account for this constancy. Embryonic stages are passed through quickly, and one might suppose that whatever agencies could interfere with the orderly and definite production of cells in the embryo would have little time to do so. Before the adult condition is reached, however, such disturbing factors might have time to operate and the number of cells be thereby altered. The fact of cell constancy in the adult, then, makes greater demand upon its explanation than does the fact of cell constncy in the embryo. I do not mean to assert that the cause of constancy in the adult is different from the causes of constancy in the cleavage stages; but an explanation which would satisfy the demands of the latter, might be inadequate for the former.

How rigidly the factors producing cell constancy must operate, therefore, is a matter of some importance. If the number of cells is absolutely invariable in the adult, as well as in the embryo, the explanation of constancy must be of one kind; if the adult number is occasionally aberrant, while the embryonic number is invariable, the explanation may be of a somewhat different order of exactitude. Regarding the invariability of the adult number one is left somewhat in doubt by the statements of Van Cleave and of Martini, chiefly because it is not usually stated just how many individuals have been studied. Van Cleave (op. cit., p. 259) states that, in his work, in no case was the number of individuals examined as small as two, and that in many cases it was as large as two hundred. He reports no variations in the number of cells in any organ, except possibly one in which the number of cells was so large that it was difficult to count them accurately. The one possible exception was presented by two individuals, studied by the reconstruction method from sections, which revealed, respectively, one hundred and eight and one hundred and nine cells in the brain, with the possibility that one of these counts was an error. Van Cleave himself raises the question whether moderate variability would invalidate conclusions drawn from cell constancy, but since he concludes that no such moderate variability was demonstrated, nor even rendered probable, in Eorhynchus, the question was not very pertinent to his own work.

Martini ('12) expresses the conviction that the number of cells in the rotifer Hydatina senta is highly invariable. He states the number of cells (or nuclei, in the case of syncitia) in each organ, adds them together, and gives the total number of cells for the whole organism as nine hundred and fifty-nine. In most of the organs, especially the smaller ones, the number of cells was never found to vary. In others there was some uncertainty. The stomach-intestine, for example, usually contains thirty cells, but in a few instances an aberrant number was found. Martini strongly emphasizes that the aberrant number were always less than thirty, and he was at first inclined to ascribe the reduced number to the probability either that the in-

dividual was a young one which had not yet undergone all its cell divisions, or that distortions concealed one or more of them. However, he finally concluded from the positions of the remaining cells, and from supposed protoplasmic remnants, that certain cells had been lost, and suggested that their disappearance was due to the diatomaceous food upon which the animals were reared. So convinced was he that the number of cells in each and every organ is constant, that in the summary of the paper cited, the possibility of exceptions is practically ignored.

Such regularity in this rotifer is surprising to one who has noted its extreme irregularity in other respects. The metabolic processes[2] which result in cell division, and determine the number of cell divisions, must needs occur with clock-like precision; while the metabolic processes that determine the rate of growth and the type of reproduction are subject to great and unaccountable fluctuations. The remarkable contrast of fixity in one set of processes, and apparent lawlessness in another, in the same animal led me to examine a very limited portion of the structure of this rotifer to ascertain whether the number of cells is as nearly constant as Martini supposed.

For the proposed test, two small organs whose cells could be easily counted were selected. These were the yolk gland and the gastric glands. The yolk gland is a large syncitial mass, in the form of a baseball catcher's mitt, closely applied to the stomach-intestine and to the ovary; it was found by Martini to have invariably eight nuclei. The gastric glands are two rounded masses, situated near the upper end of the stomach-

[2] By ascribing the initiation of cell division and the determination of the number of cell divisions to metabolic processes I do not attempt to locate the ultimate cause. I have on one occasion verbally expressed the view that in some way the number of cells is dependent upon some rhythm of the protoplasm. Here again, 'rhythm' is not supposed to name the final agent. Even if cell constancy were attributed to an accurate time relation between the rhythm of cell division and the rhythm of growth and differentiation, such that a given number of cell divisions (no more and no less) had time to occur before cell differentiation made further division impossible, the assumed rhythms would not be the ultimate cause. Doubtless something inherent (hereditary) in the organism determines these rhythms, as is almost certainly true of the rhythm in the life cycle; but this inherent something must work through metabolic processes to attain its end.

intestine, with which they are connected by short canals. They are likewise syncitia, which Martini finds always contain six nuclei.

Determination of the number of nuclei in these glands was made from serial sections. The sections had been prepared for another use in which it was important to have complete series. All imperfections in the series were therefore recorded, and for the counting of nuclei only complete series were used. Moreover, in the yolk gland, the nuclei are so large that five or six successive sections passed through each nucleus, so that any breaks in the series large enough to omit one nucleus would be easily detectible not only from the yolk gland itself, but from the surrounding tissues. In the case of the gastric gland, the nuclei are smaller, and, though imperfections in the series of sections could probably have been detected from the sections themselves, reliance was placed entirely upon the records made when the slides were prepared. In any case, incompleteness in the series of sections could never lead to an erroneous increase in the number of nuclei counted. All aberrant numbers were determined independently by two persons trained in the interpretation of serial sections, and in every case there was agreement.

YOLK GLAND

This organ was examined in two hundred and forty-five satisfactory individuals. In two hundred and thirty-five, the number of nuclei was eight. In the remaining ten, a trifle over 4 per cent of the total, aberrant numbers were found, as indicated in table 1.

TABLE 1

Distribution of the numbers of nuclei in the yolk glands of 245 specimens of Hydatina senta

NUMBER OF NUCLEI	NUMBER OF INDIVIDUALS
5	1
6	0
7	5
8	235
9	3
10	1

The commonest aberrant numbers are seven and nine, while the extremes show a reduction of 37.5 per cent below and an elevation of 25.0 per cent above the usual number (eight).

To discover whether anything in the environment caused these differences in the number of nuclei, or whether any relation between the deviation and other phenomena could be established, all recorded facts were carefully examined. Information was found concerning the following points: 1) Some of the two hundred and forty-five rotifers examined belonged to periods of many male-producers, others to periods of few male-producers; 2) some were young, some middle-aged, some old females; 3) some were reared in spring water, others in manure solution; and 4) some were male-producers, others female-producers. In each of these groups some aberrant yolk glands were found. How frequently the unusual numbers occurred under the several circumstances named, is shown in tables 2, 3, 4 and 5, respectively.

The numbers of specimens aberrant with respect to the number of nuclei in the yolk gland are, of course, too small in any of

TABLE 2

Distribution of individuals of Hydatina senta having aberrant numbers of nuclei in the yolk gland, with reference to periods of many male-producers and periods of few male-producers

PERIOD OF MANY MALE-PRODUCERS			PERIOD OF FEW MALE-PRODUCERS		
Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
93	5	7, 7, 7, 9, 10	49	3	7, 7, 9

TABLE 3

Distribution of individuals of Hydatina senta having aberrant numbers of nuclei in the yolk gland, with reference to the age of the individuals

YOUNG			MIDDLE-AGED			OLD		
Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
31	1	9	24	1	7	15	1	7

TABLE 4

Distribution of aberrant numbers of nuclei in the yolk glands of individuals of Hy-
datina senta reared in spring water and in manure solution, respectively

SPRING WATER			MANURE SOLUTION		
Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
47	2	7, 7	56	3	7, 9, 10

TABLE 5

Frequency of occurrence of aberrant numbers of nuclei in the yolk glands of male-
producing and female-producing Hydatina senta

MALE-PRODUCERS			FEMALE-PRODUCERS		
Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
47	2	5, 9	188	8	7, 7, 7, 7, 7, 9, 9, 10

these tables to justify the computation of percentages with the
expectation of making valid comparisons. There are, however,
no striking differences in the numbers of aberrant glands under
different circumstances. Yolk glands with other than eight
nuclei occur at times of many male-producers, as well as at times
of few; in young, middle-aged, and old females; in specimens
reared in spring water, and in those reared in manure solution;
in male-producers as well as in female-producers. And they
occur with what might well be equal frequency, if the numbers
examined were large enough, in each of these cases.

It seems unlikely, therefore, that any of the conditions above
enumerated have anything to do with the alteration of the num-
ber of nuclei. It is also worthy of note that the deviations from
the usual number eight are not all diminutions, as Martini (see
ante) found in the case of the stomach-intestine. It may also be
remarked that the only deviation in a young adult female was
a plus deviation (table 3), which cannot therefore be explained
by supposing that not all the usual cell divisions had yet occurred.

GASTRIC GLANDS

Degeneration of the gastric glands. It is not uncommon in this rotifer to find the gastric glands more or less atrophied. In some cases the change referred to is evidenced merely by the more homogeneous appearance of the organ, and by its failure to take the stain properly. In more marked cases the glands are plainly reduced in size, the characteristic structure is lost, and there is no longer any connection with the stomach-intestine. In extreme cases one of the glands is entirely missing, and in one specimen I was unable to find either of the glands, though the sections were apparently not damaged in the region proper to these organs. One gland was sometimes atrophied while the other was normal.

In none of these cases does the degeneration take the form of a destruction of some of the cells (here nuclei, since the gastric glands are syncitia) through high physiological activity. The whole organ degenerates simultaneously, and in the early stages the six nuclei are still recognizable.

Number of nuclei in the gastric glands. In determining the number of nuclei in the gastric glands only normal specimens were used. The degeneration described above is easily recognized, and specimens with such glands were rejected.

One hundred and twenty glands were studied. Among this number, nine had other numbers of nuclei than six. Four was the smallest number observed, seven the largest. The frequency of the various number is given in table 6.

In the case of the gastric glands, as with the yolk gland, it was possible to determine some of the conditions of age, environment,

TABLE 6

The frequency of occurrence of various numbers of nuclei in 120 gastric glands of Hydatina senta

NUMBER OF NUCLEI	NUMBER OF INDIVIDUALS
4	1
5	7
6	111
7	1

TABLE 7

Frequency of occurrence of aberrant numbers of nuclei in the gastric glands of Hydatina senta in periods of many male-producers and in periods of few male-producers

PERIOD OF MANY MALE-PRODUCERS			PERIOD OF FEW MALE-PRODUCERS		
Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
22	2	5, 5	44	5	4, 5, 5, 5, 5

TABLE 8

Frequency with which aberrant numbers of nuclei occur in the gastric glands of Hydatina senta, in young and middle-aged adults, respectively (no old adults were examined)

YOUNG			MIDDLE-AGED		
Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
17	2	5, 7	9	2	5, 5

TABLE 9

Proportion of gastric glands having aberrant numbers of nuclei in individuals of Hydatina senta reared, respectively, in spring water and in manure solution

SPRING WATER			MANURE SOLUTION		
Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
18	1	5	31	4	4, 5, 5, 5

TABLE 10

Proportion of gastric glands, in male-producing and female-producing individuals of Hydatina senta, having aberrant numbers of nuclei

MALE-PRODUCERS			FEMALE-PRODUCERS		
Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
36	0	None	75	9	4, 5, 5, 5, 5, 5, 5, 5, 7

and phase of life cycle of the individuals whose glands were studied. The facts, as far as recorded, are given in tables 7, 8, 9 and 10.

With the exception of male-producers, as contrasted with female-producers (table 10), none of the various conditions mentioned in tables 7 to 10 seem to be associated with deviations in the number of nuclei in the gastric glands. The number of nuclei deviates from the usual number six in periods of few or many male-producers, in adults of different ages, and in those reared in spring water or in manure solution. And in view of the small number of individuals studied, it is not impossible that the absence of aberrant glands in male-producers is insignificant. The minimum aberrant number is 33.3 per cent below the normal, the maximum 16.6 above the normal. It is also worthy of note that in only one specimen studied were both gastric glands aberrant, one of them having seven nuclei, the other five.

DISCUSSION

In two of the smaller organs of the rotifer Hydatina senta, an organism said to be highly constant in the number of its cells, it is now found that the number of nuclei varies. In the case of one organ, 4 per cent of those examined contained other numbers of nuclei than those claimed for it; in the other organ, 7.5 per cent were aberrant. In both organs, the highest number found is approximately double the lowest number. In one organ the nuclei are of moderate size, in the other enormous, so that they are readily counted.

If those who have found the numbers of cells in the organs of this rotifer highly constant have been misled in the case of small organs whose nuclei are easily counted, there is no reason to assume that their counts are any more accurate in the case of larger organs where the counting is difficult. If other organs are as variable as the yolk gland and the gastric glands, and if the factors which change the cell number from the 'normal' can operate in the same direction in all organs, then by rare chance the supposed nine hundred and fifty-nine cells in the whole body might be either six hundred and forty or eleven hundred and

eighteen. More likely the number would be between these, since there is nothing as yet to indicate that deviation in one organ is associated with deviation in other organs. However, the possibilities are numerous, not single. If each organ contains unusual numbers of cells in 5 per cent of the individuals, and there is no association between the deviation in various organs, the total number of nine hundred and fifty-nine would probably be realized less often than not.

It has not seemed worth while to pursue the re-examination of cell constancy in Hydatina further than the two organs named. A measure of its exactitude has been obtained, and that is all that was sought. The observations described in this paper do not destroy the problem of cell constancy; they merely make its solution easier. Even if every organ of every animal in which constancy of cell numbers has been claimed, should prove to be as variable as the yolk gland and gastric gland of this rotifer, the problem of cell constancy would remain. Although the total number of cells in an organism is the same in only a small percentage of cases, if the number in any one organ is the same in ninety per cent of the individuals, there is a problem of cell constancy which calls for solution. But there is no need of complicating this solution by assuming a degree of constancy that does not exist.

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THE EYE OF POLYCYSTIS GOETTEI (BRESSLAU)

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FIVE FIGURES (ONE PLATE)

The rhabdocoele on which this study was made has appeared from time to time during the cold months of the year in a pond near the University of Virginia. It is found in greatest abundance at a depth of about two feet when the water is at winter temperature. In the warm months of the year they must seek greater depths, for we have not encountered them in our summer collecting. Bresslau ('06), in describing the species, says: "Unmittelbar hinter der Russelbasis liegt das Gehirn, auf ihm jederseits am Vorderende ein Auge, dessen schwartzes Pigment von kugeligen Granulationen gebildet wird." (p. 417). In his figure 1 he indicates a posterior black portion and an anterior clear or colorless part in each eye. The posterior portion is evidently the pigmented part of the eye, while the clear region is comparable to what von Graff ('82) refers to in different rhabdocoeles' eyes as 'Lense.' We agree, however, with Böhmig ('87) when he says: "was von Graff als Linse bezeichnet, ist zweifellos der beim Conserviren geschrumpfte Inhalt des Pigmentbechers." (p. 487). So that the light anterior end of each eye in Bresslau's figures must be the projecting visual elements seen in profile along the margin of the mouth of the pigment cup.

Since Hesse's ('97) work, the histology of the eye of Turbellaria has been well understood. He showed that there are two sets of elements or cells entering the formation of the eye of the Turbellaria, namely, (a) pigment-cells, and (b) visual cells or retinulae.

In all cases the pigment cells form a pigmented cup about certain portions of the retinulae. These pigment cups may be unicellular or multicellular. So far as our knowledge goes

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Böhmig was the first to describe what may be called a compound pigment cup in a Turbellarian eye. Of the eye of the rhabdocoele, Vorticeros auriculatum, he says:

Der Pigmentbecher jedes Auges wird durch eine mittlere Pigmentscheidewand in eine vordern und hintern Kammer gelegt. Der Pigment besteht aus kleinen rötlichen Körnchen. Einen Plasmasaum mit Kernen den Pigmentbecher habe ich nicht auffinden können, doch soll damit nicht gesagt sein, das er in der That fehle. Durch die Pigmentscheidewand ist natürlich bedingt, dass der Pigmentbecher zwei Öffnungen besitz, von denen jede durch eine einige vor ihr liegende Zelle von linsenformigen Gesalt mit deutlichen Kern und Kernkörperchen geschlossen wird. (p. 486).

Thus it appears that there are two kinds of pigment cups associated with the visual elements of rhabdocoeles: (a) simple, in which there is but a single lumen and, (b) compound, in which the lumen of the cup is subdivided to give two secondary lumina.

In the eye of the flatworm there may be one or more visual elements which send part of their cell bodies into the lumen of the cups formed by the pigment cell or cells. In all cases the nuclei of the visual cells or retinulae lie outside of the lumen of the pigment cup. Dendy (see Benham '01) shows in his figure of the eye of Geoplana a single visual cell whose nucleus lies in the fundus of the pigment cup. This condition is so strikingly exceptional that we feel that Dendy may have mistaken another structure for the nucleus of the cell. There has recently been described a highly refractive body forming a part of the retinula of Prorhynchus applanatus Kennel (Kepner and Taliaferro, '16). The paper by Kepner and Taliaferro presents a description of the retinula of a rhabodocoele in which there is a refractive body within the cytoplasm, between the nucleus bearing portion of the cell and the end-organ or rhabdome of the cell. Kepner and Foshee ('17) showed that a striking comparison could be made between the visual element of Prorhynchus applanatus and the retinula of a vertebrate.

The pigment cup, when seen in the living specimen, is spheroidal in contour and intensely black both by reflected and transmitted light. The average diameter of the cup is twenty-five

microns or less. Beyond this no further details of the pigmented part of the eye could be recognized in the living condition nor in sections that were too thick. In series of sections cut five microns the cytology of the pigment cup could be worked out fairly well. These sections show that the pigment mass of the eye is unicellular. The nucleus of the pigment cell is oval and has a large nucleolus. These two characteristics mark it off distinctly from the nuclei of adjacent cells, such as nerve and mesenchymal cells (fig. 1, P-N). A bit of the cytoplasm is shown about the nucleus in figure 1; but, for the most part the cytoplasm is completely obscured by the presence of many black spheroidal pigment bodies (fig. 1, P, fig. 2, P). The cell body of the pigment cell forms the wall of the pigment cup of the eye, but a very striking feature of this cell body is that it forms a conspicuous partition of pigmented cytoplasm, which, standing up from the floor of the pigment cup, divides its lumen into two secondary lumina. The accessory portion of this eye, therefore, is a divided cup and presents two mouths or openings instead of one. Thus in this eye there are two principal axes instead of one as is usually the case. In short, here we have a compound eye similar to the one described by Böhmig ('87). Ordinarily one mouth of this compound cup is directed anteriorly, while the other is directed posteriorly. These mouths open dorsally as a rule. However, in our fixed material we have seen specimens in which one opening of the pigment cup was ventral to the other and the axis of each lumen was directed anteriorly. Again, we have fixed material in which the mouths lie one behind the other and the axes of the lumina are directed laterally. This shifting of the axes of the lumina of the pigment cup in fixed material is not to be considered as being due to torsion resulting from fixing; but we believe that the animal has the power to move its eye through a fairly wide range as it lies in the parenchyma. This is perhaps due to the play of the adjacent muscles of the proboscis.

A single retinula which fills the lumen of each half of the pigment cup, lies quite close to the dorsal ganglia so that it appears to arise out of the mass of cephalic nerve cells. The

shape of the retinula is that of a bent spindle. The proximal third of the cell is bent almost at right angles to the distal two-thirds. This proximal third of the cell is that part of the cell-body which projects beyond the contour of the pigment cup. The parts of the two retinulae that do not lie within the cup leave the pigment cup in opposite directions (fig. 3). This proximal third of the retinula represents one of the three regions which can be recognized in this cell. The nucleus lies within this region. The cytoplasm of this nuclear-bearing portion of the retinula shows no striation (fig. 1, M). The part of the retinula, enclosed by the pigment cup, presents two other regions. The distal of these is closely applied to the inner surface of the surrounding pigment cell. The cytoplasm of this region displays a homogeneous, or better, a uniformly finely granular texture. It is only on the slides that show the best general fixation[1] of tissues that we find the uniform texture of this part of the cytoplasm. In slides that show a little, but no great shrinkage it is suggested that there may be lines of less density present in this region of cytoplasm. These lines, due to slight shrinkage in some fixing, break, and a striated condition of part or all of this part of the cell results. That this variation, due to the method of fixing, expresses some specific condition of the rhabdome is indicated by the fact that the striae are always directed from the inner surface of the surrounding pigment cell towards the middle region (ellipsoid) of the retinula (fig. 1, R). In this distal part of the retinula we have the end organ of the visual cell—the rhabdome (figs. 1, 2, 3, R). Striated rhabdomes have been described for Turbellaria. There is, however, a third region in the retinula of this rhabdocoele, which is well differentiated by Mallory's connective tissue stain, by which the basal portion of the cytoplasm and the rhabdome take a blue color, while the third region of the visual cell has an affinity

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[1] We have used Carnoy's fluid, chrom-aceto formalin, aceto-sublimate, and Flemming's stronger solutions. These are given in the order in which we found them yielding results—the poorest fixing resulting from Carnoy's and the best from Flemming's stronger mixture. All staining was with Mallory's connective tissue stain. The sectioning was done at five microns and serially.

for the red stain. This is especially the case with material fixed
in chrom-aceto-formalin. This region, which we take to be
homologous with the 'refractive body' which Kepner and Telia-
ferro ('16) described for the eye of Prorhynchus applanatus, is
wedge-shaped. This region has been recorded for a triclad
rhabdome, and a function suggested for it by Taliaferro ('17)
who says,

The rhabdome itself shows an optically denser region in its outer
end as described in Prorhynchus applanatus by Kepner and Talia-
ferro ('16). This region because of its shape and density, must have
some effect upon the rays of light if they pass through the long longi-
tudinal axis, which it cannot have if they pass through in any other
direction.

The wider faces of the denser wedge-shaped region of the
rhabdome of Polycystis goettei are parallel to the partition of
the pigment cup (fig. 5, E) and their apices are directed towards
the fundus of the lumina of the cup (fig. 2, E and fig. 3). In
brief, we may say that there are three regions clearly differentiated
in the retinula of this rhabdocoele: (a) a proximal region
that bears the nucleus; (b) a distal region, which is the end
organ or rhabdome; and (c) a middle region arising from the dis-
tal end of the basal part of the cell and extending into the cone-
shaped rhabdome.

This three-fold differentiation of the retinula of Polycystis
goettei is homologous with the retinula of Prorhynchus applanatus
where there is a basal region which bears the nucleus and is
homologous with a similar region in the retinula of this rhab-
docoele (fig. 4, A and A'). The second region of this cell of
Prorhynchus applanatus has been seen in fresh material compressed
between cover-glass and slide to be highly refractive. In the
retinula of Polycystis goettei (Bresslau) we have a region homol-
ogous with this refractive region (fig. 4, B and B'). Finally
the end organs in the two retinulae of the two rhabdocoeles are
homologous (fig. 4, C and C').

Thus we have homologous regions in the eyes of the two
rhabdocoeles, which are strikingly analogous to, if not homolo-
gous with, the three regions that have been described in the

retinulae of the vertebrate eye.   Prorhynchus has, as the analogue of the cylindrical or conical end organ, a solid low rhabdome, while in Polycystis there is a hollow, rather high conical rhabdome (fig. 4, C, C' and C'').   Comparable with or analogous to the ellipsoid of the vertebrate, Prorhynchus applanatus has a concava-convex lens-shaped refractive body; in Polycystis goettei, a wedge-shaped body (fig. 4, B, B', and B'').   Finally, analogous to the nuclear-bearing region of the vertebrate visual cell there is the nucleated region of the retinulae of both Prorhynchus and Polycystis (fig. 4, A, A', A'').

SUMMARY

1.   There are two visual cells and one accessory, pigmented cell entering the formation of the eye of Polycystis goettei.

2.   The pigment cell is spheroidal with two lumina within it.

3.   Into each lumen of the pigment cell there enters a retinula.

4.   The retinula resembles the visual cell of Prorhynchus applanatus in that it has a third region homologous with that animal's 'refractive lens-shaped body.'

5.   This homologue of the refractive lens-shaped body of Prorhynchus is conspicuously analogous, if not homologous with, the ellipsoid of the vertebrate retinula.

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1 Section involving the pigment-cell and the two retinulae of the eye. *M*, myoid or proximal portion of the retinula, which bears the nucleus; *E*, base of the wedge-shaped region of the retinula cut obliquely; *R*, rhabdome, showing striae; *P.N*, nucleus of pigment-cell; *P*, pigment granule; *Ms*, muscle of proboscis, which passes by the eye. The plane of section is indicated by line *1* in figure 3. × 1500.

2 Section of eye, parallel to the partition of pigment-cell and to the wedge-shaped region of the two retinulae. The left side of the drawing is ventral, the right dorsal. *R*, rhabdome: *E*, wedge-shaped body (ellipsoid): *P*, pigment granules. × 1500.

3 Diagram of a meridional section of eye. *P.C*, pigment-cell with its nucleus: *R*, rhabdome: *E*, wedge-shaped region (ellipsoid) of retinula: *M*, proximal part of visual cell (myoid): *1*, plane of section of figure 1; *2*, plane of section of fig. 5.

4 Diagram of retinula of: *I*, Prorhynchus applanatus. *II*, Polycystis goettei; and: *III* vertebrate. *III* is based upon Arey's figures. Line *A-A'* connects the rhabdomes; line *B-B'*, the refractive bodies (ellipsoids): line *C-C'*, the nuclear bearing regions (myoids) of the retinulae.

5 Transverse section of a retinula involving the rhabdome, *R*: the wedge-shaped body (ellipsoid), *E*: and a part of the pigment-cell. The plane of the section is shown by broken line *2* in figure 3. × 1500.
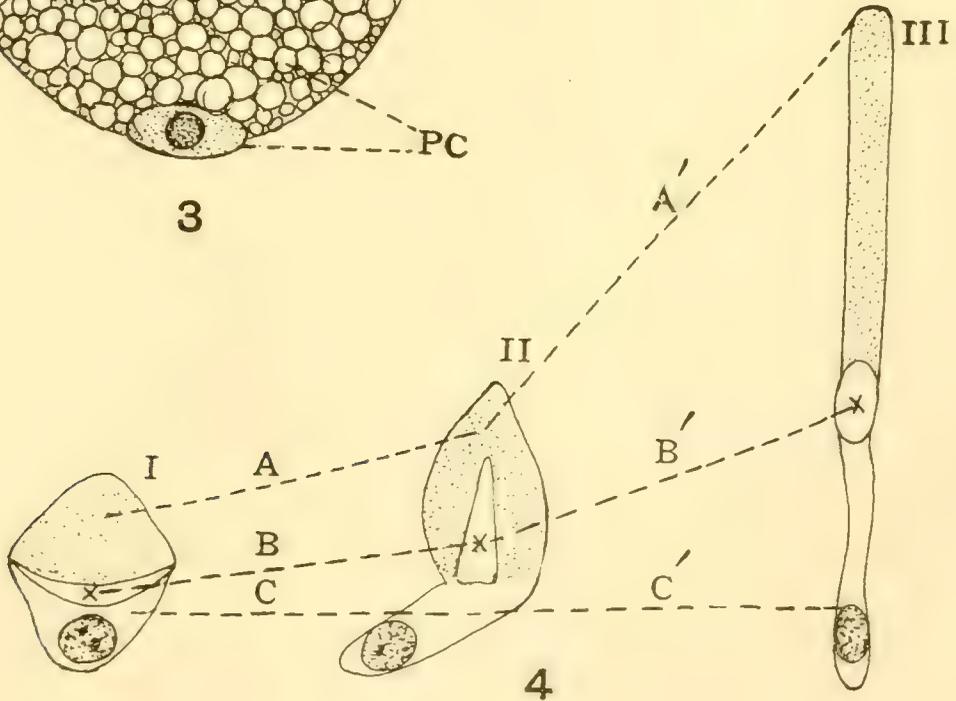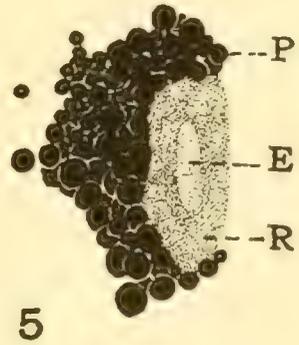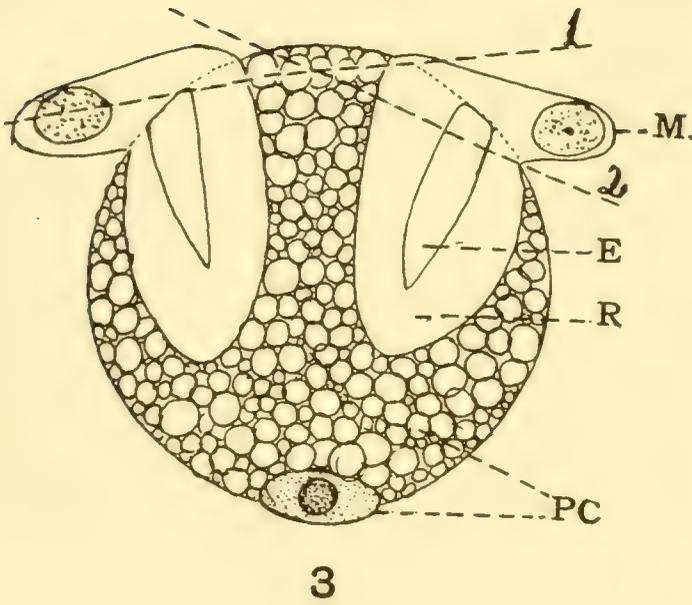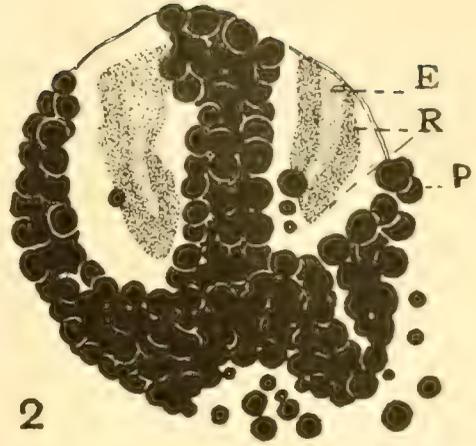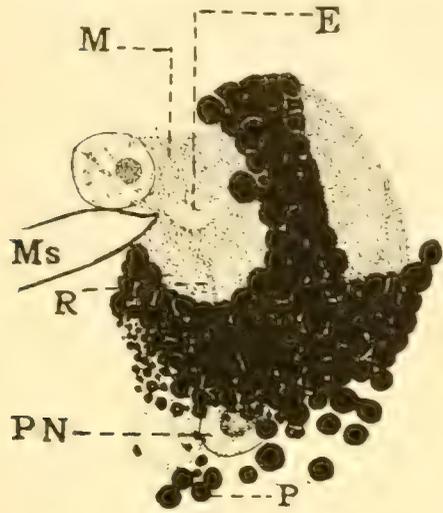
CUSHION CELLS OF THE PHARYNX OF PRORHYN-
CHUS APPLANATUS KENNEL

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ONE TEXT FIGURE AND ONE PLATE

A wide range of variability both in structure and function is presented by the pharynx and proboscis of Rhabdocoela. Of the seven families in this sub-order, the Catenulidae and Microstomidae have but simplex pharynges. In the Prorhynchidae and Dalyelliidae, the pharynx is a well-defined bulbous type. In the Typhloplanidae, there is a rosette-shaped pharynx; in some of these, however, the proboscis lacks a definite sheath. This proboscis is not concerned with ingesting food; but is an organ of both defence and offense. This organ reaches its highest development in the Polycystidae and Gyratricidae.

Prorhynchus applanatus occupies an intermediate place in this series of Rhabdocoeles, ranging from animals having relatively simple pharynges, through those presenting complex pharynges without associated proboscides, to animals in which both pharynges and highly developed proboscides are present. In the simplest pharynx there is but little muscular development and many glands present, so that, in the living specimen, the pharynx appears to be a glandular structure rather than a muscular one (e.g., Stenostoma). In the Dalyelliidae and most Prorhynchidae, the bulbous pharynx is highly muscular. The pharynx of Prorhynchus applanatus is not only highly muscular, but its muscles are so organized that in the living specimen three distinct zones are clearly recognizable, (text-fig. A). The distal zone, (a) of muscles form an 'Endkegel' similar to the terminal cone of such a highly specialized proboscis as that of Gyratrix hermaphroditus Ehrenberg. At the posterior end of the pharynx is a zone of muscles, comparable to the 'Muskel-

zapfen' of Gyratrix (*c*).   A highly refractive third zone of muscles lies between the end cone and the muscle spindle of the base of the pharynx, (*b*).   Thus, in this series of Prorhynchus which has no proboscis there is a highly muscular pharynx whose histology is as complex, if not more so, than that of the complex proboscis of a Gyratricid.
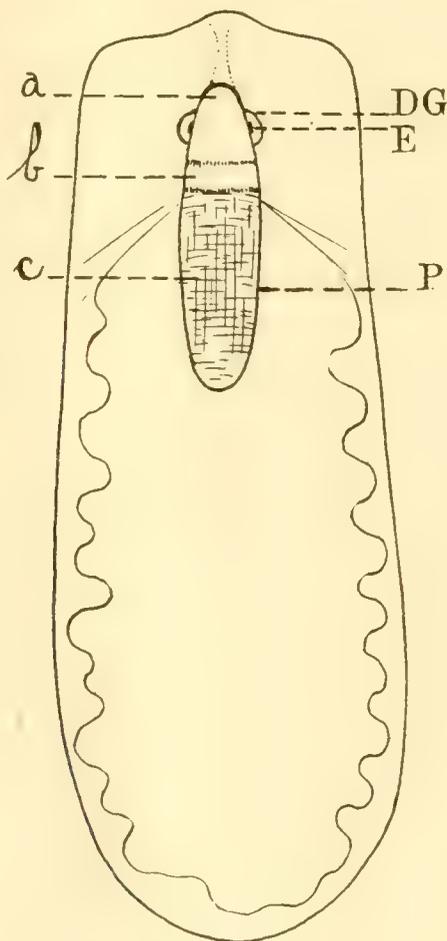
Text fig. A   Drawing of dorsal aspect of Prorhynchus applanatus. *DG*, dorsal ganglion; *E*, eye; *P*, pharynx; *a*, *b*, and *c*, the three regions of the muscular pharynx.   × 75.

The functioning of this most complex pharynx of the Rhabdocoela is not supplemented for a long period during the life of each individual by a penis which might act as an organ of defense. Animals are frequently found with the ovaries present.   Seldom are the male genitalia encountered.   These appear for but a brief period in the history of the individual.   Therefore, the

animal does not as a rule carry about with it in its pharyngeal sheath a chitinous penis which could be used defensively as do some Rhabdocoela. Prorhynchus applanatus, has of course, no defensive and offensive proboscis.

The structure of this pharynx and the conditions attending it all suggest that it is a bulbous stomodaeum which has assumed the function of a proboscis as well as that of a pharynx.

It is, therefore, interesting to see that Kepner and Taliaferro, ('16), recorded the fact that this animal uses its pharynx as a defensive organ. "Correlated with the absence of a chitinous penis, which in the other forms of Prorhynchus is used as an organ of defense, the pharynx of Prorhynchus applanatus serves as a defensive structure." Mr. W. H. Taliaferro, while working in this laboratory, was the first to make the observation that such use is made of the pharynx by Prorhynchus applanatus. While observing a specimen under a microscope, he saw it plunge its pharynx quite through another animal which passed by, and further, that its pharynx was very quickly withdrawn from the victim to retreat into its pharyngeal sheath.

This method of functioning imposes a peculiar stress upon surrounding organs, both when the pharynx is discharged at an object, and especially when its recoil is to be met. Were it not to have some special method of meeting this recoil, the surrounding parenchyma and other tissues would be greatly taxed to withstand the impact of the rebounding pharynx.

This organ is cushioned laterally by parenchyma. At its base, however, it rests upon the rim of the enteron. Thus, a more delicate situation encounters the recoil of this pharynx than is that which receives the rebound of the highly developed proboscis of the other Rhabdocoeles. The proboscis of the higher Rhabdocoeles is cushioned by an extended lateral mass of parenchyma as well as by a basal pad of mesenchyme, a connection between the enteron and the proboscis not being involved.

In Prorhynchus applanatus the pharynx, of course, communicates with the enteron. Its lumen opens into that of the enteron, while its wall is confluent with, or merges into the wall of the latter. There is thus an intimate connection between

the proboscis and the enteron. This connection between the enteron and the vigorously active pharynx must be quite delicate; for there is but a very thin tunic of muscle fibers in the wall of the alimentary canal at this thin and constricted region, and in addition to this, the wall is broken by frequent pores through which the secretion of the glands of the pharynx empty their products (fig. 1, GP).

A demand to protect the delicate connection between the enteron and pharynx, and the enteron itself from the sudden recoil of the peculiar pharynx of Prorhynchus applanatus has thus arisen. In other animals, for example, the Crustacea, "Masses of connective tissue uphold and support the various and delicate tissues about them, and protect them from the impact of surrounding organs. The largest and most regular of the cells which compose this tissue are known as Leydig's cells of the first order." page 57, Dahlgren and Kepner ('08).

As in the Crustacea, so here certain mesenchymal cells about the base of this peculiar pharynx have become large and cushion-like to meet the demand made for protecting the delicate enteron from the impact of an adjacent organ. These form a mass of conspicuous tissue which extends laterally along the posterior half of the pharynx and crowds about the base of the pharynx as it unites with the enterin. Anteriorily these cells form but a single layer about the pharynx (figs. 1 and 2cc).

These cushion cells in the fixed condition[1] first appear as irregular polyhedra. The largest ones measuring about $45\mu \times 30\mu \times 30\mu$. The most conspicuous feature of the mature cells is the greatly vacuolated condition of their cytoplasm which is for the most part disposed about the periphery of the cell. At some place this peripheral cytoplasm is piled up as an eccentrically placed mass of protoplasm around the nucleus. Figure 3 shows a section through the nucleus (N). It also shows where the large central cavity encroaches upon the peripheral cyto-

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[1] The specimens have been fixed in chrom-aceto-formalin and aceto-sublimate. The former reagent have a better result. The sections were five micra thick and were stained with Mallory's connective tissue stain or iron haematoxylin.

plasm at three regions (*a*), (*b*), (*c*). The nucleus of the mature cell is rarely found well fixed in prepared sections.

Many rhabdocoeles, for example, Dalyella, Stenostoma and Microstoma, have gland-cells projecting into the mesenchyme from the wall of the isthmus which connects the pharynx with the enteron. For a time, we had supposed that the cushion cells of Prorhynchus applanatus might be such cells, but in no case have we been able to find that they have ducts. On the other hand, places occur in which the transition from general mesenchymal cells to the typical cushion cells is apparent. In a cell which has departed little from the mesenchymal type, there is a slight vacuolization of the cytoplasm at the pole nearer the mass of more advanced cells. The nucleus of such cells has undergone but little change (fig. 4-*A*). Later the cell enlarges as shown at figure 4, *B*. The cytoplasm becomes more vacuolated, and, at this phase in the development of the cushion cell, the nucleus reaches its maximum size (fig. 4, *N*). When further growth ensues the nucleus decreases in size (fig. 3, *N*). The cushion cells of Prorhynchus applanatus therefore are not homologous with the gland cells of the pharynges of other rhabdocoeles, but are of mesenchymal origin.

In both their function and origin they suggest, therefore, the so-called Leydig's cells, which have been described for Crustacea.

SUMMARY

1. The pharynx of Prorhynchus has a double function, (a) food prehension; (b) offense and defense.

2. In correlation with this duplex functional demand there is a structural differentiation analogous to that of the proboscis of the higher rhabdocoeles.

3. There are cushion cells (Leydig-like cells) developed at the base of this highly organized pharynx, which protects the adjacent organs from the recoil of the pharynx.

4. These cells are of mesenchymal origin and are not homologous with the pharyngeal gland-cells of other rhabdocoeles.

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PLATE 1

EXPLANATION OF FIGURES

1 Frontal section involving the entire pharynx. *cc*, cushion cells; *GP*, gland pore opening into the lumen of the enteron; *1*, the approximate plane through which section, shown in figure 2, was taken. × 200.

2 Obliquely transverse section. Approximate plane of this section shown by line *1*, in figure 1. The cushion cells (*cc*) are here shown on the ventral side and extending laterally about the pharynx-base. Had the section been taken through the cells *cc* and strictly at right angles to the axis, the cushion cells would have appeared on all sides of the section of the pharynx. × 100.

3 A section of a mature cushion cell; section taken eccentric to the axis of the cell. *N*, nucleus; *a*, *b*, and *c*, diverticula of large central cavity of the cushion cell. × 1500.

4 Two phases in the growth of cushion cells. *A* younger cell, which resembles the general mesenchyme cell; *B*, older cell. At *B*, the intermediate stage of growth, the nucleus (*N*) reaches its maximum size. × 1500.
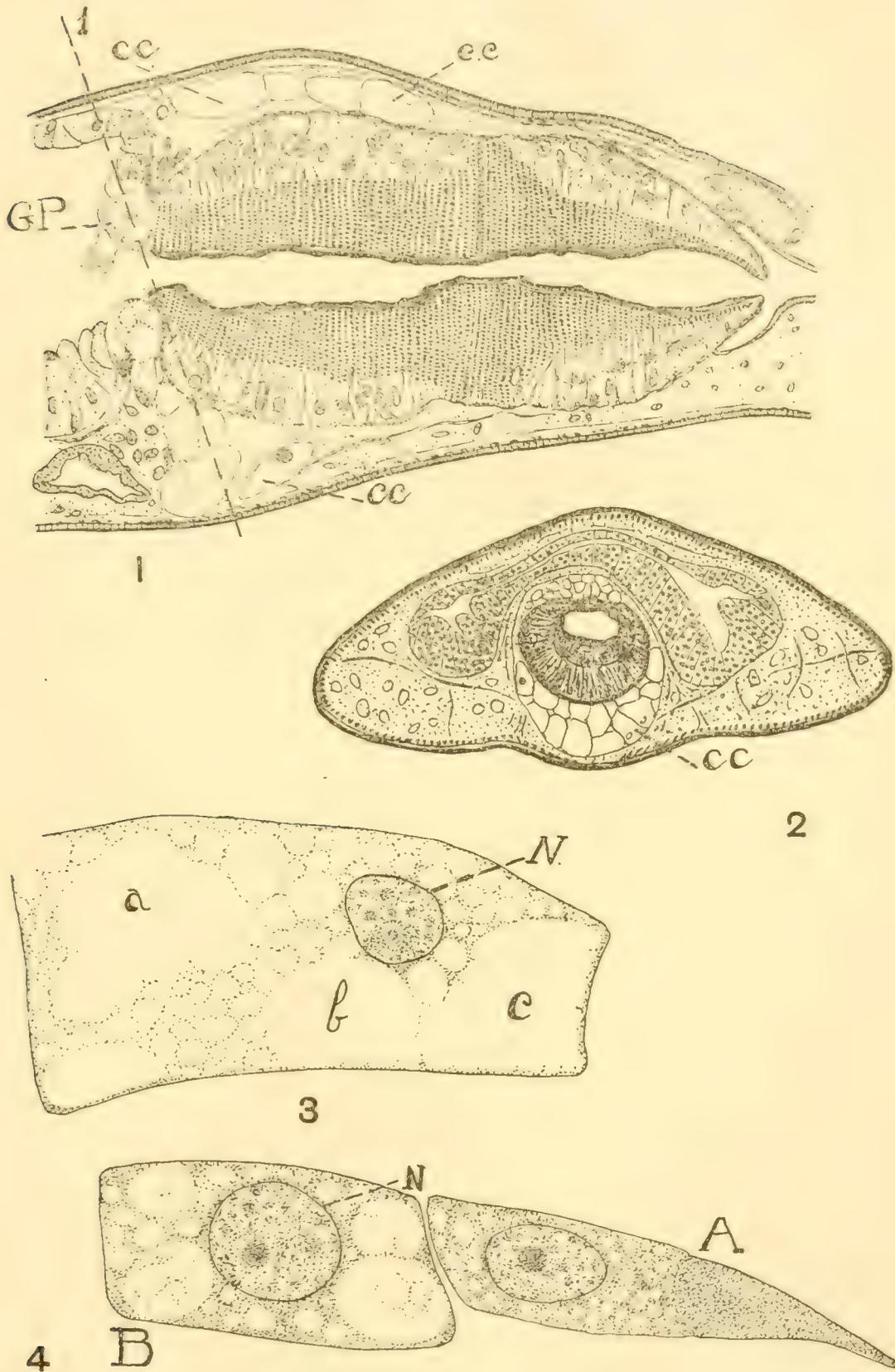
STUDIES OF AMITOSIS: ITS PHYSIOLOGICAL RELA-
TIONS IN THE ADIPOSE CELLS OF INSECTS,
AND ITS PROBABLE SIGNIFICANCE

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THIRTY-SIX FIGURES (SIX PLATES)

CONTENTS

I. INTRODUCTORY

The present paper embodies the results of my investigation on the relation of the amitotic nuclear division and metabolic activity in the adipose cells of insects, together with historical review and discussions on the general subject of amitosis. Preliminary results of the study have been published in The Anatomical Record in a brief form (Nakahara, '17).

The question of the significance of amitosis has been much debated for many years. This method of nuclear division was regarded by many authorities to be associated with highly specialized and pathological conditions, but some of the recent writers hold the view that it is related to the rapid growth and differentiation of the tissue, others believe that it is due to special environmental conditions, and still others attribute the phenomenon entirely to certain mechanical processes. The chief object of this paper is to discuss the subject in the light of the facts observed in the adipose cells of insects, and to clear up the field, if possible, by a careful review and comparison of hitherto observed facts bearing on the subject.

It is also hoped that this paper may be of some interest insofar as it is concerned with the histology and physiology of adipose tissue of insects, since, although general features of changes occurring in the cells during their activity, have been observed by many authors, cytological and histochemical studies of the subject have been rather unsatisfactory.

This work was done in the Entomological Laboratory of Cornell University under the supervision of Prof. Wm. A. Riley. My deep obligation is due to Professor Riley for his encouragement and help rendered me during the course of the work. I also wish to express my thanks to the Department of Histology and Embryology of the same Institution for the privilege of using some of the equipment.

II. MATERIAL AND TECHNIQUE

While many other insect larvae, especially lepidopterous, have been used in preliminary work, and also for comparisons, Pieris rapae is the one in which the entire subject has been worked out. A large number of larvae, representing all the developmental stages were subjected to cytological and histochemical examinations.

For general cytological purposes, materials were fixed in Bouin's picro-aceto-formol or Carnoy's chloroform-alcohol-acetic ('6–3–1'), and sections were stained with Ehrlich-Biondi-Heidenhain mixture, iron haematoxylin with or without orange

G counter-stain, or Ehrlich's haematoxylin followed by a strong aqueous solution of eosin. Some few larvae were treated with Flemming's chromo-aceto-osmic fixer and his triple stain. In some cases whole mounts of the tissue were very useful in checking up the observations on sections. In preparing such mounts, the best result was obtained by fixing pieces of the tissue in Carnoy's '6–3–1,' which dissolves the fatty contents of the tissue at the same time, and staining with Grenacher's borax carmine, followed by a differentiation with acid alcohol.

In order to demonstrate fats in the cell, very small pieces of tissue were thoroughly fixed in osmic acid and prepared as paraffin sections. Some other materials were fixed in formalin, cut, using the freezing microtome, and stained with Sudan III.

I used Gage's iodine method (Gage, '06) as the simplest and most convenient for the demonstration of glycogen, and digestion experiments by means of saliva were applied as a control.

I have been able to demonstrate mucus (?) granules in the tissue by the following technique:

1. Material fixed in picric alcohol for 24 hours.

2. Paraffin sections fastened to the slides with albumen fixative, without using water to float the sections (a rather large quantity of the fixative was used to insure the sections as free from wrinkles as possible).

3. The fastened sections treated with xylene and then with 95 per cent alcohol as usual, but without being placed in water.

4. Stained 10 to 20 minutes with alcoholic mucicarmine.

5. Sections then washed with 95 per cent alcohol and mounted.

III. THE RELATIONS OF THE AMITOTIC NUCLEAR DIVISION

A. Structural changes in the adipose cell

a. Review of Pérez's work. Structural changes in the adipose cells during the larval life of insects have been studied to a certain extent by Wielowiejsky ('83), Van Rees ('88), Karawaiew ('98), Koschevnikov ('00), Terre ('00), Anglas ('00), Berlese ('99, '01), Henneguey ('04), Philiptschenko ('07), Hufnagel ('11), Feytaud ('12) and others, especially in connection with the

problem of the internal metamorphosis of insects. The best work that has been done upon the subject, however, seems to me to be that of Pérez's ('02), dealing with the postembryonic development of Formica rufa. Since this is a very valuable paper on account of its accuracy and thoroughness, and since the works of other writers are more or less fragmentary, or not satisfactory in some other ways, it has seemed advisable to take up the French work to review for all.

In this paper the author has definitely established that the adipose cells are the seats of progressive accumulation of fatty and albuminous reserves during the larval life of insects. In the larva just hatched he observed that the adipose cells are small, 12 to 15 micra in diameter, and spherical, each with a single spherical or ellipsoidal nucleus which contains a small amount of chromatin granules. The cytoplasm is of two layers, perinuclear and peripheral, and shows a number of vacuoles, within which osmic acid preparations show blackened fat droplets.

