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A STUDY OF DESICCATION IN THE ROTIFER, PHILODINA ROSEOLA, WITH SPECIAL REFERENCE TO CYTOLOGICAL CHANGES ACCOMPANYING DESICCATION.

LOUIS MAX HICKERNELL.

CONTENTS.

I. Introduction.

The investigation upon which this report is based was begun while the author was a graduate student in the Department of Biology of Princeton University. A short summary of the earlier findings has been published in a former article ('14). The study of additional material and the completion of the present paper has been carried out in the zoölogical laboratory in the College of Liberal Arts of Syracuse University during the last year. To Dr. E. G. Conklin, who first suggested this investigation to me, I wish to express my thanks for his interest and for the many suggestions made at various times during the course of this study. I am also indebted to Dr. C. W. Hargitt for many favors during the latter part of my work.

The ability of certain rotifers, tardigrades and nematode worms to withstand periods of desiccation has been a subject of investigation for many biologists throughout a period of more than two hundred years. The first recorded observations upon desiccation phenomena are those of von Leeuwenhoek in 1701. From the gutter of a roof he took some dust which he moistened and examined with his microscope. He observed living animals swimming about actively in the water. He found that the animals, which were, no doubt, rotifers, could be deprived of moisture for many months and could then be revived by the addition of water.

After the work of von Leeuwenhoek, the problem of the drying of living things was forgotten for a time. It was not until the period between 1750 and 1775 that interest in the study of desiccation phenomena was revived. During this quarter century many forms were added to the list of animals capable of enduring desiccation.

In 1776 Spallanzani discovered certain tardigrades and nematodes which were able to endure desiccation. He worked also on rotifers and was the first to state that rotifers, when dried free from sand, could not recover from the effects of the drying process. The failure of Spallanzani's rotifers to recover has since been shown to be in no way due to the absence of sand but his experiments aroused much discussion and engaged the attention of many of the foremost naturalists of that day.

Ehrenberg (1838) maintained that the presence of sand protected the rotifers from an actual loss of water. He believed that during the apparent desiccation period all of the vital processes continued, reproduction included. In regard to the function of the sand in retarding desiccation he differed from Spallanzani since the latter believed that the sand protected the rotifer from the injurious effects of the air rather than from loss of water.

Bory St. Vincent, about this time, maintained that under no conditions could the animals survive desiccation but that their apparent revival was due to the hatching of eggs concealed in the sand.

Doyere (1842) after careful study confirmed in the main the observations of Spallanzani. Doyere found, however, that a few rotifers recovered in each lot dried on a clean slide. He disproved the theory of Spallanzani as to the fatal effects of the air, since he found that rotifers dried in air and then placed in a vacuum showed a lower mortality than those dried directly in the vacuum. He concluded that the rapidity of drying is an important factor in the effects of desiccation. He decided from his experiments with rotifers *in vacua* that the last traces of water might be extracted without destroying the power of revival, and that since life processes are impossible in the absence of water, the dried animal possessed life poentially and not actually.

About 1860 a more general interest in the subject of desiccation was revived. The advocates and opponents of the view that desiccation occurs were so evenly divided and the evidence for both views apparently so well founded that the arguments for and against desiccation were considered in 1859 by a commission appointed by the Société de Biologie of France. It was the opinion of this commission that in the dried state life existed only *in potentia*.

Up to this time no one had suggested that rotifers might secrete a water-proof membrane during the dry season. In 1873 Davis said that in *Philodina* there was a secretion of a gelatinous covering which prevented loss of the body fluids. He explained that the presence of sand prevented rapid evaporation of the water and thus gave the rotifers time to secrete the gelatinous

envelope before the last traces of moisture on the slide disappeared. This conception has been widely accepted by many up to the present day.

Zacharias (1886) and Faggioli (1891) both reverted to the view of Bory St. Vincent that the supposed revival was due to the hatching of eggs concealed in the sand.

From the time of Faggioli's paper until 1909 no conclusive work on the subject of desiccation appeared. It will at once be seen that the main questions involved in the desiccation problem had not been solved. In 1909 Jacobs, working upon *Philodina roseola* attempted to determine the real conditions attending the drying process.

Jacobs outlines the questions involved in the desiccation problem as follows: "What is the actual effect of drying on a rotifer? Is the water really removed? Does the animal secrete a protecting membrane? If the animal actually dries what is the condition of its tissues? Are life processes at a standstill or are metabolic activities going on all the time in a reduced state?"

Jacobs concluded because of the shrinkage in the tissues, because of negative results in physical and chemical tests for water in the dried animal and as a result of other indirect methods, that the rotifer body becomes truly desiccated. He found no evidence of a waterproof cyst. His answer to the question regarding the condition of the tissues in the dried animal was not very definite, at least not complete, and this for the very good reason that he made no cytological examination of the tissues of the dried rotifers. Jacobs decides that the metabolic changes probably continue in the tissues of dried rotifers.

In all the discussion and observation upon the subject of desiccation phenomena previous to the publication of the preliminary note on this work, apparently no one had considered the problem from a cytological point of view. This is true not only of the study of animal tissues, but among plants as well. Certain algæ, pteridophytes and liverworts can be dried and will subsequently recover. This fact has been known for years. Pfeffer (1903) comments upon physiological phenomena attending desiccation but mentions no changes in structure. The author published in 1914 a preliminary account of the observations recorded in this paper. Brown (1915) working with McDougal at the

Desert Laboratory at Tucson, Ariz., describes cytological changes accompanying desiccation and subsequent recovery in the cells of *Echinocactus wislizeni*. These two papers, as far as is known to the author, comprise the literature on the cytology of desiccation.

Although this paper deals with the same kind of material as was used by Jacobs and although some of the questions asked by him are of most interest here, the problem has been attacked from an entirely different angle and by cytological methods confirms and adds to the results of Jacobs which were obtained by physiological methods.

The purpose of this study is, therefore, to attempt by means of cytological examination to determine (1) the condition of the tissues of the dried rotifer; (2) the presence or absence of a protecting membrane in the dried condition; (3) the condition of metabolic activity in the tissues of the dried animal and (4) the changes attending a recovery from desiccation. In order to do this it will be necessary to compare carefully the cytological appearance of sections of animals in a condition of dryness and those of animals recovering from desiccation with sections of the normal, active animal. From the data so gathered the conclusions will be drawn.

II. Material and Methods.

Philodina roseola is one of the common rotifers belonging to the order Bdelloideæ. It occurs throughout the world and is found in depressions in rocks, eave troughs or almost any place which is moist or periodically moist and dry. It is usually associated with the unicellular alga, *Sphœrella lacustris*. Frequently one notices small individuals in hay infusions. These are, no doubt, introduced when dry hay is added to old cultures. Sometimes in infusions rotifers are found which are fully as large as those associated with the alga before mentioned. These may be encouraged to multiply if very weak hay infusion is added from time to time. The weak infusion allows sufficient bacteria to develop to replace the algæ as food and it seems not to harm the animals.

As has been stated by other authors, the behavior of *P. roseola* is dependent upon a number of factors, chief of which seem to

be the purity of the medium in which it lives, the amount of food material present and the temperature of the medium. If the water surrounding the animals does not contain too much organic material, they usually may be seen stretched out, fastened by the foot and going through the usual feeding movements. When conditions are unfavorable they contract (Pl. II., Fig. 4) and remain quiet until the surroundings become more favorable. A slight increase in temperature is usually conducive to more vigorous movements, especially swimming.

In preparing *P. roseola* for microscopic examination or subsequent sectioning some difficulty may be experienced because of the fact that the animals are generally associated with sand or other foreign material. When the animals are present in sufficient numbers the method of collection suggested by Jacobs is perhaps the best. This takes advantage of the reaction of the animals to light. If subjected to no mechanical stimulus they usually are indifferent to natural light of ordinary intensity. However, if the culture is violently agitated by shaking or stirring they become temporarily negatively phototropic and may be collected with a pipette from the darker part of the culture. When it was desirable to select individuals of any given size or condition of maturity in these experiments, or to get some absolutely free from foreign material this was accomplished by picking up the animals one by one from a Syracuse watch glass with a capillary pipette, the entire process being watched with a binocular microscope. The foregoing statement is not to be interpreted as meaning that *P. roseola* is ever covered with debris. Such is not the case. The foreign material alluded to consists of bits of sand, etc., to which the animals adhere by means of the sticky secretion of the foot glands.

When first put on the slide under a cover glass the animals are generally so active that a single individual can be kept within the field of a high power lens only with greatest difficulty. As some of the water evaporates from under the cover glass, and as the latter gently presses upon the animal, movements become less rapid and many details of organization can be made out with little trouble.

By putting the rotifer in a weak solution of neutral red some of the internal organs are stained but not with any particular

sharpness. By proper manipulation of the light through the condenser the unstained specimen becomes practically as favorable for examination as that stained in neutral red.

In preparing dried animals for sectioning large numbers of active animals, free from sand, were put in Syracuse watch glasses and the latter placed in an oven at about 40 degrees Centigrade. While the desiccation process is always fatal to a few animals in each lot which is being dried, I can affirm what Jacobs has already pointed out, namely, that in the case of animals dried on clean glass the mortality is lowest when the temperature is about 40 degrees Centigrade and the moisture is not allowed to pass off too rapidly.

In preparing undried rotifers for sectioning a large number was collected in a test tube and anesthetized with the cocaine-methyl-alcohol mixture of Rousellet. When the animals were completely relaxed the excess liquid was drawn off and the fixing fluid poured upon the entire mass. After fixation the material was washed thoroughly, passed rapidly through the alcohols, cleared in xylol and embedded in paraffine of 54–56 degrees melting point. Sections were cut 3–5 μ in thickness.

In the case of the dried animals the fixative was poured directly upon them and allowed to act from twenty to thirty minutes.

Several fixing fluids were employed. Bouin's picro-acetic formol was not very satisfactory. A solution of saturated corrosive sublimate with 5 per cent. acetic acid was used hot. This was quite satisfactory in that its action was almost instantaneous and the preservation of structures faithful in most respects. Beauchamp ('09) says that sublimate fixation was very poor in the forms he studied. He does not state the exact formula employed in his experiments but it would appear that he did not try the one mentioned above. By far the best formula for fixation is the osmic-sublimate-bichromate mixture recommended by Beauchamp.

In staining the sections iron-alum hæmatoxylin combined with various counter stains gave the best results. The iron-hæmatoxylin-eosin-lichtgrün stain of Beauchamp is very valuable. Another combination consisting of safranin, orange G and lichtgrün was used with some success. The latter is not so precise as the hæmatoxylin combination.

OBSERVATIONS. 1. *On Entire Animals.*

A. *Anatomy of P. roseola.*—Janson ('93) has carefully considered the anatomy of the Philodinidæ and I find few inaccuracies in his statements as applied to *P. roseola.* It will be necessary, however, to briefly describe the organ systems and their relationships in order to arrive at a thorough understanding of the changes in form and rearrangement of parts incident to the desiccation process.

The body of *P. roseola*, at first sight, suggests that of a segmented worm. Its segmentation (which affects only the integument and accessory structures) is, however, not so regular but that the body is easily divided into head, trunk and foot. Its outer integument is formed of a thin, more or less transparent cuticle beneath which is a granular plasma layer, the hypodermis. In the hypodermis are deposited pigment granules. These are absent in the very young animal and occur in varying number in the adult. The cuticle is soft and flexible, easily lending itself to the telescoping and folding so characteristic of this *Philodina.*

The hypodermis is a syncytium which contains nuclei distributed evenly through it. There are, in the mid-body region, longitudinal folds of the skin which are always present. Temporary cross foldings occur as a result of the vigorous movements of the animal. Externally the skin lacks spines or protuberances of any kind.