The cells become greatly enlarged in the larvae of 2.5 mm., attaining 30 to 40 micra in diameter. Binucleate cells are frequently observed at this stage, and although Pérez was rather inclined to think that this condition is due to the amitotic division of the nucleus, he did not draw any conclusion, considering the fact that Berlese claimed the occurrence of mitosis in the cells in a certain case. It is also at this stage that the eosinophile granules begin to appear in the cell-body in addition to fat droplets. These granules are spherical, 3 to 5 micra in diameter and very refractile. From all the available evidence, Pérez interpreted them as representing albuminous reserves in the cell. In 6 mm. larva the enlargement of the cells is carried still further and many of them are more than twice as large in diameter as those in the stage just examined. These large cells are filled with albuminous granules and fat droplets. The nuclei begin to be irregular in shape and a few of them are ramified. The ramification of the nucleus is carried to the extreme in the full-grown larva ready to pupate.

The series of changes, similar in the main to that noted by Pérez in Formica, has been observed by many other writers in various

insects. This seems to indicate that the changes in adipose cells are of essentially similar nature throughout all the groups of holometabolic insects.

b. *Observations on Pieris.* Tracing the series of changes in the structure of adipose cells of common cabbage worms (Pieris rapae L.), I found the descriptions by other authors on other insects hold fairly good in this case. The adipose cells from the first stage larva (fig. 1) are very small, measuring 12 to 17 micra in diameter, and each of them contains a single spherical nucleus about 4 or 5 micra in diameter. The cell-body is rich in cytoplasm and shows few vacuoles. A few fat droplets (fig. 3) and a very small quantity of glycogen (fig. 2) can be demonstrated at this stage. Very rarely mitotic figures are also to be detected (fig. 4).

In the second stage larvae, the adipose cells are almost twice as large in diameter as in the preceding stage (fig. 5). The nuclei not infrequently show peculiar shapes, indicative of different stages in the process of amitosis. A few of the cells are binucleate (fig. 6). From this stage onward, I have not seen a single mitotic figure in the tissue, but various indications of amitosis taking place rather frequently have been observed. The cytoplasmic area is much enlarged and contains abundant fat droplets (fig. 7), and here glycogen is also demonstrated in a pretty large quantity. In preparations with ordinary fixation, vacuolization of the cytoplasmic area is sometimes carried to the extreme, and in such case the whole area presents a reticulated appearance (fig. 6). In very rare cases, the ramified condition of the nuclei occurs (fig. 8). This is perhaps not a similar condition to that observed by Pérez ('02) in old larvae of Formica, because, while it is apparently related to the degenerative phenomenon in Pérez's case, the nuclei here show no sign of degenerative nature, and the ramification is not quite so extensive. Ramified nuclei occur also in later stages, but such condition never becomes very prevalent.

Late in the third stage, some of the cells begin to show peculiar spherical granules in the cell-body. These are albuminous granules. In this and in following stages the cells are pretty

large, measuring about 60 to 70 micra in diameter. Fatty droplets (fig. 9) and glycogen (fig. 10) are present in large quantities. The albuminous granules are greatly increased in amount (fig. 11), and almost all of the adipose cells are filled with such granules in the larva of complete maturity.

In full grown larva, many cells contain two or more (rarely up to five) nuclei. Some of the albuminous granules begin to show dark dots, taking basic stain, indicating that the transformation of albuminous substance into urate is beginning to take place (fig. 12). This possibly may be regarded as one of the first signs of histolytic process. Soon afterward, just before the larva enters into the prepupal stage, the nucleus loses its membrane and its structure becomes more or less indistinct (fig. 13). This is, I believe, the sign of a karyolytic process, which concludes the activity of the larval adipose cells.

B. Some plasma structures

a. Fat. This is perhaps the most important of all the inclusions in adipose cells. As pointed out in the preceding section, the increase in amount of the fat in the cell keeps pace with that of the cell in size. In other words, the amount of fatty inclusion in the cells increases with the growth of the larva.

We do not know the cytological relations of the accumulation of fat in cells. However, since it is evident that fat is not taken up by cells as such, it is necessarily true in one sense, that cells elaborate the fat within them from its modified forms. In passing, I may mention that very rarely, small fat droplets were seen lying within nuclei. Figure 14 represents such a condition.

b. Albuminous granules. The appearance of peculiar spherical granules in the cytoplasmic area of adipose cells toward the close of larval life of insects has been noted since the early year of 1864 (Weismann) by many writers. Going over the descriptions by Koschevnikov ('00), Anglas ('00), and others, it seems that some of the spherical granules which have been familiar to the earlier authors, correspond to the albuminous granules, but it was not until the time of Berlese ('99, '01) that granules of

this nature were definitely demonstrated in the adipose cells. Authorities are unanimous that the granules are reserves of albuminous substance to be used in prepupal and pupal stages for a nutritive purpose.

As to the origin of these granules, Berlese ('99, '01) held the view that it is exogenic. Seeing that the coagulated plasma in the body-cavity shows an appearance similar to the granules in the cells, he thought that the albuminous substances, set free in the body cavity at the time of the destruction of the alimentary canal, are taken up by the adipose cells, and thus constitute the granules in question. Enriques ('01) held a similar view, but assumed that the granules are derived from degenerating muscular tissue.

These hypotheses are challenged by Pérez ('10) on the ground that the albuminous granules make their appearance in the adipose cells before the insect enters into the prepupal stage and while the alimentary canal and muscles are perfectly normal. Pérez ('10) believes that the substance is absorbed by the cells in the hydrolyzed form, invisible in the preparation, and is condensed into granular form within the cell under the influence of the nucleus.

According to Hollande ('14), Pérez suggested in his later paper ('11) that, in the case of Vespid larva, the albuminous inclusion is formed at the expense of the fat in the cell, and a similar view was held by Hufnagel ('11) in her work on Hyponomeuta padella. Considering the matter from a chemical viewpoint, it seems quite possible that fat may transform into albuminous substance, or vice versa, as is said by Hollande ('14), and especially so when we recall the chemical work by Weinland ('08), who demonstrated that albuminous material transforms into fat at the approach of pupation in the larva of Calliphora. This is, however, not sufficiently adequate to base any definite conclusion upon.

Hufnagel ('11) observed in the adipose cells of Hyponomeuta, an elimination of chromatin into the protoplasm, the phenomenon which she thought to constitute "une épuration chromatique." Hollande ('14) in his work on the larvae of Vanessa,

did not note any such substance passing out of the nucleus, but he strongly maintained the endogenic origin of albuminous granules, and suggested that they may be of nuclear origin, because the granules first appear laying very close to the nucleus.

Examining the cells containing the albuminous granules in the cytoplasmic area, a different kind of granules can in most cases be distinguished from the chromatin and nucleoli within the nuclei (fig. 11). These peculiar granules are similar in appearance to nucleoli, but are distinguished from the latter in showing stronger affinity for certain acid and weaker for basic stains, and hence they may be tentatively called acidophile granules in the nucleus.

The acidophile granules in the nucleus and albuminous granules always show similar staining reactions as can be seen in the following table:

STAINS	NUCLEOLI	ACIDOPHILE NUCLEAR GRANULES	ALBUMINOUS GRANULES
Delafield's hematoxylin, eosin, and picric acid.................................	Dark olive	Orange-yellow	Orange yellow
Ehrlich's hematoxylin and pico-fuchsin.	Orange	Yellow	Yellow
Iron-hematoxylin.........................	Black	Gray	Gray
Flemming's triple........................	Red	Orange	Orange
Ehrlich's hematoxylin, strong aqueous eosin..	Pink	Pink	Pink

Still more interesting in these cells is the condition of the nucleus represented in figure 15. Here an acidophile granule is apparently migrating from the nucleus into the cell-body through the spot of the nuclear membrane, where the latter has disappeared, thus forming an opening. Since this phenomenon occurs very commonly, it may be regarded at least as one of the possible methods of formation of the albuminous granules.

As far as the evidence available indicates, the theory of the endogenic origin of the albuminous granules in the adipose cells

seems to represent the truth, and also there can be no doubt that the nuclei take a rôle of essential importance in the formation of this reserve material.

c. Urates. According to Pérez ('10), the occurrence of urates in the true adipose cells was first demonstrated by Marchal ('89), although certain special cells (urate cells) in the adipose tissue were known to respond to tests for the same substance since as early as the time of Fabre ('62).

Berlese ('99, '01) and Henneguy ('04) considered the dots, taking nuclear stains, often present in the albuminous granules, as the visible form of an enzyme, secreted by the nucleus and passed into the granules. They called such granules 'pseudo-nuclei' from their resemblance to nuclei. Pérez ('10) asserted, however that these chromatic dots in the albuminous granules are really nothing but concretions of urates. This view is strongly supported by Hollande's (14) recent study, in which he maintained that the uric concretions in the adipose cells are endogenic in origin, and that they are formed by the transformation of albuminous substances.

A consideration of the matter from a chemical point of view at once justifies the possibility of the process, and since there is no evidence showing that the cell functions in taking up urates from the blood, we may safely accept, at least from the present state of our knowledge, Hollande's opinion.

d. Glycogen. The study of glycogen in adipose cells has been very unsatisfactory. Terre ('00) cited Curvreur, who is perhaps the first to speak of this 'animal starch' in the adipose tissue of insects, and has demonstrated that fat may transform into glycogen; and the former author suggested that this process may represent the histolytic phenomenon of the adipose cells in the bee. Vaney and Maignon ('06), as cited by Deegener ('14), claimed that fat in the adipose cells of the silk-worm may change into albuminoids and glycogen. However, the fact that glycogen occurs in adipose cells from very early stages of larval life, as has been observed in the case of Pieris, seems to make the opinion of Vaney and Maignon doubtful, to say nothing of that of Terre. It is not within the scope of this work to discuss the physiological

relations of the glycogen in adipose cells. Suffice it to say that the elaboration of glycogen is one of cell activities, and it is not improbable that the nucleus may take some kind of rôle in this process.

e. *Mucus* (?). A peculiar substance, hitherto unknown in the adipose tissue of insects, has been discovered in well-grown larvae of some Lepidoptera. The substance is represented by globules (fig. 16), scattered within the cell-body and respond to some extent to histochemical tests for mucous substance. Picric alcohol preserves the globules, and the latter take mucicarmine. The sections, however, will not show any such globules, should they be placed in water or aqueous solutions, indicating that the substance is very easily dissolved in water. If the substance be really a mucus, it would seem that adipose cells have the function of secreting this substance. This, together with the question of exact periods of its appearance in the tissue, however, remain to be determined by future study.

C. *Nuclear division*

a. *Amitosis.* The occurrence of bi- and multinucleate cells in larval adipose tissue has been noted for many years, and such authors as Terre ('00) and Anglas ('00) considered them as due to amitotic division of nucleus, without a following division of cell-body, and indicating a senile condition of the cell. Pérez ('02) was first inclined to think that the multinucleate condition of the cell is due to amitosis of the nucleus, but later ('10), in his work on Calliphora, he maintained the view that it represents the incomplete separation of the cell-body after the division of the nucleus at the embryonic stage. He did not observe any nuclear figure which could be considered as representing one of the division stages. It is obvious, however, that Pérez's later interpretation must be discarded, because, while we can hardly detect a single binucleate cell in the young larva, bi- or multinucleate cells are very common in older larvae; whereas we should expect as many multinucleate cells in young larvae as in older ones, if Pérez were right in his interpretation.

Logically, bi- or multinucleate cells may possibly result in

two different ways, namely: (1) The division of nucleus without that of the cell-body, or (2), by the fusion of two or more cells. Although the spherical cells of adipose tissue are usually altered by pressure into irregular shapes, no one has ever detected any sign of their fusion, and therefore the multinucleate condition in this case must be regarded as due to the other method.

If the adipose tissue containing bi- and multinucleate cells is carefully examined, the fact may be noticed that although no mitotic figures are observable, not a few of the nuclei show such figures as might be interpreted as various stages in the process of amitosis. Since no other way of producing multinucleate cells is possible, we should conclude that this kind of nuclear division is responsible for such condition of the cell.

A number of possible stages of amitosis observed in adipose cells of Pieris larvae are figured. Figures 17 to 19 show what appears to be the ordinary process of the amitotic nuclear division. The nucleus first elongates, and then a constriction takes place across its longitudinal axis. It may be described as a development of a fold or in-pushing of nuclear membrane along that line. The two parts of the nuclei are apparently distinguished by a slight line before they separate. It is difficult to say whether this line represents the developing new nuclear membrane or is an optical image due to the constricted condition of the nuclei. When the two daughter nuclei become separate, a sort of internuclear bridge, appearing as a strand of fibers, may sometimes extend from one nucleus toward the other. This is considered by Nemikoff ('03) as representing a special substance (internuclear substance), the significance of which is not yet understood. The two parts of a dividing nucleus are not always of equal size. Figures 20 to 22 represent cases in which one of the parts was smaller than the other. An extreme case of this nature is shown in figure 23, in which one is more than twice as large as the other. Very rarely, constrictions may take place at more than two places in the nucleus, in the case where the latter is long and slender. Such a case is shown in figure 24. It seems that such nuclei may divide almost simultaneously into more than three daughter nuclei.

An interesting point to be noted is the relation of the apparent vacuolization of the cytoplasm to the form of amitosis. The nuclear division in highly vacuolated cells looks somewhat as though it were a fragmentation of the nucleus due to a mechanical interference of vacuoles, although the process is of course essentially the same as in the normal case just described. This is illustrated in figures 25 to 27.

A phenomenon of entirely different nature is represented in figure 28, where it appears as if the nucleus is divided into two by the big vacuole located in the middle; but, changing the focus, it was seen that the apparent two nuclei were continuous and really parts of a single nucleus. Figure 29 shows another case of similar nature.

It was once believed that the amitotic nuclear division is always initiated by the division of nucleolus, and that each daughter nucleus receives a single nucleolus. Since this is now known as "one of the rarest forms of cell division," it was with great interest that I observed in some adipose cells of Simulium larvae, a process of amitosis taking place in accordance with Remak's scheme. Figures 31 to 33 illustrate the main feature of the process. This is, however, not the usual method of nuclear division in this form, and the relation of the nucleolus to the division is by no means constant. Figures 34 to 36 show that one of the daughter nuclei may contain more nucleoli than does the other. Evidently there is no reason for believing that nucleoli play such a rôle as is supposed in the Remakian amitosis.

Throughout my observations, I have not been able to detect the centrosome in the adipose cell. Obviously the relation of the centrosome is not of any importance to the process of amitosis in the cell under consideration, even if this 'cell-organ' may really be present in the cell.

Lastly, I should mention that I have gone over preparations of Calliphora larvae, and have found that, contrary to Pérez's statement, no nuclear division takes place in the larval adipose cells in this form, the amitotic figure can be easily detected. One of those nuclei apparently dividing by amitosis is represented in figure 30.

b. Mitosis. Berlese ('99–'01) observed that mitotic division takes place in adipose cells during the moulting period in the silk-worm, and he maintained that adipose cells multiply by mitosis. According to Deegener ('13), de Sinéty ('01) believed that the fat cells may multiply by mitosis without showing any embryonal characters. Deegener also cites that Poyarkoff ('10) observed the mitotic division in the case of a beetle larva, Galerucella crataegi, just before it enters into moulting period.

It has already been mentioned that in Pieris the mitotic figure is observable only, and very rarely, in very young larvae. This phenomenon may be interpreted as an indication of embryonic character of the cells, because mitosis does not take place after the cells start their active functioning. Since I found very few mitotic figures, in spite of the large number of adipose cells examined, the occurrence of mitosis in larval adipose cells must be regarded as a very rare phenomenon. Of course, it is not strange that mitosis takes place when the cells are multiplying, since this is the reproductive method of cells.

D. *General consideration*

Of the nutritive deposits within the adipose cells, the droplets of fat have been known of old. Berlese and Pérez discovered another important class of deposits—the albuminous bodies. According to the results of my researches, glycogen is to be considered almost as constant an inclusion as fat, and although not satisfactorily worked out, a mucus-like substance may perhaps be counted as the fourth.

The deposits of fat and glycogen are found in the cell from an early stage larva, and these substances increase greatly in amount as the stage advances; in later stages the cells store up albuminous nutriment. The functional activity of the cells may therefore be considered to become more and more intense with the advancing ages of the larvae, until histolytic processes set in preparatory to pupation.

A very interesting fact is that the nucleus of the cell begins to divide by amitosis rather early—long before the albuminous granules start to appear, and that the nuclear division is appar-

ently not followed by the division of the cell-body. The fact that this phenomenon does not represent a senile condition of the cell may be easily seen, because the cells at these stages are still functionally very young, and it is long afterward that they begin to degenerate. Nuclear division, without following cytoplasmic division, insures the increase of nuclear surface. The fact that such increase is in close connection with the increasing functional activity of cells is seen in many cases, for example, in silk-gland cells of various insects. At once the idea that amitosis may be tributary to intense metabolic activity of cells, bringing about the increase of nuclear surface, suggests itself, and here we come face to face with one of the most debated questions in cellular biology since the latter part of last century—the significance of amitosis.

IV. THE SIGNIFICANCE OF AMITOSIS

A. Historical

Strasburger ('82) and Waldeyer ('88) regarded amitosis in the higher forms as a survival of a primitive process of direct division derived from the Protozoa. Ziegler ('87) noted amitosis in the periblast of fish-eggs and suggested an intimate connection of amitosis and the high specialization of the cell, followed by degeneration. Flemming ('89), working on the amitotic nuclear division in the epithelial cells of the bladder of a salamander, maintains that the process is, at least in this case, a pathological or abnormal one.

Chun ('90, as cited by Ziegler, '91, Flemming, '91, '92, Wilson, '00, etc.) made a very valuable suggestion that amitosis in the entodermic cells of the radial canal of Siphonophores was for the purpose of "increasing the nuclear surface as an aid to metabolic interchanges between nucleus and cytoplasm." (Wilson, '00, p. 118.)

Flemming ('91) observed that the true generative multiplication of leucocytes in lymph glands is effected only by means of mitosis, although both mitosis and amitosis occur in wandering cells, and considered those cells undergoing amitosis as being "on the road to ruin."

Vom Rath ('91, '93) finds amitosis in the spermatogonia of amphibians, but he regards those cells dividing in this way as not becoming spermatozoa, but as degenerating and later being used as nutritive material by the other spermatogonia. His view on amitosis is therefore similar to those of Ziegler and Flemming.

Meves ('91, '94, '96) says that the spermatogonia of Salmandra divide by amitosis in the autumn, but return to the mitotic division in the spring, and later they give rise to functional spermatozoa. He believes, therefore, that amitosis also falls in the generative series of cell-division.

Löwit ('91) claims that there is a generative amitosis, at least in the case of leucocytes, although a good many other cases of amitosis may be of a degenerative nature. He suggested that two kinds of amitotic divisions—generative and degenerative—might be distinguished.

Ziegler ('91) and Ziegler and vom Rath ('91) assert that amitosis takes the place in such cells as are undergoing intense secretory or assimilatory processes and have large nuclei, and that the division process is usually limited to the nucleus. They believe that this kind of nuclear division is always a forerunner of degeneration and death.

Verson ('91) observed the direct nuclear division of the large cell in the blind end of the testicular follicle in Bombyx, and he considered that this cell gives rise to true sex-cells.

Frenzel ('91), in his work on the intestinal glands of the crayfish, came to the conclusion that the amitosis occurring there is a normal method of cell-multiplication.

Flemming ('92) reviewed all of the literature on amitosis up to his time and maintained in a general way his hypothesis as regards to the significance of this process.

Gerasimoff ('92, as cited by Wasielewski, '03, '04), has shown that certain external conditions may effect the method of cell-division. He observed that mitosis in Spirogyra takes place by amitosis when this plant is placed in low temperature.

Paladino ('93, as cited by Des Cilleuls ('14), '95) observed that the new formation of the placenta in Mammalia is mainly

due to amitotic cell multiplication, and maintained the view that this kind of division is a normal physiological process.

Toyama ('94) found that the cells, including Verson's 'large cell' (Verson's cell), which divide amitotically in the testicular follicles of silk worms do not belong to the cycle of true germ cells, but finally degenerate, thus supporting the view of Ziegler, Flemming, etc.

Krompecher ('95) in his work on bone-marrow, came to the conclusion that only mitosis can be considered as generative division, and amitosis must be regarded as a breaking down of a degenerating nucleus.

Preusse ('95) arrived at the conclusion that nuclei of oogonial cells may divide by amitosis, yet afterward undergo normal mitotic division, in the case of some hemipterous insects.

Wilcox ('95) in his study of spermatogenesis of Caloptenus and Cicada, observed that 'giant spermatozoa' which are produced from spermatogonia by amitotic division are not functional, and "really come to naught," being excluded from the developmental series. He, therefore, stands in support of the degeneration theory.

Plate ('98), studying amitosis in the tracheal epithelium of Janelles, expressed his opinion that this kind of division is a process of multiplication often in the dying condition of the cell.

de Bruyne ('99) supports the view advanced by Ziegler and vom Rath in the case of amitosis in the ovarian follicle of Hemiptera and Orthoptera.

Pfeffer ('99, according to Wilson, '00, Wasielewski, '03, '04, et cetera) showed experimentally that amitosis is a result of special environmental conditions. He saw that the cells of Spirogyra placed in water containing 0.5 to 1.0 per cent of ether, undergo amitotic division, but resume mitotic division when the same individuals were transferred into normal water.

McGregor ('99) noted in Amphiuma the fact that the primary spermatogonia divided by amitosis, divide by mitosis later and produce functional spermatozoa, and thus he supports the view of Meves.

Häcker ('00) was able to induce amitosis in the eggs of Cyclops

by application of a 5 per cent solution of ether in water. He, however, doubted if such amitotic phenomenon really correspond to the normal amitosis, and proposed to call the former process 'pseudoamitosis.'

His ('00) states that the nuclear division by constriction, without any preparatory change in the nucleus does not occur in the periblast of fish, and he considered the so-called amitosis demonstrated in this tissue as a peculiar form of multipolar mitosis.

Magnus ('00) showed that in the case of certain cells of infected roots of Listera and Orchis, there is a kind of fragmentation of nucleus, which is not a dying condition but a special adaptation in the nuclear activity.

Nathanson ('00) believed in the external influence upon the method of cell division, when he experimented with Spiragyra, Closterium, and some higher plants. He claims that it is the action of ether that determines amitosis.

Regaud ('00) observed in the case of rats that the nuclei of Sertoli's cells and spermatogonia divide amitotically, but the stock of cells ultimately producing spermatozoa divide by mitosis later, so he maintained that amitosis, which is in most cases a manifestation of cellular degeneration, is not always a sign of fatal degeneration of the cell.

Wilson ('00) in his review of the subject, says that amitosis in the vast majority of cases, is a secondary process which does not fall in the generative series of cell-division, and regards Flemming's hypothesis as, in a general way, representing the truth.

Gross ('01), working on ovaries of thirteen species of Hemiptera, supported Ziegler's theory by showing that the cell which has undergone amitosis does not belong to the generative cycle of true germ cells. He also pointed out that there can be distinguished two kinds of amitosis—the degenerative and secretory amitosis.

Caminiti ('03), studying amitosis in certain liver cells, states that normally amitosis may exist as an equally important process of cell multiplication as mitosis.

Conklin ('03) observed amitosis in follicle cells in the terminal segments of ovarian tubes of the cricket, at the time when the cells are actively secreting the chorion, and the cells which have once divided by amitosis never again divide mitotically, but they degenerate after the formation of the chorion. He said that amitosis is in this case, "one of the last functions of these cells and it is therefore an accompaniment of cellular senescence and decay." (p. 674.)

Hargitt ('03) noted frequent occurrence of amitosis during regeneration in hydroids, and recognized the inadequacy of Flemming's theory regarding amitosis.

Klemensiewicz ('03) considered blood-cells produced by mitosis and by amitosis to be of equal biological value, and claims that such cells originate normally by either method.

Wasielewski ('03, '04) in his experimental work on the cells of the root-tip of Vicia, demonstrated the fact that amitosis can be induced by applying chloral hydrate to the cells, and such cells, produced by amitosis, divide subsequently by mitosis and show no sign of degeneration. He came to the conclusion that amitosis and mitosis are not fundamentally different processes, but they are to be regarded as two branches originated from a single stalk. He also pointed out that there are two types of amitosis, one initiated by the elongation of the nucleus which later takes a dumb-bell shape and finally separates into two parts, the other effected by the inward growth of a fold, which first occurs on nuclear membrane. These are designated as destructive and dissective types respectively.

Child ('04) observed the frequent occurrence of amitosis in the development of Moniezia and expressed his opinion that amitosis may mean something more than degeneration and aberration.

Osborn ('04) interpreted amitosis in the food-ova of Fasciolaria to be a futile attempt at segmentation, the cells having the impulse to divide, but being powerless to do so by mitosis, fall back on the easier mode of amitosis.

Karpoff ('04, according to Nowikoff, '08, '10), working on leucocytes, epidermis of amphibians, epithelium of the urinary

bladder of mammals, and many other tissues, suggested an idea that the initiative constriction in amitotically dividing nuclei is due to the physical property of nucleus and the osmotic phenomenon. He observed that in places where amitosis is occurring very frequently, the multiplication of cells is not effected, but that of nuclei is. The direct division of the amphibian leucocyte is the only case in which the nuclear division is followed by cytoplasmic division. Examining fresh material under the microscope, he noticed that leucocytes easily adhere to the cover glass: this makes the free locomotion of leucocytes difficult, and hence come an expansion and finally a division of the leucocyte in a mechanical way. As far as his data indicate, the normal amitosis always produces multinucleate cells, and so it cannot be regarded as a method of cell multiplication.

Gurwitsch ('04) said, in his review of the subject, that amitosis may be of more biological importance than was supposed by Flemming and others, and that the possibility of one method taking place of the other in nuclear division should also be admitted.

Glaser ('05) observed in Fasciolaria that amitosis is associated with a high vegetative activity and stated that it may contribute to such function, bringing about the great increase of nuclear surface.

Child ('07a), working out a number of cases of amitosis in various animals, both vertebrates and invertebrates, maintained the view that this kind of nuclear division is associated with conditions where the demand for material or perhaps for certain substances exceeds the supply. He believes that amitosis occurs in regions of rapid growth as well as in regions where active processes of secretion or reserve formation are concerned, and said "since orthodromic processes pushed to the extreme must always result in total destruction of the original substances, it is not strange that degeneration frequently follows amitosis, but there is no reason for believing that it must always follow." He failed in reconciling his theory of amitosis and the chromosome-theory, which he regards as very improbable as a universal hypothesis.

In another place ('07b), Child proposed a view that a certain relation between intake of material and functional transformation is the main factor of amitosis, that is, amitosis may occur when the stimulus to growth is so strong that the nucleus is forced far from a condition of equilibrium which he considers as essential to mitotic division.

Glaser ('07) discussed the process of amitosis in the food-ova of Fasciolaria as a purely pathological phenomenon, and urged the necessity of keeping separate the normal and abnormal events in the cellular life, and especially of distinguishing physiological from pathological amitosis.

Maximow ('08) believes that amitosis in the mesenchyme cells of young embryos of the dog is the normal method of development of the tissue.

Nowikoff ('08) stated that amitosis in the cartilage-cells is due to an external stimulus, causing a mechanical expansion of cells. Later ('10), he supported this view in the case of bone- and sinew-cells of the mouse embryo. Nowikoff's observations show that cartilage cells in the mouse embryo multiply by mitosis in early stage, and amitotic figures can be seen only, and very rarely, in the surface layer of cartilage anlage. In a little older embryo, both mitosis and amitosis occur side by side, but even here, mitotic figures are the more common. In the oldest embryo he examined, in which the cartilage cells are fully differentiated, he found that, while mitotic figures are very rare, abundant indications of amitosis are seen. It seems that amitosis in this case can be interpreted differently.

Patterson ('08) in the development of the pigeon's blastoderm, observed that mitosis may follow amitosis, and vice versa, amitosis apparently playing an important rôle in the rapid growth of the tissue, and made an indifferent suggestion that "amitosis is the result of special physiological conditions, which create a stimulus to cell-division."

Glaser ('08) observed that in the entoderm of Fasciolaria during the period of most active cell-multiplication, more than 1 per cent of all division is mitotic and more than 98 per cent are amitotic, while during the entire developmental period, includ-

ing the pre-cannibal, the cannibal, and the post-cannibal periods, a little over 13 per cent are mitotic and a little less that 87 per cent amitotic. He concludes from this that amitosis plays in this case "an important, if not the chief, part in the differentiation of a definitive tissue." (P. 246.)

Pacaut ('09, as cited by Des Cilleuls, '14), in his work on the epithelial cells of mammalian cornea, proposed a hypothesis that amitosis is due to the insufficient elimination of poisonous deposits accumulated in the cells during their metabolic activity.

Wieman ('10) claims that amitosis in germ cells is followed by mitosis in the ovaries and testes and nurse cells of Leptinotarsa. He believes that amitosis is due to a fluctuation in the nutritive supply of the cells brought about by a stimulus to a rapid cell division which causes a temporary derangement in the normal metabolism, and so amitosis and mitosis stand, as he conceives, for the extremes of a continued series, but representing different types of metabolism.

Foot and Strobell ('11) described in the ovaries of Protenor, the amitotic division of certain cells which later produce ova.

Payne ('12) shows that in Gelastocoris, the cells which apparently multiply by amitosis do not produce ova.

Jordon ('13 a), working on the amitotic division of ciliated cells in the epididymis of some vertebrates, considered the absence of the centrosome to be the fundamental cause of this kind of division; it having been known that the centrosome is destroyed in the formation of the cilia in such cells.

Des Cilleuls ('14), after reviewing all the more important papers on the subject, and apparently being supported in his own observations on epithelial cells of the vitrine cornea of the rabbit, asserts that amitosis is a process of the generative nature of a cell and not the sign of its senescence.

Hegner ('14) concludes, from all the evidences available, that amitosis has not been demonstrated in the germ cells. He could not demonstrate with certainty amitotic division among the oogonia or spermatogonia of chrysomelid beetles, even in the slides sent to him by Wieman.

Arber ('14), in her work on the development of the adventi-

tious roots of Stratiotes, observed amitosis in root-cap, cortex and stole, and suggested that amitosis supplements karyokinesis in this instance.

McLean ('14), recording his observation that amitosis commonly occurs in the cortical parenchyma of aquatic angiosperms, suggested that amitosis may be "the constant form of nuclear division between sister cells in all fully differentiated tissues which remain alive and continue to grow in bulk, although this does not preclude the possibility of its occurrence in meristematic tissue as well." (P. 382.)

Schurhoff ('15), demonstrating a beautiful case of amitosis in the endosperm of Ranunculus, expressed his opinion that this nuclear phenomenon does not lead to the formation of new cells, and the motive to the process seems to be the enormous growth of the endosperm nucleus.

Macklin ('16) made a very valuable observation on the amitotic nuclear division in tissues (of an embryo chick) growing in vitro. He asserted that amitosis is not a reproductive method, because he found that the process involves only the nucleus and not the cell-body, and whenever cell-division takes place, it was always by mitosis.

Saguchi ('17), working on the ciliated cells of different animals, maintained that these cells divide by amitosis only in vertebrates. Contrary to the view of Jordan, he considered "the occurrence of amitosis in ciliated cells is not owing to the lack of the centrosome; for the latter can be detected in many cases in such cells." He seems to believe that amitosis is a method of cell-multiplication and the causes underlying amitosis are due to the "degree of differentiation of the cell-plasm."

B. Discussion

In looking over the literature discussing amitosis, as briefly reviewed in the last section, one may notice the fact that many authors have based their opinions more or less entirely upon their own data, without paying much attention to the compatibility of their theories with other observed facts. Another un-

satisfactory feature in the past studies is that the results of observations have not been subjected to an analysis, so to speak, from a theoretical point of view. We had merely an accumulation of data, which does not yield a clue to the true nature of the phenomenon without being properly treated.

Before taking up other theories, I wish to say a few words about that of Karpoff ('04). According to this author, amitotic nuclear division may be a phenomenon of purely physical significance, and his observations are suggestive enough of such an idea. It must not be forgotten, however, that explanations as to the mechanism and as to the biological significance are two different things. We may be able to find the explanation of the method of the process in mechanical theories, but it does not necessarily contradict other theories regarding the 'purpose' of the process.

The old conception of amitosis as a primitive type of nuclear division derived from protozoan forms, is now universally discarded, and so I will not stop to discuss it.

Nowikoff's ('08, '10) theory that amitosis is a phenomenon of mechanical significance seems to represent the truth, as far as some particular cases are concerned, but its general application is too obviously impossible. He based his theory on the fact that the nuclei located near the surface of cartilage are very much elongated and flattened, due, as he assumes, to mechanical necessity from their location, and, since amitosis sets in these elongated nuclei, a certain mechanical stimulus can be considered as underlying amitosis. The fact that this is not the only way in which Nowikoff's data can be interpreted, is suggested in the last section (p. 35).

Equally unfortunate in its foundation is Jordan's ('13a) theory that amitosis is due to the lack of the centrosome. Many authors have described the presence of the centrosome in various cases of amitosis, and even its apparent activity in the amitotic process (Meves, '94). Jordan's cases, in which the centrosomes are destroyed in the formation of cilia before amitosis takes place, seem to be good evidence, as far as it goes, in support of the theory, but the observations of Henry ('00), Ikeda ('06)

and Saguchi ('17) demonstrate the presence of the centrosome in amitotically dividing ciliated cells.

The theory that amitosis is connected with a degeneration, aberration, or senescence of the cell, is supported by a large number of authorities, amongst whom the names of Ziegler, Flemming, vom Rath, et cetera, may be mentioned. However, as Child ('07a) has well said, it is not strange that degeneration frequently follows amitosis, because the total destruction of the original substance is the necessary consequence of the orthodromic process pushed to the extreme, but there is certainly no reason to believe that such must always be the procedure. We also have no reason for believing that the degeneration of the cell after it has undergone amitosis is due to the latter process. Why cannot this kind of nuclear division be interpreted as indicating active metabolic processes in the cell, and the subsequent degeneration of the cell as being caused by such activities? Indeed, we see that many cases of amitosis cited by various authors can be explained in this way.

With the present state of our cytological knowledge, amitosis cannot be regarded as any sort of generative process, since the daughter nuclei produced by amitosis are most probably different in the amount of their chromatin contents. This, considered in connection with numerous cases of true amitosis, would show that the direct nuclear division is concerned chiefly, if not entirely, with the vegetative function of individual cells, and the possible significance of the phenomenon would be the one suggested by Chun ('90) and more recently by Glaser ('05). There are, however, described by certain authors, at least three kinds of data which apparently speak against this theory:

First: Those presented by Meves ('94), Preusse ('95), and more recently by Wieman ('10), and Foot and Strobell ('11), showing, as it is claimed, that oogonial and spermatogonial cells in certain forms divide by amitosis, but they may subsequently undergo mitotic division and produce normal ova and spermatozoa.

Second: Those supplied chiefly by Child ('04, '07 a, '07 b), apparently indicating that amitosis is a normal method of cell-

multiplication, especially in connection with rapid growth of tissues.

Third: Those derived from experimental works by Pfeffer ('99), Nathanson ('00) and Wasielewski ('03, '04), demonstrating that amitosis can be induced by certain chemical changes in external conditions, if the experimentally produced 'amitosis, is identical with one occurring normally.

Taking up the data of such nature as the first of these, I can best cite from Hegner ('14), who concludes, after careful review of the literature as follows:

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 . . . . 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.

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 (p. 427–28).

It is indeed inconceivable that such cells as have undergone amitotic division may produce true ova or spermatozoa, unless we assume an equal distribution of chromatin substance into the daughter cells at the amitotic division. For such assumption, however, we have at least at the present, no evidence at all!

Mitosis may of course follow amitosis in some cases, as actually observed in the living condition by Macklin ('16), but here the daughter nuclei produced by amitosis do not divide by mitosis themselves, but they fuse together, their chromatin substances forming a single equatorial plate of chromosomes, and then the division takes place in a normal way. Daughter nuclei in such cases, are not independent nuclei, but they are really only parts of a single nucleus as a reproductive unit.

It may be that Meves and others observed phenomena similar to that brought out by Macklin in living cells, but the study of sectioned material did not enable the earlier authors to see the

complete series of changes. This will, however, remain as an
open question for future study.

The studies of Child ('04, '07 a, '07 b, '11, '12) and of some
others of similar nature are valuable, especially because it is
largely due to these works that theories of physiology underlying
cell-division began to develop. Their data indicate that amito-
sis occurs, not only in the regions where a secretion is elaborated
or a reserve material is formed, but also in rapidly growing tis-
sues, and it was largely this latter fact that led these authors to
conceive amitosis to be a normal, but rapid, method of cell-
multiplication. There are, however, several observations which
make us hesitate to accept the claimed occurrence of amitosis in
the regions of rapid growth. Young ('08, '10, '13), studying the
histogenesis in some cestodes, thinks that the rapid multiplica-
tion of the cells is due to what he calls 'de novo' formation of
new cells. He observed that the daughter cell is formed from
an irregular mass of coarsely granular cytoplasm, containing
numerous small, deep staining granules, around which, later,
nuclear membrane arises, and finally, together with a small mass
of cytoplasm, separates from the mother mass. Richard ('11),
in his work on Moniezia, could not affirm whether mitosis or
amitosis is the method of cell-multiplication in that form. Har-
man ('13) was also unable to decide the method of cell-multipli-
cation in her work on Taenia and Moniezia. Finally, Paxman
('15) described accurately the process of rapid cell-multiplica-
tion in the sub-cuticula of Dilepis by the development of pro-
toplasmic masses similar to that observed by Young. Paxman,
after a careful examination of 10,000 nuclei in the region, decides
strongly against the occurrence of amitosis or mitosis. It may
not be safe, therefore, to draw any conclusion from such cases of
amitosis as are claimed to occur in rapidly growing tissues, at
least not until such cases are brought under clearer light.

The data derived from experimental studies are very interest-
ing. However, 'amitosis' produced by means of certain chemi-
cals may be easily explained, if we assume, with Jordan ('13 b),
a 'stupefying' effect of the chemicals upon the kinoplasm, be-
cause this will make it impossible for the dividing cells to show

mitotic figures. The assumption seems to be supported by the fact that as soon as the effect of the chemical ceases, the cells divide by a normal mitotic method. Nuclear phenomenon of such nature may, therefore, be called an experimental, or artificial amitosis, or 'pseudoamitosis,' as suggested by Häcker ('00), and may be considered entirely apart from the normal process of direct nuclear division. Indeed, if the assumption be true, this peculiar 'artificial amitosis' must be regarded as a type of mitosis, instead of one of amitosis.

In connection with the experimental data, it may be well to call attention to the repeated experiments with chloral hydrate on Vicia, Pisum, and Allium by Němec ('04), who strongly maintains that the so-called amitosis in Wasielewski's case is really nothing but the conjugation of the nuclei.

V. CONCLUSIONS

We have seen that the nuclear phenomena which were previously known collectively as amitosis may really be of different natures. It is not surprising, therefore, to find that any one of the suggested theories is inadequate for the explanation of all.

Whatever be the true nature of 'amitosis' claimed to occur in rapidly growing tissues, and that of the 'artificial amitosis,' my observations and considerations on the subject as described in this paper, seem to lead to the conclusion that—

Amitosis, occurring in secreting or reserve-forming cells, and in other cells of similar activity, may be for the purpose of securing an increase of the nuclear surface to meet the physiological necessity due to the active metabolic interchanges between the nucleus and cytoplasm. Apparently it is not a method of cell-multiplication, nor a sign of degeneration or senescence of cells, but, whenever it occurs, it seems to indicate an intense activity in the vegetative functions of the cell.

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1   Adipose cells from 1st stage larva. $\times$ 150.
2   Do., showing glycogen (iodine preparation). $\times$ 150.
3   Do., showing fat (osmic acid preparation). $\times$ 150.
4   A mitotic figure in an adipose cell from 1st stage larva. $\times$ 300.
5   Adiopse cells from 2nd stage larva. $\times$ 150.
6   A binucleate cell from 2nd stage larva, showing reticulated appearance of cytoplasm. $\times$ 300.
7   Adipose cells from 2nd stage larva, showing fat (freezing method preparation). $\times$ 150.
8   Adipose cells with ramified nuclei. $\times$ 150.
9   Adipose cells from 4th stage larva, showing fat (osmic acid preparation). $\times$ 150.
10   Same showing glycogen. $\times$ 150.
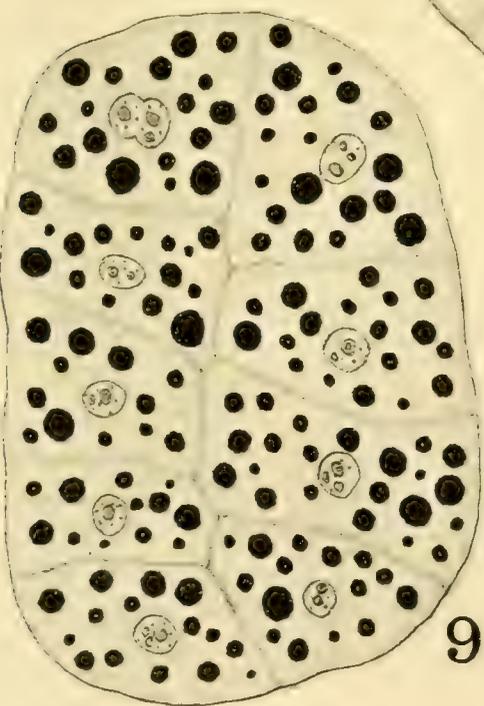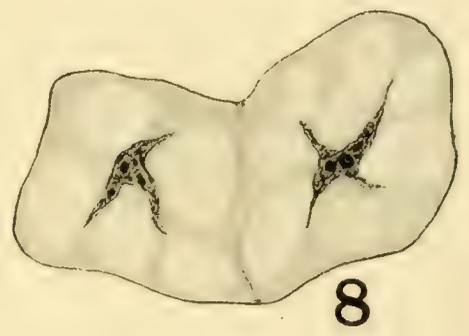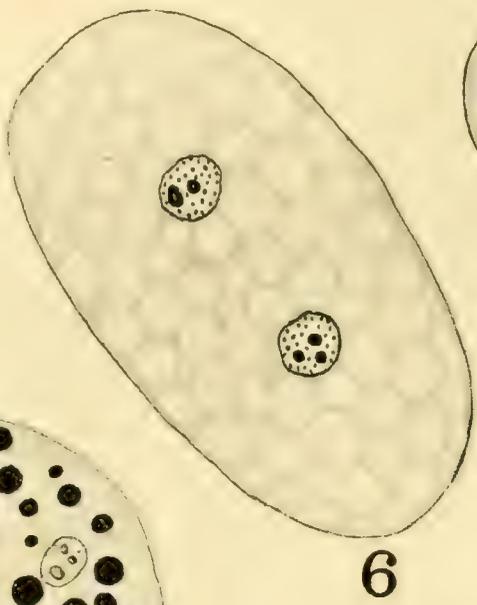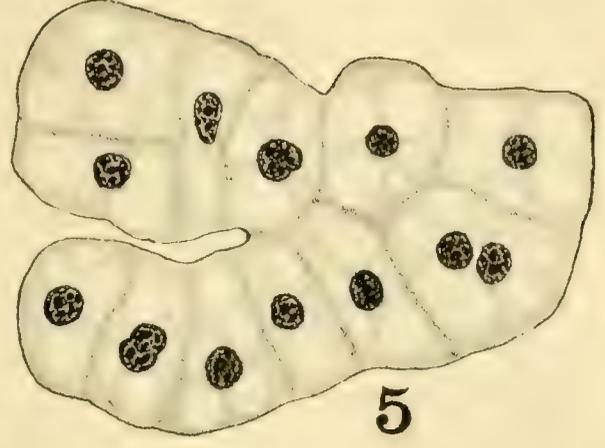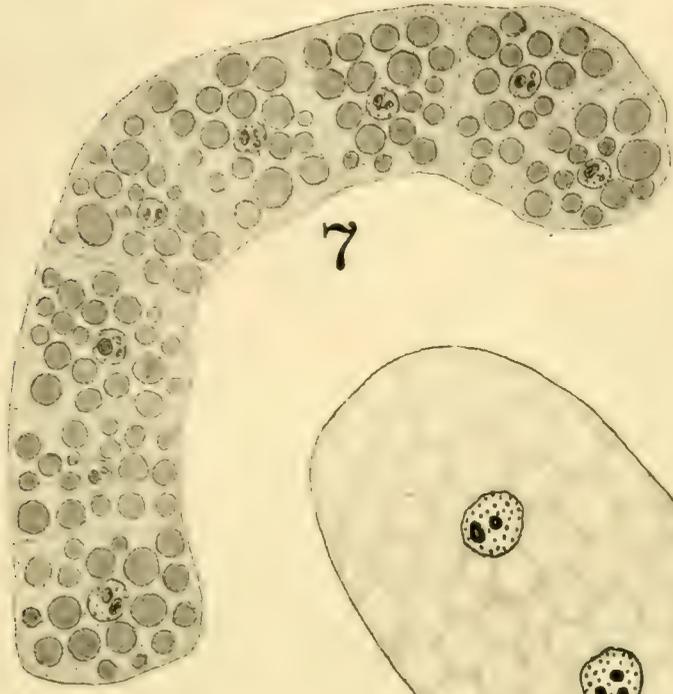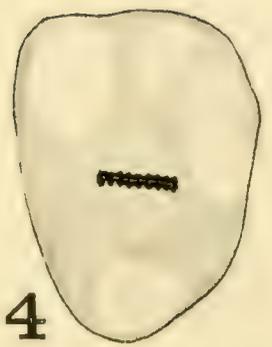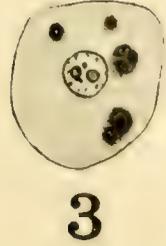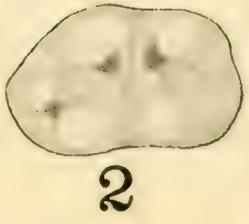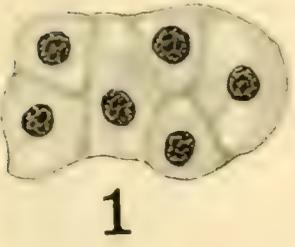
Pieris

11 An adipose cell from 5th stage larva, showing albuminous granules and acidophile nuclear granules. $\times$ 300.

12 Same from a little older larva: albuminous granules beginning to show basic color. $\times$ 300.

13 Same from a larva just before pupation, showing karyolytic condition of the nucleus.

14 An adipose cell from a mature larva (osmic acid preparation). The nucleus contains a fat droplet. $\times$ 300.

15 A nucleus of an adipose cell, showing the migration of acidophile nuclear granules into cell body. $\times$ 1200.

16 An adipose cell from a well grown larva, showing mucus (?) globules. $\times$ 300.

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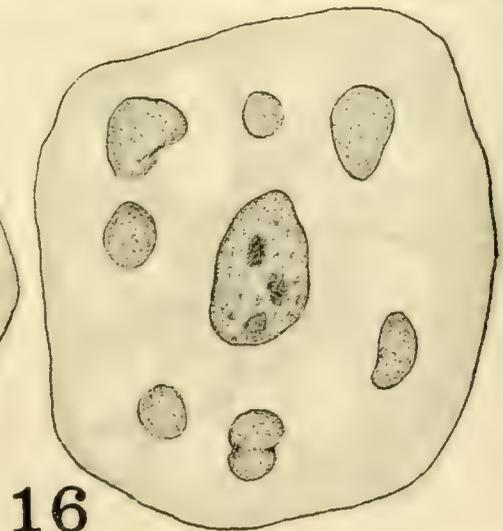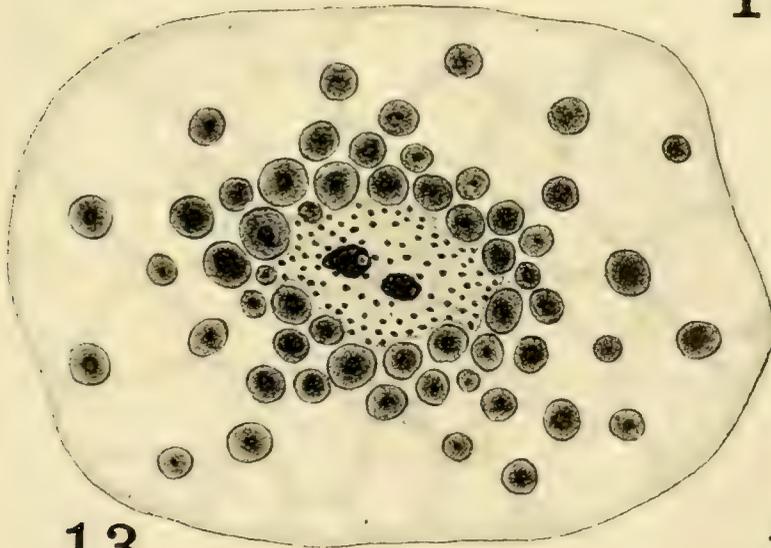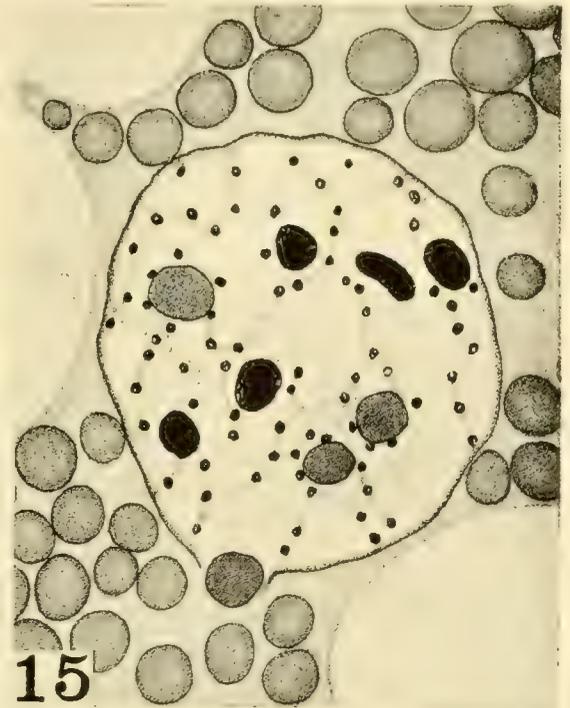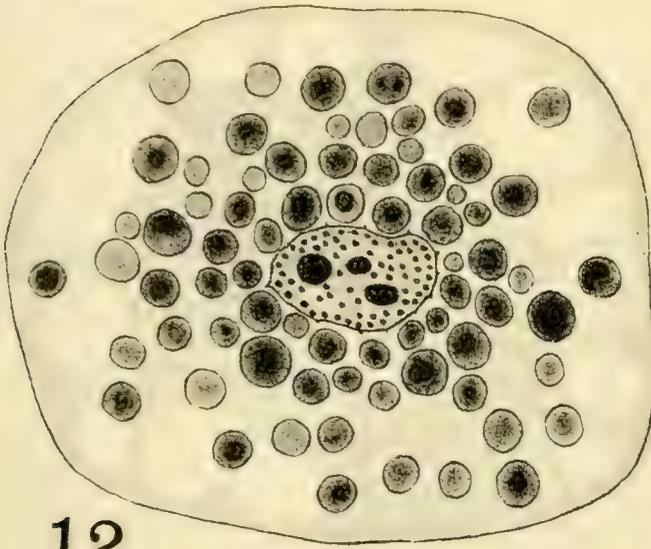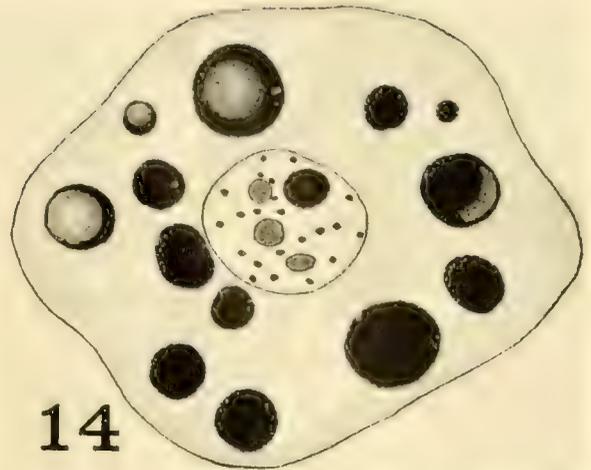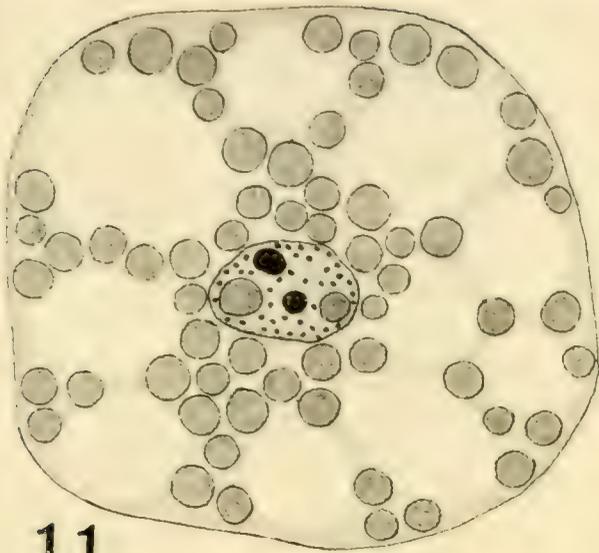
PLATE 3

Pieris

17 to 19 Normal process of amitosis. $\times$ 1200.
20 to 22 Unequal divisions of nuclei in amitosis. $\times$ 1200.
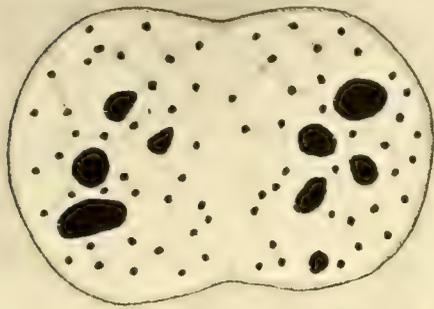
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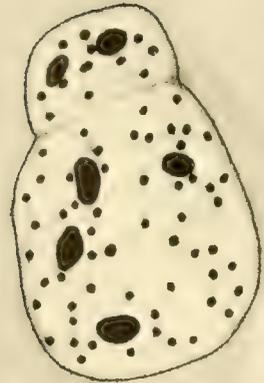
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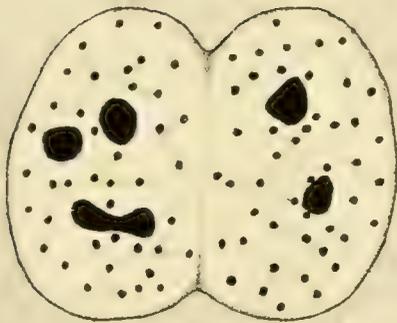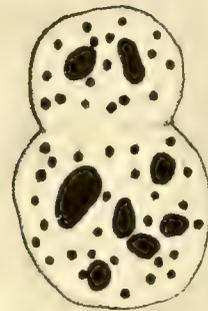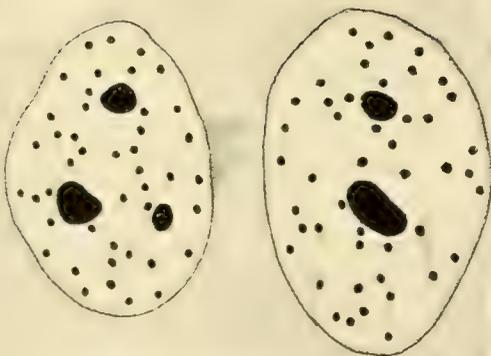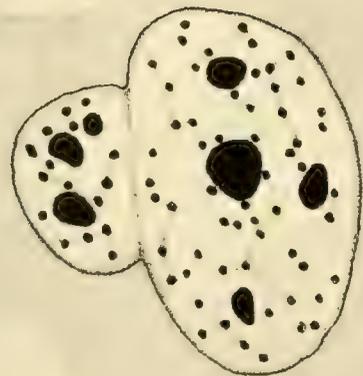
PLATE 4

Pieris

23　An extremely unequally divided nucleus..　$\times$ **1200.**

24　A nucleus dividing into three.　$\times$ 1200.

25, 26　Amitosis in vacuolated cells.　$\times$ 1200.