The head, whose most prominent structures are the trochal discs, extends as far back as the forward margin of the mastax. When the trochal discs are folded in, a blunt, ciliated projection is seen at the anterior end. This is the proboscis and it is used for tactile purposes as well as to aid in the "measuring-worm" type of locomotion so characteristic of the Bdelloideæ. The rotating organs consist of a double row of cilia, broken in the case of the anterior row, in the ventral part of the head. The more posterior ciliary wreath is made up of shorter elements and is directly continuous with the cilia which line the pharangeal cavity. The hypodermis in the head region at the base of the trochal discs is thickened, probably to furnish additional support for the rotating apparatus, and in some specimens the nuclei of this layer can be seen near the bases of the lines of cilia.

Dorsally, and arising at the posterior margin of the head, is the tactile organ (Pl. I., Fig. 1, *t. o.*) which shows from two to four segments according to its state of extension. It is capped at the end by a tuft of short cilia. Nerve fibers connect it with the brain as Zelinka has shown.

The brain (Fig. 1, *br.*) lies with its anterior margin just at the base of the tactile organ, while it extends posteriorly slightly over the forward part of the mastax. It is triangular in shape with the sharpest angle extending forward. The eyes (Fig. 1, *e*) which are red in color, lie dorsal to the brain.

The excretory canals (Fig. 2, *e. c.*) extend into the head and a terminal flame cell (*f. c.*) can sometimes be seen beating at the base of each trochal disc. These flame cells are perhaps best seen when the trochal discs are folded in. They appear very close to the margins of the folded discs (Pl. II., Fig. 3).

The trunk is almost entirely filled with the organs of digestion and those of reproduction. In its most anterior part the mastax (Pl. I., Fig. 1, *m*) is prominent. Food enters this by way of the narrow, ciliated pharanyx (Pl. I., Fig. 2, *ph.*). The mastax proper consists of two more or less crescent-shaped jaws. Transverse ridges, which at the inner margins continue as short projections, form the teeth (Pl. I., Fig. 1, *t*). In all the animals I have studied there have been two teeth on each jaw. A few authors report cases where there are three teeth on one jaw and two on the other.

The mastax is surrounded by glands which probably contribute a digestive fluid to the food as it is being crushed in the mastax. There are five of these salivary glands, two of which lie dorsally and three ventrally. The dorsal ones (Fig. 1, *s. g.*) follow the outline of the mastax quite closely, while those on the ventral side are usually larger and extend posteriorly (Fig. 2, *s. g.*). In the living animal these glands show a granular cytoplasm with nuclei of varying sizes. These nuclei appear as bright spots against the darker cytoplasmic background.

From the mastax a narrow esophagus leads to the stomach-intestine (Pl. I., Fig. 1, *st. i.*). In most cases it is difficult to see the esophagus in the living animal. This is also true of a pair of glands which lie at the anterior end of the stomach-

intestine. Both the esophagus and these glands are obscured by the ventrally lying salivary glands, and it is only when the animal is thoroughly stretched that these structures can be distinguished.

The stomach-intestine, as seen in the living animal, consists of a yellow, viscous tissue containing granules, fat-droplets and nuclei. By careful observation all of these elements may be recognized. The lumen is usually not seen except in sections. In healthy, active animals the stomach tissue shows its color distinctly. In starved animals the color is lacking in the stomach tissue as Zelinka has pointed out. In newly hatched *Philodina* the stomach is almost perfectly transparent.

Just posterior to the stomach-intestine is the "blasendarm" (Fig. 1, *bl.*) of Zelinka. This is thick-walled but not glandular. It serves for the accumulation of the undigested remains of the food. By the contractions of its walls this material is evacuated from time to time. Darker in color and more homogeneous in structure, this first division of the end gut is the most prominent structure in the posterior part of the trunk of the living *Philodina*.

The "blasendarm" leads into the rectum (Fig. 1, *r*), a narrower tube in the dorsal wall of which the contractile bladder (Fig. 1, *c. b.*) is found. This bladder is thin-walled, extensible and on either side receives the lateral excretory canals. The bladder pulsates at intervals of fifteen to twenty seconds and in so doing disposes of the accumulated waste which has been emptied into it by the excretory canals.

Lying on either side of the stomach-intestine are the reproductive organs. Since no males of *Philodina* are known, these organs are always ovaries with their accessory structures. The ovary consists of from six to ten small nuclei which lie close together in a clear syncytial ground substance on the inner margin of the vitellarium. It is not easily seen in living animals unless it contains a developing egg (Pl. I., Fig. 1, *eg.*).

The vitellarium (Fig. 1, *v*) is especially prominent in living animals. It is a spindle-shaped structure at either end of which is a connective tissue strand which fastens it to the other organs. The anterior strand is fastened to the body wall in the region of the mastax, the posterior one attaches to the digestive system

in the region of the boundary between the "blasendarm" and contractile bladder. The posterior strand looks as if it might act as an oviduct but I have never seen an egg or an embryo passing through it. This fact, together with observations of other authors who have seen embryos of *Philodina* liberated from the mother by a rupture of the body wall, makes it seem certain that if this structure ever did function as an oviduct it is now only rudimentary. The vitellarium contains eight large nuclei arranged in a row. This number may vary but this is not usually the case.

The lateral excretory canals run posteriorly through the trunk and very close to the vitellaria. They are minute, thread-like structures having flame cells connected to them at intervals. In *P. roseola* I have never observed more than five of these flame cells on each side. The canals empty into the contractile bladder as was described above.

The rectum is a narrow tube leading posteriorly from the contractile bladder and terminating dorsally in the anus (Fig. 1, *an.*) at the posterior border of the second foot segment.

The foot contains the glands which secrete a sticky substance which enables the animal to fix itself during its crawling movements. These glands are oval in outline, are uninuclear and empty by means of ducts at the base of the toes. When the animal is folded up these glands become packed closely about the "blasendarm." When the rotifer stretches out they occupy places in the four posterior foot segments. There are four toes on the tip of the foot. These aid in fixing the animal during feeding movements.

B. *The Active, Free-swimming Animal.*—*P. roseola* in its behavior shows no great difference from most of the other Philodinidæ. Zelinka ('86) and Janson ('93) have described the movements of the philodinids in general terms while Jacobs ('09) has given an account of *P. roseola* both under ordinary conditions and at the onset of desiccation. I have given the behavior of *P. roseola* much attention during the course of this work and am able to confirm Jacobs in all essential respects.

Two methods of locomotion are employed, viz., swimming by means of the trochal cilia and creeping by the alternate use of

foot and proboscis. The method of locomotion depends to a large extent upon the temperature and purity of water in which the animal lives. Other things being equal, a relatively high temperature is conducive to active swimming movements, while with lower temperatures the animal generally restricts itself to the leech-like creeping. Another factor influencing the kind of movement is the purity of the water. In cultures which contain many putrefactive bacteria the animals usually are found creeping sluggishly about, and they seldom extend the trochal cilia for any great length of time.

Swimming is the result of the rotating of the trochal cilia and involves no turning upon the body axis. The body movements during swimming are similar to those of certain infusoria as described by Jennings ('04). If any obstruction is met with in swimming the trochal cilia are suddenly retracted and a series of random testing movements occur. The cilia are then extended and swimming is resumed in a direction where there is no obstruction. Sometimes an animal will be observed to stop if it swims through a region where food is abundant. In this case it attaches itself by the foot and feeding movements begin.

In creeping the rotating organs are always retracted. The animal attaches itself by means of the secretion of the pedal glands and then after a greater or less number of testing movements, in which the body is stretched successively in several directions, it attaches itself by the end of the proboscis. This attachment of the head region is followed by the wrenching loose of the foot. The latter is then brought to a point nearer the head and the operation is complete. In changing from the creeping to the swimming movement the trochal discs are extended and the foot is loosened by a contraction of the muscles in the posterior region. The animal then moves steadily forward as long as its trochal cilia are in motion.

While feeding the animal is always attached by the foot. Currents created by the rotating organs carry bacteria, algæ, etc., in a steady stream into the open pharynx where they are further propelled by the long cilia lining the pharynx. The body may sway from side to side during feeding, in fact, the animal is frequently seen to bend its body as if to reach some

particles which were not before influenced by its trochal activity. During all this time the mastax is active. Its grinding operations are easily observed in any feeding animal.

C. *The Contracted Animal.*—Zelinka ('86) in describing the movements of rotifers of the genus *Callidina* states that the animals frequently retract the head and rotating organs without any apparent reason. He observes further that their subsequent extension may be rapid or slow and that no definite reason can be assigned for the lack of uniformity in this respect among different individuals. *P. roseola* contracts very rapidly but usually extends itself slowly. The factors which influence it to contract seem to be (1) Sudden change in temperature of the medium; (2) change in the chemical composition of the medium; (3) mechanical stimuli; and (4) desiccation.

Gradual changes in temperature in the medium in which the *Philodina* lives do not cause it to contract. Increase in temperature is conducive to more vigorous movements while a decrease causes the animal to become sluggish. If the temperature is suddenly changed by the addition of hot or cold water the animal contracts and does not extend itself for many minutes.

The addition of any active chemical to the culture causes the rotifers to suddenly contract and if the foreign substance is not removed they will remain in the contracted condition until death ensues. Philodinas kept in infusions will frequently be found to become inactive in this way. An examination usually shows that the infusion is in too great concentration or that putrefactive or acid forming bacteria have rendered the environment unfavorable.

A mechanical shock of any kind will make the animals contract completely but a contraction under such conditions usually lasts but a few seconds unless the stimulus is continuous or repeated.

When the water around a *Philodina* begins to dry up the animal creeps about rapidly apparently trying to escape from the diminishing drop. This creeping continues until movement is no longer possible. The animal then contracts into an almost spherical mass and dries.

Especially interesting to observe is the manner in which the

organs are arranged in the contracted animal. While in the extended animal there are spaces between organs, when contraction takes place every available bit of space inside the trunk integument is filled by the closely packed organs. The head and foot segments are drawn entirely within the trunk by means of the contraction of longitudinal muscles. The circular muscles at each end of the trunk may then contract and cause the entire animal to assume a shape not unlike a lemon. The large organ systems can be seen through the integument of the contracted rotifer. The head and its accessory structures can be seen just anterior to the stomach tissue. The stomach occupies the central part of the mass and is flanked on either side by the reproductive organs. In the posterior end of the trunk the foot segments containing the pedal glands can be seen. Fig. 4, Plate II., shows a *Philodina* which has been contracted but is just beginning to unfold. The foot segments are slightly protruded while the head still remains within the trunk cavity.

D. *The Dried Animal.*—When the drop of water surrounding a *Philodina* begins to dry up the animal indulges in active creeping movements until the drop becomes too small to permit further activity. The rotifer then contracts and actual desiccation begins. It is at this time, according to the older authors, that the jelly membrane is secreted. The shiny appearance described by them is indeed apparent at times but this is due not to any jelly but to the fact that the tissues of the rotifers become packed together as water disappears and since loss of transparency accompanies loss of water the light is reflected rather than transmitted by the animal. Final proof of the absence of a jelly layer in dried rotifers will be given in the part dealing with sections.

The internal organs cannot be carefully studied while the drying is going on since the integument usually folds and wrinkles as soon as drying begins. This obscures the internal structures.

Animals dried under favorable conditions all tend to assume a similar shape. The head and foot segments are drawn into the trunk as before described. The circular muscles at the end of the trunk contract giving to the animal a spindle shape. When the drying process is very slow the irregular wrinkles do not

appear but the puckering due to the contraction of the circular muscles is evident. Jacobs has pointed out this fact and my Fig. 16, a longitudinal section through a dried animal, shows the sides as being free from folds while the ends show prominent and regular wrinkles.