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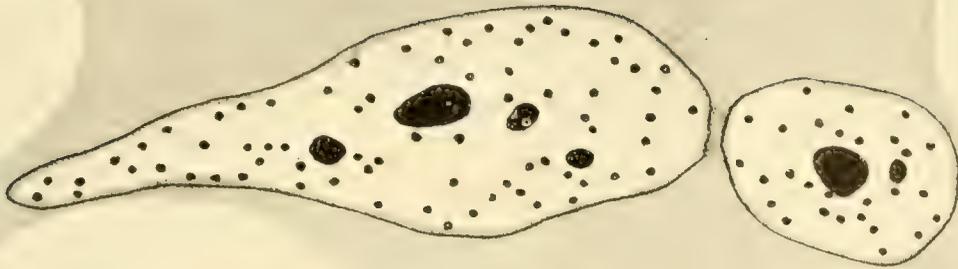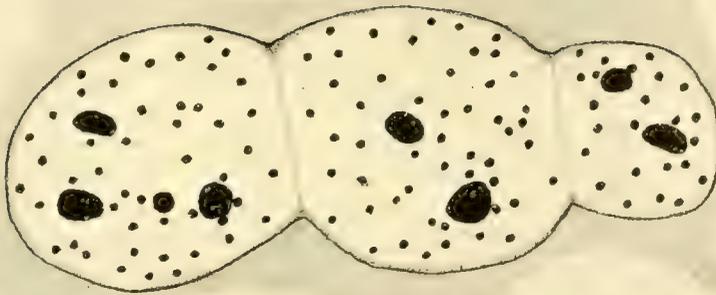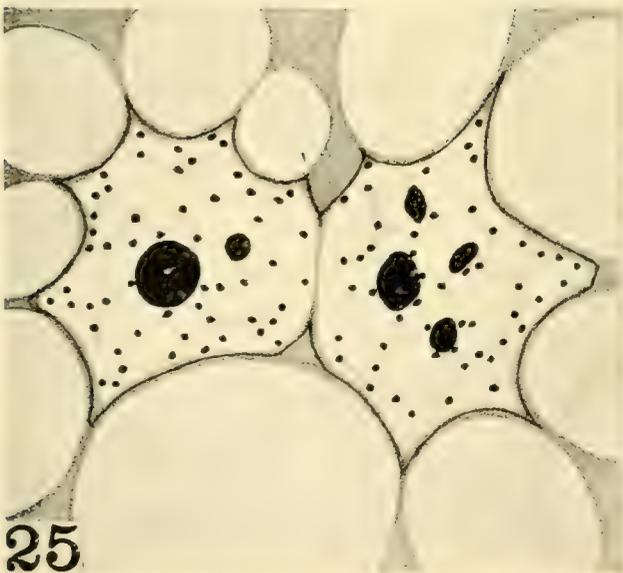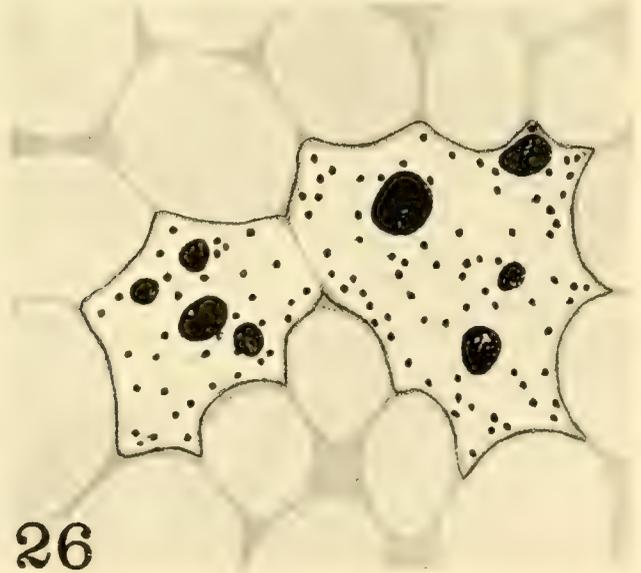
PLATE 5

Pieris

27 Last phase of amitosis in a vacuolated cell. × 1200.

28, 29 Possible cases of facultative, mechanical division of nuclei. × 1200.
Calliphora.

30 Amitosis in an adipose cell. × 1200.
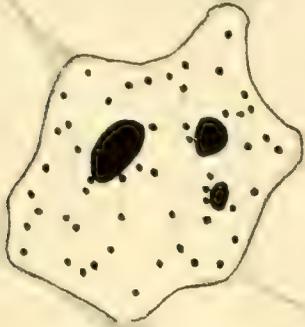
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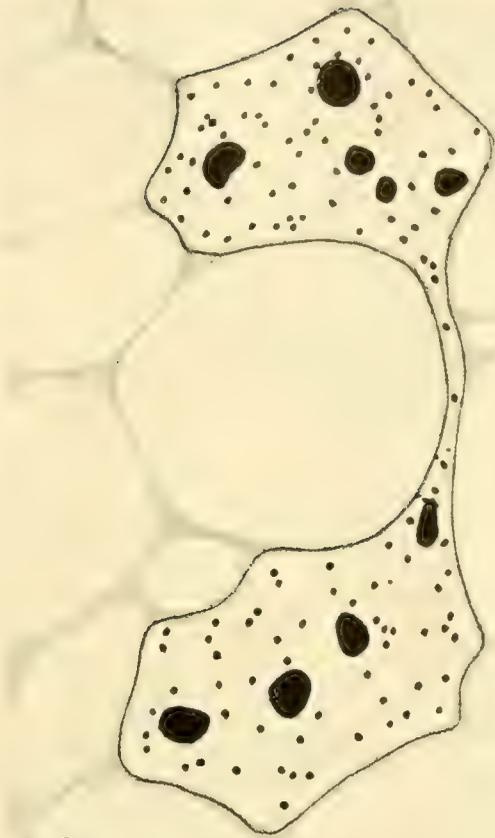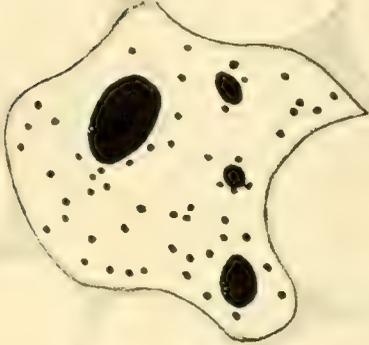
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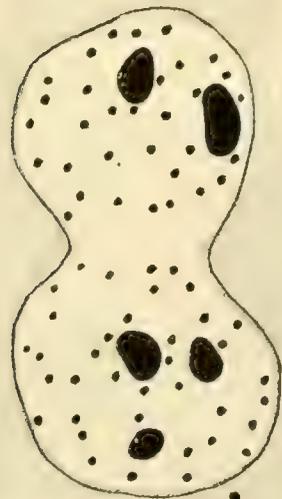
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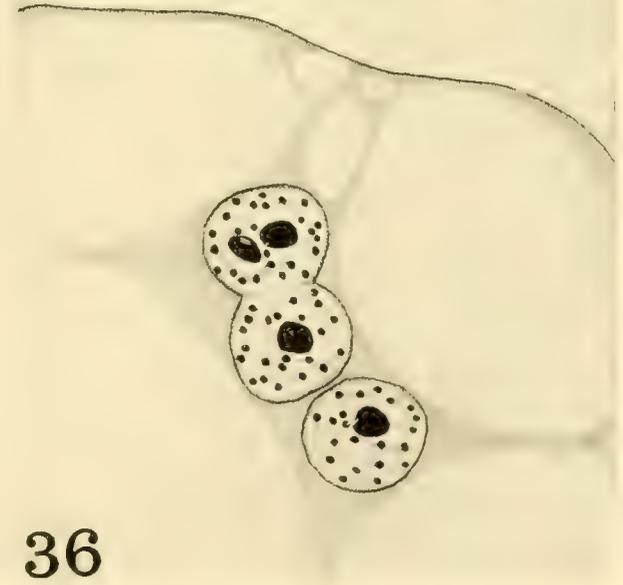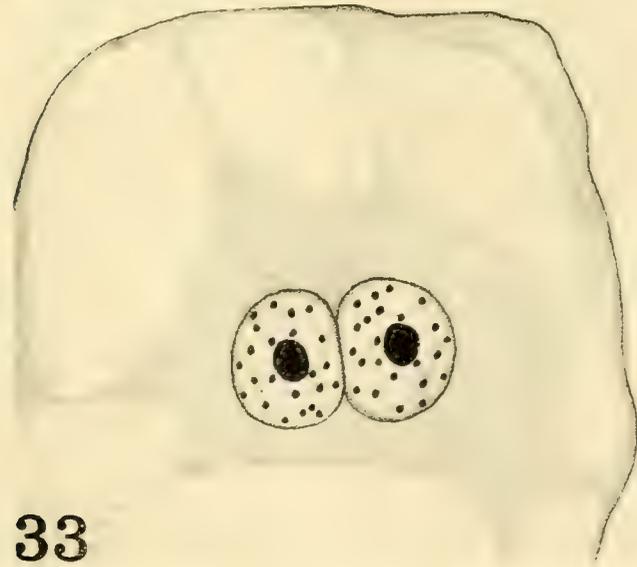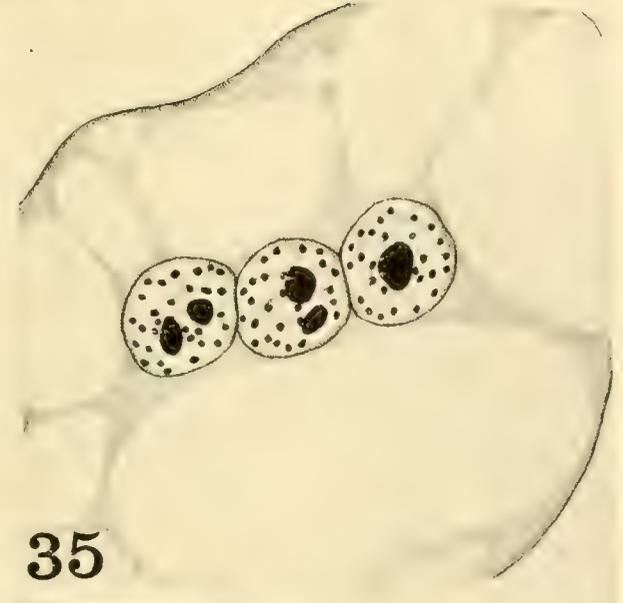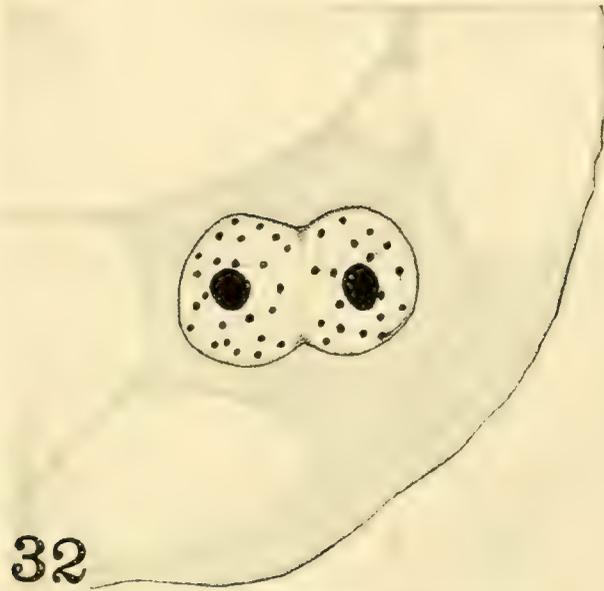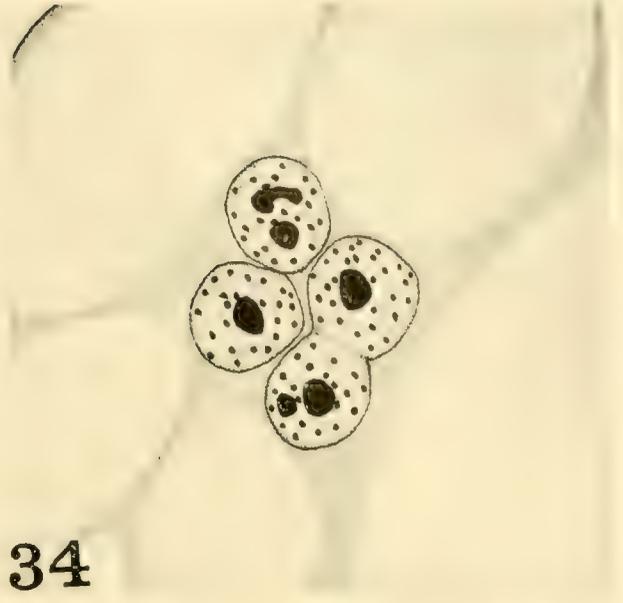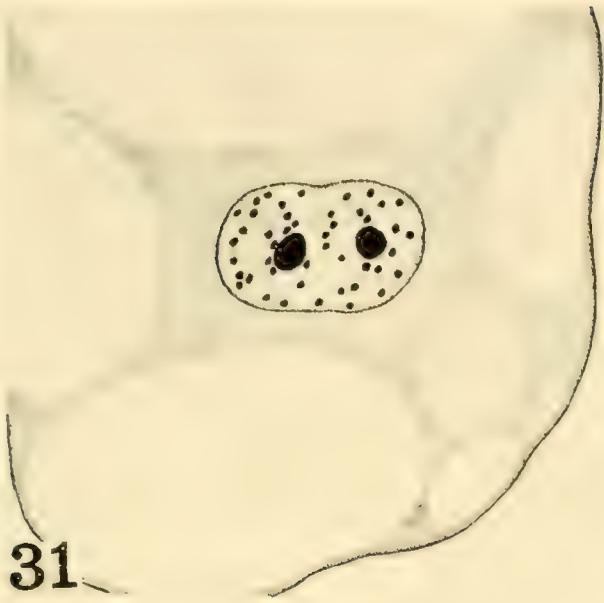
THE OOGENESIS AND EARLY EMBRYOLOGY OF ASCARIS CANIS WERNER[1]

A. C. WALTON

NINE PLATES AND ONE TEXT FIGURE

CONTENTS

I. INTRODUCTION

The work in this paper, on the oogenesis of the common nematode of the dog, Ascaris canis Werner, is a part of a broader investigation on the gametogenesis of this intestinal parasite. Previous articles (Walton, '16 a, '16 b, '16 c) have dealt with the spermatogenesis of A. canis Werner; with the systematic relationships of A. canis Werner, A. felis Goeze, and A. triquetra Schrank; and with the mitochondria of A. canis Werner, respectively. In the present paper the attempt is made to follow

the history of the development of the nuclear and cytoplasmic elements of the egg,—especially in regard to the method of the formation of the peculiar di-tetrad autosomes, and also in regard to the identity of the heterochromosome complex represented in the male by a group of six tetrad chromosomes,—and also to follow the process of fertilization and early stages of segmentation.

The material for this work was obtained exclusively from females of A. canis found in the intestines of dogs, and care was taken that only specimens of this species were employed. This precaution is important, for there is at least one other species of Ascaris (A. triquetra Schrank) found in the intestines of the dog which may be mistaken for A. canis Werner.

The writer here wishes to express his thanks and appreciation to Dr. E. L. Mark, not only for his criticism and advice in the carrying out of the investigations, but also for his assistance in obtaining material and for his care in criticizing the final form of this paper. Thanks are also due to Dr. S. I. Kornhauser, Northwestern University, for personal notes based on his examination of the material prepared by Marcus ('06) for the study of the oogenesis and spermatogenesis of an Ascaris which he wrongly designated as A. canis Werner, but which in fact was A. triquetra Schrank (Walton, '16 b). The writer is also indebted to Dr. Kornhauser for the use of slides loaned from the Northwestern University collection.

II. LITERATURE

For many years Ascaris canis Werner has been an object of study by the medical profession as a parasite in both domestic animals and man, but as a subject for taxonomic, morphological, and cytological investigation, it has been much neglected, only a few, widely separated sporadic efforts in these directions having been made. The identity of the nematode now known as A. canis Werner was for many years a mooted question among taxonomists. Nematode parasites superficially alike have been found not only in the dog and the cat, but also in other members of the families Canidae and Felidae. These were known by a

variety of names, no two workers using the same system of nomenclature. In 1872 an attempt was made to simplify the confusion by uniting all the various forms in one species, Ascaris mystax, the parasites of the different hosts being known as sub-species, and designated by terms indicative of their hosts,—for example, A. mystax, sp. canis,—or by some peculiarity of the external appearance of the worm—for example, A. mystax, sp. marginata. (For a complete list of these species and sub-species, see Glaue, '09.) The activities of Rudolphi and others in the next twenty years produced such a number of sub-species that Zeder (1803) proposed that the nomenclature be so modified as to restrict the use of the name Ascaris mystax to the intestinal parasites of the dog and the cat, the parasites of the other hosts formerly included in this species being established as distinct species. Rudolphi, discarding the name A. mystax, called the type which he believed to be peculiar to the Canidae, and perhaps the Felidae, by the name A. marginata. Schneider, in his "Monographie der Nematoden" ('66), returned to the nomenclature of Zeder, but Werner ('85) advocated the term A. canis, instead of Zeder's A. mystax, for the ascarids of the dog, the cat (A. felis Goeze), and their near relatives, the bears, wolves, foxes, lions and panthers.

It was owing to this confounding of distinct species, that the morphological and cytological results of the early cytologists when working on the intestinal nematodes of either the dog or the cat were often at variance. Regarding these several scientific names as synonomous, they unhesitatingly identified their species with that worked on by some one else, although their results varied considerably.

No attempt will be made to give here anything more than a very brief outline of the contents of any of the works cited, detailed reference to them being made throughout the present paper wherever the citations are pertinent.

Nelson ('52), Bischoff ('55), Meissner ('55), Thompson ('56), Claparède ('58), and Munk ('58) all worked with material parasitic in dogs and followed Zeder in calling the parasites Ascaris mystax. All these men worked on the sexual organs from an

exclusively morphological point of view. Nelson, Bischoff and Thompson maintained that the sperm came in contact with, but did not enter, the egg. Meissner believed that the sperm entered the egg through a micropyle, and in this view Munk ('58) concurred. Claparède ('58) denied the existence of a micropyle, and thought that the sperm could enter the egg at any point.

The work of Van Beneden ('83), ('87), Carnoy ('86) and Boveri ('86) showed that the ascarids are very favorable for cytological study. In the next thirty years practically every known species of parasitic nematodes was subjected, at least casually, to a cytological investigation, in order to corroborate or combat the evidence derived from A. megalocephala by the writers last mentioned and their numerous followers.

Carnoy ('87) was the first to examine carefully the gametogenesis of the ascaris of the dog, which he refers to as possibly being A. marginata Rud. Kultschitzky ('88 c) and Lukjanow ('89) also carefully studied dog parasites, which they believed to be A. marginata Rud. None of these three investigators arrived at the same conclusions regarding either the number of the chromosomes or the method by which reduction to the haploid number was effected, although each was supposed to have used the same species of nematodes as the other two. Carnoy ('87) found four chromosomes as the haploid number in each sex. At the formation of the first polar cell, four of the eight diploid chromosomes were eliminated. At the second division half of each of the remaining four chromosomes was cut off in the second polar cell, leaving four female chromosomes to enter into the composition of the segmentation nucleus with the four brought in by the spermatozoon.

Kultschitzky ('88 c) worked only on the oogenesis of A. marginata; he found twenty-two diploid chromosomes, which became reduced by the two maturation divisions to eleven haploid chromosomes. Lukjanow ('89) also followed Rudolphi in calling the ascaris of the dog A. marginata, but stated that A. marginata Rud. ('01), A. mystax Zeder ('03), and A. canis Wer. ('85) were all the same species. Lukjanow found that the di-

ploid number of chromosomes was sixteen, and that one-half of the number of chromosomes as well as one-half of the chromatic matter was lost at each division of the oocyte, leaving only four female chromosome elements in the mature ovum to unite with the four from the male.

No further investigations, except by medical writers, were directed toward either the taxonomy or cytology of the ascarids of the Canidae and Felidae until 1906. Marcus ('06), following the nomenclature of Werner ('85), called his material A. canis Wer. (var. mystax); but he obtained the most of his material from bears and lions, only a small portion of it being from dogs.

He maintained that the unreduced number of chromosomes in his material was twenty-two, thus corroborating the results of Kultschitzky. Marcus further showed that of these chromosomes twenty were united into pairs, but that two remained unpaired, so that each of the primary gametes, both oöcytic and spermatocytic, had ten bivalent and two univalent chromosomes. In the first maturation division the bivalents divided, each daughter nucleus receiving half of each bivalent element, while of the two univalents one passed to each of the daughter nuclei without dividing. Each daughter nucleus thus received eleven univalent chromosomes. The action of the unmated pair of chromosomes suggests that of an 'X-Y' pair, but Marcus made no mention of such possibility. These two chromosomes were the largest in the mitotic figure (Marcus, '06, taf. 29, fig. 13).

In the early spermatocytic and oocytic stages he found a single, longitudinally split, spireme thread. This contracted into the center of the nucleus and gave rise to a true plasmosome, as well as a drop of tropho-chromatin, which was entirely eliminated from the nucleus. The central mass of chromatin broke up into parallel threads that, crossing one another at all angles, united to form a reticulum, which had chromatin granules at each point of intersection; the intervening internodes being composed of linin. As these threads constricted, the granules united and formed the prophase chromosomes. Each chromosome showed distinct 'end to end' halves, connected by a linin bridge. The

plasmosome disappeared at the time of the formation of the chromosomes of the first maturation spindle. The paired, split threads upon becoming converted into compact chromosomes again divided longitudinally, at right angles to the plane of the first separation, thus forming four-parted threads. These shortened and then by telosyndesis united to form eight-parted, or octad chromosomes. In the oocytic and spermatocytic divisions the chromosomes each divided longitudinally, the second maturation division being the reduction division. There was no resting stage found between the first and second divisions. The centrosome was of intra-nuclear origin and continued from the first to the second divisions unchanged.

The two pronuclei did not fuse until after the setting up of the first segmentation spindle. The centrosomes of this spindle were derived from the one introduced by the sperm. Each of the daughter cells of the first cleavage had eleven tetrad chromosomes—the result of the pairing of the eleven dyads (each of the four elements of a tetrad dividing in the first cleavage). These tetrad divisions were longitudinal. The second cleavage showed a process of 'diminution' in the first soma cell, but none in the sister cell—the stem cell. These stem cells continued to show eleven tetrad chromosomes, while the soma cells showed twenty-two dyad chromosomes.

Medical writers had been differing greatly as to the identity of the species of intestinal nematodes of the dog and the cat, some claiming that the type found in the domestic cat was but a smaller edition, so to speak, of the A. canis Wer. found in dogs. Others maintained that it was an entirely separate species and should rightfully be called A. felis Goeze. Glaue ('08, '09, '10) made a very careful morphological and histological study of both types—A. canis and A. felis—and came to the definite conclusion that these two parasitic nematodes were entirely different species, viz. A. canis Werner and A. felis Goeze, not merely varieties of A. mystax Zeder or A. canis Wer. Schöppler und Krüger ('12) took the opposite view and called the parasites of both dog and cat A. canis Werner, in spite of the fact that the work of Edwards ('11) had shown a cytological condition in A.

felis that was very different from that reported for A. canis
(Marcus, '06). Edwards found in A. felis a heterochromo-
some group, of which he gave two possible interpretations.
One, that toward which he inclined, was that the group was
made up of an 'X-Y' pair in which the 'X' was twice as large an
the 'Y'; the other was that the group was of the simple 'X' type,
the 'X' being joined to the end of one of the autosomes. Boveri
('11), under whom Edwards worked, held to the latter interpre-
tation. In the first division each of the eight bivalent chromo-
some pairs so divided as to give each of the daughter plates as
equal share, while the univalent 'X-Y' pair gave to one plate
'X' and to the other 'Y'. In the second division 'X' and 'Y'
divided equationally (Edwards, '11, pl. 28, figs. 2, 4, 5).

The apparent morphological and cytological differences be-
tween A. canis and A. felis have been the subject of further
study by the writer (Walton, '16 a). The morphological differ-
ences given by Glaue have thus been substantiated, as was also
the cytological work of Edwards on A. felis. The work of
Marcus ('06) on A. canis, however, was utterly at variance with
the writer's results from the study of spermatogenesis. The
present writer found thirty tetrad chromosomes—twenty-four
autosomes and six idiosomes—as the diploid number in the
male, and thirty-six in the female sex cells. By pseudo-reduc-
tion these were reduced (in the male) to twelve di-tetrad (eight-
parted) autosomes and six tetrad idiosomes—eighteen in all.
At the first spermatocytic division the twelve autosomes were
halved and the six idiosomes laggingly passed to one of the
daughter nuclei, giving rise to two types of secondary sperma-
tocytes; one having twelve, and the other eighteen tetrad chromo-
somes. At the second spermatocytic division each autosome
as well as each of the six idiosomes, was again halved, giving rise
to spermatids also of two types; one having twelve dyad auto-
somes, the other having twelve dyad autosomes plus six dyad
idiosomes—eighteen dyads in each nucleus of this type. As
there was no elimination of chromatic matter during the meta-
morphosis of the spermatids into spermatozoa, the latter are of
two types, equal in number and differing from each other in the

presence or absence of the six heterochromosome dyads. The oogonia were found to have eighteen di-tetrad chromosomes as the diploid number. The heterochromosomes were present in the '2X' condition in the female.

Further work (Walton, '16 c) showed that the refractive body of the spermatozoon was purely nutritive in function, and that it arose by the fusion of the refringent granules of the spermatids. The mitochondria were shown to have had a nuclear origin and to have served for a while as centers about which the refringent granules of the yolk were built up. Later the mitochondria were grouped around the centrosomes in a cap-like mass, passing through the nuclear reduction divisions unaffected. They maintain this position in proximity to the centrosome when the sperm enters the egg and then break up and become lost to view as the spermatozoon disintegrates. Hence, they seem to have played no part as bearers of hereditary characters.

The writer (Walton, '16 b) found that A. triquetra Schrank occurred very rarely in dogs, but had been several times reported as common in bears. An examination of the gametogenesis of this parasite showed a very close correspondence with the conditions found by Marcus ('06) in A. canis. In view of the source of most of Marcus's material, and of the correspondence of cytological conditions between the form studied by him and A. triquetra, it seemed probable that the nematodes used by Marcus were A. triquetra Schrank, rather than A. canis Werner.

The work of the present writer has thus shown, not only by morphological, but also by cytological evidence, that A. canis and A. felis are different species.

The literature on other nematodes, especially Ascaris megalocephala, is so enormous that no attempt at even outlining its results has been made. Wherever pertinent, however, detailed reference to the conditions in other nematodes is made at appropriate points throughout this paper. In the study of the plasmosome, reference has also been made to certain works on the gametogenesis of insects. The work of Jörgensen ('10) has had special interest because he finds in the gametogenesis of certain Sycon sponges di-tetrad chromosomes which reduce to

the dyad form in the haploid condition, although the method of their formation was entirely unknown to him.

III. MATERIAL AND METHODS

The material was all obtained from freshly killed dogs, care being taken that only specimens of Ascaris canis Werner were employed. The worms were immediately removed from the intestine of the host, placed in normal salt solution, and kept at body temperature until they could be killed. The posterior end of the worm was clipped off and the ovaries were at once stripped out on to a glass plate and fixed, the whole process taking but a few seconds. Hermann's fluid, Flemming's fluid (strong), Bouin's fluid, Petrunkevitch's modification of Gilson's fluid, acetic alcohol, and Carnoy's fluid were used as fixatives. The most favorable fluids were those of Carnoy and of Petrunkevitch.

The best sections were obtained from material imbedded in paraffin by the benzol-chloroform method. These were cut 15μ or 30μ in thickness. Heidenhain's iron-haemotoxylin stain followed by a counter-stain of Bordeaux red or Orange G was the staining method usually employed. The Biondi-Heidenhain triple stain, safranin-licht grün, and alizarin-krystal violet stains were also used for the study of the linin and the plasmosome; likewise for the cytoplasmic structures of the maturation stages. Schneider's acid carmine was used in the study of whole mounts and smears, but was valuable only for a rapid ascertainment of the age of the cell or the number of the chromosomes.

IV. EXPERIMENTAL EVIDENCE

A. OOGONIAL DEVELOPMENT

The youngest oogonia appear as very small cells (fig. 1), closely packed in a single layer around a very fine central core, or rhachis. The diameter of the nucleus is about one-half that of the cell; its chromatin is centrally located in a single, compact, more or less spherical mass of regular outline, and is of homogeneous appearance after all the methods employed. The cyto-

plasm contains very finely granular matter scattered uniformly throughout its substance.

The division of the nucleus is clearly by the indirect method, although the chromatin, as in the spermatogonia, appears as a single mass, instead of being resolved into individual chromosomes. From this chromatin mass a nuclear plate is formed (fig. 2) in the equatorial plane of the spindle. Traces of the spindle are likewise to be seen; but the actual centrosomes cannot always be made out, owing to the extreme smallness of the elements involved. This nuclear plate divides into two equal, somewhat saucer-shaped bodies (fig. 3), which migrate, one to each pole of the spindle. Cell division follows immediately, the solid chromatin mass of each daughter cell occupying a peripheral position in the reconstructed nucleus (fig. 4). This daughter plate later moves to the center of the nucleus in all except the daughter cells of the last division. In the latter (fig. 5) it becomes differentiated into its component chromosomes while still occupying a peripheral situation. A large, intensely staining body (the plasmosome) also emerges from the common mass of material found in the earlier stages. Marcus ('06) found that the plasmosome in the dog ascarid (A. canis?) studied by him originated in the same way.

To judge from its staining reactions, this certainly corresponds to the plasmosome of the spermatogonial cells, not to the smaller of the two karyosomes, which in the male, as has been shown (Walton, '16 a), is made up of the heterochromosome group. The '2X' character of the female is not shown by a distribution of the chromosomes into separate karyosomes, as is the case in the male sex cells (Walton, '16 a, plate 1, fig. 1).

This formation of definite chromosomes and a plasmosome is recognized only after the last of the numerous oogonial divisions, which marks the end of the 'multiplication' of cells, this zone extending through one-eighth of the total length of the ovary. During this period the cytoplasm maintains its characteristic granular appearance with very slight, if any, indication of a fibrillar or alveolar structure.

Owing to the minuteness of the cells at this stage, very little

has been done on the development of the oogonia of Nematodes. Marcus ('06) in his work on A. canis (?) passes this stage by with only a remark on the smallness of the elements, and the statement that the plasmosome detaches itself from the chromatic mass, which then breaks up into chromosomes having a transverse constriction. In another dog ascarid, A. marginata, Lukjanow ('89) figures the 'stem cell', from which the oogonia originate, and the oogonial divisions as being mitotic in character. In his preparations the individual chromosomes were distinct and of thread-like character during these divisions. Kultschitzky ('88 c), also working on A. marginata, does not figure the oogonial stages.

Descriptions of the oogonial stages of A. megalocephala are much more numerous and complete. Hertwig ('90) shows that the chromosomes are formed from continuous spireme threads, which have arisen by the congregating of chromatic granules imbedded in a linin network. These chromosomes occasionally show a longitudinal splitting as a forerunner of their future plane of division. Brauer ('03), on the contrary, finds that in the spermatogonia the spireme thread is never continuous, but that each chromosome arises from a separate thread. Edwards ('10) maintains that in thirty-two per cent of the oogonia studied by him, the single idiosome is found as a separate body. In opposition to this view, Boveri ('11) and Frolowa ('12) think that the idiosome is generally attached to the end of an autosome, and exists only occasionally as a separate chromosome. Geinitz ('13) believes, as also does Kautzsch ('13), that the idiosomes, as well as the chromosomes, are multiple structures. Tretjakoff ('04 b) finds that the chromatin of the spermatogonia first exists as a central mass, which then breaks up into fine threads. These, after assuming a peripheral position, shorten into thick, rod-like bodies. By a process of parasyndesis, two of these bodies conjugate to form a true chromosome.

The nuclear wall in the case of A. megalocephala consists (Van Beneden, '83) of a layer of linin containing microsomes. The same method of formation of the nuclear membrane is reported by Goldsmith ('16) for Pselliodes cinctus. My own work

has thrown no further light on this question of nuclear-membrane formation. Goodrich ('14) finds that the oogonial chromatin in Ascaris incurva, as in A. canis, tends to divide *en masse* rather than as individual chromosomes, and that the heterochromosome group is not differentiated from the autosomes by being formed from separate karyosomes. In Ancyracanthus cystidicola (Mulsow, '12), as in A. canis, the heterochromosomes in the female are not morphologically differentiated from the autosomes, while they are in the male. Angiostomum nigrovenosum, studied by Schleip ('11), shows that the oogonial karyosomes form chromosomes while located in the nucleus centrally, instead of peripherally, the position which is common in the oogonia of most nematodes. Gulick ('11), working on Heterakis vesicularis, found that the heterochromosomes were not distinguishable from the autosomes in the oogonia, but were in the spermatogonia, coming from a separate karyosome, as they do in A. canis Walton, '16 a).

B. OOCYTES

1. *First maturation division*

a. Prophase. The prophase extends through the period of the 'growth zone' of the sex cells; it is of long duration, as is shown by the great number of cells with their chromosomes arranged in the metaphase plate. The account which follows covers both the nuclear and cytoplasmic conditions.

The nuclear contents during this period undergo a very complicated set of evolutions in the formation of the chromosomes and in the preparation for the first maturation division. However, it is not possible to identify nearly as many stages as were found in A. megalocephala by Saedeleer ('12), nor such details of chromosome structure as those given by Bonnevie ('08) and by Vejdovský ('12) for the same species. Nevertheless, it is possible to follow out the gross details of the method of the formation of the chromosome tetrads and di-tetrads.

The peripheral mass of chromatin noted after the last oogonial division (fig. 4) breaks up (fig. 5) into a large plasmosome and

thirty-six small karyosomes or 'pro-chromosomes.' These bodies become arranged near the periphery of the nucleus, while the plasmosome assumes a more central position. At first these 'pro-chromosomes' are slightly ellipsoidal (fig. 6), but later each becomes slightly constricted in a plane perpendicular to its long axis, thus assuming a dumb-bell appearance (fig. 7). Marcus ('06) interpreted this form of chromosome in another dog ascaris as evidence of a reduction of the somatic number of chromosomes by telosyndesis.

The ovary has enlarged in cross-section faster than have the oogonia. The latter, still in one layer around the rhachis, are very loosely packed and the peduncle is readily apparent Hertwig ('90, taf. IV, fig. 7) figures a similar stage for the oocytes of A. megalocephala. At the time of the appearance of the dyad-like 'pro- hromosomes,' the nucleus has doubled in diameter, while the cell body has increased its diameter by only one-fourth. The plasmosome has now become almost central in position.

The next stage in nuclear development follows quickly; it shows a fine lin n network connecting together the peripheral chromatic bodie:, which now have lost their dyad appearance and become more nearly cylindrical The plasmosome is surrounded by this network, but is not connected with it (fig. 8). To judge from its staining reactions, the plasmosome is non-chromatic and not the source of the heterochromosome group.

The network (fig. 9) now becomes more complex, and the chromatic bodies, appearing as mere knots at the angles of the network, are reduced in size.

Following this stage, the network (fig. 10) becomes coarser and the strands, much fewer become, very thick and jagged in outline, varying greatly in diameter from place to place. These network threads stain heavily in specific chromatin dyes, showing that the chromatic material is distributed uniformly throughout the threads and not concentrated at their junctions. The plasmosome is again peripheral and takes a heavy stain, especially in Heidenhain's iron-haematoxylin. The cytoplasm, while still retaining its finely granular or reticulate condition, has begun to grow in size to keep pace with the growth

of the nucleus. . Marcus ('06) finds in A. canis (?) a similar be-
havior of the nuclear elements during the formation o the net-
work and spireme from the oogonial chromosomes, except that
the threads appear to be paired, or at least to lie parallel with
one another, most often in twos. In the oocytes of A. megalo-
cephala, Hertwig ('90) finds that the doubleness of the spireme
thread may or may not be evident at this stage, although it is
generally evident n the spermatocytes. Schleip ('11) contends
that in Angiostomum nigrovenosum the parallel threads of the
spireme are due to a longitudinal splitting of one thread, not to
a pairing of two separate threads. A. canis shows no evidence
of this double nature of the spireme threads, either through
splitting or pairing.

The cells become very closely packed (fig. 11), assuming
angular outlines, and some even appear to have lost all con-
nection with the rhachis. This cord, beginning as a fine thread
in the anterior end of the oviduct, sends off, as it grows back-
ward, four primary lamellae, to which the oogonia are attached
n a single layer. The peripheral margins of the primary lamel-
lae bifurcate, and in the region of the primary oocytes
sexual cells are attached both to the four primary lamellae and
to the eight secondary ones, but always in a single layer. The
secondary lamellae quickly become so obscured by the crowding
of the rapidly growing cells (fig. 11) that they are easily over-
looked, and the oocytes thus appear several layers deep around
the primary lamellae. Careful study, however, shows that each
of the cells is in reality directly attached to the rhachis by a
fine stalk. This shortening and thickening of the primary
lamellae and the practical disappearance of the secondary lamel-
lae agree with the condition described by Hertwig ('90) for both
types of A. megalocephala, but Schleip ('11) finds that in An-
giostomum nigrovenosum the growth takes place after the break-
ing down of the rhachis and the setting free of the oocytes. In
a Sclerostomum from the horse, Kühtz ('13) shows that the
growth is completed (zone of growth) before the breaking down
of the rhachis.

Following the stage illustrated in figure 10, the chromatin

undergoes further condensation, gathering toward one side of the peripheral meshwork, leaving behind a fine linin net free from chromatin. This stage corresponds very closely to the 'bouquet' stage which Marcus ('06) describes for his dog ascaris. Hertwig ('90), Tretjakoff ('04 a), Griggs ('06), Grégoire ('10), Bonnevie ('12), and Vejdovský ('12) mention similar stages in A. megalocephala. Saedeleer ('12), however, does not find in this species any such stage. A condition similar to the 'bouquet' has been seen by Kühtz ('13) in a species of Sclerostomum from the horse.

It is during this stage that synmixis takes place, if it takes place at all, for every chromosome has lost its morphological identity in the common mass, and there is nothing to prove that the chromosomes arising from this mass are absolutely identical with the ones which enter into it.

The cytoplasm of the cell has undergone rapid growth, especially along its radial axis, and the cell has thus become shaped like a pear or a truncated cone, with the small end toward the rhachis. This change of shape is coexistent with an absorption of the lamellae and an increased linear growth of the rhachis. This allows all the cells to lie at the same level, their small ends touching the rhachis, or attached to it, and their large ends reaching the periphery of the oviduct. These cells have a somewhat less angular outline than heretofore, due to less crowded conditions in the oviduct. The nucleus is situated in the larger, peripheral end of each of the oocytes (fig. 13).

The cytoplasm has become more definitely reticulated, the granules seemingly being arranged in thread-like chains. At the outer end of the cells a few vacuoles begin to form (fig. 13), some of which are already fairly large. These increase very rapidly in number as the cell matures (fig. 15).

The nucleus (fig. 13) during these cytoplasmic changes has undergone still further condensation of the chromatin of the 'bouquet,' which becomes definitely one body, slightly irregular in outline, and having a few, rapidly disappearing linin threads diverging from its periphery (fig. 15). In many cells the lin n network has entirely disappeared (fig. 16). These radiating

threads correspond to the ones described by Marcus ('06) for his A. canis, and by Lukjanow ('89) for A. marginata. At the same time the plasmosome may show signs of degeneration. It may now (fig. 17) break up into a few small, deeply staining granules (Heidenhain's iron-haematoxylin), surrounded by a more faintly staining ground substance, and then shortly disappear; or it may persist as a compact body until the final reformation of the chromosomes before it entirely disappears.

The concentrated mass of stained material, consisting of chromatin and linin, begins to show a differentiated appearance in the majority of cells before the network entirely disappears (fig. 17), thus indicating that the linin network, as well as the chromatin, had been involved in the condensation process, and that it had not become invisible merely by becoming diffused generally throughout the nucleus. This differentiation proceeds rapidly (fig. 18), dark bodies of chromatic material being easily distinguishable against the less deeply staining linin when iron-haematoxylin is used. With safranin, followed by licht-grün, the contrast is sharp between the reddish chromatic bodies and the greenish substratum of linin. The karyosomes thus differentiated are thirty-six in number, the diploid number characteristic of the sex cells of the female. The plasmosome is in some cases included in the condensation mass as a definite body, and as such is again released upon the dissolution of the mass; but in other cases it is not so included.

The linin ground-work becomes rapidly diffused, and disappears, leaving the karyosomes lying free in the karyoplasm. At first closely grouped, the karyosomes soon spread throughout the peripheral region of the nucleus (fig. 19) and then gradually a few of them take up a more central position. These bodies are ellipsoidal, not as yet showing the definite constriction perpendicular to the long axis that was noted in the chromosomes formed in the early prophasic nuclei (fig. 7) after the last oogonial division. This method of the formation of the chromosomes differs markedly from that ordinarily described for the Ascaridae. It corresponds, however, exactly with that of the formation of the diploid chromosomes in the primary spermatocytes

of the male of A. canis, as shown by the writer (Walton, '16 a). However, in the male sex cells two masses of chromatin and linin instead of one are found. One of the two contained the components of the heterochromosome group, while the other contained all the autosomes. In the primary oocytes the components of the heterochromosome group, which is of the '2X' character, are not so differentiated from those of the autosomes as are those in the male; but all are united into a single group, in which it is impossible to recognize individual elements. From analogy with the male one might infer that it is these heterochromosomes that, after their differentiation, are the first to move toward the center of the nucleus, just as the heterochromosomes are known to do in the case of the male sex cells; but no satisfactory evidence is at hand to support this hypothesis.