Frequently several animals are carried together by the diminishing drop as it evaporates. In this case each rotifer tends to assume a hexagonal shape as a result of mutual pressure. Fig. 5, Plate II., shows an animal which was dried on a clean slide at room temperature and drawn nine days after the desiccation began. It will be seen at once that the integument has become much folded and the internal organs indistinct, but the vitellarium, foot and stomach are still recognizable.

E. *Animals Recovering from Desiccation.*—When water is added to dried animals, such as that described in the last section, they usually regain their normal size within ten minutes, often before. The rapid swelling of the animal causes any wrinkles which may be in the integument to disappear. Active movements may occur within a short time if the conditions under which they are dried are favorable. Sometimes movements may not occur for several hours, even a whole day. This fact may account for the negative results of the desiccation experiments of some of the older authors. These investigators probably did not give their rotifers sufficient time to recover, as Jacobs points out.

As the animals recover they resume their usual activities. I have noticed that rotifers recovering from desiccation are lighter in color than they were before the process began. Further comment will be made upon this point in a later paragraph.

2. *Study of Sections.*

The cytological details of the structure of rotifers has been described in comparatively few cases. Of the Bdelloideæ, *Discopus synaptæ* has been described by Zelinka ('86). From the study of the figures of sections through the principal body regions as represented by Zelinka one can see many points of similarity between this form and *P. roseola.* In the case of *P. roseola* I find no record in the literature which tells of the cytological structure of its tissues. The accompanying figures

and descriptions are therefore presented here for the first time.

The finer cytological changes accompanying the process of desiccation and subsequent recovery can only be shown after describing the conditions present in a normal, undried specimen. I shall therefore now describe the arrangement of organs and their cytological peculiarities in both extended and contracted normal animals, after which comparisons will be made with sections of animals which have been desiccated and also with sections of animals recovering from desiccation.

A. *The Normal Extended Animal.*—The relationship of the internal organs is well shown in transverse sections of the extended *Philodina*. Fig. 7, Plate III., shows a section through the posterior part of the head. The pharyngeal cavity leading to the mastax is lined with cilia. Its wall consists of thick cells with large nuclei. The brain, with outer cellular elements and inner homogeneous zone, appears close to the pharyngeal tube. The integument surrounding the region of this section is much thinner than it is in the mid-body region. This is to be expected when one remembers the flexibility of the head segments as compared with those of the trunk. There is a marked similarity in the arrangement of the elements here described and those in a transverse section through the anterior part of *Discopus synaptæ* as described by Zelinka ('88).

In *Discopus*, Zelinka shows the integument as being much thinner than I have found it in *Philodina*. The hypodermis of *Philodina* appears as a definite layer much thicker than the cuticle and containing nuclei embedded in its syncytial groundwork. This feature is seen in the figures of the sections throughout all parts of the animal's body. In *Discopus* the nuclei are flattened rather than round and appear as swellings upon the cuticle. Furthermore, Zelinka figures the hypodermal nuclei as being without a nucleolus. Such a condition is not usually found in *Philodina*. Another point of variance between the two forms concerns the brain. Zelinka figures the brain of *Discopus* as being composed of an outer ganglionic layer and a central "punktsubstanz." In *Philodina* the same divisions appear but the cells of the ganglionic layer have distinct walls as distinguished from the syncytial condition in the brain of *Discopus*. The

walls of the pharynx with their internal ciliation are not markedly different in the two forms.

A section through the esophagus is shown in Fig. 8, Plate III. As was mentioned before, the esophagus is difficult to observe in the living animal because it is generally concealed by the salivary glands which surround it. The section shows it to be a narrow, thin-walled tube. Surrounding it are the salivary glands whose cytoplasm is uniformly granular but not divided into distinct cells. The nuclei appear scattered irregularly and have the characteristic structure found in like elements of other tissues.

The esophagus in section in *Discopus* shows about the same relative size and position as it does in *Philodina*. It is likewise surrounded by the salivary glands so that it probably is not visible in the living animal. The salivary glands of *Discopus* do not appear to be as dense or deeply staining as those of *Philodina*. The cytoplasmic granules are less closely packed together and the nuclei appear to be less chromatic. The most noticeable difference between the figures of sections of corresponding regions in the two animals is the lack of hypodermal tissue in *Discopus*. While it is to be expected that the integumentary structures would be thinnest at the points of greatest flexibility, it seems that the total absence of hypodermis in this and some other of Zelinka's figures must have been a result of an oversight on his part.

A section through the middle portion of the trunk (Fig. 9, Plate III.) shows the reproductive glands and the middle portion of the digestive system surrounded by a somewhat thicker ring of the integument. The plasma of the vitellarium is a syncytium made up of granules of varying sizes. The granules are surrounded by small, clear areas containing a cell-sap which is lost during desiccation. The nuclei of the vitellaria (*v*) are the largest to be found in the rotifer body. These consist of a single karyosome surrounded by a clear homogeneous area and having at the periphery a distinct nuclear membrane. This is the "nucleolar nucleus" of Carnoy and is characteristic of most of the cells of the rotifer tissues. The ovary (*ov*) is a small elongated structure lying in a depression of the inner border of the

vitellarium. It never attains very great size. It consists of from five to twelve intensely chromatic nuclei surrounded by a glassy, homogeneous plasma. A section of a typical ovary is shown in Fig. 10, Plate III. Both ovary and vitellarium are surrounded by a delicate membrane.

Zelinka does not figure a section of the ovary of *Discopus* containing more than four nuclei. This small number is frequently observed in *Philodina* but the average number is slightly larger. The clear, non-staining cytoplasmic portion of the ovary seems to be similar in both forms but Zelinka does not figure a membrane separating ovary and vitellarium. Whether this last point was due to improper or insufficient staining is hard to say but it seems that a limiting membrane should be present. In *Philodina* a definite ovarian capsule can be easily demonstrated in sections through the proper region of the normal, free-swimming animals.

Another point which leads me to believe that the staining methods employed by Zelinka were insufficient to demonstrate all structural details is the fact that he does not figure a nuclear membrane in the nuclei of the vitellarium. The karyosome is surrounded by a clear area as in *Philodina* but he figures no limiting membrane about the entire structure. It is possible that he thought the karyosome to represent the entire nucleus. However this may be, there is, in all cases, in the vitellarium of *Philodina* a definite nuclear membrane surrounding the clear, outer zone of the nucleus.

In cross-section the stomach-intestine (Plate III., Fig. 9, *st*) shows as a thick-walled tube with a narrow lumen. From the inner wall cilia project into the lumen while at the base of the cilia are deeply staining granules, which Beauchamp, in other rotifers, interprets as cross-sections of longitudinal muscle fibers. My own observations lead me to believe that this interpretation is correct. The stomach tissue is of a spongy consistency, the ground substance appearing as closely packed vacuoles. No cell walls can be demonstrated but nuclei are scattered at intervals throughout its extent. The nucleus has a karyosome surrounded by a homogeneous plasma which stains with acid dyes. Scattered irregularly through the stomach tissue are large, deeply

staining granules which are masses of reserve food material. Janson ('93) says that these masses are kept by the rotifer for use during the dry periods when feeding must of necessity cease. Beauchamp ('09) shows sections of the stomach of *Hydatina senta* in which these granules appear. He shows further that in a section of the stomach of an individual of the same species which had been starved for twenty-two days, these granules had disappeared and were replaced by vacuoles. In sections of very young *Philodinas* no such granules are present and I shall show later that after periods of desiccation in *Philodina* these granules become fewer or disappear entirely, thus furnishing cytological evidence in support of the theory of Janson and Zelinka, which by them was based entirely upon observations upon the entire, living rotifers.

The stomach tissue is most pliable and the lumen seems normally to be able to occupy almost any position in it. This fact has been remarked by Beauchamp in *Callidina socialis*.

The stomach tissue of *Discopus* as figured by Zelinka lacks some of the elements which I find in *Philodina*. I refer to the granules of reserve food material just mentioned. In none of his figures does he show these deeply staining aggregations. It may be that the digestive processes of *Discopus* differ from those of *Philodina*. The difference in the habits of the two forms might account for this. On the other hand these elements might have been present but not differentiated by the stain. The latter condition does not seem probable, however, for I have found that most of the nuclear stains have an affinity for the food granules.

Another point of difference is in the structure of the ground substance of the stomach tissue. In *Discopus* this ground substance is represented by Zelinka as being made up of fine granules loosely packed together. In *Philodina* the ground substance of the stomach tissue is composed of alveoles closely apposed. This difference could perhaps be accounted for upon the basis of fixation.

Fig. 11, Plate III., shows a section through the most posterior part of the trunk at the point where the stomach-intestine joins the "blasendarm." The contrast in the structure of the two

tissues is well marked. The "blasendarm" contains none of the reserve food granules and its plasma has only occasional nuclei scattered through it. Zelinka does not show a section through this region of *Discopus* but there is probably no great difference here between the two animals.

Fig. 12, Plate III., is a transverse section through the second foot segment. It shows the pedal gland (*f. g.*) whose cytoplasm consists of fine alveoles and whose cell-walls are distinct. A single nucleus is present in each cell. The form of this nucleus is subject to little variation in the glands of the undried animal. It consists of a karyosome surrounded by a clear space and a well defined nuclear membrane.

The foot-glands of *Discopus* differ but slightly from those just described. The individual cells are perhaps more nearly round than those of *Philodina* and the chromatic part of the nucleus more nearly spherical. The general arrangement and appearance of these cells is, however, not much different from those of *Philodina*.

Ventral to the pedal glands and separated from them by a narrow space, lies the cloacal cavity. This section throws some light upon the nature of the contractile bladder itself. It has been assumed by Huxley, Claus, Vogt and Yung, Hudson and Gosse, that the contractile bladder is merely the enlarged ending of the lateral canals and must therefore be considered as a part of the excretory system in a strict sense. Semper thought that the contractile bladder merely forced the excretory fluid into the end-gut and that other contractions of the cloacal wall were necessary before the fluid reached the exterior.

In Fig. 12 the cloacal chamber is shown to be composed dorsally of a thin membrane such as one would expect in a structure as distensible as the contractile bladder is known to be. Ventrally the wall is thicker and in all respects similar in its texture to that of the "blasendarm" (Fig. 11). On one side and at the junction of the thin dorsal and thick ventral walls there is a break which I interpret as the entrance of the excretory canal. The large cell lying beside the entrance of this canal is part of the sphincter which prevents the fluid from re-entering the lateral canals at the time of the contractions of the bladder.

This sphincter has been described by other authors but sections of it in *Philodina* have never been shown.

It is evident then, that the contractile bladder is not an independent structure connected to the posterior part of the gut by a narrow neck but is only a portion of the wall of the gut modified to permit its distension. Its periodic contractions force the collected liquid waste to the exterior without further contractions of other parts of the cloaca. The more solid waste from the digestive canal is forced out of the "blasendarm" by independent contractions of the latter structure.

B. *The Contracted Animal.*—The closely packed condition of the organs in the folded animal is shown in Fig. 13, Plate IV. The cilia of the trochal discs and some of those lining the pharynx will be noticed. On either side of the infolded trochal cilia are the ends of the large glands connected with the mastax and with the anterior end of the stomach-intestine. The edge of the chewing apparatus is shown in the middle of the section, while surrounding it will be seen the stomach tissue and a small part of its lumen. The folding of the integument of the animal is well shown here, especially at the anterior end where the convergence of the longitudinal folds shows in section as a rosette. It is apparent that in the contracted condition most of the water which circulates in the body spaces is lost, for the organs lie close to each other in contrast to the condition shown in Figs. 1, 2 and 3 where the spaces, especially those between the integument and internal organs, are very large. Fig. 14, Plate IV., shows a section through a slightly different plane in an undried, contracted animal. In the central part of the section the cells of the outer part of the brain are shown while around the periphery various gland cells appear. This section also goes through one of the vitellaria and shows its characteristic form and structure.