The direct formation of the chromosomes from the chromatic mass, that is without the intercalation of spireme threads, also occurs in the case of A. marginata (Kultschitzky, '88 c, p. 673), where the chromosomes are described "als Resultate dessen ein progressives Zerfallen des Chromatinklümpchens in ein Häufchen einzelner Körner . . . . sich einstellt." Although Kultschitzky makes no statement as to the number of chromosomes formed, he pictures twenty-two in the majority of the cases figured, namely figures 11 to 14. Lukjanow ('89) also worked on A. marginata, but came to an entirely different conclusion both as to the method of the formation of the chromosomes, and as to their number. However, in agreement with Kultschitzky, he states that the spireme stage is entirely missing. He finds that eight bivalent (dyad) chromosomes are formed by the secretion of 'hyaloplasm' around small chromatic karyosomes which appear suddenly in the ground substance of the nucleus. One member of these dyad-like pairs is always slightly larger than its mate. The presence of the dyads is due to a process of 'pseudo-reduction,' by synapsis, to the haploid number, which occurred before the definite appearance of the chromosomes.

Marcus ('06, p. 445) describes a single spireme thread "der deutlich längsgespalten ist" even before it enters into a clumped

or synaptic stage similar to the one described above for A. canis. This clumped mass breaks up into a tangle of interlacing fibers, each one of which is longitudinally split, and has its contained chromatin unevenly distributed along its length instead of being deposited in definite chromatic bodies. These threads radiate in pairs from the nucleolus as a center, and the chromatin collects into rods on each thread, thus giving pairs of rods; but each rod is itself already longitudinally divided, owing to the double nature of the thread around which it is formed. Each chromatic body, or chromosome, is therefore made up of four parts, closely bound together by linin. A transverse constriction appears soon after (taf. 30, fig. 38),—at the time of the disappearance of the linin threads,—leaving the chromosomes, now octads in form, lying free in the nucleus. During this period, according to Marcus, the nucleus loses its limiting membrane and assumes a remarkable star-shaped appearance, with one of the eleven (reduced number) octads occupying the outer extremity of each ray. The chromosomes are now ready for the maturation divisions.

By the time that the chromosomes of the prophase in A. canis have become differentiated into definite tetrads, the cells have lost all connection with the rhachis and lie free in the oviduct, not very closely packed, and have a slightly rounded contour. While the cells still show, in longitudinal section, approximately pear-shaped outlines, the nucleus no longer occupies the larger end of the cell, but lies near the middle of the longitudinal axis. The cells now gradually assume a more nearly spherical outline (figs. 20 and 21).

The thirty-six chromosomes (fig. 20) begin to show a transverse constriction, becoming dumb-bell shaped. The plasmosome, when present, is at a little distance from the nuc'ear membrane. The cytoplasm clearly shows a granular reticulum, interspersed with numerous medium-sized vacuoles.

Soon after the appearance of the transverse constriction (*Querkerbe* of Haecker), a longitudinal split or a constriction (fig. 22), at first very faint, appears in each chromosome. Differences in the sizes of the chromosomes also appear (fig. 22);

twelve being smaller than the remaining twenty-four. Study of the corresponding stages of the primary spermatocytic chromosomes showed twenty-four large and six smaller tetrad-like bodies. As the six small chromosomes were there (Walton, '16 a) shown to be the heterochromosome group of the 'X' type, and as the female is of the '2X' type, it does not seem illogical to conclude that these twelve small primary oöcytic, tetrad-like chromosomes are the members of the heterochromosome group.

The next step in the formation of the mature chromosomes is one of pseudo-reduction, by which the number of chromosomes is reduced to one-half the original number, that is to eighteen. This is the haploid number, and appears before the maturation divisions; for this reason the term pseudo-reduction, in the sense of Gregóire ('10), is applied here to this process. The true reduction comes later, at the time of the maturation divisions, so that the reduction of the prophase chromosomes, that is the pseudo-reduction, is a reduction in number only.

The tetrad chromosomes begin to pair, possibly according to their sizes, although no differences in size, except for the general difference between the autosomes and the heterochromosomes mentioned above, can be seen, and therefore no members of definite pairs can be recognized before their union. The two members of a pair (fig. 23) come to lie with their broad faces in contact, that is with the planes of the two tetrads parallel to each other. An achromatic bridge of connecting material is built up between them. The union is thus one by parasyndesis, and places A. canis in the class with A. megalocephala.

Sabaschnikoff ('97) was the first to trace, in A. megalocephala, the formation of the tetrad as the result of the conjugation of two longitudinally split chromatin threads, though this had previously been predicted by Boveri ('87) and O. Hertwig ('90). Tretjakoff ('04 b) definitely proved their formation in A. megalocephala, and contended that parasyndesis was the method of their formation. In the later works of Boveri ('99, '09 a, '09 b) and in those of Brauer ('03), Bonnevie ('08, '12), Vedjovský ('07, '12) and others, this view has been adopted. Griggs ('06) also supports this explanation of the formation of the tetrads,

1

2

c

3   a b

4

5   b a

6   a b

7   b a c

8   a b

9   b a

10   b a

11
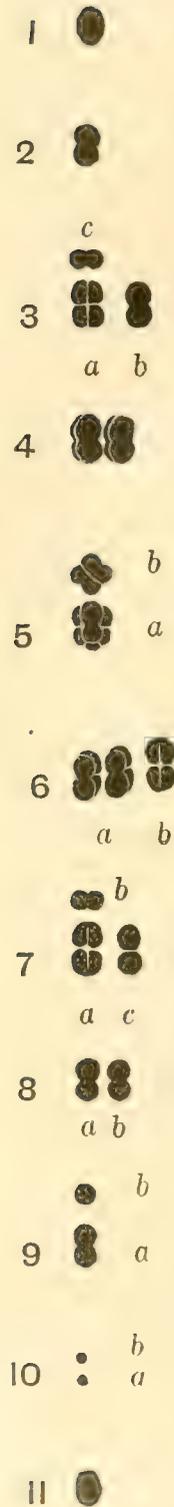
Fig. A Chromosome cycle in Ascaris canis. *1*, monad chromosome of last oogonial stage; *2*, dyad chromosome of early prophase, first oocyte, showing Querkerbe; *3*, tetrad chromosome of later prophase, showing Querkerbe and first longitudinal constriction; *a*, face view; *b*, side view; *c*, end view; *4*, two tetrads, late prophase first oocyte, undergoing parasyndesis; *5*, di-tetrad formed by the parasyndesis of two tetrads; *a*, side view; *b*, end view; *6*, first maturation division, along plane of first longitudinal constriction; *a*, two daughter tetrads separating; *b*, daughter tetrad, face view; *7*, daughter tetrad, second oocyte; *a*, face view; *b*, end view; *c*, side view; *8*, second maturation division, along plane of parasyndesis; *a*, *b*, two daughter dyads; *9*, dyad chromosome, found in ootid, and in 'stem' cells of embryo; *a*, side view; *b*, end view; *10*, monad chromosome, found in soma cells of embryo after 'diminution'; *11*, monad chromosome, found in propagation cells of embryo (Querkerbe suppressed).

but he believes that they are produced by the folding on itself of a single longitudinally split chromosome. Hertwig ('90) alone favored the telosyndesis view.

In A. canis, by a like process of parasyndesis, as I have stated, one eight-parted chromosome is formed out of two tetrad chromosomes. Marcus ('06), working on A. canis (?), found similar eight-parted chromosomes, but he believed them to be formed by the telosyndesis of two chromosomes, each of which later showed two longitudinal splits, in planes at right angles to each other. These chromosomes Marcus designates as bivalent tetrads or octads. Gregóire ('10) has pointed out that, as the chromosomes are the result of a pseudo-reduction process, the term 'octad' is a misnomer, for the resultant eight-parted chromosomes are in reality not two conjugated (that is involving synmixis) chromosomes, but only two loosely joined, semi-independent tetrads, and he suggests the name 'di-tetrad' as a better appelation. In accordance with this conception, the term di-tetrad, instead of the term octad, is used throughout this paper to denote the eight-parted chromosomes of the prophase and metaphase of the first maturation division.

The same type of chromosome is formed in the spermatocytes (Walton, '16 a), but there, the process of formation could not be followed clearly enough to show that the method of union is by parasyndesis; yet the similarity of the process in the spermatocytes to that in the primary oocytes is so close as to force one to conclude that the method of formation is the same in both sexes. After the union the di-tetrad chromosomes look like tetrads in either a side or a polar view (fig. 24), but by careful observation the plane of union between the two tetrads can be seen to be unlike that of the cross and longitudinal constrictions of the original tetrads (text-fig. A, 5 b).

The presence of eight-parted chromosomes has been reported for several Nematodes, and by Jörgensen ('10) in sponges (Syconen). Bonnevie ('08) describes a faint indication that the chromosomes of A. megalocephala before the first maturation division (Muttertetraden) are really octad in nature, and are reduced to tetrads by this division. She finds the same to be

true in the case of Allium and also of Amphiuma. Mulsow ('12) describes ten tetrads in the prophase of the spermatocytes of Ancyracanthus cystidicola, which unite to form five bivalent tetrads. The idiosome, however, remains as a mono-tetrad. This condition is similar to the one found by the writer (Walton, '16 a) in the spermatocytes of A. canis. In the oocytes of A. cystidicola the same process occurs, but the idiosomes (2X type) do not pair to form a bivalent tetrad; each member remains as a mono-tetrad.

In most of these tetrads and octads (bivalent tetrads) there appears a transverse constriction, which may or may not coincide with the plane of one of the maturation divisions. Haecker ('95) and his pupils maintain that this transverse constriction, or 'Querkerbe' (Haecker, '95, p. 586), is the forerunner of a maturation division, and Haecker ('11) states that its appearance is a proof of metasyndesis (telosyndesis). Agar ('12) and Kornhauser ('15) have both shown that the Querkerbe found in the chromosomes of copepods, nematodes, various molluscs, and even certain vertebrates, cannot be taken as a proof for either para- or telosyndesis. Neither is the Querkerbe a definite forerunner of a future maturation division. Its real origin and nature are so obscure that as yet no satisfactory conclusion can be reached as to its significance. In A. canis, as has been stated already, the Querkerbe is not an indication of telosyndesis, nor does it indicate, as will be shown later, the plane of a maturation division.

In A. canis, soon after the formation of the di-tetrads (fig. 24), the plasmosome breaks up and finally disappears, if it has not already done so. This homogeneously staining mass of achromatic material breaks up into many minute, deeply staining granules, which are at first imbedded in a faintly tinged matrix, but soon become scattered throughout the nucleus. Some of these granules eventually penetrate the nuclear membrane and emerge into the cytoplasm of the egg before losing their identity. A few of them break down *in situ* in the nucleus, but sometimes the plasmosome gradually diminishes in size without breaking up into granules (fig. 25). As has been shown by its staining

reactions and by its behavior, the plasmosome is entirely achromatic in nature, and is not a storehouse for either chromatin in general (Marcus, '06), or the chromatin of the heterochromosomes (Medes, '05; Boring, '07; Dederer, '07; Randolph, '08; Stephens, '08, '10; Payne, '09; and Goldsmith, '16).

Following the formation of the di-tetrads (figs. 23 and 24), the autosomes lose their peripheral position and mix with the already centrally located members of the heterochromosome group, from which they cannot now be distinguished.

After the most careful study of this and closely related stages, the writer has failed to see any sign of a centrosome system. Such a structure is first noted when there are two centrosomal bodies which have taken up their positions at the opposite poles of the nucleus. A cell of this stage is shown in figure 26 with the fundaments of the maturation spindle in process of forming, and the nuclear walls already breaking down at its poles. Whether this apparent absence of the early stages of the centrosome is due to an inherent inability to take stains, or to gaps in my material, has not as yet been determined. Coincident with the setting up of the maturation spindle, the chromosomes gather into the equatorial region of the nucleus (fig. 26) to form the metaphase plate (fig. 27) in preparation for the first maturation division.

During these nuclear changes the cytoplasm has become much vacuolated. The majority of the larger vacuoles are arranged near the periphery of the cell. The alveolar nature of the cytoplasm can be clearly seen, but the alveoli apparently do not tend to form rows radiating from each centrosome,—by which the astral figures are emphasized,—as they do in the corresponding stage of the spermatocytes (Walton, '16 a). The cell wall is still exceedingly thin, appearing in sections as a very faint line.

b. Metaphase. A very noticeable change of achromatic, as well as of chromatic, material is noted in the early metaphase. The cell wall thickens and appears to be of a structureless, jelly-like nature (fig. 27). The cytoplasm immediately surrounding the centrosomes has lost its alveolar structure, leaving them imbedded in a small sphere of undifferentiated material. There

is, however, no evidence of astral rays. The spindle seems to be made up of many strands, or bundles of fibers, each bundle being associated with only one chromosome (fig. 27). Besides these mantle fibers, there are others (fig. 28), which extend from centrosome to centrosome without having any connection with the chromosomes.

The chromosomes are re-oriented on entering the metaphase plate. The spindle fibers are attached to the sides of the chromosomes and cause them to rotate so that in their new positions they lie with their long axes in the equatorial plane (fig. 28). Hence the plane of the division must lie along one of the longitudinal splits of the di-tetrad, and the division must be longitudinal. When seen in side view (fig. 27), the chromosomes show a long axis parallel to the spindle fibers, but it is only of a secondary nature. Figure 29 shows a much enlarged view of the spindle at this stage. The bands of fibers attached to each chromosome are clearly distinguishable. The centrosomes show as very faintly staining granules at each pole of the spindle, but without traces of an astral system. The chromosomes (fig. 29) all clearly show a decided polar elongation preparatory to division, and in one, the halves are already pulled slightly apart.

The nuclear elements remain in this condition until the entire figure has migrated bodily from the center to the periphery of the cell. Here it remains in an undivided condition until after the entrance of the sperm and the formation of the fertilization membrane. This membrane is formed as a secretion of material by the cytoplasm, incited by the chemical influence of the spermatozoon, and is deposited just inside of the original cell wall, which remains distinctly separated from the apparently homogeneous fertilization membrane.

The nuclear mass arrives at the periphery of the cell with the long axis of the spindle parallel to a tangent to the nearest part of the cell wall. Here it undergoes a rotation of ninety degrees and comes to rest with one pole close against the cell membrane (figs. 31 to 33 and 35). The chromosomes may or may not have begun to pull in two by the time this final position of the spindle is reached, but the action goes no further unless the egg has been

fertilized.   No cases were found in which the first maturation division occurred before insemination, and in all eggs which failed to become fertilized the nucleus broke down without any further development.

Kultschitzky ('88 c) and Lukjanow ('89) find that in A. marginata the sperm always enters the egg before the first polar cell is given off and often before the egg nucleus has migrated to the periphery of the cell.   The same is true for A. lumbricoides, and also for A. megalocephala, although here the first polar cell may have been ready to be cut off before the sperm has entered (Van Beneden, '83; Carnoy, '87; Boveri, '88; Hertwig, '90; Zacharias, '12 d; *et al.*).   Zacharias ('12 d, p. 357) definitely states:—"Bei unbefruchtet gebliebenen Ascariseiren kommt es überhaupt zu keiner Ausstossung von Richtungskörpern. . . . ."

The grouping of the spindle fibers for each chromosome now disappears, so that no differentiation between mantle fibers and central ones can be noted (figs. 35 and 37).

From the time of insemination until well into the segmentation stages, the alveolar nature of the cytoplasm is very apparent, the walls of each alveolus being heavily granulated.   Figures 36 and 37 show under higher magnification face and edgewise views, respectively, of the metaphase plate just as division begins.   The face view shows the Querkerbe, no longer a constriction in the short axis, as a very faint line, best seen at the periphery of the chromosomes.   The deep cross constriction marks the line of the original 'side by side' union of the prophase tetrads.   Figure 38 is a side view of the spindle in the late metaphase, showing the chromosomes pulling apart, but still connected by linin bridges.

The conditions of the formation and division of di-tetrads hitherto described in other animals are as follows: Marcus ('06, fig. 40) has shown that in the metaphase plate of his A. canis (?) the octad chromosomes arrange themselves with their long axes at right angles to the spindle fibers, just as in my own observations on the true A. canis.   These fibers are arranged in bundles, one of which is attached to each side of every chromosome.   At

division the octads are separated into two daughter tetrads by a splitting along one of the previous longitudinal constrictions. In this case the Querkerbe corresponds with the plane of telosyndesis. In a Sycon sponge, likewise, a similar longitudinal division of the double tetrads is found (Jörgensen, '10). In A. megalocephala the first division is generally regarded as being longitudinal, and perhaps as being foreshadowed by the longitudinal split noted by Bonnevie ('08). Kühtz ('13) reports a longitudinal division of the chromosomes at the formation of the first polar cell in several species of the Sclerostomum of the horse.

c. *Anaphase.* The daughter chromosomes (fig. 38), as they begin to separate, have their long axes in a plane parallel to the equatorial plate. As division progresses (fig. 39) each chromosome changes its orientation by rotation around an axis which is perpendicular to the axis of the spindle, until its long axis becomes parallel to the spindle fibers. This fact seems to argue against the active influence of the spindle fibers in the division, as they were originally attached to the sides, not the ends, of the chromosomes. The fibers are somewhat more accurately to be regarded as being mere passive formations of the alveolar walls of the karyoplasm acting in response to forces such as might operate between two electrical centers, that is, the centrosomes. That the eighteen daughter chromosomes of each plate are tetrads, is shown by a comparison of edgewise (fig. 40) and face (fig. 41) views of nuclear plates of the same stage, in which they appear respectively as tetrads and as dyads. As a result of this division into daughter chromosomes the Querkerbe, without any shifting of its position in relation to the chromatic matter, now lies in the plane of the short axis rather that in that of the long axis of the tetrad; but by the ninety degree rotation of the tetrad its plane is now perpendicular to the axis of the spindle, whereas in the beginning it was parallel to it. That constriction in each of the tetrads which is parallel to the axis of the spindle is very plainly marked (fig. 40) and represents the original plane of parasyndesis. Inasmuch as this first division is longitudinal, and each daughter tetrad is, as far as can be determined, exactly

like its mate, the division is not a reductional one in the Weismannian sense, but must be equational. Owing to the pseudo-reduction by conjugation in the prophase, the longitudinal division along a plane which does not separate the conjugated halves—and such is the case in A. canis—can only cause a reduction in the mass, not in the qualities of the chromosomes.

d. Telophase. As the chromosomes come near the poles of the spindle (fig. 42), the outer end of the latter, lying in contact with the fertilization membrane, is cut off, partly by constriction and partly by the formation of a dividing membrane (figs. 43 and 44). The chromosomes remaining within the egg substance do not ordinarily clump together and lose their individuality, as do those which enter the polar cell (fig. 44). The group of eighteen daughter tetrads in the egg organizes a new nucleus with only a very transient nuclear membrane. Rarely the remnants of the old interzonal fibers can be seen (fig. 44). The centrosome divides, its halves move apart, and, as the nuclear membrane fades away, take up their position at the poles of the new spindle (figs. 45 and 46), which at first lies with its long axis parallel to a tangent to the surface of the egg at that point. This procedure differs from that followed in the spermatocytes of this species, and usually also in both types of A. megalocephala, in that the intermediate stage here possesses a nuclear membrane. This newly formed spindle rotates through an angle of ninety degrees and assumes a position perpendicular to the tangent to the surface of the egg at that point (fig. 47) and is ready for the second maturation division.

During the formation of the first polar cell, the numerous large, peripheral vacuoles in the cytoplasm fuse and cause it to shrink away from the fertilization membrane. When this shrinkage occurs, the first polar cell is left attached to the inner surface of the fertilization membrane. This shrinkage also may allow a slight rotation of the cytoplasm within the membrane, so that the second polar cell may be given off apparently at another position on the surface of the egg. This rotation may reach a magnitude of twenty or more degrees (figs. 47 and 48), and rarely the second polar cell seems to lie at the pole of the egg opposite that at which the first was formed.

2. Second maturation division

The telophase of the first division merges imperceptibly into the prophase rearrangement of the chromosomes of the second maturation spindle, there being no pause to mark the separation between the two stages. The new spindle (fig. 46) is at once set up, and the eighteen tetrad chromosomes arrange themselves in the metaphase plate. The spindle then undergoes a rotation of ninety degrees and comes to rest with its long axis perpendicular to a tangent to the surface of the egg at that point (fig. 47). In the formation of the second maturation spindle no centrosomes can be made out—only the centrioles, and these but faintly.

As was the case in the first division of the oocyte, and also in the case of the second spermatocyte (Walton, '16 a , the division is longitudinal (fig. 48). The centrioles are faintly visible and the spindle fibers are uniformly distributed (fig. 49). The second division is clearly shown to be longitudinal. This division is along the plane of the original parasyndesis of the first oöcyte prophase chromosomes, and hence separates parts that are not alike—unless an improbable synmixis has taken place. Such a separation would result in a distribution of unlike parts to the daughter chromosomes, and so would be a reduction in the Weismannian sense, a reduction such as is denied for Nematodes, especially for Ascaris megalocephala, by most writers, Griggs ('08) and a few others being the only exceptions. Griggs favors a pre-reduction after metasyndesis. Tretjakoff ('04 b) finds post-reduction after parasyndesis in the male of A. megalocephala, and both Fick ('08) and Meves ('08) believe a true reduction occurs in the prophase. Vejdovský ('07) and others contend that there is no true reduction in A. megalocephala. A Weismannian reduction by means of post-reduction (transverse division) after telosyndesis is asserted by Marcus ('06) to occur in A. canis (?). Study of material from what is believed to be the same species of dog ascaris that Marcus used shows that, though Marcus was right in the idea of the nature of the reduction, he was probably mistaken in his idea of telosyndesis.

In Sycon sponges, according to Jörgensen ('10, p. 204), there is a pseudo-reduction followed by a pre-reduction, but not in the Weismannian sense. The true reduction occurs in the oogonia, a conclusion which he states in the following words:—

In einigen Fällen aber schien es mir so, als ob die etwas längliche Muttertetrade—die wir uns aus zwei länglichen parallel konjugierten Chromosomen, deren jedes aus zwei Chromomeren zusammengesetzt ist, entstanden denken können—mit ihrer Längsachse in die ѕpindelachse eingestellt würde. Nach der Verdoppelung der Tetrade zum viersäuligen Prisma erfolgt die Teilung gemäss dem Querspalte. Diese Teilung würde hintereinandergelegene Chromomere eines Chromosome trennen, würde also eine Reduktionsteilung im sinne Weismanns. Bei beiden Oogoniengenerationen kann—nach meinen Beobachtungen—diese Reduktionsteilung auftreten.

Each of the two daughter plates of the second maturation division in A. canis (figs. 50 a and 50 b) contains eighteen dyad-like chromosomes, each chromosome being constricted transversely by the Querkerbe (figs. 50 and 51). The daughter plates move apart (fig. 51) and the peripheral one with its enveloping cytoplasm comes to lie in the space between the egg and its fertilization membrane (fig. 52). The interzonal fibers then break down and the detached body secretes a membrane, thus completing the formation of the second polar cell. Meanwhile the group of chromosome retained within the egg cytoplasm is converted into the female pronucleus (fig. 53). I all the material examined the chromosomes of both the polar cell and the female pronucleus are apparently in the clumped condition during the late telophase. This apparent clumping of the chromosomes of the female pronucleus is not an actual one, but is due to the very close crowding of the chromosomes, which it becomes very difficult to resolve after staining.

In all cases the second polar cell remains on the surface of the cytoplasmic mass, while the first one is attached to the inner surface of the fertilization membrane.

The further development of the female pronucleus will be traced in connection with that of the male pronucleus in the following section.

C. THE FERTILIZATION PROCESS

The fertilization of the nematode egg, especially that of A. megalocephala, has been studied carefully by many observers, the chief among whom are Schneider ('83), Van Beneden ('83, '87, '88), Nussbaum ('84), Boveri ('87 b,'87 d,'88 a, '90), Zacharias ('87 a, '87 b, '87 c, '87 d, '12), Kultschitzky ('88 a, '88 b), Hertwig ('90), Sala ('94), Meyer ('95), Carnoy et Lebrun ('97), Erlanger ('97), Retzius ('12 b), Held ('12), Romeis ('12), and Vejdovský ('12). Fertilization in the dog ascarid A. mystax was early studied by Nelson ('52), Bischoff ('55), Meissner ('55), Thompson ('56), Claperéde ('58), and Munk ('58). Later Carnoy ('87), Kultschitzky ('88 c), and Lukjanow ('89) studied the same process in A. marginata, and Marcus ('06) in "A. canis (sp. mystax)," which he believed to be identical with the A. marginata of the earlier writers. Glaue ('08) has maintained that the names A. canis, A. marginata, etc., are all synonomous with the older term A. mystax Zeder; however, it seems extremely doubtful whether A. canis and A. marginata are identical species.

The work of the early observers on the ascarids of the dog was, of course, confined to the more superficial events of fertilization. Nelson ('52), Bischoff ('55), and Thompson ('56) maintained that the spermatozoon merely came in contact with, but did not enter, the egg, and by this contact initiated segmentation. Meissner ('55) believed he saw the sperm enter the egg through a micropyle. He also was the first to suggest that the refractive body of the sperm was of a nutritive character. This has recently been shown (Marcus, '06; Wildman, '12; and Walton, '16 c) to be the correct interpretation. The existence of a definite micropyle for the entrance of the sperm was denied by Claperède ('58), but he supported the idea that the sperm entered the egg, claiming that the sperm was passively forced into the egg substance by a contraction of the oviduct wall. Munk ('58) defended the existence of a micropyle and also maintained that the sperm entered the egg through its own activities. Carnoy ('87), Kultschitzky ('88 c), and Lukjanow

('89) all deny the presence of a micropyle for the entrance of the sperm into the egg in these ascarids. They all state that the male and female pronuclei unite to form a fusion nucleus before segmentation can take place. According to these authors the male pronucleus is formed by a rearrangement of the elements of the sperm nucleus, and the female pronucleus is made up of what remains of the egg nucleus after the two polar bodies are given off.

Marcus ('06) confirms for A. canis (?) the opinion of some of the earlier writers that the sperm is amoeboid, and may enter the egg at any point of its surface. Once within the egg, the refractive body, or what is left of it, breaks down and becomes lost in the egg cytoplasm. The compact nucleus of the sperm in forming the male pronucleus breaks up into the characteristic haploid number (in A. canis (?), eleven) of dyad chromosomes. The centrosome brought in by the sperm sets up an astral system in the cytoplasm of the egg, and functions at the first segmentation division. According to Marcus the two pronuclei, each with eleven dyad chromosomes, fuse before the first cleavage of the egg. At division each of the dyads so divides that each daughter nucleus acquires twenty-two dyads (eleven from each pronucleus), which unite in pairs, making eleven tetrads. This union of the dyads to form tetrads completes, in the opinion of Marcus, the act of fertilization. The second cleavage shows the process of 'diminution' in the soma cell, but not in the 'stem' cell. The stem cells continue to show eleven tetrad chromosomes, whereas the soma cells show twenty-two dyads.

In the following account a careful study has been made of the formation of the male pronucleus from the spermatozoon, and of the female pronucleus from the inner half of the second maturation figure.

1. The formation of the male pronucleus

The sperm in this species (A. canis) is mature at the time of copulation. and nourishes itself up to the time of its entrance into the egg by feeding on the nutriment accumulated in the refractive body (Walton, '16 a). Depending upon the interval

between coitus and insemination, more or less, therefore, of the refractive body may be present at the time of union of the egg and sperm. The amoeboid spermatozoon, ascending the oviduct, meets the descending oocyte and enters the egg, blunt end foremost, at the first point of contact—as is to be inferred from the fact that there is no apparent relation of the point of entrance and the position of the egg spindle. The amoeboid method of penetration and the relation to the egg membrane are well shown in figure 30, where the spermatozoon is found partly within the egg. It is very narrow at the point of entrance, and very much enlarged just within the membrane. The dark bodies in the cytoplasm of the sperm are mitochondrial granules. The centrosome (fig. 31) occupies a depression in the side of the sperm nucleus. In the case here figured, a small remnant of the refractive body is still visible.

The entrance of the sperm affords the necessary stimulus for the completion of the process of maturation. The spermatozoon, after entering, migrates to the center of the egg before beginning its metamorphosis. Its cell wall then disappears (fig. 32) and its cytoplasm gradually merges with that of the egg (figs. 33 to 35).

As shown in a former article (Walton, '16 c), the mitochondrial bodies of the sperm become distributed through the cytoplasm of the egg and, apparently without further participation in the process of fertilization, are lost to view.

The centrosome (fig. 32) divides, leaving a 'centrodesmus' between its halves (figs. 33 and 39), as has been shown by Boveri and others in the case of A. megalocephala. Figure 34 shows the two centrosomes so far separated from each other that their central connection is lost. No astral figure is apparent. The separation of the halves of the divided centrosomes in this case was slightly precocious, for the centrosomes ordinarily remain closely connected until after the sperm nucleus has undergone greater development than is indicated in this case.

Figure 47 shows the nucleus lying free in the cytoplasm of the egg, with the centrosomes slightly at one side, surrounded by a remnant of the sperm cytoplasm. The chromatic mass of the sperm nucleus, hitherto apparently homogeneous, now begins to

be resolved into its component chromosomes (figs. 47, 48, and 52), which lie imbedded in a matrix of non-chromatic material. This matrix gradually fades as the nuclear membrane is formed, leaving the chromosomes (fig. 53) lying free within the karyoplasm. Each chromosome is dyad-like in form, not differing in appearance from the ones which united to form the compact nuclear mass of chromatin in the late spermatids.

2. The development of the male and female pronuclei

At this stage (fig. 53) the two pronuclei are of exactly the same appearance, each having eighteen dyad-like chromosomes. Though alike, the two pronuclei can be distinguished by their positions in relation to the polar bodies and the centrosomes. The female pronucleus lies near the place where the second polar cell was formed, while the male pronucleus generally is close to the centrosomes, which, as we have seen, are of male origin. These aids in distinguishing between the male and female pronuclei are necessary only when the sperm nucleus contains eighteen chromosomes, namely the heterochromosome complex of six idiosomes in addition to the twelve autosomes. When fertilization is accomplished by a sperm lacking the six idiosomes, the male pronucleus, containing only the twelve autosomes (fig. 54), is easily distinguishable from the female pronucleus with its eighteen chromosomes.

The development of both types of male pronuclei is so similar that the details will be followed in only the one containing the heterochromosome group.

The male pronucleus is slightly more precocious in its development than the female (fig. 55). The chromosomes of the former break up into small granules, which are grouped into a single mass near the nuclear membrane; from this mass radiate many fine linin fibers. There appear from two to twelve nonchromatic nucleoli, or plasmosomes, which apparently are entirely unconnected with the linin meshwork. The granular mass of chromatic material (fig. 55) soon becomes less granular (fig. 56), and looks like a tangle of short threads.

Meanwhile the female pronucleus (fig. 56) has begun to form a similar linin network and soon (fig. 57) establishes a localized center for the chromatic granules which result from the breaking down of the chromosomes.

In the male pronucleus the linin network assumes a wide-meshed condition, and then (fig. 57) each strand becomes much thicker. The chromatin at the same time migrates outward from the central mass along each strand, and becomes so widely distributed that it loses the blue-black appearance characteristic of massed chromatin when stained with iron-haematoxylin. The development of the male pronucleus does not proceed beyond this condition (fig. 58) until the female pronucleus has reached the same stage. At this period it is not possible to distinguish one type of male pronucleus from the other, nor either from the female pronucleus.

Up to this time the pronuclei have been widely separated, but now they come to lie side by side. The centrosome, meanwhile, has divided and become surrounded by a mantle of slightly denser alveolar cytoplasm. This method of formation of the astrosphere has been shown for A. megalocephala by Erlanger ('99), Retzius ('12), Vejdovský ('12), and others; it does not support the 'archoplasm' theory of Boveri. The closely joined centrosome 'couplet' occupies a position midway between the two pronuclei, and when the latter approach each other its two components move asunder (figs. 55 to 59) until they are as far apart as the diameter of the pronucleus and about equally distant from a line joining the center of the two nuclear bodies.

During the movement of the two pronuclei toward each other the chromatin of each collects into numerous large, irregular clumps at the junctions of the linin meshwork (fig. 59), and the plasmosomes fuse into a few, larger, peripherally located bodies. As the two pronuclei come to lie side by side (fig. 60), the linin network begins to disappear, leaving the karyosomes lying free in the karyoplasm. The karyosomes now have the number characteristic of the chromosomes, that is there are either twelve or eighteen for the male, and eighteen for the fe-

male pronucleus. These chromatic bodies develop directly into the ellipsoidal chromosomes (fig. 61), which later (figs. 62 and 63) assume the characteristic dyad-like form.

3. Fertilization

The pronuclei, as is the case in A. megalocephala (Vejdovsky '12; Zacharias, '12; et al.), do not ordinarily fuse before the first segmentation division, although occasionally they may do so (fig. 63). Ordinarily the walls of the two pronuclei break down first on their adjacent faces (fig. 61), and afterwards on the sides directed toward the poles of the spindle (fig. 62), the chromosomes meanwhile grouping themselves into the equatorial plate and the spindle fibers making their appearance (fig. 64); but in the cases where fusion takes place, only the adjacent portions of the nuclear membranes break down, the two nuclei thus uniting into one 'fusion' nucleus, which contains either thirty-six (fig. 63), or thirty dyad-like chromosomes, depending upon the character of the sperm which entered the egg.

The process described above is essentially the same as that observed in A. megalocephala by Zacharias ('12) and the majority of other workers. According to Zacharias, however, the formation of either a 'fusion nucleus' or a 'fusion spindle,' by the two pronuclei does not constitute fertilization. If one defines the term fertilization as meaning the intermingling of the parental constituents of the nucleus by a process of actual fusion of the chromosomes, then the conditions observed in A. canis do not warrant the statement that fertilization has taken place before the first segmentation division occurs. If this is the correct definition of fertilization,—as Zacharias ('12) and others maintain,—then in A. canis, as well as in A. megalocephala, true fertilization occurs only in the 'resting nuclei' of the daughter cells of the first cleavage. It is at this time that the individuality of the chromosome is first lost, and it is during the stages of the subsequent reorganization of each daughter nucleus that the opportunity is afforded for a complete interchange of such hereditary characters as are borne by the chromosomes. In case qualities are carried by the non-chromatic portions of the

nuclei, their intermingling is possible at any time after the flattened membranes between the two pronuclei have broken down, and in case the cytoplasm of the sex cells is a bearer of any hereditary characteristics, these could intermingle at any time after the sperm enters the egg and loses its separate identity.

4. Early cleavage

The first cleavage spindle (fig. 64) arises immediately after the juxtaposition, or the fusion, of the two pronuclei. When a 'fusion nucleus' (fig. 63) is produced, the process is similar to the formation of any mitotic figure; the chromosomes arrange themselves in the equatorial plate (fig. 64) and proceed to divide in the usual manner. The more common process, in which the two pronuclei do not fuse until the spindle is formed, is somewhat different. The spindle fibers arise upon the disappearance of the portions of the nuclear membranes which are contiguous with each other and those which face the centrosomes. The chromosomes arrange themselves into a single equatorial plate (similar to the one shown in figure 64), but owing to the morphological similarity of the chromosomes, it is not possible to locate the male and female groups in definite portions of the spindle.

The close association of the chromosomes in fixed material often gives the appearance of division *en masse* (fig. 65), each daughter group receiving half of the chromatic material. Direct evidence as to the deportment of the individual chromosomes during this division is lacking, but some indirect evidence seems to suggest that the plane of separation coincides with the long axis of the chromosome, for in some cases the daughter cells exhibit chromosomes smaller than those of the mother nucleus, but apparently of the same number and form. This could be the case only when the division was longitudinal. A transverse constriction, still evident in the daughter chromosomes, is probably the Querkerbe. Other daughter cells, both those resulting from the first division, and those from later divisions of the soma cells, show a curious behavior that may afford an explana-

tion of the Querkerbe. These cells (fig. 66) exhibit a great number of chromosomes,—between sixty-five and seventy-five,—perhaps more correctly called karyosomes, which are almost spherical, and show no constriction in any plane. It seems probable that these chromatic bodies result from the splitting or fragmentation of the chromosomes along the plane of the Querkerbe. Thus the Querkerbe may indicate the plane of division of the complex chromosomes of the sexual cells into their ultimate components, such division into simple chromosomes occurring only in cells about to undergo the process of 'diminution.' The variation in the number of these ultimate chromosomes is probably due to the sex of the embryo, depending upon the number of male chromosomes—twelve or eighteen—added to the eighteen of the egg.

In A. canis, as was shown in A. megalocephala by Boveri ('88), the stem cell (figs. 68, 69, and 71) is ordinarily the smaller of the two daughter cells of the first cleavage division. When the two cells are in the resting condition (fig. 68), this criterion is the only one available for distinguishing the soma (so.) from the stem cell. After the nuclear rearrangements, the chromatin of the two-cell stage is gathered into discrete chromosomes (fig. 68) of the same number and form as those which enter the daughter plates, that is there are either thirty or thirty-six dyad chromosomes in each nucleus.

As the second cleavage commences, a further difference between the two cells is manifested. In practically all cases, the soma cell (figs. 69 to 72, so.) is the one which begins to divide first. The axis of its spindle figure is parallel to the first cleavage plane; but the spindle of the stem cell, formed later, is perpendicular to that plane (fig. 73).

It is a feature of all the cleavage cells, first manifested in the two-cell stage, that the centrosome retains its identity from cell generation to cell generation. Examination of the cells at any time between mitoses shows the centrosome persisting as a discrete, clearly distinguishable granule (figs. 72, 74 to 76) surrounded by a small envelope of cytoplasm, which is slightly more refractive than the rest of the cytoplasm.

Boveri ('88, '92, '99), Hallez ('85), Meyer ('95), zur Strassen ('96, '98), Ziegler ('95), and Zoja ('96) all have shown that in A. megalocephala the first soma cell divides before the stem cell and, with the exception of Zoja ('96), all state that the phenomenon of 'diminution,' or 'chromatin reduction,' takes place in the first division of the soma cell. With the exception of Boveri and zur Strassen, these authors all state that only four soma cells are produced in succession by the stem cell and that each of the four undergoes a 'diminution' process at its first division. Boveri, on the contrary, believes that there are five such cells exhibiting 'diminution' divisions, and that it is the sixth, not the fifth, division which results in pure propagation cells. Zur Strassen holds that there are six such 'diminution' divisions in cells produced in succession by the stem cell. In A. canis the evidence is not complete enough to warrant a positive statement as to the exact number of 'diminution' divisions but embryos of about the forty-cell to fifty-cell stage show a 'diminution' spindle in the cell adjoining the stem cell, and that is an argument for the view that this had been separated from the stem cell at the previous (fifth) generation, and that it was dividing to form a sixth generation of cells.

Zoja ('96) states that the 'diminution' process in the case of the first soma cell is often delayed until its second division, namely, that of the daughter cells of the first soma cell. Thus two 'diminution' spindles are often seen at the same time as the embryo goes from the four-cell to the six-cell stage. As the division resulting in the formation of the second soma cell does not take place until somewhat later, there is no stage showing three 'diminution' divisions at one time. In A. canis this delay, which is an occasional feature in A. megalocephala, is as frequent as the earlier 'diminution.'

'Diminution' consists, as was first shown by Boveri, in the breaking up of the chromosomes and the extrusion into the cytoplasm of portions of the chromatin occupying the ends of each chromosome thread. The middle portion of each prophase chromosome breaks into a number of small granules, which become arranged in a metaphase plate (figs. 69 to 76), while the

rest of the chromosome, one-half to two-thirds of its total volume, becomes massed with corresponding parts of other chromosomes into a few larger clumps, which occupy the periphery of the equatorial plate. The small granules, or karyosomes,—sixty to seventy-two in A. canis,—are arranged in the middle of the plate. As division proceeds each of the centrally placed karyosomes divides into equal parts, and the two daughter plates, consisting of these halves, migrate toward the poles of the spindle (figs. 70, 72, 73, 75, 77), leaving the large clumps of useless (?) chromatin behind. As the new cell wall forms, these clumps of chromatin divide in a plane coinciding with that of the chromosomal division and go, half to each daughter cell, where they are seen lying in the cytoplasm close to the newly formed cell wall. In this position they undergo dissolution without further activities.

As already shown, in A. canis the larger (soma) cell divides first. In half of the cases the division is one of 'diminution,' various stages of which are illustrated semi-diagrammatically from actual cells in figures 69, 70 and 72. In the other fifty percent of the cases, the division is by ordinary mitosis (fig. 71), in which the same number of dyad chromosomes occurs as in the stem sells. The stem cell, dividing in a plane at right angles to that of the soma cell (fig. 73), gives rise to the vertical part of the 'T-shaped' embryo (fig. 74), which is peculiar to the nematodes. The division of the stem cell is here somewhat precocious (fig. 73), as the soma cell is ordinarily completely divided before the spindle of the stem cell is set up. The embryo pictured in figure 74 is one in which the 'diminution' process has been delayed, and hence all four nuclei are alike. The cell at the end of the upright of the 'T' is the stem cell of the second generation.

The stem cell (strp.) rotates upon the second soma cell in such a manner that it comes to lie in the angle between the second soma cell and one of the daughter cells of the first soma cell, thus forming a typical lozenge-shaped embryo (side view, fig. 75; face view, fig. 76). Figures 75 to 77 represent the condition in passing from the four- to the six-cell stage, the soma cells dividing before the stem cells. In all three cases figured, the

'diminution' division of the first soma cell had been delayed, and appears in its two daughter cells. In two of these embryos (figs. 75, 76) the stem cell (*strp.*) is shown with the fundaments of the spindle forming around the centrosomes, but the nuclear membrane is still intact.

The six-cell stage (figs. 78, 79) shows the four soma cells, descendents of the first soma cell, with resting nuclei, which contain a small amount of chromatin. The chromatin lost at 'diminution' can be seen lying free in the cytoplasm. In figure 78 the metaphase plate of the stem cell is seen edgewise, and figure 79 shows the appearance of the same plate when seen in face view. No evidences of 'diminution' are ever found in the stem cells. Figure 80 shows a six-cell embryo with normal division occurring in the stem cell (*strp.*) and a 'diminution' division in the second soma cell (*so.*). By the division of these two cells, the embryo passes to the eight-cell stage. Figure 81 shows a median section, which is perpendicular to the spindle of the dividing stem cell, in which can be seen the difference between the resting nuclei of the soma cells and the nucleus of the stem cell. The segmentation cavity has already made its appearance and continues to grow in size with the increase in the number of blastula cells. A typical morula stage is not found.

In embryos of two to four cells, four to eight cells, eight to twelve cells, and sixteen to twenty cells, it was easy to distinguish the soma cells produced by the first, second, third, and fourth divisions of the stem-cell series (since these were undergoing 'diminution') from their sister stem cells, which showed no signs of it. Each of the other soma cells, of course, possessed a nucleus containing the diminished amount of chromatin. One embryo containing forty to fifty cells showed the fifth soma cell undergoing 'diminution' while its sister stem cell showed normal division. In the few older embryos at hand, it was impossible to find evidences of the active process of 'chromatin diminution;' but the two stem cells could be distinguished from the soma cells by the condition of their nuclei, for the latter were rich in chromatin while those of the soma cells were not. No embryos were found which had advanced far enough to show

any evidences of gastrulation or even of the ingrowth of the propagation cells into the segmentation cavity.

These observations, while scanty, go to prove that Ascaris canis agrees with Ascaris megalocephala (Boveri, '99), in that there are five stem cells which give rise to a soma cell and a stem cell, the sixth stem cell giving rise only to pure propagation cells. Each of these five soma cells, or its immediate daughter cells, undergoes the process of 'chromatin diminution'. Therefore, after the division of the soma cell derived from the stem cell of the fifth generation, there is no further evidence of any 'diminution' processes of any kind, the soma cells multiplying by ordinary mitoses as the propagation cells also do. Yet the soma cells are distinguishable from the propagation cells by their smaller and more numerous chromosomes.

V. DISCUSSION

The writer (Walton, '16 a) has shown that in the spermatogonia of Ascaris canis Werner there are thirty tetrad chromosomes—twenty-four autosomes and six idiosomes—as the diploid number. By a process of pseudo-reduction in the prophase of the first spermatocytes, this number is reduced to eighteen—twelve di-tetrad autosomes and six tetrad heterochromosomes. Both spermatocytic divisions are longitudinal. By the first spermatocytic division, two types of second spermatocytes are formed, one type having twelve tetrad autosomes and six tetrad idiosomes, while the other has only the tetrad autosomes (twelve). These spermatocytes, by division, form spermatids, likewise of two types, having either eighteen or twelve dyad chromosomes. By direct metamorphosis the spermatids thus give rise to two types of sperms. Further work ('16 c) has shown that each sperm is provided with a refractive body of nutritive material, upon which it subsists until union with an egg is accomplished. The sperms also have cytoplasmic granules (mitochondria), which are not 'plasma bearers of heredity.' The spermatozoa mature rapidly at regular intervals, and copulation occurs immediately if a female is present; if not, the spermatozoa are ejaculated into the surrounding media.

The present paper has shown that in the ogoonia of A. canis there are thirty-six tetrads—twenty-four autosomes and twelve idiosomes—the diploid number. Through a pseudo-reduction by parasyndesis, occurring during the prophase of the first oöcyte, the number of chromosomes is reduced to eighteen, all di-tetrads in form. By means of the two maturation divisions, the mature state is reached. But maturation does not proceed beyond the formation of the first spindle, and its subsequent migration to the periphery of the egg, unless one of the amoeboid sperms has entered. As soon as a sperm has penetrated, a thick homogeneously staining, highly refractive cell-wall is secreted around the egg, just within the original cell-membrane. This wall prevents the entrance of further spermatozoa.

One of the female centrosomes is eliminated at the second maturation division, and all trace of the other is lost. The sperm carries with it a centrosome imbedded in its nucleus; and as the latter breaks up into its component chromosomes, the centrosome divides into halves, which separate and lie free within the cytoplasm of the egg, often in a position midway between the two pronuclei. It is the centrosome of male origin, therefore, which functions in the cleavage spindles.

The chromosomes of the male and female pronuclei, each enclosed within a nuclear membrane, become broken up into minute granules arranged on a linin network. These granules gather toward one pole of the nucleus in a manner somewhat resembling the 'bouquet' found in the corresponding stages of cell division in many other animals. From this mass the individual chromosomes—either twelve or eighteen in the male pronucleus, and eighteen in the female pronucleus—are formed, and take up peripheral positions within the nucleus.

The two pronuclei approach each other, the portion of the wall of each which is adjacent to the other breaks down and either their contents intermingle in a single 'fusion nucleus,'—the less common method,—or the polar as well as the adjacent walls of the pronuclei break down and the chromosomes, thus set free, immediately become arranged in the spindle set up between the halves of the divided male centrosome—the more common method.

The cleavage divisions follow in rapid order, exhibiting the characteristic nematode 'diminution' of chromatic material in the first division, or the following one, of each soma cell after it has been cut off from the stem cell.   The three-cell and four-cell embryos show the curious 'T' shape first seen in the embryos of A. megalocephala.   In the four-cell stage, a rotation of the stem cell occurs, giving a lozenge-shaped embryo.   In the propagation (stem) cells, the chromosomes retain their dyad character, being either thirty or thirty-six in number.   In the soma cells, after 'diminution', the chromatin has the form of monad chromosomes—sixty or seventy-two in number depending upon the sex of the embryo.   The propagation cell is one of the cells in the upright of the 'T' embryo.   Often the division of the first soma cell is unaccompanied by the process of 'diminution,' this phenomenon being delayed until the division of the two daughter cells of the first soma cell, the three-cell embryo thus showing two, instead of one, 'diminution' spindles at the same time. Since the second stem cell divides before the second soma cell, four-cell embryos are occasionally found which show the two 'diminution' spindles and also the normal spindle figure of the dividing stem cell.

The present paper on the oogenesis of A. canis, together with that on the spermatogenesis of the same species (Walton, '16 a), gives adequate grounds for stating that the dog ascaris studied by Marcus ('06) was not Ascaris canis Werner, although Marcus so regarded it, but was most probably (Walton, '16 b) Ascaris triquetra Schrank—also an occasional intestinal parasite in dogs; for A. triquetra differs both in gross anatomy and in cytological details from the conditions found by the writer in A. canis, but agrees cytologically with the conditions set forth by Marcus ('06) for A. canis.

The results of the present study have led to several questions of interest.   The chromosome cycle of A. canis is one of these. Study of the spermatogenesis had shown that A. canis Werner is one of the few Nematodes which posses a peculiar eight-parted chromosome at the beginning of the first maturation division. The two spermatocytic divisions result in a chromosome, which

is apparently dyad in nature, since it is constricted by the 'Querkerbe' of Haecker. The present work on the oogenesis has shown the method by which this type of chromosome is formed in A. canis, and has, perhaps, also thrown some light upon the nature of the 'Querkerbe.'

Beginning with the last generation of the oogonia (fig. A, *1*), we find the chromosomes emerging from the clumped condition of the chromatin at first in the form of ovoid bodies, which almost immediately show indications of a transverse constriction (fig. A, *2*), the 'Querkerbe' of Haecker ('95, p. 586). In the prophase of the first oocytes and spermatocytes, this dyad chromosome exhibits a longitudinal split (fig. A, *3 a*), thus becoming a tetrad in structure. A little later in the prophase of the first oocytic division, the tetrads unite into pairs—the di-tetrads—by a process of parasyndesis (fig. A, *4*). The results in the male are so similar to those in the female that there seems no reason to doubt that the method of di-tetrad formation there, also, was a case of parasyndesis.

Careful study shows that in the di-tetrad chromosomes the original plane of parasyndesis is clearly distinguishable from the 'Querkerbe.' It is the more apparent of the two constrictions (fig. A, *5 b*), and coincides with the long axis of the tetrad (fig. A, *6 b; 7 a*) resulting from the first oocytic division. At the first division (fig. A, *6 a*) the chromosomes separate along the plane of the longitudinal constriction previously noted (fig. A, *3 a*). This division is therefore equational amd simply reduces the quantity, not the qualities, of the chromatic material. In the male there were found twenty-four tetrad autosomes and six tetrad idiosomes before the pseudo-reduction—by parasyndesis—took place. After syndesis, there were twelve di-tetrad autosomes and six tetrad idiosomes; the chromosomes of the 'X' group (idiosomes) not uniting in pairs, as do the autosomes. In the female, however, the twelve idiosomes ('2X' group), as well as the twenty-four autosomes, unite in pairs, the result being eighteen di-tetrad chromosomes. In the first spermatocytic division the 'X' group goes undivided to one pole, so that for the autosomes the division is equational, whereas for the

idiosomes it is reductional, one daughter cell receiving none of the 'X' group, the other, all six of its members. In the female the first division is clearly equational for the majority of the chromosomes, and apparently so for the rest. Although the idiosomes can not be accurately distinguished from the autosomes, by any criteria as yet found, it is entirely possible that the cases where it is difficult to locate the plane of division may be those of the idiosomes. In these chromosomes it is often difficult to distinguish the plane of the original longitudinal split, in each of the two tetrads forming the pair, from the plane of parasyndesis, also longitudinal in relation to the axes of the resultant di-tetrad. From the evidence now available, it seems most probable that in all of the chromosomes the first maturation division is equational in character, though the possibility that the division of the idiosome pairs may be along the plane of parasyndesis— hence reductional, as in the case of the male—can not be disregarded. It is hoped that future study may clear up this seeming anomaly, in which the 'reducing' division in the male 'X' group does not occur at the same stage that it does in the female, whereas the 'reducing' division of the autosomes does occur at corresponding stages in both sexes.

As a result of the first division, therefore, the chromosomes of any one of the second oocytes are like those of all the others, the chromatin matter in each cell, however, being reduced to one-half of its former amount. Each resultant chromosome (fig. A, 7 a) is a tetrad, similar in appearance to those found in the early prophase of the primary oocytes (fig. A, 3 a). It shows a definite longitudinal constriction as well as a transverse one, the Querkerbe. The second division (fig. A, 8) is, like the first, along a plane parallel to the long axis of the tetrads, but perpendicular to the plane of the first one, and hence coincides with the original plane of parasyndesis. By thus separating into dyads along such a plane, true reduction of chromatic qualities in the Weismannian sense occurs. The resultant chromosomes contain one-fourth the original chromatic material and, theoretically at least, two sister dyads are qualitatively different while quantitatively alike.

The ootids and the spermatids thus possess the haploid number of dyad chromosomes (fig. A, *9 a*). After the formation of the first segmentation spindle, there is once more the diploid number of chromosomes, last seen in the oogonia and spermatogonia. The two first cleavage cells thus have either thirty or thirty-six dyad chromosomes—depending on the type of the fertilizing sperm, that is whether it had twelve or eighteen (twelve plus the 'X' group of six) chromosomes—each of which has the characteristic transverse constriction or Querkerbe (fig. A, *9 a*). In the cleavage cell which is the forerunner of the propagation cells, the chromosomes do not change, but retain their dyad form during subsequent divisions. During the first division of each of the soma cells of the first five generations after being separated from the 'stem' cell, a very different phenomenon appears. Each of these soma cells, in their first (or second) division after separation from the 'stem' cell, undergoes 'diminution' of chromatic material. From one-third to one-half of the chromatin material is cast out into the cytoplasm and disintegrates. The remaining chromatin, however, does not resume its original state in thirty or thirty-six smaller dyad chromosomes, but appears in the form of sixty or seventy-two monad chromosomes, practically spheroidal in shape (fig. A, *10 a; 10 b*). It seems most probable that these small spheroidal chromosomes have arisen by a process of division, or fragmentation, of the original chromosomes along the plane of the Querkerbe. Thus it may be that the Querkerbe, since it does not represent a former plane of telosyndesis, or parasyndesis, nor yet the plane of a future maturation division,—Agar ('12) and Kornhauser ('15) having shown that Haecker ('95, '11) and his students were wrong in supporting such views,—is an indication of the plane along which the final division of the chromosomes into their simplest components will take place. The presence of the Querkerbe may, then, perhaps, be taken as an indication of the presence of plurivalent chromosomes in the sex cells of animals, perhaps also in plants. As yet, however, the actual presence of plurivalent chromosomes has not been demonstrated in all forms which show the Querkerbe. Another fact, which may or

may not have theoretical importance for the question, is the behavior of the chromosomes of the sex cells after the soma cells have been definitely separated from them. In these cells, resulting from the division of the sixth generation of stem cells, the chromosomes (fig. A, *11*) gradually lose their dyad appearance and become ovoid monads. They have the same appearance when first recognized in the spermatogonia and oogonia, which appear in the young individuals soon after hatching. It seems to the writer that this disappearance of the Querkerbe has no real significance, as the structure always reappears before the oögonia or spermatogonia undergo further development, and hence the physiological potentiality of the Querkerbe is always present, even if it is morphologically lost to view. The same argument can not be applied to the soma cells, for the Querkerbe never reappears in any of them. They are always characterized by the larger number (sixty to seventy-two) of small, spheroidal chromosomes, thus showing that not only the morphological, but also the physiological identity of the Querkerbe is lost.

There is another possible interpretation of the Querkerbe, viz., that it is the indicator of the plane along which the chromosomes will be divided at the time they undergo the process of 'diminution.' This interpretation, however, is open to the very serious objection that the Querkerbe is present in forms which do not undergo a process even comparable to 'diminution;' therefore, although it is possible that such an interpretation might hold among the nematodes, it is highly improbable that it could be accepted in any other group of animals.

Another question brought to our attention by this study is that of the origin and the behavior of the centrosome 'couple' which functions in connection with the first cleavage spindle. From the study of the spermatogenesis of A. canis, we find that the sperm brings into the egg at the time of its entrance a centrosome, imbedded in its nuclear substance, and also that this centrosome soon becomes released from the sperm and lies free in the egg cytoplasm. The present case is of considerable interest because it is one of the few instances in which the male centrosome has been definitely followed, first, in the spermatid

through all the stages of metamorphosis into the mature spermatozoon; secondly, afterwards during the entrance of the sperm into the egg, and then, thirdly, as the centrosome which appears after the entrance of the sperm and functions at cleavage. While the kinetic continuity of the male centrosome has long been known to exist, evidences of its morphological continuity have been very scanty. We find that the centrosome becomes imbedded in the side of the nucleus of the metamorphosing spermatid,—perhaps in a similar way to the imbedding of the anterior centrosome in the nucleus of a mammalian sperm,—and is carried into the egg by the sperm. Here it divides, and its halves, still connected by a 'central-desmus', migrate out from the sperm nucleus as the latter forms into discrete chromosomes. Taking up a position in the egg cytoplasm at some distance from the sperm, it becomes surrounded by a faintly distinguishable aster, the origin of which—whether from the sperm or the egg cytoplasm—is as yet not definitely determined. From careful observation, however, it seems most probable that the immediately surrounding cytoplasm of the aster is mostly of male origin and the more peripheral portions mainly female. There is no definite line of demarcation, since the transition from the cytoplasm of one source to that of the other is very gradual. The later separation of the sister centrosomes, concomitant with the approach of the two pronuclei towards the center of the egg, and the final setting up of the cleavage spindle with the two centrosomes, both of male origin, is entirely normal in character.

The entrance and subsequent metamorphosis of the sperm before the giving off of the first polar cell may well give rise to questions as to the time and manner in which fertilization is accomplished. If we adhere to the definition of fertilization as the intermingling of the chromatic material of the egg and sperm, such an action can take place only after the formation of the resting nuclei of the first two cleavage cells. It is here, and here only, that the chromatic material of the two gametes has a chance to intermingle and that the process of synmixis takes place. This definition is advocated by Zacharias ('12) and by Vejdovský ('12). The latter has furnished good arguments for his view,

based on conditions in A. megalocephala. This interpretation rests upon the supposition, however, that only the chromosomes carry hereditary qualities. If other portions of the nucleus carry such qualities, we must admit that fertilization can at least begin as soon as the two pronuclei break down to form the 'fusion nucleus' or the 'fusion spindle.' The probability of this, at least in the case of A. megalocephala, is somewhat diminished by the work of Vejdovský ('12), in which it is shown that from cell generation to cell generation only the chromosomes persist, not the nuclei. From these chromosomes are derived, not only the new chromosomes, but also the entire new nucleus. Hence all hereditary qualities of the nucleus must necessarily reside within the chromosomes. However, this condition is not shown to exist in the case of A. canis, so that nuclear (as distinct from chromosomal) transmission of hereditary characters from generation to generation still remains a possibility.

Meves ('11) and others have argued that the mitochondria are ' plasma bearers of heredity,' but since the work of Vejdovský ('12), Wildman ('12) and Walton ('16 c) has shown that the mitochondria are of nuclear origin, and do not continue from cell generation to cell generation, these bodies may be disregarded in looking for nuclear bearers of hereditary qualities. The works of Boveri ('95, '03) on the fertilization of enucleated egg fragments of sea-urchins, and of Lillie ('12) on Nereis, have brought additional evidence that the mitochondria are not functional as hereditary bearers. In the case of sea-urchins the female mitochondria are present, but apparently do not function. In the case of Nereis the male mitochondria do not even enter the egg, but remain outside in the mid-piece of the sperm.

Turning now to the purely cytoplasmic structures of the sex cells, we find considerable evidence, at least in the egg, of ability to carry hereditary traits—mostly physical in nature. The work of Conklin, Wilson, Lefevre and others in the last ten years, has shown that the cytoplasm of the egg may play an important rôle in the transmission of at least physical traits and characteristics. Organ-forming substances are localized before fertilization, and injury to such a portion prevents the later

complete development of the potential organ so localized. The work of Loeb on artificial parthenogenesis has shown that eggs can develop, apparently in a normal manner, without the presence of a spermatozoon. Normal or facultative parthenogenesis also is of somewhat common occurrence. From this it would seem that all the necessary traits of the organism are borne in the egg itself, and the traits, if any, borne by the sperm are superfluous. The fact that practically no male cytoplasm ordinarily enters the egg with the sperm seems to indicate that it has little or no function in fertilization. However, in Ascaris, as in all nematodes, the sperm nucleus is surrounded by a comparatively large amount of cytoplasm, the possible rôle of which in the fertilization process is yet to be demonstrated, but can not *a priori* be denied simply because it has not yet been found. Parthenogenetic nematodes are frequently found, however, and this may be an argument for the non-functioning of the sperm cytoplasm as a necessary factor in the transmission of hereditary traits.

From these facts it seems that the conception of fertilization as expressed by Marcus ('06), Zacharias ('13), and others—that is, fertilization taking place only in the 'resting nuclei' of the daughter cells of the first cleavage division—is too limited. Fertilization may, and perhaps does, begin the instant the sperm cytoplasm comes in direct contact with that of the egg by the breaking down of the sperm-membrane—even earlier, if we accept the osmotic theory of cytogamous fertilization as shown by some amoebae—and may continue until the chromosomes of the two daughter cells of the first cleavage division have re-formed after their 'resting' condition. Fertilization is thus a process which may extend over a long interval, and can not be limited to any one definite period or place in cell development.

Cleavage, incited by the centrosome brought in by the male, may thus begin before the process of fertilization is completed. As is commonly the case in most nematodes, the two pronuclei in A. canis do not ordinarily fuse into one definite 'fusion nucleus' before the first cleavage spindle is established. Of the hundreds of cells observed, only two such 'fusion nuclei' were found. The more common method of procedure was that of direct

spindle formation by the two pronuclei without their first fusing to form one nucleus. The membranes of the two pronuclei, which lie side by side somewhat eccentrically and nearer the animal pole of the egg, disappear, not only on the adjacent faces of the nuclei, but also in the regions nearest the centrosomes. Thus the chromosomes of the two origins come to lie in different portions of the cleavage spindle. Whether this separation is a permanent one, as is the case in A. megalocephala, is a question not as yet answered, for there is not sufficient morphological difference between the chromosomes of male and female origin in A. canis to allow of their identification when together in the same spindle. The process of fertilization is completed during the reorganization of the nuclei of the two daughter cells of the first cleavage division.

Apparently the first two cleavage cells are exactly alike, save for a slight difference in size, and the difference between the soma and the stem cell is seldom recognizable until the second cleavage division, and often not until the third. This difference lies in the fact that 'diminution' takes place in the soma cell at its first division after separating from the stem cell. As shown by Zoja ('96) in A. megalocephala, this 'diminution' process may be, and in A. canis very commonly is, deferred until the division of the cells derived from the original soma cell. Thus two 'diminution' figures appear when, in the four-cell stage two soma cells divide to produce the six-cell stage. There is one rather unreliable means of distinguishing between the first stem cell and the first soma cell even when both divide by ordinary mitosis (that is, without 'diminution' in either cell), for the soma cell is ordinarily the first to commence division.

The ordinary 'T-shaped' embryo of the four-cell stage is formed, with the propagation cell at the free end of the upright of the 'T'. This cell rotates upon the second soma cell (also in the upright of the 'T') until the embryo assumes a lozenge-shape with the stem cell at one of the acute angles. In the cases where the 'diminution' process has been delayed, the division from the four- into the six-cell stage shows two 'diminution' spindles. This process of 'diminution' takes place in each

of the soma cells arising by the first five divisions of the stem cell, and except for the soma cell of the first generation, the process always occurs in their first subsequent division. The amount of chromatin thus lost is about equal to the amount retained, and the extruded masses of material can be seen for some time, since their disintegration in the cytoplasm is slow. These masses are commonly located next to the cell wall which was formed in the plane of the equatorial plate of the 'diminution' spindle.

The sixth division of the stem cell gives two purely propagation cells, whereas each of the five previous divisions had given rise to a stem cell and a soma cell. From the two propagation cells come all the sexual cells of the embryo and the adult. As no 'diminution' takes place in the propagation cells at any division, and as all the soma cells have arisen from cells which have undergone this process, it is easy to recognize the primitive germ cells from the body cells, not only by their greater amount of chromatin, but also by the fact that they still have only thirty or thirty-six (sexual difference) dyad chromosomes, whereas the soma cells all have sixty or seventy-two small monad chromosomes.

VI. SUMMARY

The following points have been brought out in this study of the oogenesis of Ascaris canis Werner.

1. The diploid number of chromosomes is thirty-six; the haploid eighteen.

2. There is a heterochromosome group of the '2X' type, consisting of twelve tetrad chromosomes.

3. Each of the chromosomes of the oogonia—diploid number—is potentially a dyad and becomes a tetrad by a longitudinal division during the early first oöcytic prophase.

4. By a process of pseudo-reduction through parasyndesis these thirty-six (diploid number) tetrad chromosomes are reduced to eighteen di-tetrads (haploid number) in the late prophase of the first oöcyte.

5. Of the two oöcytic divisions, the first is equational, the second reductional, in the Weismannian sense.

6. The mature ootid contains eighteen dyad chromosomes, which unite with the twelve or eighteen dyads of the sperm nucleus to form respectively:—(a) a zygote (male individual) with thirty dyad chromosomes, or (b) a zygote (female individual) with thirty-six dyads.

7. Neither maturation division takes place before insemination, that is the penetration of a spermatozoon.

8. There is a definite interkinetic period between the two maturation divisions during which a nuclear membrane is formed.

9. The cleavage centrosomes are traceable directly, through the one brought in by the sperm, to the centrosome that arose with the spermatid.

10. Fertilization is possible from the time that the sperm enters the egg, up to the time of the reorganization of the nuclei of the first two cleavage cells and the final union of the male and female chromosomes.

11. Of the five soma cells immediately derived from the stem-cell series, all except the first one undergo 'diminution' at their first division; the first soma cell also does in fifty per cent of the individuals, but in the remaining fifty per cent the division is deferred until the division of its daughter cells.

12. The descendants of the sixth generation of the stem-cell series are entirely propagational in nature.

13. The Querkerbe, always present, at least potentially, in all the chromosomes of the stem and propagation cells, disappears entirely in the chromosomes of the soma cells after 'diminution' has occurred. The Querkerbe is probably the sign of a plurivalent condition of the chromosome, and never indicates the plane of a syndetic union or that of a maturation division.

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VIII. EXPLANATION OF PLATES

All the figures in plates 1 to 9 were drawn with the aid of a camera lucida, the projection distance being 410 mm. Spencer 2 mm. oil immersion, and 4 mm. dry, objectives, and compensating oculars 5, 10, and 15 were used.

The following abbreviations are used:—

so., 'soma' cell, derived from the previous division of the 'stem' cell.

strp., 'stem' or 'propagation' cells.

PLATE 1

1 Oogonial cell showing plasmosome and resting condition of chromatin. (× 4050)

2 Side view of metaphase spindle of oogonial division. (× 4050)

3 Side view of anaphase spindle of oogonial division. (× 4050)

4 Telophase of last oogonial division, showing the peripheral position of the chromatin. (× 4050)

5 Resolution of discrete chromosomes from the peripheral chromatic mass in the telophase of the last oogonial division. The plasmosome is also shown. (× 4050)

6 Peripheral distribution of chromosomes in early prophase of the first oocyte. (× 4050)

7 Cell showing attachment to rhachis during growth period. Chromosomes showing Querkerbe. Chromosomes peripheral, plasmosome central in cell. (× 3200)

8 Early prophase of first oocyte, showing formation of spireme and the breaking down of the chromosomes. (× 3200)

9 Early prophase of first oocyte, showing loose skein with chromatic granules at the nodes of the linin network. (× 3200)

10 Early prophase of first oocyte, showing condensation of network into thick, jagged strands; plasmosome distinct from network. (× 3200)

11 Cross-section of oviduct, showing arrangement of oocytes around the rhachis. (× 550)

12 Early prophase of first oocyte, showing chromatin gathering toward one side of the nucleus and becoming slightly vacuolated. (× 3200)

13 Early prophase of first oocyte, showing still further condensation of the chromatin into one mass. Vacuoles very apparent. (3200)
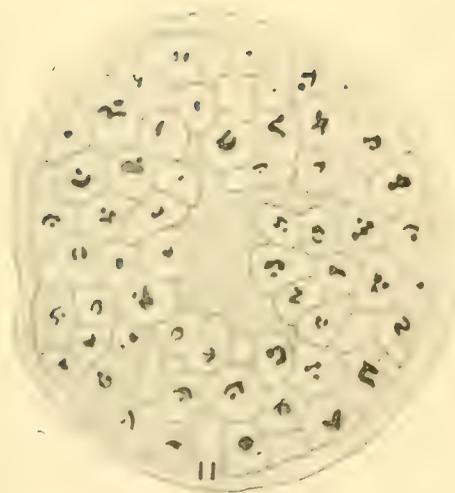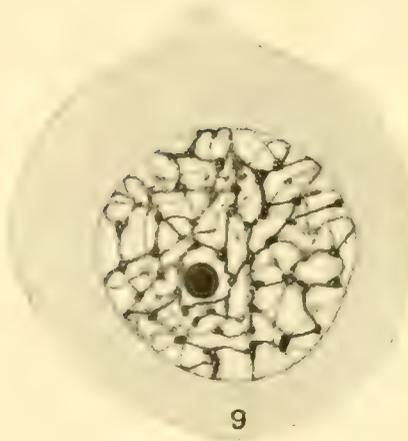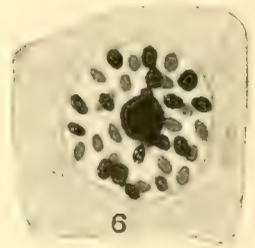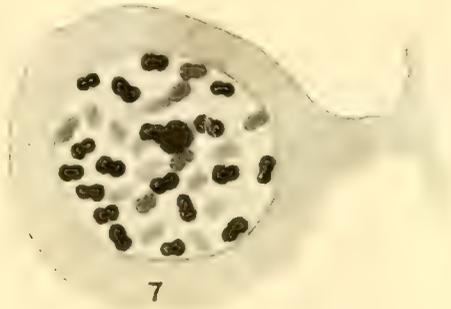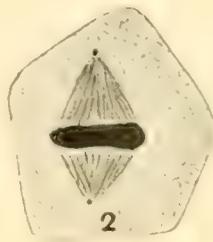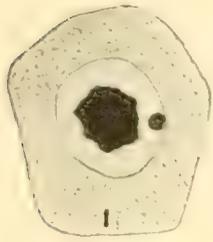
All figures on this plate are of primary oocytes

14 Cross-section of oviduct, showing single layer of primary oocytes with peripherally placed nuclei. Rhachis degenerating. (× 550)

15 Chromatin of prophase nucleus condensed into one peripheral mass, the linin network disappearing, and vacuolation of the cytoplasm begun. Plasmosome discrete. (× 1700)

16 Prophase nucleus, with linin network entirely broken down. Plasmosome degenerating. (× 3200)

17 Prophase nucleus, with slight evidences of vacuoles in the chromatin mass. Linin network and plasmosome degenerating. (× 3200)

18 Prophase nucleus, showing resolution of discrete chromosomes from chromatin mass. Plasmosome granules at the left. (× 3200)

19 Prophase nucleus, with fully formed monad chromosomes and a discrete plasmosome. (× 3200)

20 Prophase chromosomes with a Querkerbe and increased number of vacuoles in the cytoplasm as the cell becomes more nearly spherical. (× 1700)

21 Slightly older than that of figure 20. (× 3200)

22 Prophase nucleus, showing beginning of longitudinal split in the thirty-six dyad-like chromosomes. (× 3200)

23 Prophase nucleus, showing union of thirty-six tetrads by parasyndesis to form eighteen di-tetrads. (× 3200)

24 Prophase nucleus, showing degeneration of plasmosome and the extrusion of some of its elements into the cytoplasm. (× 3200)

25 Nucleus, of same age as the one shown in figure 24, with the plasmosome degenerating and disappearing *in situ*. (× 3200)

26 Beginning of the first maturation spindle and the arrangement of the chromosomes into an equatorial plate. Nuclear membrane breaking down at poles of spindle. First appearance of centrosomes. (× 1700)
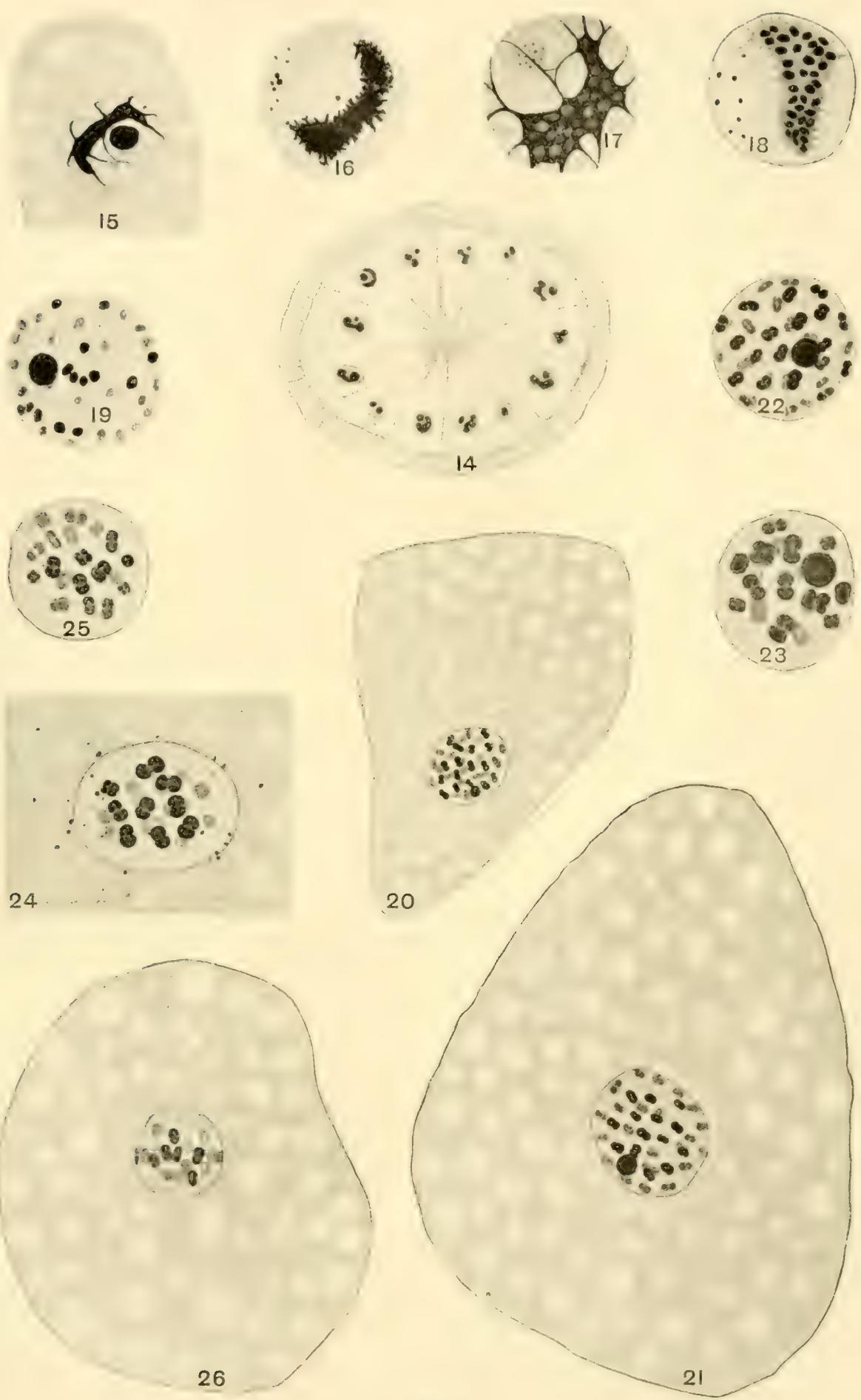
All figures on this plate are of primary oocytes

27 Primary oocyte, first maturation-division spindle established. Spindle still centrally located in egg mass. ($\times$ 1700)

28 Polar view of metaphase plate of first maturation spindle, showing eighteen chromosomes. ($\times$ 1700)

29 Lateral view of late metaphase plate of first maturation spindle. Spindle fibers arranged in bundles for each chromosome. ($\times$ 4050)

30 Amoeboid sperm penetrating vitelline membrane of primary oocyte. ($\times$ 1700)

31 Sperm almost completely within the egg. Spindle figure migrating toward the periphery of the egg. Male centrosome seen imbedded in side of sperm nucleus. ($\times$ 1700)

32 Spindle at periphery of egg. Fertilization membrane formed. Sperm beginning to lose its outline. Large vacuoles in oöcytic cytoplasm becoming peripheral in position. ($\times$ 1700)

33 Spindle lying parallel to a tangent to the surface of the egg. Male centrosome divided and its halves connected by a 'centro-desmus.' ($\times$ 1700)

34 Polar view of metaphase plate of first maturation spindle. Sperm losing its sharp outline. Centrosomes widely and somewhat precociously separated. ($\times$ 1700)

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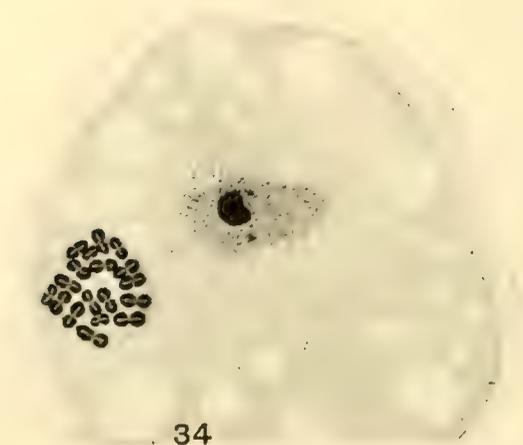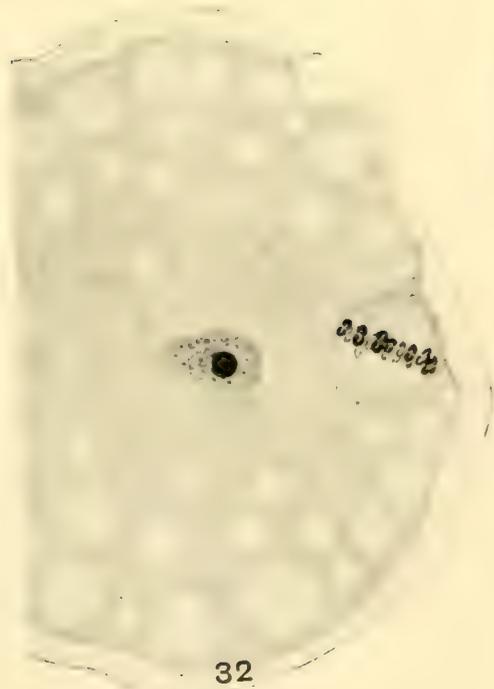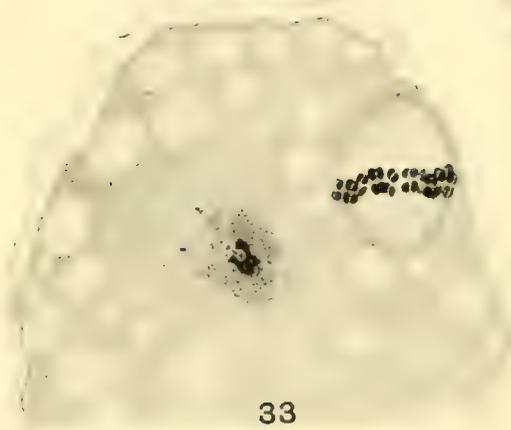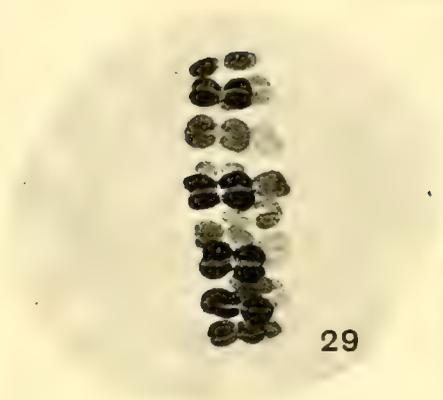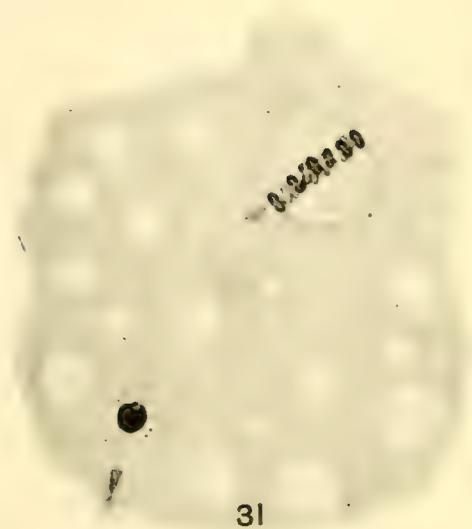
PLATE 4

35 First maturation spindle rotated so that its long axis comes to lie perpendicular to a tangent to the surface of the egg at its point of contact. In this case the male centrosome is undivided. ($\times$ 1700)

36 Polar view of metaphase plate of first maturation spindle, showing eighteen chromosomes. ($\times$ 4050)

37 Lateral view of spindle of the same age, showing di-tetrad nature of the chromosomes. Spindle fibers no longer in bundles. ($\times$ 4050)

38 Lateral view of very late metaphase plate showing longitudinal division of the chromosomes. ($\times$ 4050)

39 Primary oöcyte, showing the spindle in the anaphase condition and the sperm accompanied by the male centrosome in process of division. ($\times$ 1700)

40 Lateral view of anaphase spindle of first division, showing tetrad nature of the daughter chromosomes. ($\times$ 4050)

41 Polar view of anaphase group of the first maturation division. ($\times$ 4050)

42 Late anaphase of the first maturation division. ($\times$ 1700)

43 Cutting off of the first polar cell. ($\times$ 1700)

44 First polar cell completed. Reorganizing of inner half of spindle figure. ($\times$ 1700)

45 'Interkinetic period.' Formation of a transient nuclear membrane and the division of the still persistent female centrosome. ($\times$ 1700)

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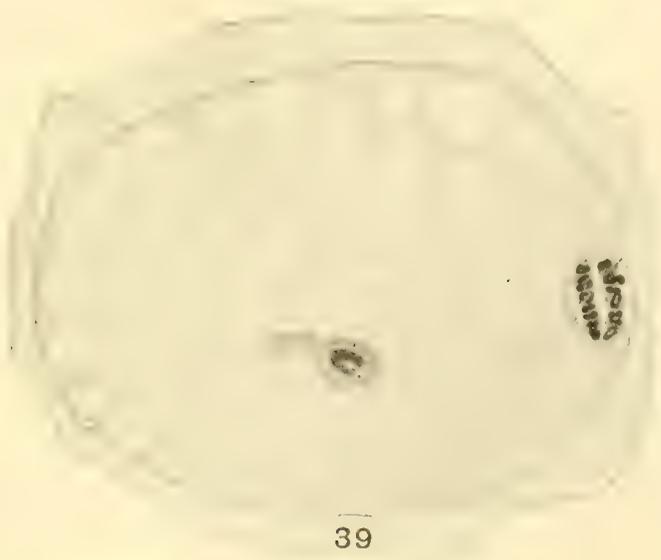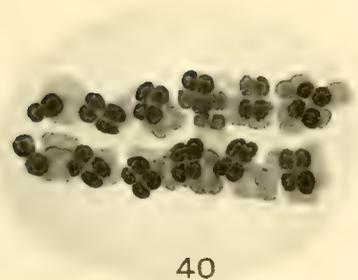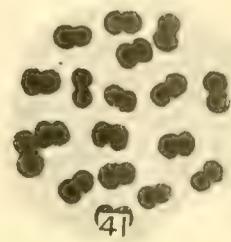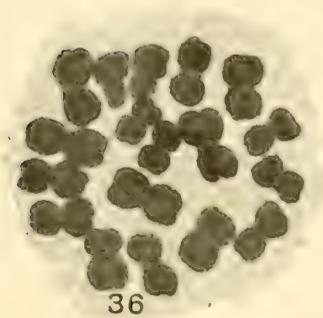
46 Second maturation spindle lying with its long axis parallel to a tangent to the surface of the egg. Perivitelline space formed around egg cytoplasm. First polar cell attached to fertilization membrane. ($\times$ 1700)

47 Second maturation spindle rotated so that its long axis is perpendicular to a tangent to the surface of the egg. Sperm nucleus beginning to break up into chromosomes. Male centrosomes, surrounded by denser cytoplasm, at one side of the sperm nucleus. ($\times$ 1700)

48 Secondary oöcyte showing early anaphase spindle, side view. Sperm nucleus breaking up to form discrete chromosomes. ($\times$ 1700)

49 Same spindle as figured in 48, showing longitudinal (reductional) division of tetrad chromosomes. Centrosomes very indistinct. Spindle fibers not in definite strands. ($\times$ 4050)

50 Polar view of sister plates of the same anaphase spindle, showing dyad nature of the eighteen daughter chromosomes. ($\times$ 4050)

51 Late anaphase of second maturation spindle, side view. ($\times$ 1700)

52 Formation of second polar cell. Chromosomes of male origin clearly differentiated. Mitochondrial granules showing distinctly. ($\times$ 1700)

53 Male and female pronuclei organized, each with eighteen dyad chromosomes. Centrosome couple of male origin with its surrounding aster well differentiated. ($\times$ 1700)

54 Male pronucleus with only twelve dyad chromosomes, the female with eighteen. ($\times$ 1700)

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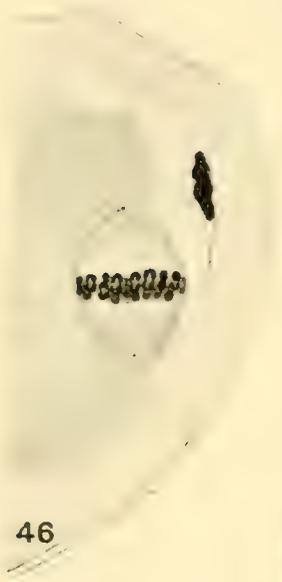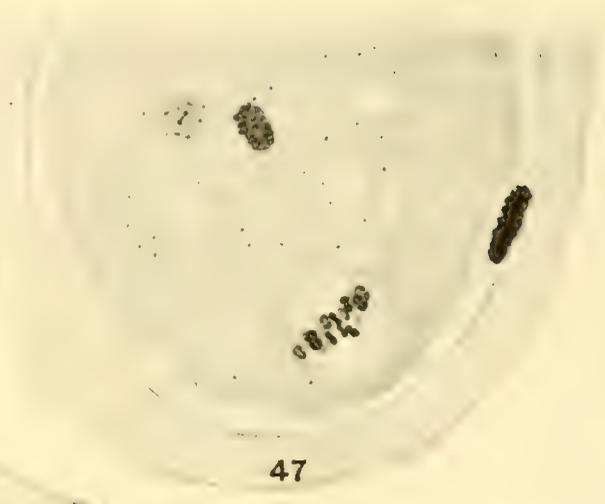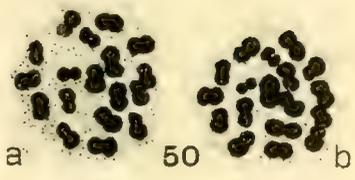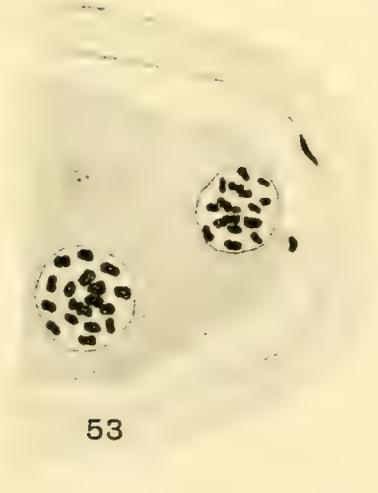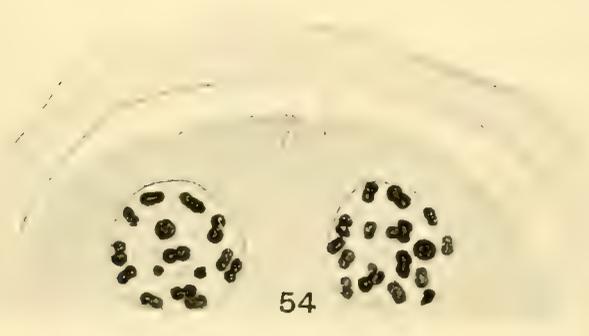
PLATE 6

55   Formation of 'bouquet' stage in male pronucleus.   Appearance of several plasmosomes.   ($\times$ 1700)

56   Chromosomes of female pronucleus beginning to break up.   ($\times$ 1700)

57   'Bouquet' formed in both pronuclei.   The centrosomes of male origin taking up a position midway between the two nuclei.   ($\times$ 1700)

58   Formation of diffuse spireme in both pronuclei.   Centrosomes beginning to separate.   ($\times$ 1700)

59   Two pronuclei lying side by side in the egg.   Centrosomes widely separated.   ($\times$ 1700)

60   Two pronuclei in apposition.   Chromatic network breaking down and discrete chromosomes being formed.   Fusion of plasmosomes into one large body.   ($\times$ 1700)

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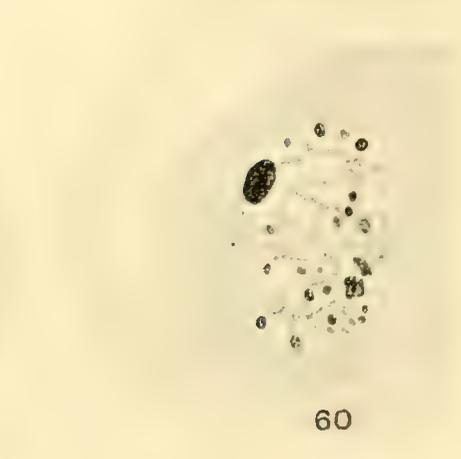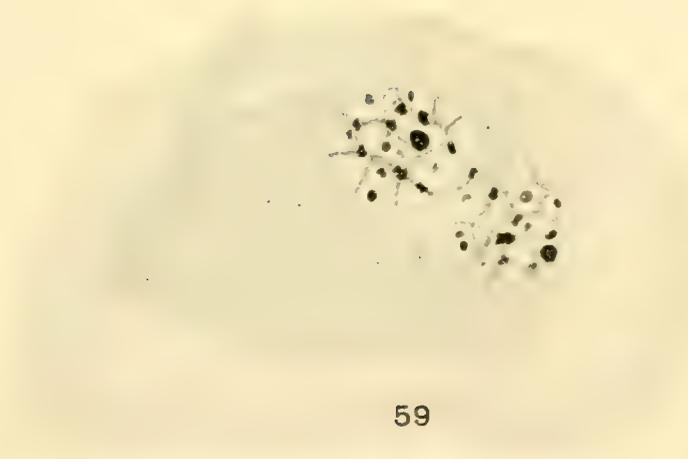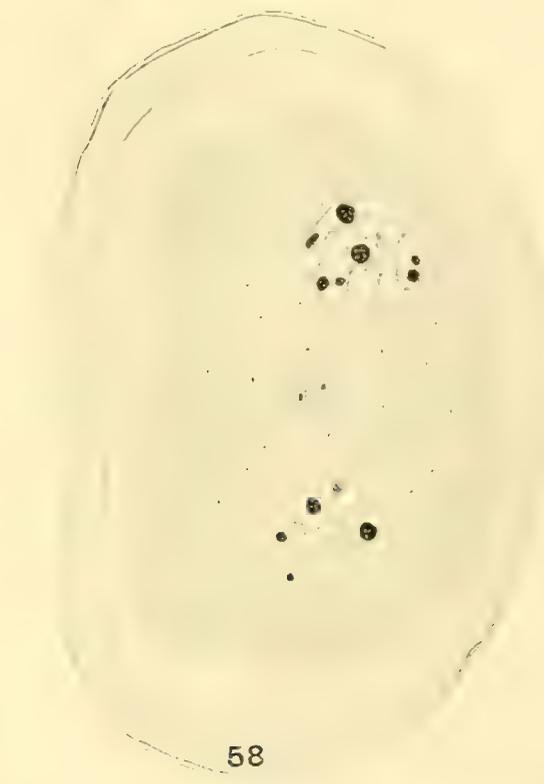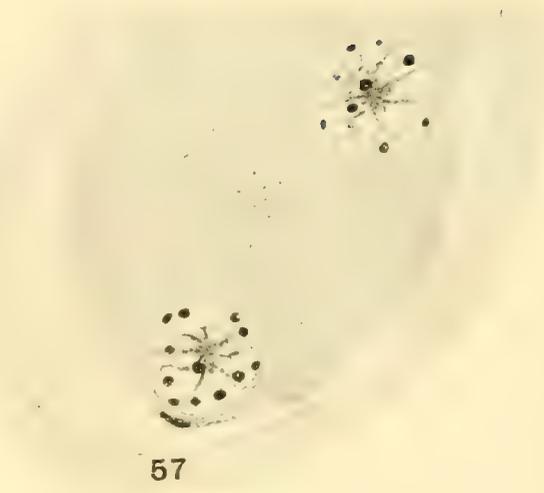
61   Fusion of two pronuclei to form a 'fusion nucleus.'   ($\times$ 1700)

62   Fusion of two pronuclei to form a 'fusion spindle.'   Nuclear membranes breaking down, both between the two pronuclei, and also in the regions turned toward the centrosomes.   ($\times$ 1700)

63   'Fusion nucleus,' showing cleavage centrosomes in position.   ($\times$ 1700)

64   Side view of first cleavage spindle.   Polar cells also shown.   Diploid number of dyad chromosomes present.   ($\times$ 1700)

65   Anaphase of the first cleavage spindle, side view.   ($\times$ 1700)

66   Polar view of metaphase plate in a soma cell which has undergone 'diminution,' showing double the diploid number of monad chromosomes.   ($\times$ 1700)

67   Side view of metaphase spindle of a soma cell which has undergone 'diminution.'   ($\times$ 1700)

68   Two-cell stage, showing diploid number of dyad chromosomes in each nucleus.   ($\times$ 1700)

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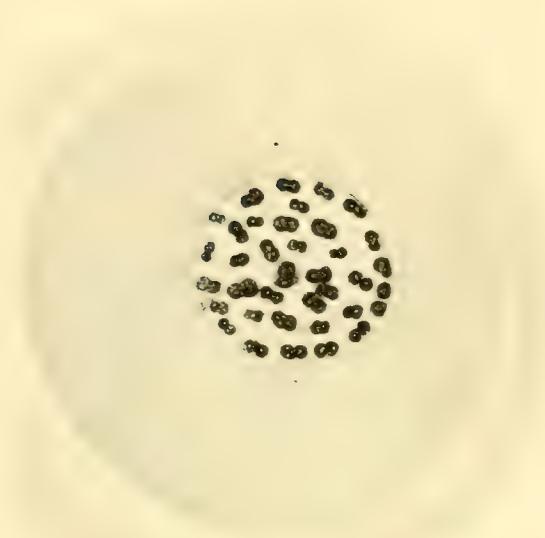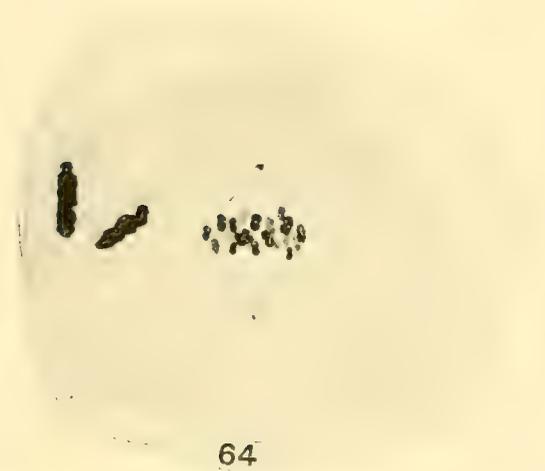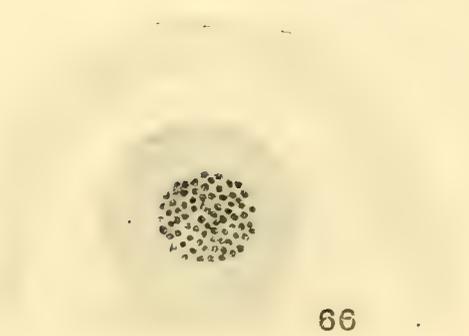
69   Two-cell stage, showing 'diminution division' in the soma cell (*so.*)   ($\times$ 1000)

70   Two-cell stage, showing anaphase of the 'diminution spindle' in the soma cell (*so.*).   ($\times$ 1000)

71   Two-cell stage, showing first soma cell (*so.*) dividing by ordinary mitosis. ($\times$ 1000)

72   Three-cell stage, showing late anaphase of the 'diminution division' in the first soma cell (*so.*).   ($\times$ 1000)

73   Formation of the 'T'-embryo.   Soma cell (*so.*) dividing by a 'diminution' process, and the stem cell (*strp.*) by ordinary mitosis.   ($\times$ 1000)

74   'T'-embryo of four cells, in which the first two divisions have both been by ordinary mitosis.   Stem cell (*strp.*) at the end of the upright of the 'T'.   ($\times$ 1000)
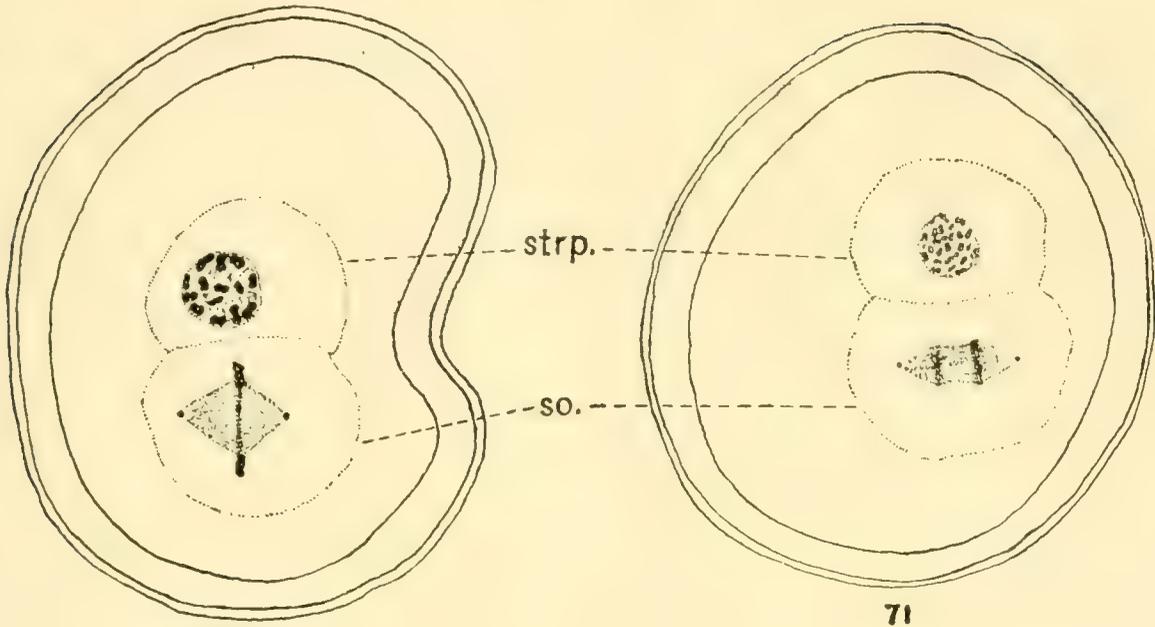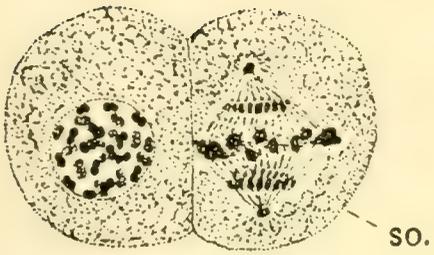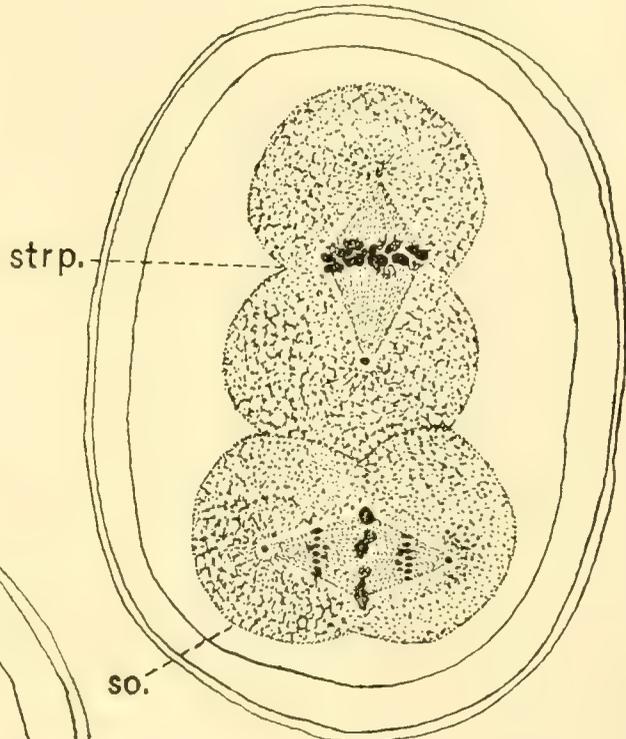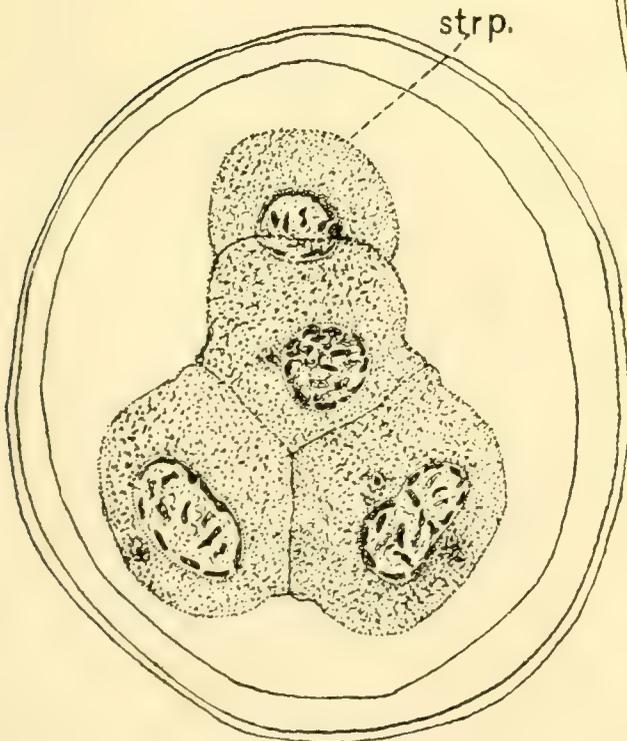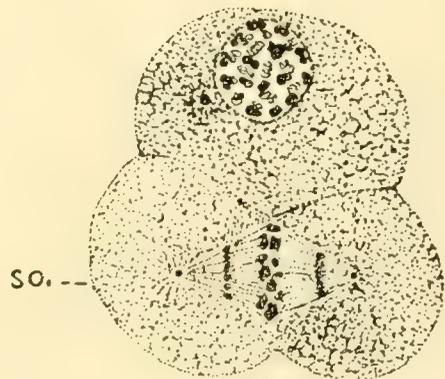
EXPLANATION OF FIGURES

75 Side view of four-cell embryo showing diminution divisions in the daughter cells of the first soma cell, the latter having failed to undergo 'diminution' at its first division.   The stem cell is *strp*.   (× 1000)

76 Top view of 'lozenge-shaped' four-cell embryo, showing polar views of the 'diminution spindles' in the daughter cells of the first soma cell.   Second soma cell at *so.*, and the stem cell at *strp*.   (× 1000)

77 Side view of the 'diminution spindles' in the daughter cells from the first soma cell.   (× 1000)

78 Six-cell embryo, showing the nuclei of the soma cells which have undergone 'diminution,' and the stem cell (*strp.*), which is dividing by ordinary mitosis. Extruded mass of chromatin lying free in cytoplasm of the soma cells.   (× 1000)

79 Six-cell stage, showing the stem cell with the diploid number of dyad chromosomes, while the soma cells have all descended from cells which have undergone 'diminution.'   (× 1000)

80 Six-cell embryo in which a "diminution division" is occurring in the second soma cell (*so.*), and a normal mitotic figure in the stem cell (*strp.*).   (× 1000)

81 Median section through an eight-cell embryo, showing at the right the stem cell, in which a 'diminution division' never occurs.   (× 1000)

75

77

76

78

strp.