C. *The Dried Animal.*—In the desiccated animal the arrangement of organs is in no way different from that in the previously described contracted, normal ones. The organs are still more closely apposed and the folding of the integument is closer and better marked.

Fig. 16, Plate IV., shows a section of a rotifer which was dried

at room temperature and then kept in an evacuated calcium chloride desiccator for eighteen days. At the end of this time it was fixed and sectioned. It will be observed that the cilia still show as individual fibers and have not fused into a homogeneous mass as some authors assert must be the case when all moisture is removed. There is no sign of fusion or other abnormal condition in these elements. Each cilium preserves its identity as well as would those of an animal living in its natural environment.

Of especial interest is the structure of the integument in the dried state. It has been asserted, and for some time quite generally believed, that just before actual drying takes place the rotifer secretes a jelly-like capsule through pores in the skin and this capsule hardens to make a water-proof cyst which remains during the dry season and is dissolved again upon the addition of water. Janson ('93) thinks it is not unreasonable to suppose that a gelatinous covering is secreted. He reasons that since the pedal glands (which are undoubtedly derived from the hypodermis) are known to secrete a sticky substance and since the tube-dwelling forms secrete a slime from their skins which helps to make the tube, then the forms which survive desiccation might easily do the same. He admits, however, that he has not found the actual secreting tissues.

Jacobs ('09) found, as a result of staining reactions with methylene blue, that the integument in the trunk is undoubtedly different in its chemical nature from that of the head and foot. He says: "The fact that that part of the cuticle which alone is exposed at the time of drying should be of a different nature from the remainder is probably significant." Jacobs is convinced that no water-proof cyst is secreted and he suggests that the thickened integument of the trunk region may be a means of preventing a too rapid evaporation as dryness comes on.

While I have no cytological evidence to show that the suggestion of Jacobs is correct or incorrect, certain it is that there is no thickening by a secretion or otherwise of the integument in the dried condition. Fig. 16 shows that instead of the integument being thicker it is actually much thinner than it was in the undried condition. The cuticle shows no great change in thick-

ness. This would be expected in a non-protoplasmic structure. The hypodermis, however, which in the undried animal is from two to four times as thick as the cuticle, has shrunk until it is scarcely thicker than the cuticle itself. The nuclei do not shrink perceptibly but cause swellings on the hypodermis at the points where they occur. I have examined sections of hundreds of specimens dried in various ways and know that this is the characteristic condition of the integument of the dried rotifer. I believe that these observations should effectively dispose of the arguments of those who maintain that the drying animal secretes a water-proof cyst for protection during the dry periods.

In the vitellaria the drying process affects the cytoplasmic portion in less striking fashion than it does the nuclei. The granular material of the cytoplasm appears almost the same as in the undried organs. The spaces between granules are less noticeable. These spaces are probably filled with cell-sap in the active animal and it is to be expected that with loss of water they will largely disappear, permitting the more solid granules to pack closely together. The membrane surrounding the vitellarium and ovary shows no marked change. It is of practically the same thickness and consistency in the dried as in the undried animal.

In the nuclei of the vitellarium noticeable changes have occurred. As was stated before, a section of the nucleus in the vitellarium of an undried animal shows a central, densely staining karyosome surrounded by a clear space, and around the clear space a definite, but not usually thick, nuclear membrane. The nucleus in the dried vitellarium loses, for the most part, its affinity for stains. The karyosome may entirely disappear but usually there are remnants of it distributed here and there through the nuclear space. Taking the place of the karyosome is a more or less regular reticulum which reaches to the nuclear wall. The latter has become thickened during the process of drying; whereas in the nucleus in the undried condition the densely staining material was aggregated in the center and the clear area around the periphery, in the nucleus in the dried condition these relationships are just reversed. What remains

of the chromatic material has collected close to the nuclear wall and the central area shows a more or less clear condition. This change in the position of the chromatic material in the nucleus is not the only one incident to the drying process in the vitellarium or in the other organs as a comparison of the figures will show, but it is by all means more frequent than the other changes.

The condition of the ovary in the dried animal is best shown in Fig. 21, Plate V. It will be remembered that the cytoplasm of the undried ovary usually shows as a glassy, homogeneous ground substance and that the nuclei appear as dense, spherical bodies closely packed in this syncytial ovarian cytoplasm. In the dried ovary the cytoplasm becomes more deeply staining and assumes an appearance not unlike that of the vitellarium. The nuclei become less dense and the chromatic material collects in a peripheral ring as was described for the vitellarium nucleus.

In Fig. 21, Plate V., a trough-like depression is seen at the margin of the vitellarium which lies nearest the integument. This is caused by one of the band-like, circular muscles, which, drying in the contracted condition, caused a deformation in the vitellarium tissue which shows thus in longitudinal section.

At this point it might be well to comment upon the fact that many of the muscles in the rotifer body actually dry while contracted. It would seem that when the normal moisture content was removed from a muscle that it would have a tendency to relax and in this way cause the animal to become more loosely folded during the later stages of drying. That this is not true is evident from the figures and descriptions given. The rotifer remains tightly contracted during the most complete conditions of desiccation.

This condition may be due to two factors. In the first place the integument is the first structure to dry. The moisture evaporates from the surface more quickly than from the internal structures. This integument is chitinous in its outer layer but is of such consistency that while moist it is very pliable. When the moisture is removed, however, the cuticle becomes more rigid. In this respect it might be likened to gelatin and such a resemblance is indeed noticeable. Now with the withdrawal of

moisture the cuticle could form a rigid capsule which would retain the original shape of the folded animal even if the muscles did relax.

On the other hand it would seem that if the maintenance of the shape of the folded animal was due to the action of the cuticle the muscles would be somewhat flattened in cross-section as a result of the mutual pressure of internal organs and integument. This last condition, however, is not true, for the muscles in the dried animal still show in cross-section something of their oval outline. It seems probable that there is a limit to the amount of drying which a muscle may undergo and at the same time continue to exert a contractile tension. But the amount of drying necessary to cause a muscle to reach this limit would at the same time withdraw enough water from the cuticle to cause it to become rigid. I think, therefore, that we can account for the lack of relaxation at the time of drying by assuming that both of the factors mentioned above acting in succession produce the given result.