79

80

81
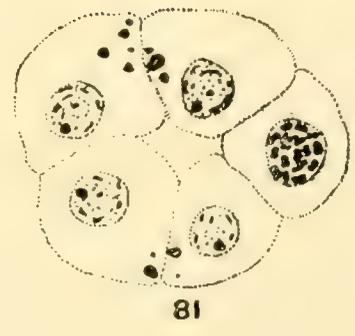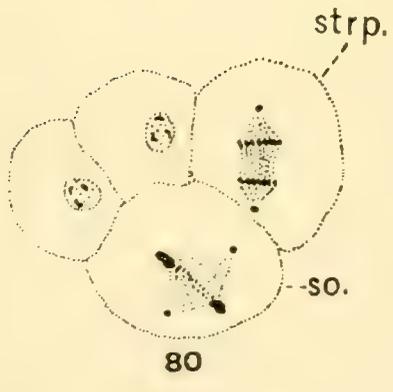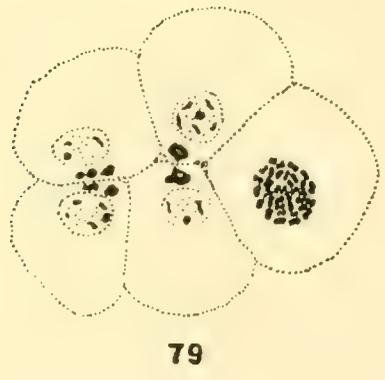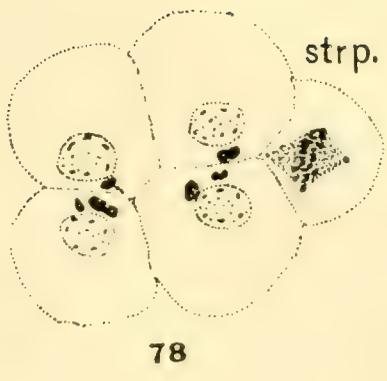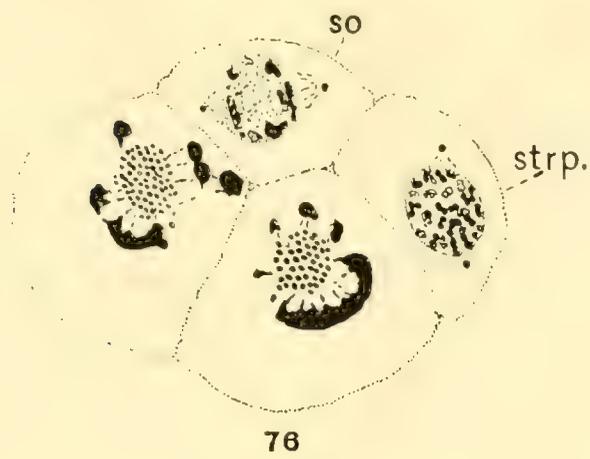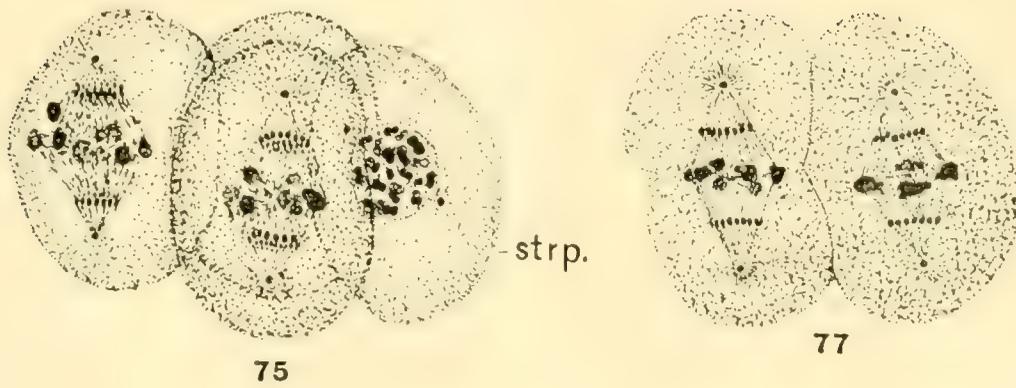
PHARYNGEAL DERIVATIVES OF AMBLYSTOMA[1]

FRANCIS MARSH BALDWIN

FORTY-SEVEN FIGURES (TWO PLATES)

CONTENTS

[1] Contributions from the Zoological Laboratories of the University of Illinois No. 103.

I. INTRODUCTION

The recognition, within recent years, of the parts played by certain of the structures arising from the walls of the human pharynx, in health and disease, renders these bodies of great importance, and hence every fact which throws light upon their morphology is of value. It was from such considerations that the study detailed below were outlined, for, while a knowledge of their development and structure in amphibia may have no immediate practical value, the close relations of that group to the ancestors of the mammals gives every addition to our knowledge an interest which would not be expected with such groups as the teleosts and birds.

The literature of the subject, while not great, is rather difficult to follow, because of the lack of unanimity of opinion as to homologies, and also from the conflicting nomenclature of the subject, the same structures often having different names even in the same minor group. In some groups pharyngeal derivatives are found which apparently do not occur in the amphibia or at least in the urodeles. These have not been considered here, and the present work is restricted to the series of 'glands' et cetera which

are developed in the pharyngeal region of Amblystoma, the list including the thyreoid, and thymus glands; the so-called post-branchial body; the so-called epithelial bodies or gill-remnants; and also the carotid gland, although it is not a true pharyngeal derivative.

Since but little recent work has been done on the developmental side of these structures in amphibians—Maurer ('88), Livini ('02), Greil ('02–06) and Marcus ('08) excepted—a detailed study of their morphogenesis in Amblystoma has some value.

The work has been carried on in the zoological laboratory of the University of Illinois, under the direction of Professor Kingsley, to whom the writer is deeply indebted for invaluable aid during its prosecution. My thanks are also due Professor Ward, for laboratory facilities and numerous other courtesies.

Materials and methods

This research is chiefly based upon the study of serial sections of larvae of Amblystoma punctatum, beginning with 5 mm. stage, and including successive stages through the 45 mm. larvae, and following these with stages in transformation, and sections of adult heads. Supplementing the study of the slides, gross dissections were made of adults, using Amblystoma tigrinum as a basis. The young larvae were fixed in Smith's fluid, stained in toto in borax-carmine, dehydrated, imbedded and mounted in the usual way; older larvae and adult heads were fixed in formalin (heads decalcified), dehydrated, imbedded, sectioned and doubly stained on the slide with borax-carmine and Lyons blue.

For stages in metamorphosis, I am greatly indebted to Professor Kingsbury of Cornell University, for the use of his excellent slides, and my thanks to him for his kindness are here given. The figures representing the various structures are drawn by the aid of the camera lucida.

II. OBSERVATIONS

1. *Thymus bodies*

A. Amblystoma larvae, 8 mm. long. At the 8 mm. stage (figs. 4 and 5) the mouth is not open, the place where it is to form being occupied by an extensive oral plate, in which the exact limits of entoderm and ectoderm can be recognized only uncertainly and with difficulty (Kingsley and Thyng '04, and Johnston '10). The pharyngeal lumen (*ph.*) extends from behind forward into this plate. The pharyngeal region is a flattened tube throughout. It gives off on either side, the visceral pouches—the future gill clefts—which have not yet broken through. The most anterior of these pouches, the hyomandibular, lies about the level of the eye in the series on which these observations are based. The outer surface of its entodermal walls does not quite reach the external ectoderm of the head, while below and in front of it is a mass of mesoderm, the ventral prolongation of the third head somite. A conical projection, consisting of a few cells at the posterior dorsal angle of this pouch is the anlage of the first thymus body. The projection is some distance from the ectoderm, between which and the anlage are cells, some of which stain more darkly than the surrounding cells. These darker cells are apparently those which Drüner has interpreted as the ectodermal contribution to this thymus body, concerning which more will be said below.

This first thymus anlage (fig. 6), lies about mid-way between the eye and the ear. Dorsal to it is the developing facial (*VII*) ganglion, while the internal carotid artery (*i.c.*) lies medial to it and sends a small twig (the quadrato-mandibular artery) forward and ventrally to supply the anterior region of the body, the latter passing just in front of the thymus anlage. The anlage on the left is seen in three, and the one on the right, in four successive sections (each 10 micra), but it is difficult to limit its extent exactly.

The second pouch (first branchial) is much like the hyomandibular in its general features. Externally it extends in the longi-

tudinal direction of the body about 200 micra, measured from the caudal side of the hyomandibular pouch and ending with the caudal, lateral projection, just under the anterior wall of the ear. The anlage of the second thymus body is formed from the dorsal angle of this pocket at its caudal extremity, in the same way as the first body was formed from the hyomandibular pouch. This anlage (t_{II}, fig. 8) is not as well defined as the first, so that it is difficult to fix its exact limits. The conical projection is pronounced in but a single section, but it is probable that cells of entoderm in front and in back of this contribute to its formation. In addition to the dorsal region, the lateral region of this pouch thickens and extends toward the ventro-lateral wall where the ectoderm is to split to form the first (br.1) branchial cleft. The anlage of the body, as noted above, lies just below the anterior wall of the ear; the glossopharyngeal (IX) ganglion, and the internal carotid artery are medial to it. Near the anlage of the second thymus body are numerous more darkly stained cells, similar to those mentioned in connection with the first. In the ventral region of the body, the structures are not clearly defined, although the pericardium and the truncus arteriosus are recognizable.

The third pouch (second branchial) is nearly 160 micra long, its caudal extremity lying just posterior to the ear. The anlage of the third thymus body (t_{III}, fig. 9) is formed by a lobe of cells directed dorsally from the distal tip of this pouch, the lateral portion of which extends toward the ectoderm of the body wall to form the second (br.2) branchial cleft. This anlage is more clearly defined than that of the second, and occurs in three successive sections (each 10 micra). The vagus (X) ganglion is just above this anlage, the more darkly stained cells of Drüner intervening between the two. The pericardial chamber in the ventral region of the body is here of considerable size, and the heart is flexed.

The anlagen of the fourth and fifth thymus bodies (t_{IV}, t_{V}, figs., 10 and 11), are formed in a similar way as solid outgrowths from the dorsal extremities of the fourth (third branchial) and fifth

(fourth branchial) pouches respectively. The fourth pouch is about 70 micra long, and the fifth, about 40 micra these two being now comparatively close together. The anlage of the fourth thymus body is fairly well defined. It is a lobate body, about 20 micra in diameter, extending dorsally from the caudal dorsal end of the third pouch and, in one section, shows a tendency to be constricted from the other cells of the pouch. The darker cells are somewhat more compact about the dorsal tip of this anlage, than in the case of the others. The vagus (X) ganglion is dorsal and a little medial to the fourth anlage, while below and medial to it are the pericardial chamber and the heart. It is difficult to determine the exact extent of the anlage of the fifth thymus body. It is a lobate, illy defined mass of cells extending in a dorsal direction from the fourth branchial pouch towards the lateralis ramus $(L.)$ of the vagus nerve.

The general positions of the thymus anlagen at this stage in Amblystoma agree in most essentials with the description of the bodies in Siredon and Triton by Maurer ('88), and in Siredon by Drüner ('04) and Maximow ('12). Concerning the interpretation of the darker cells which are found in close proximity, dorsal and lateral, to the bodies there is some differences of opinion. Drüner ('04), in his work on Siredon, was the first to suggest that they are derived from the ectoderm, and that they contribute to the formation of the thymus bodies. To these groups he gave the name 'Ektodermzapfen.' Maximow ('12), p. 573, however finds them, (der Drüner'schen Thymus ectodermalis) very sharply set apart from the thymus bodies, and more closely associated with the anlagen of the nerve ganglia of the different regions. He says, "Ob diese Zellansammlungen wirklich dem Ektoderm entstammen, wie es Drüner will, vermag ich nicht anzugeben; jedenfalls hängen sie alle unmittelbar oder durch einen kürzeren oder längeren Zellstrang mit der Masse der betreffenden Gehirnganglien zusammen und sind von deren Zellen nicht scharf abzugrenzen." In my material, it is far from certain that there is any such ectodermal contribution as Drüner claims. I find, indeed, cells, differing in staining reaction from those of the other entodermal cells in this very region, but I can trace no connection of these at any

stage with the ectoderm. In addition, at no time in the subsequent stages do these darker cells, so far as I am able to judge, unite either directly with the thymus bodies or with the nerve ganglia, and therefore, I find no good reason for regarding them as other than mesodermal cells of the region.

B. Amblystoma larvae, 9.5 mm. long. In 9.5 mm. larvae, the mouth is not yet open, but the pharyngeal pouches have extended laterally so that the entoderm of their distal walls has fused with the cells of the ectoderm of the body, although the clefts are still closed. The pharyngeal entoderm is now lighter in color, due to its less amount of yolk content, so that the morphological relationships of parts are more easily followed.

The anlage of the first thymus body (fig. 12), has not greatly changed in its relative position. The first pouch (hyomandibular) has extended laterally and at the same time a little caudad, the thymus body being carried a corresponding distance in the same direction. It is now a somewhat spherical knobule of entodermal cells (about 30 micra in diameter), still connected with the dorsal wall of the pouch, extending dorsally just lateral to the facial (*VII*) ganglion. Of three successive sections which pass through it, only in the second is it connected with the pouch by a thin stalk of cells. The internal jugular vein is just above, while the internal carotid artery is medial to it. The darkly staining cells of Drüner form a somewhat loose mass between it and the facial nerve. In front of the thymus, the internal carotid artery sends the mandibularis branch to the region of the jaw, while caudad to it, the facial ganglion gives off two rami; one (ramus palatinus) courses medially, the other, a large single trunk (which breaks up into the ramus hyomandibularis, the ramus alveolaris and buccalis), passes lateral and enters the jaw region (Herrick; Strong; Coghill).

The second pouch (first branchial) is much like the first; its caudal portion meets the ectoderm of the side of the body, below the posterior wall of the ear, where the gill cleft is to open. The anlage of the second thymus body in this stage is a group of cells, (fig. 14, t_{II}) similar in color to those of the dorsal wall of the pouch

from which they have separated. This group is located well to the side of the body, in the region of the glossopharyngeal ganglion and lies equidistant between the medial surface of the levator arcuum branchiarum muscle of the first gill arch, and the internal jugular vein. It is just in front of the point where the pretrematic ramus of the glossopharyngeal nerve enters the gill arch. The body is almost spherical in shape, and is about 30 micra in diameter. The darkly stained cells are now less in evidence; only one or two appear in each section.

The caudal region of the third pouch (second branchial) lies about 90 micra caudad to the second. The anlage of the third thymus body (t_{III}, fig. 15) is not yet completely separated from the dorsal cells of the pouch, having a very narrow connecting stalk of cells. This body is very small, between 15 to 20 micra in diameter. It lies just posterior to the second efferent branchial artery on its course from the arch to the radix aorta. Caudad to the gland, the first branchial nerve of the vagus passes into the arch. The jugular vein lies in the same relative position as before, but the internal carotid artery does not extend as far back as this region. The darkly stained cells in the region of this thymus body have disappeared. Maximow found the third thymus body in Siredon, 9.5 mm. long, free in the mesenchyme, dorsal to the dorsal wall of the pharynx, and in close connection with the vagus ganglion, and medial to the large muscle ('wahrscheinlich den M. levatores arcuum branchialium'), and somewhat larger (40 micra in diameter) than the one I have described in Amblystoma of this stage.

The caudal extremities of the fourth and fifth pouches (third and fourth branchial) are more dorsal in position than any of the others, so that the points from which the fourth and fifth thymus bodies develop, lie above the level of the notochord, and close under the dorso-lateral wall of the larval body.

The caudal distal extremity of the fourth pouch, from which the fourth thymus body is developed (t_{IV}, fig. 16), lies 60 micra caudad to the third thymus body. Its anlage is an elongated, ovoid group of cells still in connection with the dorsal portion of the

pouch. It is between 35 and 40 micra in diameter and lies just ventro-medial to the levator arcuum branchiarum of the third arch, in the region of the vagus ganglion.

The anlage of the fifth thymus body (t_v, fig. 17), is a small bean-shaped group of cells still in connection with the caudal distal end of the fifth pouch (fourth branchial) and is 120 micra caudad to the fourth body. It is about 20 micra in diameter, and protrudes dorsally into the space between the levator arcuum branchiarum muscle of the fourth arch, and the medially placed dorso-laryngeus muscle. Since the pouch from which this body is derived has been pushed laterally and caudally as noted above, its caudal extremity in section is completely separated from the central pharyngeal tube. In the intervening space, the internal jugular vein passes ventrally, and, medial to the vein, the strands of the dorso-laryngeus muscle extend from the region of the larynx to the lateral wall of the neck region. The small lateralis ramus of the vagus nerve passes backward just above the anlage of the thymus body, the scattered darkly stained cells lie in the space between.

C. Amblystoma larvae, 10 to 12 mm. long. Since larvae of these lengths are very similar in developmental details, a single description of the thymus bodies in them will answer for all. Growth has been rapid, so that parts which were only outlined as loose cell masses in the earlier stages, are now well defined. This rapid development has its effect upon the thymus bodies as well as upon the other organs and tissues, so that some irregularities occur in their size, shape and general position.

The first thymus body is now (fig. 18), reduced in size, and although still attached to the pharyngeal wall, it protrudes dorsally as a finger-shaped stalk, just ventral to the facial ganglion and medial to the postero-lateral surface of the quadrate anlage. Its distal end is now even smaller than its stalk, the whole body being only about 20 micra in thickness, and occurs in but two successive sections. The mandibular trunk of the internal carotid artery passes to the lateral region of the jaw just cephalad of the thymus body, and the hyo-mandibular ramus of the facial ganglion passes to the region of the jaw just caudad to it. This

anlage (fig. 19) is composed of numerous large, loosely packed epithelial cells (separated by a light gray matrix), similar in size and shape to the cells of the pharynx with which they are in intimate relation. They are surrounded by a thin membrane—a sort of membrana propria—apparently a continuation of that of the upper surface of the pharyngeal wall. It is evident that this body is undergoing degeneration. Maurer found the body on both sides, in an Axolotl 1 cm. long, reduced to two or three yolk-filled cells completely cut off from the pharynx and lying in the connective tissue in about the same relative position as in Amblystoma. Maximow ('12), however, in 10 mm. Siredon larvae found the body still in connection with the pharyngeal epithelium, occupying a position ventral to the anterior wall of the ear, between the quadrate anlage and the facial ganglion. "Sie war in einem solchem Fall auf neun aufeinanderfolgenden Schnitten von 8 μ Dicke zu sehen und war hier sogar noch mittelst eines dünnen Stiels mit dem Pharynxepithel verbunden." On the other hand, he found the body reduced in 11 mm. larvae, to several irregular epithelial cells completely separated from the pharynx and which occurred in only 2 or 3 serial sections. Drüner, in 11 to 15 mm. stage of Siredon, found the first body still in contact with the pharyngeal cells.

The second thymus body in 10 mm. larvae is not readily found, as it is reduced to a group of loose epithelial yolk laden cells, situated just cephalad and a little medial to the point where the post-trematic branch of the glossopharyngeal nerve enters the gill arch. These cells, on the right side of the body, are four or five in number and occur in but two successive sections (10 micra each), and are closely applied to the nerve tract. I am unable to find cells corresponding to these on the left side, in 10 mm. larvae. In 11 mm. larvae, no trace of the second thymus body is found on either side of the body.

The third thymus body in 11 mm. larvae (on either side of the body) is free from the pharyngeal entoderm, and lies as a rather compact mass in the connective tissue, dorsal to the cartilage

anlage of the second gill arch, and medial to its levator arcuum
branchiarum muscle, just caudad to the posterior wall of the ear.
The body is a very small spherical mass of epithelial cells, be-
tween 25 to 35 micra in diameter. It is composed of several
(5 to 9) large epithelial cells, separated from each other by a con-
siderable amount of intercellular substance, there being no sharp
marginal membrane. Although no attempt is made here to
describe the histological relationships of the constituent parts of
the thymus bodies, it is of interest to note Maximow's results in
10 to 11 mm. Siredon larva, since the facts in Amblystoma accord
very closely to the description given by him in that form, p. 578.

Nachdem sich die Knospen abgeschnürt haben, sieht man aber im
Folgenden, dass der histologische Charakter des Thymusepithels und
des Pharynxepithels bald sehr verschieden wird. Was das Pharynx-
epithel betrifft, so sind schon bei 10.5 mm. langen Larven die Dotter-
körnchen darin viel spärlicher als früher und die Zellen selbst sind in-
folge rascher Wucherung kleiner geworden; ihre Grenzen sind viel
deutlicher. . . . .
Die Epithelzellen der Thymusknospen bewahren hingegen das ur-
sprüngliche Aussehen viel länger. Die Wucherung verläuft hier zun-
ächst sehr träge, Mitosen sind vorerst nur äusserst selten zu finden.
Zellgrenzen sind nicht zu unterscheiden und die ganze Knospe sieht wie
eine einheitliche Protoplasmamasse aus, die durch und durch mit
groben, verscheiden grossen Dotterkörnchen erfüllt ist, zwischen denen
eckige, stark deformierte, blasse Kerne zusammengepresst liegen. Die
Kerne werden mit der Zeit noch etwas heller, besonders im Vergleich mit
den Kernen der Mesenchymzellen, weil ihre Chromatinteilchen feiner
und spärlicher werden und auch die Nukleolen an Grösse abnehmen.
Die Kernmembran ist äusserst zart und ihre Konturen sind zwischen
den Dotterkörnchen nicht überall genau zu definieren. Zwischen den
letzteren liegen einzeln oder Häufchen Pigmentkörnchen zerstreut.
Eine Membrana propria fehlt an der Oberfläche der Thymusknospen
vollständig—man sieht hier keine scharfe Begrenzungslinie.

The fourth and fifth thymus bodies are similar in character,
size and position (on the two sides of the body), to that of the
corresponding bodies in 10 mm. larvae, and, like them, these are
completely separated from their respective pouches, and lie as
small rounded masses imbedded in the connective tissue close to
the lateral wall of the body. The fourth is 60 micra caudad to the
third and is almost spherical, being about 30 micra in diameter.

The fifth is some distance caudad (about 120 micra) to the fourth and is slightly oval in form, extending through three successive sections, having a minor diameter of 20 micra. In both fourth and fifth bodies, the epithelial cells retain the light yolk laden character of the cells of the pharynx and there is no well defined outer membrane.

D. *Amblystoma larvae, 13 to 15 mm. long.* Except for the persistence of the anlage of the first body on the right side in one larva 13 mm. long, both the first and second thymus bodies on either side have now degenerated. The persisting anlage is reduced to a short finger-like stalk of cells extending dorsally from the pharynx at a point between the medial ventral surface of the quadrate cartilage and the facial ganglion, just under the anterior wall of the ear. Its epithelial cells are small and thinly scattered, and the light yolky character has disappeared. In the 15 mm. larva, the degeneration of the first two bodies is complete, no trace of them being found in subsequent stages. This early degeneration of the first two bodies in Amblystoma, agrees in the essential points with facts recorded by Maurer ('88), in Axolotl, and Drüner ('04) in Siredon, and Maximow ('12) in Siredon. Drüner, however, found an epithelial follicle in two of his later stages (36 mm.), which he interpreted as remains of the first thymus body. He also found a similar follicle in an older larva (the exact length of which he does not state). Careful examination of the region of the first thymus body in Amblystoma in subsequent stages has thus far failed to show such a follicle as Drüner describes.

Shiftings of the surrounding parts, due to growth, are taking place more slowly now, so that the third, fourth and fifth thymus bodies are relatively little changed in position. All are round or slightly oval in form, and vary slightly in size from each other and from their fellows on the opposite side of the body. The margins of the bodies, as in the earlier stages, are not well defined, the cells being imbedded in an intercellular matrix, there being no membrana propria. The contained yolk, so conspicuous in the early stages is now rapidly disappearing, being replaced by a more

darkly staining substance, and the large epithelial cells have divided into numerous smaller ones.

The third body (about 100 micra caudad to the ear) is almost spherical, about 40 micra in diameter, and is slightly flattened dorso-ventrally. It has moved slightly dorsally and laterally, so that it lies dorsal and lateral to the cartilage of the first branchial arch, and immediately dorsal to its levator arcuum branchiarum muscle.

The fourth body is close behind the third (about 40 micra), but is a little dorsal and medial to it, directly above the second branchial cartilage and lateral to its levator arcuum branchiarum muscle. Like the third it is almost spherical, varying in diameter between 37 and 43 micra.

The fifth body is some distance (about 110 micra) caudad to the other two, and is more dorsal than either, lying close to and a little above the level of the middle region of the brain. It is oval and measures 44 x 53 micra.

E. Amblystoma larvae, 19 or 20 mm. long. Since the thymus bodies take positions which remain practically constant for all succeeding larval stages and their morphological relationships are well defined, the description is given in some detail. As a basis for this I use a wax model of a 19 mm. larva.

The three bodies have increased somewhat in size, but retain, on the whole, their general spherical or slightly oval form. The third and fourth are close to each other (about 95 micra apart), the fifth, however, is quite a distance caudad to these two (nearly 285 micra). The fact that the fifth body is relatively farther caudad to the fourth, than the fourth is from the third is noted both by Maurer ('88, Axolotl) and Maximow ('12, Siredon); neither however give the exact distances.

A dorsal view of the reconstructed parts, showing the three thymus bodies on the right side of the body is shown in figure 41. In size, the third and fourth bodies are about equal, being 90 x 75 micra and 75 x 60 micra respectively, while the fifth is somewhat larger than either, measuring 90 x 130 micra. It is interesting to note here that the fifth body in Amblystoma at this stage of development is nearly the same size as that given for the fifth

body in 17 mm. Siredon larva described by Maximow (136 x 87
micra). The other two, however, are a little smaller in Ambly-
stoma than those of Siredon.

The third thymus body (fig. 26) begins 140 micra caudad to the
posterior wall of the ear, where it lies in the connective tissue in
the region above the pharynx, a little medial and anterior to the
posterior caudal tip of the cartilage of the first branchial arch.
The first efferent branchial artery emerges from the medial sur-
face of the first branchial cartilage near its caudal tip, and passes
forward and medially, coursing just below the third thymus body,
and, after giving off the internal carotid artery, turns medially to
become the radix aortae. A little ventral to the anterior end of
the thymus body—between it and the blood vessel just described
—the glossopharyngeal nerve (IX, fig. 41) passes to the lateral
surface of the caudal tip of the first branchial cartilage, where it
divides into its pre- and post-trematic rami. In a similar manner,
a ramus (the first branchial nerve of the vagus, n_1,) passes
laterally, caudad and a little ventrally to the posterior end of the
third thymus body, and breaks up into its pre- and post-trematic
branches.

The fourth thymus body (fig. 27) bears the same relation to the
caudal tip of the cartilage of the second branchial arch, as the
third thymus body does to the first, but, since the arches turn
upwards and inwards at their caudal extremities, this thymus
body is a little higher as well as a little more medial in position.
The second efferent branchial artery passes from the medial sur-
face of the caudal tip of the second arch, just back of and a little
below the fourth thymus body, and courses medially to join the
radix some distance in front. The second branchial nerve (n_2) of
the vagus, passes into the gill region just back of the caudal end
of this body.

The fifth thymus body (fig. 28) is somewhat dorsal and caudal
to the junction of the third and fourth branchial cartilages at their
most caudal ends, and, since these are directed medially as well as
dorsally (see also fig. 41), this body has been forced into the space
between them and the muscles, especially the dorso-laryngeus

(*d.l.*), muscle of the side of the neck.   The lateralis nerve of the vagus passes caudad just medial to it.

All three bodies are similar in their histological appearance. They are loosely imbedded in the connective tissue, the matrix of which is composed of fine fibers.   Within the bodies, the margins of which are not clearly defined, are the larger epithelial cells, and, in addition to these, numerous other cellular elements which conspicuously modify the general appearance of the bodies. Nearly all of the yolk has disappeared, and the cells uniformly take a darker stain.   There is no membrana propria, the larger marginal cells being apparently held in place by a loose reticulum, which penetrates not only the spaces between the outer cells, but also extends inward throughout the spaces in the central area. The size and shapes of the epithelial cells differ in different sections.   (For further detailed description of the histogenesis of the thymus bodies of this stage, refer to Maximow '12, 19 mm. Siredon larva, p. 597).

F. Amblystoma larvae, 25 to 45 mm. long.   As noted above, the position of the thymus bodies in the succeeding larval stages up to metamorphosis (46 mm.), changes but little, so that only a brief consideration need be given them here.

There is little change in size and position of the bodies in a 26 mm. larva, from that just described.   The third, on the right side, is almost spherical, about 90 micra in diameter, and lies just dorsal to the first efferent branchial artery; the fourth is slightly oval (87 x 75 micra); and the fifth (110 x 130 micra), lies some considerable distance caudad.   No great difference in the histological appearance is noted.   In section (fig. 29) the fifth body (which is typical of the others also) has an outer layer of rather large epithelial cells, which enclose a central cavity of some size. There is no membrana propria, the cells being separated one from another by strands of connective tissue.

A wax reconstruction was made of the three bodies in the 35 mm. larval stage of development, but this shows little difference from that of the 19 mm. stage.   The bodies have increased in size; the fourth is larger than the third, and the fifth is the largest of all.   The third is oval (102 x 93 micra); the fourth is the same

shape but larger (118 x 95); and the fifth (slightly flattened medially) is 135 x 105 x 80 micra. In histological appearance however, two noticeable changes have taken place. The epithelial cells are now much smaller and more numerous and, except for the central area, are scattered loosely throughout the mass (fig. 30). The outer margin of the mass is now surrounded by a loose connective tissue envelope, strands of which pass between the small epithelial cells.

Aside from considerable increase in size no great difference is noted in the bodies in the 39 to 40 mm. larvae. The three bodies are oval; the third being 200 x 155 micra, and the fourth and fifth measuring 152 x 122 micra and 175 x 135 micra respectively.

In the last stage studied before the larva undergoes its transformation (45 mm. long), all of the thymus bodies have increased markedly in length, but no great change in diameter has taken place. The third is now 220 x 164 micra, and lies about 125 micra caudad to the ear; the fourth is 180 x 240 micra and the fifth is 285 x 350 micra. Because of this increase in length, the caudal end of the third, and the cephalic end of the fourth are close together (separated only about 40 micra). These two lie caudad and a little lateral to the levator arcuum branchiarum muscle of the second arch. The fifth is about 200 micra caudad to the other two, it is somewhat elongate and its cephalic end is inserted into the space between the distal tips of the third and fourth branchial cartilages, and the medially placed dorso-laryngeus muscle, its caudal end tapering to a point close to the lateralis ramus.

G. *Transforming Amblystomae.* The caudal extremity of the gill region in the early transforming larvae is undergoing most modification, so that the fourth and fifth (third and fourth branchial) clefts are closed, the pharyngeal entoderm of these persisting as mere rudiments. This readjustm   the caudal region of the gills has its effect upon the three thymus bodies, especially the fourth and fifth, which, as earlier pointed out, were intimately associated with these pouches in their genesis. The following description is based upon a wax model of the three bodies in this stage.

The third thymus body (fig. 32) is 120 micra caudad to the ear, and lies as an elongate, ovate body of considerable size (240 x 160 micra) dorsal and a little medial to the caudal tip of the first branchial arch. As yet its relation to the blood vessels and nerves of the region is little changed; the first efferent branchial artery passes to the dorsal aorta from the gill just below it, and the rami of the glossopharyngeal and vagus nerves pass into the gill region, one in front the other behind the body. The condition of the pharyngeal pouches in this region however show that the clefts are closed to the outside.

The fourth thymus body (fig. 33) is a smaller oval structure (150 x 100 micra), and is nearly 180 micra caudad to the third, but, because of the reduction of the distal end of the second arch, it lies for the most part caudal to its posterior tip. The second efferent branchial artery, has moved forward, so that now its trunk leaves the medial side of the cartilage well in front of and below the thymus body. The pharyngeal entoderm which formed the gill pouches (third and fourth branchial) is now reduced to two short blind tubes on either side, which extend caudally and laterally but a short distance from the central cavity of the pharynx. With the closure of the gill slits, and the reduction of the caudal extremity of the arches, the ramus of the vagus to this region has also disappeared.

Like the fourth, the fifth thymus body (fig. 34) lies well dorsal and for the most part caudal to the cartilage of the fourth arch. It is the largest of the three (360 x 120 micra) and lies about 60 micra behind the fourth.

In the late transforming stage all of the slits are closed, their corresponding pharyngeal pouches being recognized as mere stubs of entoderm, and the branchial arches are rearranged to form the hyoid apparatus of the adult. As a result of this extensive shifting of parts, the three thymus bodies have been forced somewhat caudad to the ear, the third being nearly 400 micra from it, the fourth and fifth lying closely behind the third.

The third thymus body is now considerably flattened laterally, having been forced by shifting into the somewhat limited space above the first branchial cartilage, between the dorso-laryngeus

and the trapezius muscles and the skin of the lateral surface of the body. It is about 240 micra long (its long axis parallel to the long axis of the body), but is flattened laterally, its shortest axis is less than 100 micra.

The fourth, similar in shape and position, is close behind the third (only one or two sections intervene, about 60 micra), but it is a little dorsal to the latter and is flattened against the dorsolaryngeus muscle. It is about 270 micra long and 60 micra in minor diameter, and somewhat narrowed at its cephalic end.

The fifth thymus body is close behind the fourth (about 60 micra), and is flattened laterally and closely pressed against the muscles of the region. It is the largest of the three, discus-shaped, measuring 500 micra in its major, and about 75 micra in its minor diameters.

H. The thymus gland of the adult. Since the thymus gland in certain adult Amblystomae has been the subject of several descriptions, from both the morphological and histological standpoints (Simon, '45, Axolotl; Leydig, '52; Siebold and Stannius, '56; Fischer, '64, Siredon; Bolau, '99, A. tigrinum; Dustin, '11, Axolotl and others) the description here is brief. In addition to two series of microscopical slides through the head and neck region of A. punctatum (one just metamorphosed, the other an older adult), several specimens (A. tigrinum, 15 cm. and 22 cm. long; A jeffersonianum, etc.) were dissected. Although minor differences occur in the location and appearance of the gland in the individuals, on the whole its relationships are fairly constant. Variability in the gland was noted by Bolau ('99) who says, "Ein Constanz im Auftreten der Thymus der Zahl nach habe ich bei Amblystoma tigrinum selber oder bei der weissen Varietät nicht finden können. Die Zahl der Drüsen wechselte nicht nur individuell, sondern ich fand auch Verschiedenheiten bei demselben Thier auf den beiden Seiten." The following description is based upon the dissection of A. tigrinum.

The gland is exposed (fig. 37) by removing the skin from the side of the neck caudal and dorsal to the angle of the jaw. It is generally a light gray, somewhat elongate, flattened, three-lobed

structure of considerable size (3 to 6 mm. long, and 0.5 to 1.3 mm. broad), lying in the loose connective tissue just dorsal to the caudal region of the first branchial cartilage, and closely pressed against the deeper lying dorso-laryngeus muscle (*d.l.*). (In some cases the surrounding connective tissue is deeply pigmented in the region of the gland so that its light color is masked, in others this pigmented area is some distance below, enveloping then the carotid gland and epithelial bodies, described below).

The three lobes, one behind the other, are usually in a line, a little oblique to the longitudinal axis of the body. The anterior lobe (probably the third thymus body of the larva) is a flattened bean-shaped body (between 1 and 2 mm. long, and 0.3 mm. broad) lying in the angle between the mandibular-pectoralis (*m.p.*) and the cephalo-dorsalis (digastric) (*di.*) muscles, its cephalic tip being partly covered by the caudal margin of the latter. The second lobe (probably the fourth larval body) is almost spherical (about 1.5 mm. in diameter), and lies a little dorsal to, but close behind the first. The posterior lobe (probably the fifth larval body) is a flattened ovoid body and is the largest of the three.

The large blood vessels (figs. 36 and 40) in this region—the internal jugular vein, the internal carotid artery and the aortic arches—are exposed by removing the digastric, mandibular, and pectoralis muscles, and reflecting the cartilage and muscles of the hyoid apparatus. The connective tissue in which the gland is imbedded is well supplied with blood. A small branch from the second aortic arch and one from the external carotid artery enter the region. (For the details of the vascularization of the thymus gland in adult Axolotl and a discussion of its histology, see Dustin '11).

The region of the gland is innervated by several rami of the glossopharyngeal (*IX*) and vagus (*X*) nerves. These emerge from the region just caudad to the ear (can be seen after reflecting the muscles in this region), the gland itself and its surrounding connective tissue being innervated by a small twig from the ramus cutaneus (*IX*) as Fischer ('64) long ago pointed out in Siredon.

2. Thyreoid

The anlage of the thyreoid is first recognized in Amblystoma, as in a number of the other amphibians in which its development has been studied, (Triton, Maurer '88; Muthmann, '04; Necturus, Miss Platt '96; Hypogeophis, Marcus, '08; and Axolotl, Maurer, '88; and Muthmann, '04) in the late embryo and early larval stages. Muthmann ('04) describes its earliest appearance in embryos of Triton and Axolotl of 21 somites, but carries his study only to the hatching stage, 30 somites (probably about 4 mm. long). In the following account the description of it begins with embryos 5 mm. long, thus roughly corresponding to the last stage described by Muthmann.

A. *Amblystoma larvae, 5 mm. long.* At this stage, the general features of the pharyngeal region are not much different from those earlier described in dealing with the thymus bodies. The place where the mouth is to form is easily recognized, being indicated by an extensive oral plate, into which there extends from behind, the now spacious pharyngeal lumen, lined throughout by a comparatively thick layer of yolk-filled entodermal cells. The caudal end of the lumen is continuous with the rest of the primitive alimentary tract, which dips ventrally in the region caudad to the heart to form the anlage of the liver. As yet the pharyngeal pouches are indicated by mere grooves along the sides of the pharyngeal tube, the dorsal wall or roof of the tube being composed of a rather uniform layer of cells. The thyreoid body arises from certain cells lying in the median floor of the pharyngeal tube, in the region between the oral plate and the heart.

In the region of the early anlage of the hyomandibular pouch (not the second branchial, as Maurer '88 described in Triton), and in front of the anterior limits of the pericardial wall, the entoderm in the mid-line of the floor of pharynx thickens and at the same time folds in such a way as to form a very shallow cuplike depression which protrudes ventrally and a little caudal between the mesodermal cells of the third somite of the head below. This is the early anlage of the thyreoid, and, because of its close proximity to the heart region in the earlier developmental stages,

was interpreted by Brachet ('98) as a part of the heart (see Muthmann, '04, fig. 31, which was taken from Brachet's fig. 8, p. 34) his error being later corrected by Muthmann.

The marginal limits of this anlage are not well defined. Figure 1 represents a section taken as nearly as possible through the medial sagittal plane of the thyreoid anlage of this stage. On either side of this section, the ventrally directed fold of entoderm is less pronounced. Transversely, the anlage is cut in five successive sections (10 micra each), the general appearance of the anlage is shown in figure 2, and somewhat in more detail in figure 3. On either side of the anlage, mesodermal cells are developing the mandibular arteries, but there are no other structures yet formed in this region. The approximate lateral limits of the anlage in this stage are indicated by r,r.

From the phylogenetic standpoint much interest attaches to the question as to whether the early anlage of the thyreoid in the amphibia is solid or hollow. In Amblystoma, it is evidently solid, and thus is similar to the structure found in Triton, Siredon, Salamandra by Maurer ('88); Salamandrina by Livini ('02); and Triton by Muthmann ('04). Miss Platt ('96), on the other hand, claims that not all the urodeles have a solid thyreoid bud, but some may have a hollow one, as was the case in Necturus described by her. Marcus ('08), finds a hollow anlage in Hypogeophis, a Gymnophionian. For the anurans, contradictory statements occur in the literature. In Bufo and Rana, Maurer ('88) claims it is first vesicular and two days after hatching it becomes solid, but W. Müller ('71), observed a solid one in Rana temporaria and R. platyrrhinus, in which the first lumen appeared in 25 mm. larvae, after the gland had divided into halves.

In Amblystoma, the cells of the solid distal end soon proliferate and form an elongated cylindrical bud in the stage next to be described.

B. Amblystoma larvae, 8 mm. long. Before proceeding to the account of the thyreoid anlage in this stage, certain shiftings due to growth of the surrounding parts may be described. By marked increase in size of the brain and spinal cord above; the forward and upward growth of the pericardial chamber and its

contents below; and the lateral growth of the walls of the pharynx to form the pharyngeal pouches; the pharyngeal cavity has been greatly modified, being depressed dorso-ventrally. Below, and to the sides of this pharyngeal tube, in the region between the thickened oral plate (the mouth being still closed) and the anterior wall of the pericardial chamber, numerous accumulations of mesodermal cells occur, which have a considerable effect upon the position of the thyreoid anlage in this and subsequent stages. Figure 45 represents a reconstructed portion of the thyreoid anlage in this stage, and shows its relation to the pharyngeal entoderm, and the accumulations of mesodermal cells which are to form the copula and the cartilages of the branchial apparatus.

The thyreoid anlage is now a solid, cylindrical rod of cells (*tr.*, fig. 45) of considerable length (about 100 micra long) connected anteriorly with the cells of the medial ventral floor of the pharynx. Its distal end extends in a horizontal direction toward the anterior wall of the pericardial chamber, but stops short of the latter by about 40 micra. The fact that the thyreoid outgrowth in Amblystoma does not reach the pericardial wall in this, and in later stages, is a point of difference in its development from that recorded by Miss Platt in Necturus. In describing the thyreoid outgrowth in a 13 mm. larva, which from her figure 6 (p. 562), corresponds closely to the stage here under discussion, she says, ''From the floor of the alimentary canal, the thyreoid outgrowth extends backward as a solid bar of tissue to the anterior wall of the pericardium with which its posterior cells come into intimate contact.'' This fact she further emphasizes in her conclusions, ''it remains in contiguity with the pericardium as long as its union with the floor of the branchial cavity is retained.'' In none of the developmental stages of Amblystoma have I seen any intimate relation of the thyreoid anlage to the anterior wall of the pericardium.

Figure 4 is a medial sagittal section through the thyreoid anlage (*tr*) in this stage. At the stomodeum (*st*), the entoderm touches the ectoderm and forms an oral plate of considerable thickness where later the mouth is to break through. In the

space above the thyreoid anlage and between it and the ventral wall of the pharynx is an accumulation of numerous darkly staining mesodermal cells (co), which form the early anlage of the copula of the branchial arches, while below the anlage or a little to either side of it, close to the ventral surface of the body wall, are the early anlagen of the genio- and mylo-hyoideus muscles. (The last named structures are shown in transverse sections, see figure 7, which is taken in the plane indicated by the line d-d of figure 4.)

Figure 6 is through the most caudal lateral extremity of the hyomandibular pouch (taken in the plane indicated by the line c-c of figure 4), and shows not only the thyreoid anlage (tr) at its point of junction with the pharyngeal entoderm of the middle line of the floor of the pharynx, but also the anlage of the first thymus body as already described. The anlage of the genio-hyoideus muscle is a mass of illy-defined cells at the lateral side of the thyreoid, while the anlage of the mylohyoideus muscle (m.hy) is somewhat more plainly outlined immediately below it.

Figure 7 is about 75 micra caudad of the one just described (taken in the plane indicated by the line d-d of figure 4) and shows the thyreoid in this region completely isolated from the pharynx. A broad sheet of mesodermal cells to form the copula and branchial arches is now interposed between the thyreoid anlage and the pharyngeal floor, while below is the anlagen of the muscles, which are much the same as in the other section.

In this stage a differentiation in the appearance of the cells of the different germ layers has begun. Those of the pharyngeal wall and of the thyreoid outgrowth are still filled with yolk granules and are much lighter in color than those of the mesoderm in which the yolk is rapidly being absorbed. This differentiation is, however, more pronounced in the stages immediately following. Microscopically there is little difference between the cells of the pharynx and those forming the distal region of the thyreoid anlage, bothhaving large round or oval nuclei.

C. *Amblystoma larvae, 9.5 to 11 mm. long.* This stage may be characterized as the period of most rapid development, both for

the general structures, and for the thyreoid anlage. The mouth is now open except in the 9.5 mm. larvae; the gill pouches have fused with the ectoderm, and the slits are open. The ventral mesoderm forms well defined structures—the branchial arches, and blood vessels—in the region below the pharyngeal tube.

The anlage of the thyreoid in the 9.5 mm. larvae, is slightly longer (about 140 micra) than that in the 8 mm. larvae, the proximal end is still connected with the pharyngeal entoderm, while the distal end is divided for about 40 micra into right and left halves, thus giving to the whole a Y-shaped appearance, with the forks of the Y much shortened. Figure 13 is a section through the caudal region of the anlage in this stage, the right and left halves lying just dorsal to the geniohyoid muscle. In the region above the thyreoid anlage, the mesodermal cells plainly show the relative positions of the anlagen of the copula (co) in the medial region, and the hyoid (hy) the and first branchial arch (c.br.1), the relations of which will be detailed in 10 mm. larvae, below. The inferior jugular vein lies a little lateral to the thyreoid anlage, while the external carotid artery is smaller and is latero-ventral to the jugular.

In the 10 mm. larvae the relationships differ considerably from those just described. The thyreoid anlage has severed its connection with the floor of the pharynx, and in addition is divided completely into right and left halves, which assume a position a little lateral to the geniohyoideus muscle of either side, the cephalic ends of each being about in the plane with the junction of the first branchial arch with the copula. The relative positions of the thyreoid anlage and the developing copula and branchial cartilages in the present and previous stages are the result of differential growth. The thyreoid anlage is carried backward below the copula, the keel of undifferentiated tissue extending ventrally from the copula (possibly the anlage of the external rectus muscle of the hyoid or the first branchial cartilages) intervening between the halves. Maurer ('88, p. 360), in describing this stage in Triton says,

Dann (two or three days after hatching) beginnt die Differenzirung des knorpeligen Kiemenapparates und die Kiemenspaltem öffnen sich.

Die Schilddrüsenanlage wird von ihrem Mutterboden weiter entfernt, durch die Copula des Hyoidbogens. Zugleich beginnt sie sich zu theilen, so dass an fünften Tage die beiden Hälften nur durch einen dünnen Isthmus zusammenhangen.

The division of the thyreoid anlage described above leads to the brief consideration of two points; the fate both of the cells which formed the connection between it and the pharynx (the duct of the other glands) and of those which connected the halves before their separation. Miss Platt ('96), in Necturus, found cells from the anterior part of the 'broken outgrowth' (thyreoid) distributed upon the dorsal surface of the mylohyoideus muscle, which were finally lost in the muscular tissue. She also found a few scattered cells from the anterior part of this outgrowth, above the level of the geniohyoideus muscle, the fate of which she could not follow, but in her material they did not develop into accessory thyreoids.

Maurer ('83) in Triton, on the other hand, found cells in this region persisting for a considerable length of time, which he interpreted as accessory thyreoid. These he thought were originally part of the isthmus, which eventually formed a group of four or six cells containing colloid, lying in the mid-line between the gen ohyoideus muscle of either side. He says, p. 361,

In Folge dieser raschen Lageveränderung der Schilddrüse, Kurz nachdem sich die oft unregelmässige Theilung vollzogen hat, bleiben häufig, besonders weiter vorn in der Medianlinie zwischen den Musculis geniohyoideis, Theile des ursprünglichen Isthmus liegen, welche später als vordere unpaare aus vier bis sechs Acinis beste hende colloidhaltieg Nebenschilddrüsen sich erhalten.

In Amblystoma, I am unable at any stage to locate cells in this region which may be interpreted as an accessory thyreoid. There are, to be sure, a few scattered cells, lying in the space between the geniohyoideus muscles and the copula in this region, but these are similar in appearance to the numerous mesenchymatous cells of his same region, and are not sharply differentiated from them. If these s attered cells can be looked upon as the remains of the isthmus of the two halves, it is clear that they are rapidly degenerating, and have lost the staining quality which is still retained

by those of the thyreoid, and are being dispersed into the surround ng connective tissue in a manner similar to that described by Miss Platt in Necturus, and do not form persisting follicles like those which Maurer interpreted as accessory thyreoid in Triton.

Each half of the thyreoid is now an elongate ovate, mass of epithelial cells (about 150 micra long), lying in the connective tissue, its long axis paralleling the sternohyoideus muscle of either side, the anterior end of the thyreoid appears in the same section with the anterior wall of the ear, and the caudal end stops short of the anterior wall of the pericardial chamber by 60 or 70 micra. Although somewhat interrupted and irregular in places, the cells of which the halves are composed, are, for the most part solidly packed as in the earlier stages, and they retain to a considerable degree, the yolk-filled character as do the cells of the pharyngeal wall at this stage. The principal blood vessels of the region (the inferior jugular vein, and the external carotid artery) although faint in outline, are fairly easily recognized; these, and their branches running for the most part through the region lateral, and a little dorsal to the thyreoid of either side. As development proceeds, the cells of each half of the thyreoid pass laterally and dorsally, so that they soon come to lie close to the medial wall of the inferior jugular vein.

D. Amblystoma larvae, 13 mm long   In the 13 mm. stage, the cells of the thyreoid have changed noticeably in their appearance and arrangement. They no longer form solid anlagen, but are more scattered throughout the surrounding connective tissue. For the most part they are free from one another, but are arranged in a broken line on either side of the body, paralleling the inferior jugular vein for a distance of about 300 micra. It is difficult to make more than general statements of the arrangement of these cells throughout their extent, since this varies greatly on the two sides of the body. As a rule, the cells have migrated laterally and dorsally, and now are arranged about the wall of the inferior jugular vein, and occupy in places, the spaces between the walls of the jugular and its junction with numerous smaller branches, some even lying in the space between the jugular and the lingual

branch of the carotid artery which lies some distance lateral and dorsal to it.   In color, and general form and size, these cells are well differentiated from the surrounding mesenchyme on the one hand, and from the pharyngeal cells from which they have had their origin on the other.   These differences in color and shape are interesting, since Miss Platt ('96), in her description of this stage in Necturus remarks that, "they are distinguished from the surrounding mesenchyme merely by their closer grouping." Maurer ('88) notes the condition of the thyreoid cells in Triton in this stage in the following terms (p. 362),

So lange die Zellen der Schilddrüse Dotterblättchen enthalten ist das Organ leicht zu erkennen.   Zwei bis drei Wochen nach dem Verlassen des Eies verschwinden die Dotterblättchen und est hält dann eine Zeit lang schwer, die Schilddrüse aufzufinden.   Sie besteht jederseits aus einem wenig vortretenden soliden Zellschlauch, der an dem bezeichneten Platze, vor dem ersten Arterienbogen, gerade hinter dem zweiten Keratobranchiale, zur Seite des Sternohyoideus liegt.

E. Amblystoma larvae, 15 mm. long.   Aside from the continued growth of the surrounding parts, but little change is noted in the relationships of the thyreoid cells from that of the 13 mm. stage. Although distributed over practically the same area and the same region of the body (occurring for 380 micra along the inferior jugular vein, and extending from the point of junction of the first branchial cartilage with the copula to the region of the anterior wall of the pericardium), the cells have increased in numbers and are more compact; a few (three to six) in a group, and several groups usually being cut in the same section.   This arrangement of the cells in groups is the first indication of the formation of follicles, which are developed rapidly in the stages immediately following this.

Contradictory statements are made as to the exact manner in which the thyreoid follicles are developed from the primary anlage.   W. Müller ('71), claimed that the thyreoid gland in all vertebrates passes through three stages: (1) a severing of the anlage from the pharynx; (2) the formation of a network of tubes of glandular epithelium; and (3) the formation of follicles from these tubes.   Stockard ('06), in Bdellostoma and Gudernatsch

('11), in the teleosts have shown that the second stage may be suppressed or absent. In the trout, Maurer ('86) says that the formation of follicles is very simple; solid buds form on the primary vesicle (which is here hollow instead of solid as in the urodeles), central cavities soon form in these, each then separates. L. Muller ('96), believes that new follicles originate in the teleosts from the older as buds from the epithelium which subsequently become free. Gudernatsch ('11, p. 721) says,

The reptiles, birds and mammals are said by the majority of observers to show a vesicular thyreoid anlage, which changes into a compact organ from which follicles later originate. Kölliker, however, observed in the rabbit a thickening in the ventral wall of the pharynx from which a wart-like solid process was cut off. Born also records the same for the pig.

It would seem, from facts recorded above, that the formation of the follicles in Amblystoma differs slightly from Maurer's account of the urodeles he studied (Triton, Salamandra atra and Siredon), in that the solid cylinder of epithelial cells forming the thyreoid an age (right and left) breaks up into groups of cells which become scattered loosely about the wall of the inferior jugular vein, or in the connective tissue of the region, and subsequently these isolated cells increase in number by mitosis and form the walls of the follicles. Although Miss Platt, ('96) in her description of the development of the thyreoid of Necturus does not enter the discussion of this point, it is very evident that she noted a similar condition when she says,

One finds in place of the solid thyreoid outgrowth (17 to 18 mm. larvae), two lines of cells extending obliquely outwards . . . forming neither a solid mass, nor walls of closed vesicles (follicles). These cells of the thyreoid migrate dorsal wards . . distinguished in an embryo of 19 mm. as a group of cells lying on each side, between the lateral surface of the sternohyoideus. . . In an embryo 21 mm. one or two small vesicles (follicles) are found where the group of cells (th.) lie in figure 8. These vesicles increase in number as the embryo grows until in a little amphibian of 46 mm., they constitute a considerable mass of vesicular tissue.

The relative position of these cells groups (which we may now call follicles) differ not only in individuals, but on the two sides of

the body in the same individual, so that only a general description is necessary The most anterior group on the left side is cut in the same section with the anterior wall of the ear. It consists of six to eight cells lying close against the dorsal wall of the inferior jugular vein, a small group on the right side (the most anterior of this side) occur some distance back of this (about 60 micra), the cells of which are pressed against the latero-ventral surface of the sternohyoideus muscle of this region. From this point caudad for 350 to 80 micra on either side, various other thyreoid follicles occur, the exact details of the positions are omitted here.

These developing follicles in this stage are also variable in form, Some are globular or elliptical, with a well defined margin of outer cells and have a lumen, while others are very irregular in outline, and are solid masses of cells with no cavity. The first of these types usually occur as isolated ollicles, lying singly in the supporting tissue, while the others occur in regions of greater congestion and crowding. So far as I am able to say, there is no enveloping membrane (membrane propria) in this stage, either surrounding the individual follicles, or enveloping collectively the follicles to form the gland.

F. Amblystoma larvae, 19 mm. long. In the 19 mm. stage, and all subsequent larval stages studied, the thyreoid follicles are so massed as to form a well defined structure on either side of the body, the definitive thyreoid gland, which changes but slightly in its position. The follicles here, as in the 15 mm. larvae, are irregular in size, form and relative distribution about the wall of the inferior jugular vein, and extend in a longitudinal direction a little more than 400 micra on each side. As before, the most anterior follicles are cut in section with the anterior wall of the ear, the most caudal occur about 80 micra in front of the anterior wall of he pericardium. Typically, the follicles in this stage are relatively small, spherical or slightly ovate bodies, varying between 19 and 36 micra in major diameter, and showing in section, a layer of cuboidal cells about the outer margin, enclosing a conspicuous cavity, which contains a fluid of some sort but which as yet, does not have the staining properties of a colloid. In some cases a

slight marginal membrane about the layer of cuboidal cells is noticeable, in others apparently there is none.

The follicles are more numerous throughout the central region of the gland than at either extremity, and they are separated from each other by conspicuous spaces (large or small in comparison to the individual follicles of the region) in the connective tissue in which they are imbedded. These spaces for the most part are lymph vessels, although here and there are small veins (especially those returning blood from the geniohyoideus and sternohyoideus muscles), which unite with the inferior jugular vein in this region as pointed out above. This complex of follicles, lymph spaces, blood vessels and loose cells and strands of connective tissue, is maintained in the central portion of the gland, but at the two extremities, these relations are not so conspicuous.

So far as I have observed, there is no good evidence that either the inferior jugular vein, or the smaller twigs from the muscles break up into a 'rete mirabile' in this region, a fact which, when taken in connection with conditions in later larva stages is of considerable interest, and concerning which more will be said below. No attempt is made here to describe the histological relationships of the parts in the thyreoid gland. The gland changes but little in the succeeding larva stages, and hence but little space need be given to it.

G. *Amblystoma larvae, 26 mm. long.* In the 26 mm. larvae the gland is distributed along the inferior jugular vein for a distance of 350 or 400 microns. A few isolated follicles lie in the same section with the anterior wall of the ear, and on the right side these lie some distance medial to the inferior jugular vein. There is no regularity in the arrangement of the follicles on either side. Most follicles have conspicuous lumina, but as yet these contain no colloid. The lymph spaces are somewhat larger and more prominent, but otherwise there are no great differences from the 19 mm. stage.

H. *Amblystoma larvae, 35 mm. long.* The thyreoid in the 35 mm. larva has elongated, now being between 450 and 500 micra in

length on either side, the anterior end occurring in sections with the anterior wall of the ear, the caudal region extending to within 60 or 75 micra of the anterior wall of the pericardium. In addition to this increase in length, the gland is now enveloped in a very thin connective tissue sheath, which surrounds not only the follicles and lymph and vascular spaces, but also includes the large trunk of the inferior jugular vein for a considerable distance in its course through this region. The inferior jugular vein enters this sheath, but in this and later stages, it does not break up into a 'rete mirabile.' The external carotid artery, which courses lateral to the gland, is not enveloped in the connective tissue sheath of the gland and has no intimate connection with the thyreoid in this stage. The lumina of the follicles are now larger in comparison than before, but as yet there is no colloid.

I. Amblystoma larvae, 39 mm. long. In the 39 mm. larva, the thyreoid gland is a well defined ovate, somewhat flattened body, a little less than a millimeter long, lying dorso-medial to the inferior jugular vein on either side of the body. The connective tissue envelope is here more pronounced, and a few of the follicles contain colloid. Figure 47 represents a wax reconstruction of the gland and the cartilages of the branchial apparatus in this stage. The follicles are rather large throughout, and are more numerous in the central and caudal region.

J. Amblystoma larvae, 45 mm. long. In the 45 mm. stage (the last to be described before transformation), the gland is about a millimeter long. Nearly all of the follicles are now filled with colloid. In a section through the central region of the gland, (fig. 31), eleven follicles are cut; these almost completely surround the inferior jugular vein, which in this region is within the connective tissue sheath. The follicles are separated from one another by vascular and lymph spaces (*v.v.*). Nearly all the follicles in this section show an outer lining of cuboidal cells with spacious lumina within.

K. Transforming Amblystomae. In the early stages of transformation, the gland is noticeably shorter, and, with the greatly increased size of the surrounding muscles (genio- and sterno-

hyoideus and adductors) it has a more lateral and ventral position close to the ventral wall of the body. It is now between 800 and 900 micra long, this reduction continuing in the two succeeding stages, to between 712 to 775 micra and 550 to 660 micra respectively. To compensate for this shortening, the follicles are crowded together, those at either extremity having apparently been pushed into the central portion by the growth of the surrounding parts, the result being that the central portion of the gland is now greatly enlarged and elliptical in section, measuring between 475 to 625 micra in major, and between 225 to 390 micra, in the minor diameter of the ellipse.

The following description is based upon a wax reconstruction of the gland in this stage, (fig. 43). The follicles are few (fourteen or fifteen in all), but have increased in size; some being now about 100 micra long, and between 50 and 75 micra in diameter. The anterior extremity of the gland consists of a single large follicle, which lies dorsal to the wall of the inferior jugular vein, and, like all the others, is full of colloid. Just behind and a little lateral to this, is a second follicle of about the same size, similarly placed upon the wall of the jugular. Following these, numerous other follicles are irregularly arranged about the vein so that in the caudal region of the gland, the vein is completely surrounded by them. The spaces between the follicles are occupied by small blood vessels and the lymph sacs.

Later, when the distal ends of the second, third and fourth branchial arches are greatly reduced in length, and the corresponding pharyngeal clefts are greatly modified (the third and fourth closed, and represented by short lateral processes of entoderm; the second still open but reduced); these changes, although not affecting directly the thyreoid, modify the hypoglossal and the hypobranchial musculature and blood vessels, which, in turn, have a direct effect upon this structure. The gland on either side is short and ovate, measuring in the long axis of the body 712 micra, on the right, and 775 micra, on the left, and in section in its central region varying from 390 to 510 micra in diameter. It lies, as before in the reduced space between the now much enlarged sterno- and mylo-hyoideus and adductor muscles, and is close

upon the wall of the inferior jugular vein. The gland appears in the same section with the anterior wall of the ear, but the anterior wall of the pericardium is about 250 micra caudad to its posterior end. This relationship is interesting, since in the next stage there is a difference in the relative position of the heart and the gland.

When transformation has proceeded so far that the arrangement of the hypoglossal muscles, branchial arches, blood vessels and other parts is practically that of the adult, only the hyoid and the first and second branchial arches remain, the latter (second arch) is much reduced in size and length; the gill-slits are completely closed, only mere rudiments of pharyngeal entoderm marking their former position. Simultaneously with this rearrangement, the thyreoid gland of either side has been converted into a compact, egg-shaped mass of follicles, and has migrated laterally and a little caudally, so that it now lies lateral to, and a little in front of the pericardial chamber, in the space just back of the caudal tip of the second branchial cartilage, between the adductor muscle of the first branchial cartilage, and the large medial sternohyoideus muscle. The position of the gland relative to the cartilages and blood vessels is shown in figure 46.

The follicles of the gland vary greatly in size and shape, and in the color of the colloidal contents, some follicles being light gray, others a brownish or yellowish hue. The differences in the number and size of follicles and the variety in their histological appearance, when compared to those of the later larval stages, and to those of the adult, would indicate that the gland itself is undergoing a metamorphosis of some sort. Although it is not the purpose to enter here a discussion of the histology of the gland, it is very evident that the larger follicles are giving rise to smaller ones by budding, and that this process of follicular formation, begun in the late transforming stage, continues in the adult. Different opinions have been expressed as to the method of formation of follicles throughout the vertebrates (Baber, '76; Anderson, '94; Forsyth, '08; and Gudernatsch, '11).

L. Adult Amblystoma. The relations of the thyreoid gland to the structures in the neck region of several adult urodeles, has

been described by a number of investigators (Maurer, '88; Bolau, '99; Drüner, '04; and others) but little is said concerning the morphology of the gland in Amblystoma. Maurer, after describing the development of the gland in Triton remarks, (p. 363) "bei Siredon pisciformis findet die Anlage der Schilddrüse . . . . in der gleichem Weise bei Triton taeniatus statt," but he says nothing concerning the later larval and adult stages. Although, in the main, the facts of development accord with Maurer's description of both Triton and Siredon, Amblystoma differs in one or two points from either. I therefore give here a brief description of the gland, based for the most part upon the gross dissection of the gland in several adult Amblystomae, chiefly A. tigrinum.

By removing the skin and caudal portion of the mylohyoideus muscle of the ventral surface of the neck the gland is seen (tr., fig. 36) imbedded in rather loose connective tissue, in front of and a little lateral to the pericardium, flanked medially by the geniohyoideus, laterally by the hyoideus internus of the first branchial arch, and dorsally by the deeper lying sternohyoideus muscles. It is a rather flattened ovate body (between two and three millimeters long, and from one to two and a half millimeters broad), differing in shape and size in individuals, and on either side of the same specimen. It is gray or yellowish or even sometimes brownish in color. Usually the cephalic end is pointed and the caudal blunt. Although somewhat variable in their relation to the gland, the blood vessels are very conspicuous. The inferior jugular vein is now large and prominent, and passes lateral to the gland as it courses toward the heart; the external carotid artery parallels it closely as it passes forward from the carotid gland.

The gland and blood vessels were removed from several individuals and were cleared and mounted. Four glands were thus treated; both right and left glands of A. tigrinum 15 cm. long, and one gland each from other specimens of the same species, 17.5 cm. and 22 cm. respectively. The blood supply of the gland is variable. In most cases, the interfollicular net-work of the gland is formed by the small venous twig which passes caudad

from the lateral surface of the geniohyoideus muscle; this enters the anterior medial region of the gland and emerges from its posterior medial surface to join the large trunk of the jugular vein some distance caudad. In one instance the venous connection of the gland with the jugular vein is made by a small twig coming from the sternohyoideus muscle. In none of the preparations does the large jugular trunk itself break up to form the complex 'rete mirabile' which has been described by Maurer in Triton alpestris (see his figure 22, a and b).

The gland is rarely connected with the carotid artery. In only one case could I trace a small thyreoid artery into the vascular net-work of the gland. Such a condition in Amblystoma accords with Maurer's description in Triton. In the live Triton, he showed that the circulation of the blood continued in the gland when, with forceps, he closed the carotid artery just caudad to the gland. On the other hand, he was able to bring the circulation to a standstill when he closed the twig of the jugular vein in front before it entered the gland. He therefore concludes:

dass die Schilddrüse der Tritonen in den venösen Kreislauf eingeschaltet ist, eine Thatsache die ich nirgends erwähnt finde und welche wohl auch für die Schilddrüse einzig dasteht. Von mechanischem Wert für die Blutcirkulation des Kopfes kann dieses Verhalten nicht sein, weil sehr häufig nur ein Ast der Jugularvene diese Wundernetzbildung zeigt. Auch die Ernährung der Schilddrüse leidet nicht darunter, da aus vielen Hautkapillaren Blut, welches somit die Hautathmung durchgemacht hat, in den Stamm der äusseren Jugularvene übergeht und somit der Sauerstoffgehalt immer noch ein genügender ist, zumal ja auch das Arterein blut hier ein gemischtes ist.

It is also probable that it is not necessary that the thyreoid in Amblystoma be connected with the carotid artery in order to carry on its metabolic processes, since the blood in the jugular twig, returning from the capillaries of the skin contain sufficient oxygen for the functioning of the gland.

3. Postbranchial body

In its development and morphological position, the post-branchial body in Amblystoma is 'postbranchial' in the sense

Maurer used the term (in Triton, Siredon, and Salamandra), and not 'suprapericardial' (van Bemmeln) as Miss Platt found it in Necturus. The body is caudal to the last gill-pouch (fifth visceral or fourth branchial) in about the position where one would expect to find a sixth visceral pouch (fifth branchial), and not cephalad to the posterior pouch as in Necturus. As a rule, the body in Amblystoma is asymmetrical in its development, usually occurring on the left side (in this respect agrees with Triton and Salamandra) but in one individual (19 mm. larva, see below) it appears on the right side as well, and in this stage more closely agrees with the conditions described for Necturus (Miss Platt '96) and Hypogeophis (Marcus '08), although the body on the right is smaller, and possibly persists but a short time.

A. Amblystoma larvae, 8 mm. long. The earliest stage in which the anlage of the body is recognized with certainty in my material is in larvae 8 mm. long. Maurer distinguished it earlier in Triton, even in an embryo before hatching, but Miss Platt, begins her description of it in Necturus, with a much larger stage, (15 mm.). Since the region of the body where the anlage occurs has been described in some detail in the discussion of the thymus, it is only necessary to say here that the first appearance of the postbranchial body is in sections which pass through the ganglion of the vagus. Here, on the left side, a portion of the ventral floor of the pharynx, between the anlage of the fourth branchial pouch (fifth visceral), and the anlage of the glottis, becomes slightly thickened. The exact extent of this thickening —both laterally and anterio-posteriorly—at this stage is impossible to define, as it fades out on all sides, but cells in its central region begin to extend ventrally into the connective tissue above the pericardial wall there to form the anlage of the postbranchial body. Figure 10a is through the thickened portion where the ventral cells forming the postbranchial anlage is most pronounced. The lateral limits of this anlage are marked roughly at points *r-r*, medial to which is the anlage of the glottis, and lateral to which is the anlage of the fourth branchial pouch, while four or five large procartilage cells below them mark the position of the anlage of the fourth branchial arch. At this time there is no other notice-

able differentiation between cells of the pharynx and those of the postbranchial anlage, both containing a considerable amount of yolk.

B. *Amblystoma larvae, 9.5 mm. long.* In this stage there is considerable advance in the development of the postbranchial body. By proliferation, cells have been pushed in a ventral direction from the thickened anlage, so that they now form a well defined, short-stalked, cylindrical body, protruding vertically downward toward the dorsal wall of the pericardium. This anlage is about 40 micra long, and between 25 and 30 micra in diameter. The cells of the anlage are closely packed; the body is solid; and as yet no histological differentiation is apparent. That this early anlage is solid in Amblystoma, and remains so for a considerable length of time after the pharyngeal pouches have opened to the exterior, would seem to be a point (though not conclusive) in favor of Maurer's interpretation of it as a 'post-branchial' structure, and not a rudimentary sixth pouch, a view which Greil ('05) opposes. On the other hand, this solid structure in Amblystoma is quite different from the early anlagen of the bodies described by Miss Platt in Necturus, where she finds them—one on either side—forming ''small vesicles immediately below the floor of the branchial chamber, with which they are still connected by stems. . . . . They are formed by a single layer of yolk-laden cells whose nuclei are near the surface of the vesicle, while the yolk granules are aggregated at the centre.'' Figure 15 is a transverse section through the postbranchial anlage (*pb.*) at this stage, and shows its relation to the surrounding structures. This section is just caudal to the posterior wall of the ear, and cuts the glossopharyngeal and vagus ganglia, as well as the internal jugular vein and the first and second efferent branchial arteries. It also shows the lateral extensions of the successive pharyngeal pouches (third, fourth and fifth visceral) which extend to varying distances toward the ectoderm of the lateral surface of the body, and between which occur the anlagen of the branchial cartilages.

C. *Amblystoma larvae, 10 to 11 mm. long.* In the 10 mm. larvae, the anlage has lengthened (now about 60 micra long) and reaches

nearly to the dorsal wall of the pericardium, and at the same time it is reduced in its minor diameter so that it is a solid finger-shaped spur of entodermal cells, reaching from its point of contact with the pharynx toward the pericardial chamber. It is shown in figure 20 *pb*. As yet it has no lumen, and its cells show no marked histological differentiation from those of the pharyngeal wall.

In the 11 mm. larvae, although still connected with the pharyngeal wall, the axis of the anlage is changed noticeably, the distal end now turning medially and caudally, so that in five successive sections, only the first and second show the connecting stalk, the remaining three pass through the distal portion of the anlage, which now lies almost parallel to the long axis of the body. The cells of the anlage have changed somewhat in their histological appearance, especially in their distal region, where they are much darker in color (due probably to the loss of yolk), and where the tissue is less compact. A few cells in this region have separated from the others and lie scattered in the connective tissue; some lying close to the medial wall of a small twig from the fourth branchial artery, and others some distance from it.

D. Amblystoma larvae, 13 to 15 mm. long. The postbranchial anlage now has a position parallel to the long axis of the body, this change being due probably to the increase in size of the surrounding parts. The connection with the pharynx is lost and the body lies completely isolated—an elongate, irregular, illy defined mass of cells in the connective tissue, between the medial aditus laryngeus muscle and the more lateral cartilage anlage of the fourth branchial arch, and the dorsal wall of the pericardium. A few scattered cells in its anterior region persists as a remnant of the connecting stalk; these extend upward toward the ventral floor of the pharynx, while cells in its caudal region are scattered in the connective tissue and are poorly defined. The central region is solid and is nearly 50 micra long and about 10 micra in average diameter, although in places it is very irregular as already noted. This stage corresponds roughly to that described by Maurer in Triton (p. 362), "Schon vier Tage später hat sich dieser epithelia le Zellzapfen von der Schlundwand abgeschnürt

und liegt als solide Zellkugel direkt unter der Schlundwand, seitlich vom Kehlkopfeingang."

E. *Amblystoma larvae, 19 to 20 mm. long.* In a single specimen, 19 mm. long, contrary to the usual condition, two postbranchial structures are developed, one on the right, and the other on the left side of the body. Both have the same general structure, and occur in the same relative position on the two sides, although the right is much smaller. The right body is about 105 micra long, and about 20 micra in average diameter, while the structure on the left is 165 micra long, and nearly 50 micra in average diameter. A transverse section through the central portion of the body on the left side is shown in figure 25. Both, for the most part, are compact, but here and there throughout their extent each shows the presence of small cavities, which have the appearance of lumina, but which do not contain colloid. These cavities are not continuous with one another; they may appear in one section and in the next disappear.

A wax reconstruction of the postbranchial body of the right side is represented in figure 38. The cephalic end is bent ventrally, so that it lies close to the dorsal wall of the pericardium, and is insinuated between the lateral surface of the aditus laryngeus muscle, and the fourth afferent branchial artery, while the caudal extremity is turned slightly in the opposite direction and lies close to the ventral wall of the pharynx. In addition to a small twig of the fourth afferent branchial artery, which, as mentioned above passes to the ventral wall of the pharynx in this region, numerous lymph spaces now occur, which in places nearly envelop the postbranchial body, especially in the region of its caudal extremity.

Except for the appearance of the body on the right side, as noted above in a single individual, the general position and structure of this body in Amblystoma, at this stage of development, is similar to that recorded by Maurer in Triton, p. 362.

In den nächsten (second) Wochen wächst dies Gebilde in Länge, behält seine Lagerung bei und liegt medial von der Knorpelspange des vierten Kiemenbogens. Sehr früh tritt schon ein feines centrales Lumen darin auf; indess macht bei älteren Larven, von 2–3 cm. Länge,

das Organ noch den Eindruck eines soliden Epithelschlaüches und erst nach Metamorphose bildet sich ein weites Lumen, das jedoch niemals Colloid enthält.

The appearance of a postbranchial body on the right side as well as on the left in this specimen, is unusual, since in none of the other stages or specimens is it present. The fact however, is of interest, for it indicates that the development of this body may vary within a single genus of urodeles, as well as among different families, as has been pointed out by Miss Platt.

F. Amblystoma larvae, 26 mm. long. In the 26 mm. stage, the postbranchial body on the left side is a fairly compact structure, little different in position and general appearance from that of the 19 mm. larva, but in the subsequent stages, several irregularities may occur. In a wax reconstruction, (fig. 44) it is an irregular, elongate body, rather tapered at the anterior end, and somewhat blunt caudally. On its surface, here and there, are numerous lobules, resembling follicles, but which are unevenly distributed. The cephalic end lies close above the dorsal wall of the pericardium between the aditus laryngeus muscle and the cartilage of the fourth branchial arch, and the caudal end is just below the ventral wall of the pharynx. It has a length of about 150 micra, and for the most part it is solid, only here and there do the sections show a lumen.

G. Amblystoma larvae, 35 to 40 mm. long. In larvae 35 mm. long, the body has divided into numerous smaller components which are distributed over a little greater longitudinal area. Portions of it occur in 18 sections (10μ thickness each), so that it is about 180 micra long. The anterior third is follicular, a layer of cuboidal epithelial cells enclosing a fairly large lumen. Behind this is another third, which is oval in shape and solid and is somewhat flattened dorso-ventrally, with irregular bud-like groups of cells on its surface. The caudal end has separated into several cell groups, with large lymph spaces and connective tissue between them. The caudal extremity, as before, lies just below the floor of the pharynx, and the cephalic end close to the dorsal wall of the pericardium.

Drüner's description of the body in 36 mm. stage of Siredon

(p. 508) differs in several points from that of Amblystoma, especially in being still in contact with the pharyngeal epithelium. I quote very briefly as follows:

Der Suprapericardialkörper . . . . bestand in der einen aus einer kleinen, ampullenförmigen Einsenkung des Pharynxepithels, . . . Diese kleine Ampulle zeigte das gleiche Epithel wie die Pharynx-wand, auch Schleimzellen wiesen sich an ihr durch die Färbung aus. Von hier aus setzte sich ein aus kleinen Zellen bestehender Epithelstrang, welcher streckenweise ein feines Lumen zeigte, in ventraler Richtung fort und er streckte sich so in den von Bindegewebe erfüllten Raum zwischen Herzbeutel und dem (interbranchial) Muskel. Hier löste er sich in zahlreiche kleiner Zellenstränge auf, welche mit Unterbrechungen . . . verfolgen waren. Hier bildeten sie dorsal von der Wurzel der 3 Kiemenarterie einen grössern Zellenhaufen.

In another series of sections however, Drüner was unable to find any trace of the ampullar structure.

H. Late larval and early transforming stages. Although several stages (40, 45 mm. and early transforming) were studied, the postbranchial body changes but little in its morphological relations in any of them. A wax reconstruction of the body in a 40 mm. larva shows it a fairly compact structure, somewhat flattened dorso-ventrally, and very irregular in outline (its total length is now about 300 micra). As in the early larval stages, the cephalic end insinuates itself between the lateral margin of the aditus laryngeus muscle and the fourth afferent branchial artery, the caudal end taking a more dorsal position. Internally it contains several poorly defined intercommunicating cavities, which run for the most part in a longitudinal direction.

The body in the early transforming stage, although not changed in position, is pressed closely against the medial wall of the fourth branchial arch, by the growth of the now much enlarged aditus laryngeus muscle. This flattens the body laterally so that the lumina are obliterated throughout the greater part of its length. At this stage the body is about 400 micra long, and as in the previous stages, its anterior end is close to the wall of the pericardium, its caudal end close to the floor of the pharynx.

I. Late transforming stage. In this stage, the parts in the immediate region of the postbranchial body, as well as the general

gill region, have changed considerably. The cartilages of the third and fourth arches have now disappeared, except for a small medial portion (basibranchial), and with this modification, the blood vessels of the corresponding gills have been affected. By a flattening of the ventral surface of the body of the larva, all structures included in the space between the ventral floor of the pharynx, and the ventral wall, present a much crowded appearance. As a result of this, the postbranchial body is now forced into a very limited space lateral to the aditus laryngeus muscle and between the ventral wall of the pharynx and the lateral strands of the sternohyoideus muscle of the left side of the pericardial wall, where it is reduced to a mass of poorly defined epithelial cells, which, contrary to the usual statements for the other urodeles, contains no conspicuous lumen. This solid mass of epithelial cells is much reduced in length (now about 90 micra long).

J. The adult postbranchial body. The position and anatomical condition in the adult is somewhat variable. The body is rather inconspicuous and it is practically impossible to recognise it in gross dissection. In transverse sections through the adult head, it is not greatly different from that of the late transforming animal, being a fairly compact oval structure, somewhat irregular in outline, composed of quite large cuboidal cells with a spacious lumen. In position it is somewhat more lateral to the larynx, and the aditus laryngeus muscle, and lies just caudal to the second aortic arch as it turns dorsally to join the dorsal aorta on the left side. I am not able to locate the structure with certainty, in sections of an older head. Whether this be due to the fact that the body has degenerated and disappeared, or whether it has (as is claimed for it in some forms) become closely associated with the thyreoid (lateral thyreoid) I am not able to say, although I incline to the first of these views.

4. Carotid gland and epithelial bodies

Although there is no evidence that the carotid gland in Amblystoma arises from cells derived from the entodermal lining of the

pharyngeal tube, its appearance in the larva at the time of metamorphosis, and its close relationships to the epithelial bodies in their development, leads to its consideration here.

Different views are held regarding the origin of this gland in the anurans and the urodeles. Maurer ('88), described its development in anurans from the first branchial pouch, and was inclined to believe it had a similar origin in the urodeles. Zimmermann ('98) considered it simply as an enlargement of the walls of the blood vessel in anurans, and Boas ('83) earlier suggested this was the case in Salamandra. Maurer ('02, p. 152) says:

Dies Organ ist hier anzuschliessen. Nach der Auffassung vieler Authoren ist sie eine blosse Gefässbildung, durch Wucherung der Gefässwandung enstanden. Da Andere aber eine Beteiligung von Schlundspaltenepithel angaben, so ist sie hier zu erwähnen. Bei Anuren fand ich im Bereich der 2. Schlundsplate eine epitheliale Knospe, die sich genau so verhält wie die Epithelkörperchen der 3. und 4. Spalte, aber eben durch ihre sehr bald erkennbare Beziehung zur Kiemenarterie sich eigenartig erweist. Nach Zimmermann ist eine Epithelknospe nicht vorhanden, und nur eine Wucherung der Gefässwand bildet die Drüse.

In Amblystoma the carotid gland and the two epithelial bodies begin their development during metamorphosis. The carotid gland is the first to appear, its anlage forming on either side, in the region where the first afferent branchial artery enters the gill. The anlagen of the two epithelial bodies of either side appear at first some distance caudad and medial to one another and to the carotid gland; but as development proceeds they are brought closer and closer together so that in late transforming and adult stages, one lies posterior to the other.

A. *Transforming Amblystoma larvae.* In the early transforming stage, the caudal pharyngeal pouches are being reduced, the fourth and fifth slits being completely closed to the exterior. The carotid gland is just beginning as a slight enlargement of the wall of the first afferent branchial artery in the region where it enters the first gill-arch, this enlarged portion being augmented by the union of the external carotid artery in front, and the internal carotid artery close behind. At the same time there is a noticeable thickening of the walls of the enlarged portion (anlage

of the gland), the origin of the cells of which is difficult to determine. The exact extent of the anlage is difficult to determine, but it is about 150 micra long. Just caudad and somewhat medial, the ventral portion of the second branchial pouch extends downward, separating the first and second branchial cartilages ʿrom each other. A similar ventral portion of the first branchial pouch, separates the hyoid and first branchial arches.

The anlage of the first epithelial body in this stage ($e.b._1$, fig. 39), is an irregular longitudinal tract of entodermal cells, of considerable length (a remnant of the ventral portion of the third branchial pouch), lying between the cartilages of the second and third arches. The cephalic end of this sheet is about 700 micra caudad to the carotid anlage, and lies just back of the point where the second afferent branchial artery enters the second gill-arch. The caudal limit is difficult to determine.

The anlage of the second epithelial body ($e.b._2$, fig. 39), is a similar tract of entodermal cells lying between the cartilages of the third and fourth arches, the cephalic end of which lies just caudad to the point where the third afferent branchial artery enters the third gill-arch. The cephalic end of this anlage is 360 micra caudad to the cephalic end of the first, but the anlagen of both bodies parallel one another, so that in transverse section the cephalic end of the second is cut with the caudal end of the first.

Maurer ('88) in describing these structures in Triton, was unable to find any trace of the carotid gland in the early stage of metamorphosis. He describes however, the anlagen of the epithelial bodies. "Auf Querschnitten zeigte sich zwischen dem zweiten und dritten, so wie zwischen dem dritten und vierten noch vorhandenen knorpeligen Kiemenbogen die Reste der vierten und fünften Kiemenspalten in Form unregelmässiger länglicher Gebilde, die sich aus Epithelzellen zusammensetzen und nur durch Aneinanderlagerung und Schrumpfung der Kiemenplatten entstanden sein können." It is possible that the Amblystoma larva of this stage in my material is somewhat farther advanced in its metamorphosis than was that of Triton described by Maurer.

In the late transforming larvae the gill region has been so far modified that it is similar in many respects to the adult condition.

The cartilages of the arches are altered to the hyoid apparatus of the adult; the afferent and efferent branchial arteries now form functional aortic arches; the muscles of the ventral pharyngeal region are greatly enlarged, and as a result, the carotid gland and epithelial bodies are carried somewhat caudal and lateral.

The carotid gland is now an enlarged, ovate body (489 x 200 micra) situated close behind and a little lateral to the caudal end of the thyreoid gland. It is enveloped in a rather heavy connective tissue covering. The first afferent branchial artery enters it medially, a little toward the caudal end, while the two carotid arteries leave it anteriorly.

The first epithelial body is a well defined oval structure (180 x 100 micra), composed of closely packed epithelial cells surrounded by a conspicuous connective tissue covering, situated close behind (about 150 micra) and a little dorsal to the carotid gland, and just lateral and caudal to the second aortic arch. The second epithelial body is similar to, but somewhat smaller (120 x 70 micra) than the first, and lies close behind (between 60 to 70 micra) and a little lateral to it. The third (very small) aortic arch, passes from the ventral aorta, just in front of the epithelial body, (the epithelial bodies appear in section with the third and fourth thymus bodies, and they lie some distance below them).

B. *Carotid gland and epithelial bodies of adult.* These structures present only minor differences from the other stages. In a wax model of the parts, (fig. 42) on the left side, just after metamorphosis, the carotid gland (*c.g.*) and epithelial bodies (*e.b.*) lie close behind one another, imbedded in the connective tissue medial to the first branchial cartilage. The first aortic arch (*1*) passes slightly caudo-laterally, as it leaves the ventral aorta, and enters the carotid gland as before. The second aortic arch (*2*) is large, and, after taking a latero-caudal direction, turns dorsal and cephalad to join the dorsal aorta. The third aortic arch has now disappeared, while the fourth (*4*) is modified to function as the pulmonary artery (*p.a.*), a small ductus arteriosus persisting. With these changes in position and function of the blood vessels, the two epithelial bodies are not closely associated, but are more lateral and somewhat more caudal in position. In transverse

section at this stage, (fig. 35) the epithelial body ($e.b._2$) lies just lateral to the point where the second aortic arch bends dorsally to join the dorsal aorta and is a little below the level of the branchial cartilage (1).

The structures are exposed by gross dissection in fully adult animals ($e.b._{1,2}$, fig. 40), by removing the skin and superficial muscles from the neck region. They lie in the connective tissue medial to the distal tip of the first branchial cartilage. The carotid gland ($c.g.$) is a very evident oval body of small size, into which the first afferent branchial artery enters, and from which the external and internal carotid arteries pass forward. Close behind and a little lateral to it, are the two small, whitish, or yellowish white epithelial bodies. These are slightly oval, nearly equal in size (varying from 200 to 300 micra in diameter); they lie one closely behind the other and are enveloped in connective tissue which contains considerable pigment. By carefully dissecting and clearing the parts, it is evident that the blood supply of this region is by two small arteries, one passing back from the second aortic arch, and the other from the external carotid artery. I was unable to trace the origin of numerous small nerve twigs, but it is probable that the region is innervated by branches (probably the third and fourth) of the vagus nerve.

There is little histological difference between the adult epithelial body and that of the late transforming stage. The epithelial cells are solidly packed, separated only by a very fine matrix, the whole surrounded by a well marked layer of connective tissue. Such a compact condition differs slightly from the structure of the body in Triton and Salamandra where (Maurer) they were composed of irregular masses of epithelial cells separated one from another by strands of connective tissue.

III. REVIEW OF THE LITERATURE

A considerable literature deals with the structures derived from the pharyngeal wall and gill-pouches of the amphibians, and, since various authors have described identical structures in the same species and homologous ones in related forms under different names, the results are sometimes confusing.

Thyreoid. Although the general anatomy and position of the thyreoid gland in certain adult amphibians was noted by a few workers in the latter half of the last century, notably—Leydig ('53) in Triton punctatus, 'Landsalamander,' Proteus, Caecilia annulata, Rana and Bufo, and Wiedersheim ('79) in Caecilia, Siphonops and Epicrium, and later ('84) the same author noted its position in representatives of both anura and urodelia—the first investigator to describe it from the developmental side was W. Müller ('71) who followed its ontogeny in Rana temporaria and R. platyrrhinus. Soon after this, Göette ('75) described its development in Bombinator igneus, and a little later, de Meuron ('86), worked it out in both Rana and Bufo. According to Maurer ('88), no work had been done on the development of the thyreoid gland in the urodeles before he wrote, and what was known of it was learned from macroscopic study of the adult animal. As a basis of his study, he used series of Triton taeniatus, T. alpestris and T. cristatus (stages in metamorphosis excepted), supplemented by series of Salamandra maculata and young and old axolotls. He also studied representatives of the anurans—Rana esculenta and temporaria; Bufo vulgaris and variabilis; Hyla viridis and Bombinator igneus. His results are briefly as follows:

In Triton taeniatus the thyreoid anlage appears before the gill-pouches have united with the ectoderm, as a solid epithelial process, extending ventrally from the floor of the pharynx, between the second gill-pouch and the pericardial cavity. During the first day after hatching (5.5 mm. long), the anlage separates from its point of origin, as an ovoid mass of yolk-filled cells. By the growth of the copula and the other structures in its region, the anlage gradually splits longitudinally into right and left halves except for a small portion in front which persists for some time as the 'isthmus,' holding the two halves together. Eight days later, (larva now 7 mm. long), division is complete, the two halves being now separated by the developing sternohyoid muscle. From this point on irregularities occur, both in size and in details of the two halves, and the color of yolk content of the cells. A few cells (from four to six) may group themselves in an irregular

clump in the mid-line in front of the halves; these Maurer inter-
prets as remnant of the isthmus and calls it 'Nebenschilddrüse' or
'accessory thyreoid.' For a time it is difficult to follow the two
halves in their subsequent development because of their loss of yolk
content. (The writer has had the same difficulty in his material
of Amblystoma of this stage). Cells arrange themselves in a solid
cord on either side of the sternohyoid muscle. Later, (three or
four weeks) these cell cords develop into follicles which show the
presence of colloid. The external jugular vein lies a little ventral
to the thyreoid in the adult, but the wonderfully complex network
of blood vessels, of the adult does not develop in the larval stages.

He found the development of the thyreoid in Siredon pisci-
formis and Salamandra maculata similar to that in Triton
taeniatus, and hence he concludes that the latter species fur-
nishes a good example of the development of the organ in the
urodeles in general.

Miss Platt ('96), finds one or two points of difference in Nec-
turus maculosus, from the description given for Triton by Maurer.
In Necturus, the thyreoid arises from the ''base of the hyo-
mandibular pocket, (not from the second, as stated by Maurer)
directly above the oral fusion, and extends backward below the
ventral aorta to a point where the mesoderm of the hyoid and
mandibular arches unite in the medial plane'' (p. 561). In addi-
tion, ''the cells separate from the anterior part (isthmus of Maurer)
of the outgrowth, are taken into the mylohyoideus muscle and do
not form accessory thyreoid'' (p. 567). The cells of the posterior
portion are divided into two lines, and form neither solid masses or
closed vesicles, and later (46 mm. larva) they constitute a mass of
vesicular tissue (thyreoid proper).

Bolau ('99) gives a description of the position of the thyreoid
and thymus glands in adult amphibians, but has no account of
their development. Livini ('02), describes the development of
the thyreoid gland in Salamandrina perspicillata, which, in its
essentials, closely accords with Maurer's account of Salamandra
maculata. He confirms the solid condition of the early anlage,
typical of the urodeles. Maurer ('02) in Hertwig's Handbuch,
gives briefly the results of his earlier work ('88), and mentions, in

addition, that the gland· had since been studied in Necturus (probably referring to Miss Platt's work), but other than this makes no further comment. Muthmann ('04), repeating some of the earlier work by Brachet ('98), followed the very early development of the thyreoid anlage in a number of urodeles — (Triton alpestris, Axolotl, Salamandra atra) and also certain anurans (Bufo vulgaris and Rana temporaria) with special reference to its position relative to the heart anlage. In Triton alpestris the anlage of the gland is laid down very early in the embryo (as early as the endothelium of the heart), and in a 20 to 21 somite specimen, it was a solid bud of cells on the floor of the pharynx, just in front of the bulbus arteriosus; later (25 somites), this elongated slightly and lay between the right and left mandibular arteries. In the 30 somite stage the caudal end had elongated a little, but the proximal end was still connected with the pharynx, and remained so for the first four days of larval life. Muthmann made no serious attempt to follow its development beyond this stage, remarking, (p. 43), "Genauere Beschreibung dieser Vörgange geben Maurer und Livini."

Drüner ('04) notes the position of the thyreoid with reference to certain adult structures in Siredon pisciformis, but contributes nothing to our knowledge of its development. Marcus ('08), has described the development of the thyreoid gland in Hypogeophis, where he found, instead of the solid early anlage (as in urodeles and some anurans), a hollow one, similar to that described by W. Müller ('71), for Rana, and Göette ('75)· for Bombinator.

Finally, Mrs. Thompson ('10), in a paper on the thyreoid and parathyreoids throughout the vertebrates, gives a very brief study of this gland in the amphibians. She bases her study on a single species of urodele (Spelerpes ruber, adult) and on the frog (mentions no genus nor species), and since she gives no details of its development, only passing mention need be made here. She shows however, a drawing (pl 10, fig. 4) of the histological structure of the adult gland in Spelerpes, which she claims is the first to be given for any urodele.

Thymus. The position of the thymus in the adult amphibians,

and something of its morphology in the various forms has long been known. Simon ('44), observed it in Necturus, Amphiuma and Cryptobranchus, and described its appearance and position in these forms. Soon after, Leydig ('53), in addition to the forms studied by Simon, described the structure in Proteus, Siredon, Caecilia annulata and a number of anurans. Its position, according to Leydig, is, in both the anurans and the urodeles close behind the angle of the jaw, superficially placed beneath the skin. In the Caecilians, he noted four small bodies, one behind the other just behind the angle of the jaw. The nerve supply of the thymus was first noted by Fischer ('64), especially in Siredon pisciformis, as the cutaneus ramus of the vagus.

Göette ('75) was the first to study the development of the thymus in amphibians. He described it in Bombinator igneus as developing from the dorsal portion of the second gill-pouch, but at first he did not believe this to be the true thymus, preferring to call it the 'Halsdrüse.' Later, de Meuron ('86) described the development in Bufo and Rana, as an epithelial body arising from the dorsal region of the second gill-pouch. This early separates from the rest of the epithelial cells, and soon contains cells which, in nature and appearance resemble those of the connective tissue. This he called the true thymus, and homologized it with the first thymus body of selachians. Maurer ('88), was the first to follow the development of it in the urodeles (Siredon pisciformis, Salamandra maculata and Triton taeniatus). Briefly stated, he found that in Siredon, five epithelial bodies were concerned in its genesis, the first two of which early degenerated and the three posterior ones remained. In Salamandra, three such epithelial bodies developed, which he thought could be looked upon as the homologues of the three persisting bodies in Siredon. In Triton, the anterior two early degenerated, while a single large bean-formed body remained some distance behind, which he was unable to follow in its developmental stages. Whether this was formed by the fusion of the three posterior bodies, as in the case of Siredon, or whether it was formed solely from the last he could not say. ''Jederfalls ensteht die Urodelenthymus aus dorsalen Epithelknospen hinterer Kiemenspalten, während die zweite Knospe,

die bei Anuren die Thymus hervorgehen lässt, sich sehr früh mit der ersten Knospe rückbildet." Later, in Hertwig's Handbuch, ('02) Maurer reviews briefly the development and position of the thymus bodies in Triton and Siredon, in which he corrects one or two points of his earlier work; these are noted below in connection with Drüner's discussion.

Contrary to Maurer's description of the development of the thymus in the other urodeles, Livini ('02) finds the permanent thymus in Salamandrina to be the fifth larval body, formed from the fifth gill-pouch, and states that the bodies of the third and fourth as well as the first and second pouches degenerate early without leaving a trace.

In his work on the musculature and gill-region of the urodeles, Drüner ('04), touches upon the developmental relationships of the thymus in Siredon. He found, as did Maurer, five thymus elements arising from the dorsal region of all five gill-pouches; of these the first two degenerate early, and the last three form the permanent thymus. In one or two cases he found a small follicular-like body persisting in the relative position of the first thymus body, which he interprets as a remnant of the same. According to him also, not only entoderm from the gill-pouches is concerned in the formation of the five primitive bodies, but ectoderm also contributed to their formation. On the other hand, he agrees with Maurer ('02), in locating the first body as lateral to the facial, rather than the Gasserian ganglion, and the second body lateral to the glossopharyngeal instead of the facial, as was stated by Maurer ('88). He also locates the third and fourth bodies with reference to the lateral branches of the branchial nerves of the vagus, rather than to the ganglion itself.

Marcus ('08, p. 737) found thymus thickenings on the dorsal portions of six gill-pouches of Hypogeophis, those on the first and sixth, however, were rudimentary and degenerated early, while the other four pouches formed thymus bodies, which, after separating from the pharyngeal epithelium, became in the adult a four-lobed thymus gland, the lobes of which were separated by thin strands of connective tissue.

Of the two more recent workers in the field of the histogenesis

of the thymus gland in amphibians, Dustin ('11, on Axolotl, and '13, on Rana fusca) and Maximow ('12, on Siredon pisciformis and Rana temporaria), only the latter gives a brief description of its morphogenesis. He finds the five early epithelial bodies in Siredon in essentially the same position as described by Maurer ('02), and, although he observes darkly staining cell groups (what he calls the 'thymus ectodermalis' von Drüner) closely associated with the thymus bodies on the one hand and with the corresponding ganglia on the other (in the early stages—7.5 to 15 mm. larvae), he is not certain of the origin of these, and he says (p. 573), "Ob diese Zellensammlungen wirklich dem Ektoderm enstammen, wie es Drüner will, vernag ich nich anzugeben . . ." The true epithelial character of the bodies is retained but for a short time (from the 7.5 to the 9 mm. stages), there soon appearing other elements (p. 576), which increase in numbers in the 11 mm. and subsequent larval stages. He also substantiates the results of the earlier workers (especially Maurer '88, and Drüner '04) concerning the early degeneration of the first two epithelial bodies, and the persistance of the last three to form the definitive gland, although he carries his work only through the 25 mm. stage, appending the remark (p. 603), "späteren Entwicklungsstadien der Thymus beim Axolotl boten für mich kein Interesse mehr . . . ."

Postbranchial (suprapericardial or ultimobranchial) body

This structure, which has been given different names by different authors, was probably first described in the anurans (Rana and Bufo), by Leydig ('53) as parts of the true thyreoid. (Greil, ('05), says that Leydig described them as 'Glandes thyreoides accessories,' but in Leydig's paper, I can not find that he mentions this name, and it is probable that Greil took this information from de Meuron who uses this term, see p. 542.) Van Bemmelen ('86) was the next worker who described the structure (in selachians) and from its position in that group gave to it the name 'suprapericardial body.' Later, de Meuron ('86), p. 541, gave a brief account of its development in Bufo. According to him, it is

symmetrical in development, arising from either side of the pharynx, as a small diverticulum which soon forms isolated cysts lying on either side of the trachea, above the pericardium. In the adult, the cyst lying on either side was close to the thyreoid and was thus considered by de Meuron as 'accessory thyreoid,' although he was unable to follow the details of development.

Maurer ('88), was the first to trace the development of the body in both Anura and Urodela, and, because of its developmental position with reference to the branchial pouches, he gave it the name 'postbranchial body.' In the anurans (Rana and Bufo), the body develops symmetrically behind the fifth pouch, just lateral to the aditus laryngeus muscle, and consists of either single or complex follicles, which never contain colloid. They retain their early position close to the thyreoid, but, according to Maurer, never unite with that gland in anurans. In the urodeles (Triton and Siredon), he found the body arising as a solid epithelial bud from the floor of the pharynx on the left side; this, after elongating, becomes separated from the pharynx, and takes a direction parallel to the long axis of the larval body. Late in development it acquires a lumen which never contains colloid. It does not in any way, according to Maurer, resemble the thyreoid, and therefore in his view can not be considered as an 'accessory thyreoid' as was de Meuron's belief. Maurer believed de Meuron described correctly the origin of the postbranchial body in Anura, but thought he confused its later development with the epithelial derivatives of the pouches, which later come to lie close to the adult thyreoid gland. Maurer agrees with de Meuron that the structure in Anura is homologous with that of the selachians. The unilateral position of the body in the urodeles, which he studied, and the persistance of the connecting stalk with the pharynx, suggests to him that perhaps it represents a remnant of the ductus oesophago-cutaneus of Bdellostoma.

Miss Platt ('96), found that the body developed symmetrically on either side in Necturus, and thus pointed out that Maurer's conception of the asymmetrical development in the urodeles as a whole to be erroneous. In addition to this she found that the structure arises as a small vesicle (in 15 mm. larva), from the ven-

tral floor of the pharynx on either side, not posterior to the fifth
pouch (as was described by Maurer), but between the fourth and
fifth pouches. Thus, the position of the body in Necturus as
described by her, opposes the view of van Bemmelen, on the one
hand, that they be regarded as 'rudimentary branchial clefts,'
and that of Maurer, that they are 'postbranchial' on the other.
She therefore prefers to call them 'suprapericardial' rather than
'postbranchial.'

Maurer ('02), p. 143, briefly reviews the development of the
structure in the amphibians, and while he contributes no new
evidence of his own, he emphasizes the fact that it is to be re-
garded as a 'postbranchial' structure. He says, ''Da er hier
hinter der letzten Kiemenspalte liegt, eine Beziehung zum Peri-
card nicht besteht habe ich das Organ als postbranchialen Körper
bezeichnet. Er liegt stets *hinter* der letzten Kiemenspalte mag
diese nun die 4, 5 oder 6 sein.''

Drüner ('04), p. 508, describes the postbranchial body in but a
single stage of Siredon (3.6 cm.) where it was a small ampulla-
like structure, on the left side only, formed by the insinking of the
pharyngeal epithelium. In other series of the same form, he
failed to find it.

Greil ('05) describes its development in Rana, Bufo and Hyla,
and disagrees with Maurer's view that it is 'postbranchial,' since
it arises from the ventro-caudal region of a rudimentary sixth
pouch, and is thus 'ultimobranchial.' The early anlage, accord-
ing to him, is not formed by an out-pocketing of the pharyngeal
epithelium, but by a simple thickening, which later develops a
lumen. He agrees with van Bemmelen, that in the selachians,
the body represents a rudimentary seventh pouch, but in the
amphibians, where the seventh pouch is lost, the formation of the
structure is assumed by the sixth, and so on in the higher groups
as in mammals, the formation of the body is assumed by the last
gill-pouch, the fourth.

In Hypogeophis, Marcus ('08), finds that the body develops
symmetrically, but is derived from the seventh pouch instead of
the sixth as in the other amphibians; the sixth in this form appar-
ently degenerating (see p. 733). He retains the name 'ultimo-

branchial' body and interprets it as a structure derived from the branchial, rather than from the postbranchial region of the pharynx.

At the Leipzig meeting of the Anatomische Gesellschaft ('11), Maurer defends his view in his discussion with Rabl in the following way (p. 161), "Während die von mir eingeführte Bezeichnung 'Epithelkörperchen' sich gans eingebürgert hat, ist die Bezeichnung 'postbranchialer Körper' in die des ultimobranchialen Körpers umgeändert worden. Ich kann diese Aenderung nicht für glücklich halten. Es ist das betreffende Organ bei *allen* Wirbeltieren *hinter* der letzten Kiemenspalte vorhanden und zeigt aufsteigend eine fortschreitende Weiterbildung, im Gegensatz su den Schlundspalten, die ganz schwinden. Es ist also das Gebilde etwas von den Schlundspalten Verschiedenes und liegt immer hinter der letzten Schlundspalte, darum ist es weniger präjudizierend, wenn man es als postbranchialen Körper bezeichnet."

The most recent contribution on the structure is that of Kingsbury ('14), who, although studying its development in mammals, especially man, gives a discussion of its significance and homology in the other vertebrates. He says, p. 609, "Three points remain indefinite—(1) its origin—whether it arises from branchial or post-branchial region of the pharynx. Dependent upon the answer to this question is the decision as to its designation as ultimobranchial, telobranchial, or postbranchial. (2) Its fate; whether it persists within the thyreoid—(3) its value and interpretation as an organ or structure." He gives Grosser's view (a ductless gland which has become rudimentary), and objects to this on two points, namely: (1) that, "with the exception of the ultimobranchial body in birds, no gland has been found in the forms below the mammals with which the ultimobranchial body may be homologized," and (2), "that the ultimobranchial bodies—if we use that term—of the different classes of vertebrates cannot themselves be directly homologized." He then cites Greil ('05, anurans), Tandler ('09, man) and Grosser ('10, man), where all agree that it is a derivative of a gill-pouch—the first that of the sixth pouch, and the two latter that of the fifth pouch. "It is

obvious that upon the interpretation of the ultimobranchial body as a branchiomeric organ, as a derivative of a rudimentary fifth pouch, the 'ultimobranchial' structures of the lower vertebrates cannot represent it, since the fifth pouch may be a functional gill-pouch in the amphibian." Rabl's ('11, '13) suggestion that i is the representative of both fifth and sixth pouches in man, meets with Kingsbury's objection, since, although it satisfies the homology between man and amphibians, "it fails as applied to the elasmobranchs where the sixth pouch is a functional gill cleft caudad of which occurs the suprapericardial body which appears to be an ultimobranchial body (van Bemmelen ('89), Greil ('05))."

"For those who view this structure as a vestigial ancestral gland of some kind, Maurer's term and interpretation inherent therein —postbranchial body— presents no such logical difficulty, since, as Maurer ('11) said in defense of his term at the Leipzig meeting of the Anatomische Gesellschaft, these structures might then be homologized throughout the vertebrate series in the forms in which they occur." The evidence, however, as Kingsbury sees it, "indicates strongly that the structures . . . . belong to the branchial region and are not 'postbranchial.'" The only way then, "whereby these pharyngeal structures may be interpreted as ultimobranchial and also directly homologized in the different vertebrates would seem to be the assumption,—that it is the last branchial pouch which in the form of ultimobranchial body or represented by it as a derivative has retained its individual existence while the reduction in number of branchial pouches has been brought about by the elimination of the gill clefts that proceeded it in the series.

"The double assumption of this pharyngeal derivative as an 'ultimobranchial' body and as a vestigial gland representing an ancestral organ . . . . can not be true on any morphological basis of homology."

Kingsbury thus concludes that in man, "no reason is seen for considering the ultimobranchial body so called either as representing an ancestral gland, vestigial in mammals, or representing any specific pouch, either V or VI, but merely formed by a continued growth activity in the branchial entoderm."

Epithelial bodies and gill-remnants.   Maurer ('88) was the first to describe the genesis of the small epithelial-like structures in close proximity to the glandular structures in the neck of the adult amphibians, and was first to suggest that possibly these could be looked upon as the homologues of structures in the higher forms.   He found that the epithelial bodies develop during the time of the formation of the inner gills, from the ventral ends of the third and fourth pouches of the tadpole of the frog, while the gill remnants, ' Kiemenreste,' develop from the anterior ventral region of the branchial chamber at the time of the reduction of the gills.   The carotid gland in Rana, according to Maurer, develops in a similar manner, from the ventral end of the second pouch, and was therefore considered by him as an epithelial structure.   (This origin of the carotid gland has been doubted by other workers, especially Schaper ('96) and Zimmermann ('98)).

Norris ('02), studying the development in Rana fusca of the so-called ventral Kiemenrest of Maurer, is unable to agree that it is related in any way in its genesis with the branchial apparatus, but says that it arises (during metamorphosis), in the region of the body previously occupied by parts of the basi-hyobranchialis muscle of the tadpole.   In the urodeles, no such gill-remnants as occur in anurans have yet been described.

Maurer ('88) was the first to work out the development of the epithelial bodies in the urodeles.   In Triton taeniatus, he found the bodies developed from the ventral portions of the third and fourth pouches, not during the larval stage as in the case of anurans, but during the metamorphosis, and in the adult those came to lie close to the lateral (convex) wall of the aortic arches. Maurer observed the formation of the carotid gland in the region of the second pouch, during this same period, but whether it developed from the cells of the transforming pouch he was unable to say, although in the anurans th's seemed to him to be the case.

Since Maurer wrote, very little work has been done on the development of the epithelial bodies and gill-remnants in the amphibians.   Verdun ('98), refers to Maurer's work, and Mrs. Thompson ('10), gives but very brief discussion of them in her

paper, where she prefers to call them parathyreoids. In the only urodele she studied (Spelerpes ruber), she found but a single body on either side, and she does not trace its development, but merely remarks on its small size (she was not able to find it with the naked eye) and gives only a brief description of its histology. She refers to Maurer in her account of the development (p. 101) of this body in the anurans, but contributes nothing further of her own.

Morphology of the pharyngeal derivatives in other vertebrates

As yet the problems of the homologies of the pharyngeal derivatives in the different groups of vertebrates are not settled and only brief statements of fact can be made here in the light of our present knowledge.

Thyreoid. This, of all pharyngeal structures, is the easiest to homologize in the vertebrates, since, in all, it develops from the epithelium of the floor of the pharynx. Whether it is paired or unpaired, solid or hollow in its early anlage, and whether the morphology, physiology and histology of the adult gland is the same in all cases is not settled. Its development in the cyclostomes has been recently followed by Reese ('02), Cole ('05), Schaffer ('06) and Stockard ('06). In the ammocoete stage of the lamprey, it retains its connection with the pharynx, and it was homologized by W. Müller ('71), with the endostyle of the tunicates. In the later stage of the lamprey, as well as in the adult myxinoids the duct is lost, and the gland becomes follicular and its parts scattered in the region below the pharynx (Stockard, '06, in Bdellostoma).

In the elasmobranchs, Mrs. Thompson ('10) finds a compact structure which she claims is partly epithelial, and partly adenoid in character, and suggests that possibly the adenoid portion corresponds to the 'parathyreoids' of the higher vertebrates. In the teleosts (Gudernatsch '11), it has the same tendency to become broken into small isolated groups and scattered in the connective tissue, and is not an anatomical unit and is therefore not a gland in the sense of a compact structure.

In the reptiles, the gland is a single, lobate, follicular structure, which contains colloid and lies close to the trachea, but in the birds, it is paired, the halves lying close to the blood vessels and the side of the neck (Verdun '98) large areas of which may be devoid of colloid (Mrs. Thompson '10), and its vesicles are usually closely compact. Whether the anlage is single or double in the mammals, is not yet altogether settled, although Maurer ('02), states that the evidence seems to be in favor of the single origin, which would make it homologous with the gland in other vertebrates.

The parathyreoids, according to Verdun ('98), have their homologues in the 'epithelial bodies' of the amphibians, the latter having first been described by Maurer ('88), and with this identification I agree. (Concerning these structures more will be said below.)

Thymus. Whether the cyclostomes, like the gnathostomes, have a true thymus is not settled. J. Müller ('43), p. 115, was the first to describe a paired structure situated behind the gills, in the region of the heart in the myxinoids, which he, at that time, called 'Nebennieren.' Later, in an editor's note to Stannius' ('50) work on the thymus gland of the fishes (p. 507) he suggests that the 'Nebenieren' previously described by him corresponds to the thymus of the fishes, and thus it was that Stannius ('54) in his Handbuch (p. 256) says, "Die paarige Thymus is bischer bei den Myxinoiden . . . . beobachtet worden," and in a footnote on the same page he remarks, "Bei den Myxinoiden ist dies gebilde aufgefunden und beschrieben von J. Müller, der es zuerst als Nebennieren, später als Thymus deutete." The statement of Beard ('94, p. 485), that "the only positive statement we possess emanates from Stannius," is not therefore exact, although his interpretation of this organ as a degenerate pronephros in Myxine, may be correct. Stockard ('06), p. 99, in his study of the thyreoid in Bdellostoma, found no evidence of a thymus gland in any of the stages of development, and doubts its existence.

On the other hand, Schaffer ('94) described the development of seven small bodies on the dorsal as well as the ventral region of as many pharyngeal pouches in Petromyzon, which he interprets

as the thymus bodies in that form. Maurer ('02), however, points out that there is some doubt as to the homologies of these, and says, p. 132, "Ob sie der Thymus höherer Wirbeltiere homolog sind, ob nur die dorsalen dafür anzusprechen sind, die ventralen aber als Epithelkorperchen zu deuten sind, ist nicht zu entscheiden: ja es ist möglich, dass sie nur den Cyclostomen zukommende Bildungen eigener Art darstellen, welche auf gnathostome Wirbeltiere nicht übergegangen sind."

In the gnathostomes, structures which have been interpreted as thymus bodies occur. Although arising from the endodermal epithelium of various number of gill-pouches (usually from the dorsal angle), these have been fairly well homologized in the different groups of vertebrates (mammals perhaps excepted). In the elasmobranchs, small thymus bodies develop from the dorsal angle of several (second to sixth and possibly the spiracle) visceral pouches. In the few teleosts in which the development has been studied, and in the caecilians (Hypogeophis, Marcus, '08), these develop from the second to the sixth pouches. In the urodeles, they arise from pouches one to five; the first two usually degenerate, the last three persist to form the adult thymus. In the anurans, only the first and second pouches form such bodies, the second alone persisting to form the definitive structure in the adult.

Comparatively little is known of the development of the thymus gland in the reptiles, but in the groups which have been studied, some differences are found. In certain of the Lacertilia, (Maurer ('99); Anguis, Gongylus, Lacerta, Saint-Remy, and Prenant ('04), etc.) the thymus bodies develop from the dorsal region of the second and third pouches, while in certain of the ophidians (Coluber, Tropidontus), the third and fourth and possibly the fifth pouch form a thymus lobule. (For further details, see Maurer, '02, p. 136.)

In the birds, a similar condition prevails. According to de Meuron ('86) and Mall ('87), only the third pouch forms a thymus body in the chick, while van Bemmelen ('86) and Verdun ('98) believe the fourth also forms thymus elements, and the latter author thinks the fifth pouch contributes as well.

The thymus bodies in the mammals are developed from the ventral (rather than the dorsal) portion of the third and fourth pouches, and this act throws considerable doubt on the homology of these organs in mammalian and non-mammalian groups. In some cases also the ventral region of the second pouch (rabbit) has been described as forming a thymus body. Although it is beyond the scope of this paper to enter into a discussion of the intricate points of homology, brief reference may be made to the discussion by Kingsbury ('15), pp. 359–371. I quote his conclusions.

"Recognizing that the thymus-forming factors are not intrinsic, but extrinsic, i.e., partly a function of position and relation, it is no longer necessary directly or completely to homologize thymus bodies in different forms, since it is obvious that different growth conditions may determine thymus development from quite different portions of the branchial epithelium, and portions that in one form may persist and undergo thymus transformation, in others may degenerate and disappear without the characteristic reaction appearing."

"In closing . . . . it may be stated that there is a widespread tendency to thymus-formation in the branchial region, characterized by a persistence and growth of epithelium with a characteristic (though not peculiar) reaction with adjacent tissues, under conditions that are not yet fully analyzable. What these conditions are and what determines the development of a thymus or thymus bodies is unknown, and any attempt to determine them awaits further analysis of growth conditions of the region, particularly in the lower forms."

Postbranchial body. This structure has not been described in the cyclostomes. It appears in all elasmobranchs with the exception of Heptanchus, but is absent from all the teleosts yet studied. In the other gnathostomes, its appearance and position is variable, and its significance in these forms has led to wide-spread discussion, as is evidenced by the different names and interpretations given it by various workers, as stated above. Its phylogenetic significance is not clear. Some have suggested that it is the representative of the ductus oesophago-cutaneus of

Bdellostoma; some think it represents a rudimentary gill-pouch, or that it is an ancestral gland of some sort; while others think that it is formed merely by continued vegetative growth of the branchial entoderm.

In the elasmobranchs it develops behind the sixth pouch, and is sometimes paired and sometimes single. In the amphibians it usually develops behind the fifth pouch, and is usually paired (anurans) or may be single (urodeles, Necturus excepted). In Bombinator, according to Greil, '05, it is not developed on either side. In the Lacertilia, it is sometimes single (van Bemmelen '86), sometimes paired (Maurer, '99), while in the snakes, it may be entirely absent (van Bemmelen). The relative position of its origin is a point upon which some differences are expressed; some authors interpreting it as developing from the pharynx behind the fourth pouch, and others, behind the fifth. In the birds and the mammals also, great diversity of opinions are expressed as to its position, significance and fate, but since certain phases of this have already been discussed (see pp. 657–661), a repetition here is not necessary.

Epithelial bodies and parathyreoids. In all the gnathostomes thus far studied (elasmobranchs excepted, and if Mrs. Thompson's '10, contention be correct, in these forms as well), certain structures occur, which Maurer ('88) discovered in amphibians, and to which he gave the name 'epithelial bodies,' the homologues of which, according to certain authors, have since been discovered in higher forms, but described under different names; parathyreoids (Sandstrom), glandules thyreoidea (Gley), et cetera. These structures develop from the ventral region of the third and fourth visceral pouches in the higher vertebrates, (reptiles, birds and mammals), and their homology to the epithelial bodies of the amphibians become at once apparent. Although enbryologically and anatomically quite distinct and different in every way from the thyreoid, yet topographically, in the higher forms at least, the parathyreoids become intimately associated with the former. This fact is strongly emphasized by Mrs. Thompson ('10, p. 127), who says, "Thyreoids and parathyreoids are to be looked upon as structures of somewhat different embryological origin, which

are anatomically separate and distinct in the lower vertebrata but which come into very intimate anatomical and physiological relationships with each other in the mammalia." Their functions, so far as they are known, are very different.

IV. GENERAL SUMMARY

1. The anlagen of the thymus bodies are recognized in Amblystoma larvae 8 mm. long, as epithelial thickenings at the caudal dorsal extremity of five pharyngeal pouches (counting the hyomandibular pouch as the first). These thickenings soon become solid epithelial bodies, which lose their early connection with the pharyngeal entoderm, and lie in the connective tissue dorsal to their respective pouches.

2. The darkly staining cells which are scattered or grouped about in the region close to the thymus bodies are regarded as mesenchymatous in origin and are not ectodermal contributions to the thymus bodies.

3. Of the five primitive epithelial thymus bodies, the first two degenerate early; this degeneration takes place with some irregularity in different individuals.

4. The three remaining bodies are, at first, small, structures of about equal size; during the later larval stages they increase gradually in size and become more elongate.

5. The three bodies persistent during the larval stages, lie close behind one another and caudo-lateral to the posterior wall of the ear. The third and fourth are usually close together, while between the fourth and fifth bodies there is a much larger interval.

6. In the adult, the thymus gland is a three-lobed flattened structure (probably formed by the union of the three larval thymus bodies) lying in the side of the neck, caudad and a little dorsal to the angle of the jaw. It is surrounded by loose connective tissue richly supplied with vascular vessels, and is innervated by rami of the glossopharyngeal and vagus nerves.

7. The anlage of the thyreoid gland is recognized in larvae 5 mm. long, as a very shallow cup-like depression in the medial floor of the pharynx in the region of the hyomandibular pouch be-

tween the thickened oral plate, and the anterior limits of the pericardium.

8. As development proceeds, this cup-like anlage proliferates cells from its ventral surface forming a 'solid cord of cells which extends caudally toward the anterior wall of the pericardium, but it does not unite with the latter in any way. A little later the distal extremity of this cord divides into right and left halves, the division being completed in 10 mm. larvae, and in addition, the connection with the pharynx is lost; the two halves lie lateral to the geniohyoideus muscle of either side.

9. I find no evidence that the cells which formed either the isthmus of the two halves of the thyreoid, or those which formed the connecting stalk persist to form accessory thyreoids.

10. After division into right and left halves, each half loses its solid and compact condition. The epithelial cells scatter loosely into the connective tissue and arrange themselves irregularly about the dorsal wall of the inferior jugular vein of either side and from these loosely scattered cells the follicles of the gland subsequently arise by mitotic division.

11. Follicles first appear in larvae 15 mm. long; they are either globular or elliptical, with a well defined outer layer of cuboidal cells enclosing a conspicuous cavity, probably containing a fluid of some sort but no colloid. They vary greatly in size, but, as a rule, they enlarge as development proceeds.

12. A membrana propria appears late in the larval life; it surrounds not only the follicles and the intervening lymph spaces, but also envelops the inferior jugular vein in the region of the thyreoid gland.

13. A so-called rete mirabile of the inferior jugular vein fails to develop during the whole of the larval period, and the external carotid artery has no direct connection with the gland in any of the larval stages.

14. Colloid appears rather late in the larval stages, the first evidence of it being seen in larvae 39 or 40 mm. long.

15. With the transformation of the ventral gill-region, the thyreoid gland is pushed a little laterally and caudally, so that it lies in the space just in front of the anterio-lateral wall of the

pericardium, and, in the adult, it is flanked medially by the genio-hyoideus, laterally by the hyoideus internus muscle of the first branchial arch, and dorsally by the sternohyoideus muscles.

16. From the condition of the follicles in the late transforming and early adult gland, it is evident that new and smaller follicles are being budded from the larger ones.

17. The blood supply of the adult gland is from small venous twigs coming from either the sternohyoideus or geniohyoideus muscles (never from the large inferior jugular trunk). These, upon entering the gland, break up into numerous smaller vessels ('rete mirabile') and join the jugular vein some distance caudad. A connection with the external carotid artery is very doubtful, in only a single case did a small twig (probably the thyreoid artery) pass into the vascular network of the gland.

18. The anlage of the postbranchial body is recognized in larvae 8 mm. long. It develops as a rule on the left side (in single individual, 19 mm. long, on the right side as well). At first it is a thickening of a portion of the pharyngeal floor, between the anlage of the fourth branchial (fifth visceral) pouch and the an-lage of the glottis, and protruding slightly ventrally toward the dorsal wall of the pericardium. It is thus 'postbranchial' as Maurer claimed for the similar structure in Triton, and appears in the relative position of the sixth pouch.

19. In 9.5 mm. larvae, the anlage makes a solid cylindrical stalk of cells extending vertically downward from the floor of the pharynx. It soon elongates, loses its connection with the pharynx and as a irregular, poorly defined mass of cells extending in an anterio-posterior direction lies medial to the aditus laryngeus muscle.

20. In one 19 mm. larva, a postbranchial body was found on the right as well as on the left side; the left being the larger. This unusual condition is of interest since it shows variability within a genus of urodeles. It is also important as bearing upon the questions of homologies of these structures.

21. In the later larval stages the postbranchial body becomes very irregular; in some regions it is solid, in other parts it shows follicular structure, with cuboidal cells forming a fairly definite

layer enclosing a conspicuous lumen, but which in no case contains colloidal material.

22. During transformation, because of increase in size of the surrounding structures (especially the laryngeus and sterno-hyoideus muscles) the postbranchial body is flattened laterally and reduced to a sheet of poorly defined cells which now contains no lumen.

23. The structure and anatomical position of the postbranchial body in the adult is variable. In sections of young adult heads, it is a fairly compact oval body composed of fairly regular cuboidal cells enclosing a spacious lumen. In sections of old heads, I was not able with certainty to locate the structure.

24. The carotid gland and epithelial bodies in Amblystoma begin their development at the time of metamorphosis. There is no evidence to show that the epithelial cells of the degenerating gill-pouches are concerned in its formation.

25. The anlagen of the epithelial bodies (two on either side) are irregular longitudinal sheets of entodermal cells (remnants of the ventral portions of the fourth and fifth visceral pouches) extending caudad from points where the afferent branchial arteries enter their corresponding gills in the early transforming stage.

26. As transformation proceeds, the sheets of cells become resolved into compact oval bodies (two on a side). In the late transforming stage, these lie one behind the other, close caudad to the carotid gland, each being enveloped by connective tissue. They now take a position lateral to the second aortic arch.

27. The two epithelial bodies in the adult lie imbedded in connective tissue on the side of the neck in the region just below the thymus gland, just caudad to the carotid gland, although their position may vary slightly in different individuals.

28. The blood supply of the epithelial bodies is from small twigs of the second aortic arch, and from the external carotid artery, while they are innervated (probably) by a small ramus from the vagus ganglion.

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ABBREVIATIONS

a.a. $_{1-4}$, aortic arches

a.b.a. $_{1-4}$, afferent branchial arteries

a.l., aditus laryngeus muscle

ao., dorsal aorta

l r. $_{1-5}$, branchial pouches

c.br. $_{1-4}$, branchial cartilages

c.g., carotid gland

co., copula

d.c., Cuverian duct

d.i., gastric muscle

d.l., dorso-laryngeus muscle

e., epithelial cells

e.b. $_{-1,2}$, epithelial bodies

e.b.a. $_{1-4}$, efferent branchial arteries

e.c., external carotid artery

e.j., inferior (external) jugular vein

fol., thyreoid follicles

g.hy., geniohyoideus muscle

ht., heart

hy., hyoid

hy.m., hyomandibular pouch

i.c., internal carotid artery

i.j., internal jugular vein

l., lateralis nerve of the vagus

lat.d., latissimus dorsi muscle

l.br., levator branchiarum muscles

l.s., levator scapularis muscle

ma., mandibular artery

mas., masseter muscle

m.c., Meckel's cartilage

m.hy. mylohyoideus muscle

m.p., mandibular-pectoralis muscle

n., notochord

n. $_{1,2,3}$, branchial nerves

p.a., pulmonary artery

pb., postbranchial body

ph., pharynx

pro-h., procoraco-humeralis muscle

st., stomodeum

s.v., sinus venosus

t. $_{1-5}$, thymus bodies and gland

te., temporalis muscle

tp., trapezius muscle

tr., thyreoid anlage or gland

v.a., ventral aorta

v.v., vascular vessels

VII, facial ganglion

IX, glossopharyngeal ganglion

X, vagus ganglion

1, cartilage of the first arch

2, cartilage of the second arch

3, cartilage of the third arch

4, cartilage of the fourth arch

PLATE 1

1. Medial sagittal section of 5 mm. larva showing relation of the early thyreoid anlage (*tr.*) to the oral plate and the pericardium. (× 50)

2. Transverse section on line *a* of figure 1, showing the thyreoid anlage (*tr.*) projecting between the mandibular arteries of the two sides. (× 50)

3. Enlarged portion of figure 2. (× 200)

4. Medial sagittal section of 8 mm. larva, showing the now elongated thyreoid anlage and its relation to the ventral mesoderm (*co*) and the pericardium. × 50)

5. Transverse section along line *b-b*, of figure 4, showing cells of the stomodeum and the thickened oral plate. (× 50)

6. Transverse section of 8 mm. larva, along line *c-c*, of figure 4; the anlage of of the thyreoid (*tr.*) still connected with the pharynx. Anlage of first thymus body (t_1) is at the dorsal lateral angle of the hyomandibular pouch, and below the facial ganglion (*VII*). (× 50)

7. Transverse section of 8 mm. larva, along line *d-d*, of figure 4, showing the thyreoid anlage (*tr.*), ventral mesoderm (*cc.*) and anlage of the geniohyoideus musmuscle (*g.hy.*) of either side. (× 50)

8. Transverse section through the anlage of the second thymus body (t_{II}) of 8 mm. larva. It arises from the entodermal cells of the dorsal angle of the first branchial pouch (*br.1*), and lies lateral and ventral to the glossopharyngeal ganglion (*IX*). (× 50)

9, 10, 11 Transverse sections showing the anlagen of the third, fourth and fifth thymus bodies of 8 mm. larva respectively (t_{III}) (t_{IV}) (t_V). (× 50).

10 a Transverse section of 8 mm. larva, showing the anlage of the postbranchial body at this stage. (× 200)

12 Transverse section through the anlage of the first thymus body (t_I) of 9.5 mm. larva. This section also shows the unpaired thyreoid (*tr.*) between the genio-hyoid muscles; the copula lies just above it in the medial line. (× 50)

13. Transverse section through the caudal extremity of the thyreoid of 9.5 mm. larva, showing its division into right and left halves in this region, the copula dipping ventrally between the two. (× 50)

14 Transverse section showing the second thymus body (t_{II}) of 9.5 mm. larva completely severed from the pharyngeal entoderm, and lying dorsal to the pharynx, medial to the levator muscle of the second arch. (× 50)

15 Transverse section showing the third thymus body (t_{III}) of 9.5 mm. larva in connection with the pharyngeal epithelium in the region of the dorsal extremity of the second branchial pouch. The anlage of the postbranchial body (*pb.*) is also shown. (× 50)

16, 17 Transverse sections showing the position of the fourth and fifth thymus bodies (t_{IV}) (t_V) of 9.5 mm. larva in relation to their respective pharyngeal pouches. (× 50)

18 Transverse section showing the now much reduced finger-shaped first thymus body (t_I) of the 10 mm. larva. It protrudes dorsally from the pharynx and reaches almost to the ventral surface of the facial ganglion, which lies just above it in this region. ($\times$ 50)

19 The same thymus body enlarged ($\times$ 200), showing large epithelial cells similar to those of the pharynx. It is connected directly with the pharynx by a slightly constricted stalk.

20 Transverse section showing the postbranchial body of 10 mm. larva. ($\times$ 50)

21 Transverse section through the fifth thymus body of 11 mm. larva. ($\times$ 50)

22 Section through the fifth thymus body of 11 mm. larva much enlarged ($\times$ 200), showing the large epithelial cells imbedded in the surrounding connective tissue.

23 Transverse section through the anterior region of the thyreoid gland of a 19 mm. larva, showing the position of three thyreoid follicles placed about the dorsal wall of the inferior jugular vein. The external carotid artery is somewhat dorso-lateral. ($\times$ 95)

24 Section showing the three thyreoid follicles of figure 23, in more detail. ($\times$ 200)

25 Transverse section showing the relative position of the postbranchial body, (pb.) of 19 mm. larva. ($\times$ 50)

26 Transverse section through the third thymus body (t_{III}) of a 19 mm. larva. ($\times$ 50)
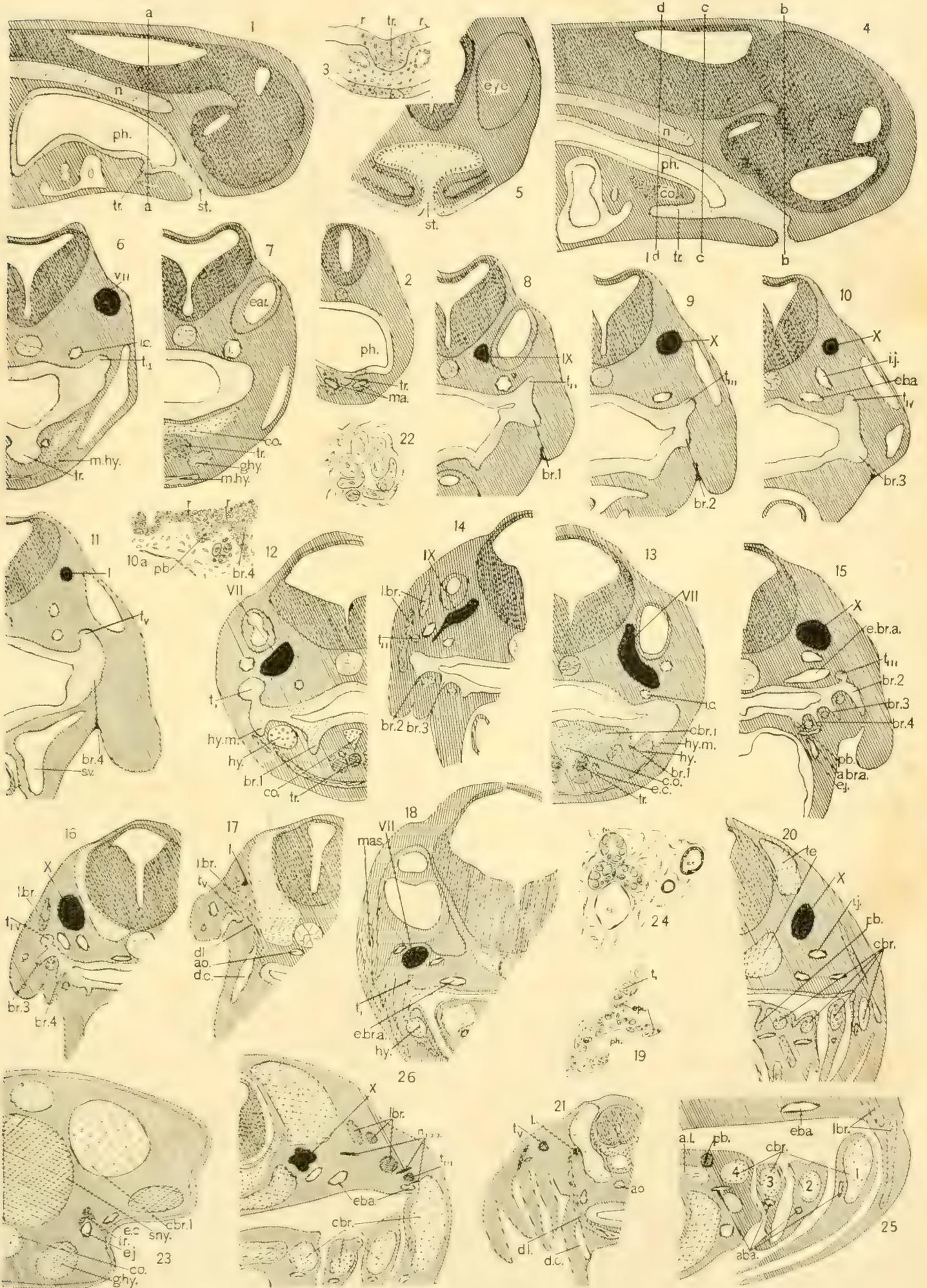
PLATE 2

27, 28 Transverse sections showing the relative positions of the fourth and fifth thymus bodies (t_{IV}) (t_{V}) of a 19 mm. larva. ($\times$ 50)

29 Transverse section through the central region of the third thymus body of 25 mm. larva, showing the comparatively few large epithelial cells forming the margin of the body, within which is a conspicuous space. Note there is as yet no connective tissue envelop about the margin, the cells are simply imbedded in loose connective tissue cells. ($\times$ 200)

30 Transverse section through the central portion of the third thymus body of a 35 mm. larva, showing the now scattered smaller epithelial cells surrounded by a connective tissue envelop. ($\times$ 200)

31 Transverse section through the central region of the thyreoid gland of a 45 mm. larva, showing the arrangement of the thyreoid follicles (*fol.*) to one another, and to the vascular and lymph vessels (*v.v.*). Note that the inferior jugular vein (*e.j.*) is almost surrounded by the follicles, and is enclosed like them with the 'membrana propria' of the connective tissue, while the external carotid artery (*e.c.*) is not thus enveloped. ($\times$ 200)

32, 33, 34 Transverse sections through the third, fourth and fifth thymus bodies (t_{III}) (t_{IV}) (t_{V}) of an early transforming larva. Note the condition of the now transforming pharyngeal pouches. ($\times$ 15)

35. Transverse section through the head of a young adult, showing the position of the second epithelial body ($e.b._2$), and the thymus gland (*t*). ($\times$ 15)

36 Schematic ventral view of the head of a young adult, showing the relative positions of the thyreoid (*tr.*), thymus (*t*) glands and epithelial bodies (*e.b.*), to the blood vessels and the muscles of the region. ($\times$ 4)

37 Lateral view of adult showing the position of the three-lobed thymus gland. ($\times$ 4)

38 Wax reconstruction showing the relationships of the postbranchial body (*pb.*) of a 19 mm. larva. ($\times$ 50)

39 Transverse section showing the early anlagen of the epithelial bodies ($e.b._{1,2}$). ($\times$ 25)

40 Schematic lateral view of the head of adult, showing the position of the thymus (*t*) and thyreoid (*tr.*) glands, epithelial bodies (*e.b.*) and the blood vessels. ($\times$ 4)

41 Wax reconstruction of the three thymus bodies of a 19 mm. larva (dorsal view of right side), showing their relative position to the branchial cartilages, (*1, 2, 3, 4*) blood vessels, and nerves of the region ($n_{1,2,3}$). ($\times$ 50)

42 Wax reconstruction of the region of the epithelial bodies (*e.b.*) of a young adult on the left side, showing their relation to the blood vessels, carotid glands, thymus and thyreoid glands, etc. ($\times$ 50) (See also fig. 35, in section.)
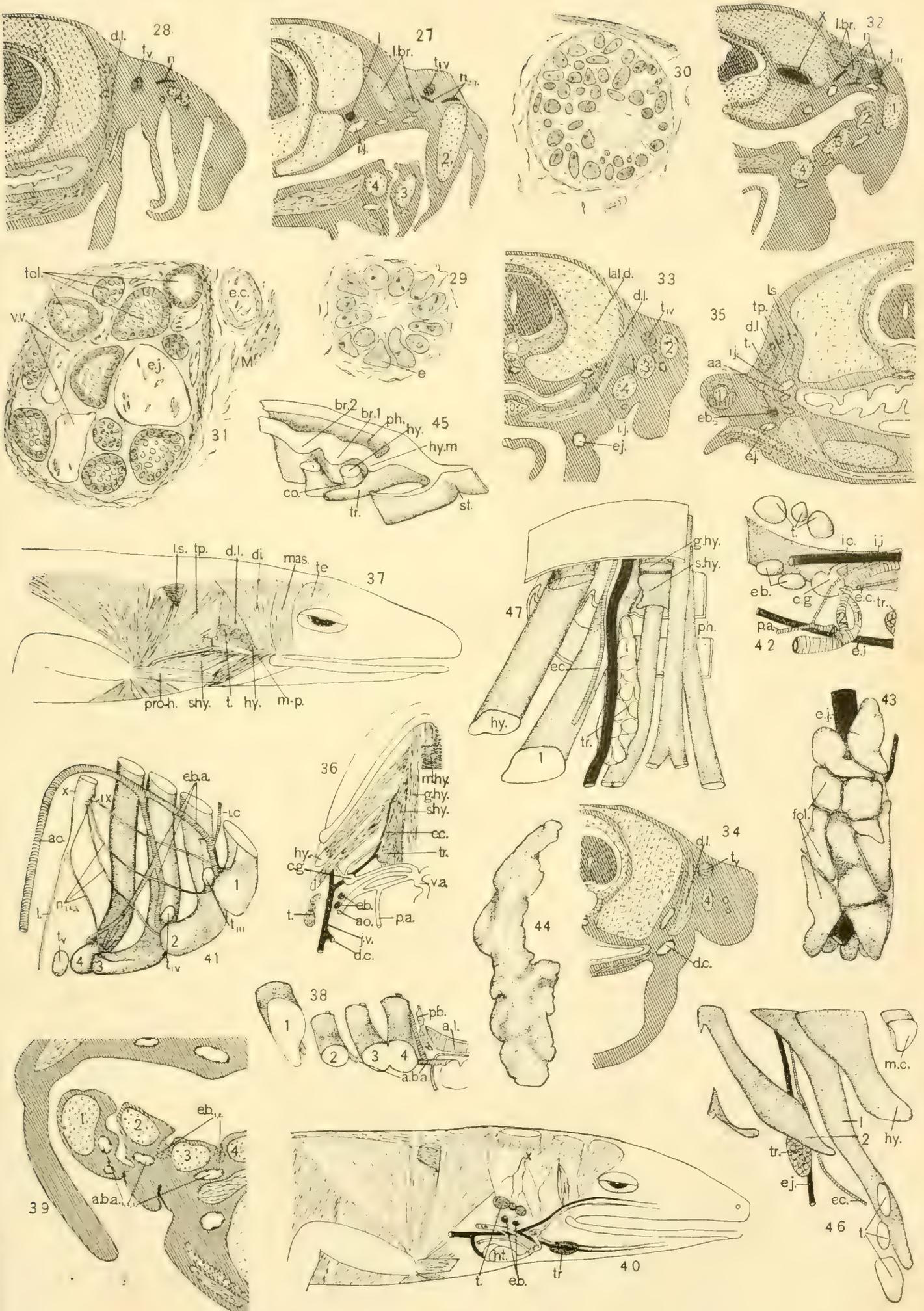
43   Wax reconstruction of the thyreoid gland of an early transforming larva, showing the relation of follicles one to another, and to the external (inferior) jugular vein, and the small medial twig from the sternohyoideus muscle, which forms a rete mirabile within the follicular mass.   Note the large size of follicles. (See fig. 31, which is a cross section of this gland for details of the internal relationships.)   ($\times$ 62)

44.   Wax reconstruction of the left postbranchial body of 26 mm. larva, from above.   ($\times$ 200)

45   Wax reconstruction of the thyreoid anlage of 8 mm. larva, showing its connection with the pharyngeal epithelium anteriorly at the point where the hyomandibular pouch is extending laterally.   Notice also, the position of the copula (co.) and the hyoid and first branchial cartilage anlagen just dorsal to it.   ($\times$ 50)

46   Wax reconstruction of the hyoid apparatus of the late transforming larva which is almost that of the adult condition, showing the relative positions of the thyreoid (tr.) and thymus (t) glands to the cartilages.   ($\times$ 50)

47   Wax reconstruction of the ventral pharyngeal region of 39 mm. larva, (right side), showing the position of the thyreoid gland in this stage to the branchial cartilages and blood vessels.   (about $\times$ 30)

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