The appearance of the mastax and its surrounding salivary glands in the dried animal is shown in the central part of Fig. 16, but perhaps better in Fig. 25. Upon comparison with Fig. 24, which is a section of the undried mastax and glands, it will be seen that the entire structure has collapsed and decreased perceptibly in size. The gland tissue is more dense as to its cytoplasmic content while the nuclei, although just as prominent as before, show the same rearrangement of chromatic material as was described for the nuclei of other tissues in an earlier paragraph. The details of this chromatic movement given for the nuclei of the vitellarium and ovary will apply here and need not be repeated. The same is true of the foot-gland cells although I have observed certain cases where the nuclei of the latter do not assume the usual ring form. This condition is shown in Fig. 19.

The stomach of the dried animal shows, perhaps, the most remarkable changes. In Fig. 16, lying below and at the side of the mastax, is figured the stomach in its dried condition. The cytoplasmic syncytium still preserves its alveolar appearance. The lumen is shown winding about through the syncytium, while

nuclei, made up of the chromatic ring, with central clear space, are scattered irregularly about. The densely staining granules of food material, so prominent in the sections of the undried stomach, are not present here. I have examined large numbers of sections of dried animals, and while the food granules are not always entirely absent, as figured here, yet they are absent in many cases, and certainly in all cases they are much less numerous than in sections of the stomach of undried animals. This point is of importance as bearing upon the question of metabolism in the dried state. As was previously stated, the identity of these granules as particles of food material can hardly be questioned. Zelinka ('88) has called attention to the fact that they do not appear in sections of the stomach of the newly hatched rotifers. Beauchamp ('09) has shown that in sections of the stomach of *Hydatina senta* which had been feeding regularly these granules were present in great numbers, but in sections of the stomach of starved individuals they do not appear. Many authors have commented upon the fact that among those rotifers which survive desiccation the stomach tissue in gross appearance is generally lighter in color after recovery than it was before drying commenced. This observation I have repeated many times and find it to be correct. I have not, however, been able to find in the literature figures of actual sections of the stomach of rotifers recovering from desiccation. Fig. 16, which is but one of many that I might show, by the absence of all food particles demonstrates that metabolic or at least katabolic activities must go on in the stomach tissue of the dried animal.

Of great importance in connection with the question of the nuclear changes is the determination of the exact time at which the chromatic rearrangement takes place. It is a reasonable supposition that it could not easily be brought about while the moisture is entirely absent from the tissues. Since the protoplasm is always more or less fluid in nature in its normal condition, the changes described would certainly have to take place very slowly if they occurred in the dried state. It would seem, upon theoretical grounds, that the more favorable time for the changes to take place would be during the short period just

before actual drying occurs. At this time the tissues contain the usual amount of moisture but the approach of the dry condition is, at the same time, apparent to the animals. That there can be no doubt about the preparation of the animals for the dry state is evidenced by their behavior at the onset of desiccation, before described.

Sections of animals killed at the moment when the last visible traces of moisture were disappearing, confirm the supposition outlined above. The nuclei of the cells of the different tissues plainly show the remarkable changes which are going on and which I have described in an early part of this section. While in many cases the rearrangement of chromatin is not so complete as in some of the sections of dried animals here shown, yet it was definite and uniform in the different tissues and indicates beyond question that the transitional period for chromatic rearrangement occurs just before the last traces of moisture are removed.

The movement of chromatin just before drying is interesting for the light it throws upon the question of the lability of the nuclear material. Chromatin undergoes changes of form, position and chemical structure at the time of mitotic activity but there are few cases recorded where such changes occur during the resting condition. In the present instance, however, we have a marvelous rearrangement of the nuclear materials which occurs as a vegetative rather than a reproductive process, the essential steps of which may take place in a few minutes and the new internal conditions so established enabling the animal to resist an unfavorable environment for years.

In this connection also lies a clue to the solution of the question of mortality among rotifers which have been dried. Since under the most favorable conditions of drying some few animals never survive, it seems that there should be some definite cause to account for the fact. Comment has been made concerning the tearing of the organs of a rotifer which might result from too rapid drying. In cases where this last factor cannot enter there are still some fatalities and these cases seem to admit of explanation upon the basis of lack of time or vitality to bring about the internal rearrangement of cell elements necessary in resisting the dried condition.

A further confirmation of the fact that the changes in cell-structure take place early in the desiccation process is found by comparing sections of animals which were dried and kept in the open air with those of animals kept days or weeks in an evacuated desiccator. The essential features of cell structure and arrangement are the same in both cases. This certainly seems to indicate that the extent of the adaptive, structural response to the new condition is not directly proportional to the intensity of the stimulus.

The nuclear membrane undergoes no disintegration or other visible change in structure during the drying process. It might be supposed, from a consideration of the movements of the chromatic material into the cytoplasm, that the membrane would break up during the process. Such is not the case. Not only does it remain intact during the drying but it actually thickens. This thickening is of course due to the migration of chromatic material which normally occupies the middle of the nucleus. The chromatin proper cannot be distinguished from the nuclear membrane, however, since both react alike to stains. The nuclear membrane, then, is a persistent cell organ and does not atrophy as a result of the abnormal conditions.

It is interesting to speculate whether the material which the nucleus imbibes upon recovery from desiccation is the same as that which passed through the nuclear membrane at the time of drying. I have not been able to make any of the finer microchemical tests upon this substance but it would seem that the material which passes from nucleus to cytoplasm takes part in the oxidations discussed in a later section. The chromatin remaining in the nucleus probably manufactures new nuclear material from elements imbibed from the cytoplasm upon addition of water.

The conditions described in the last paragraph show that there are probably two kinds of chromatic material present in the nucleus, one of which is able to pass out into the cytoplasm and one which is not able to do so. Judging from the varying amounts of chromatic material which are present in different cells of the same kind when subjected to similar conditions it seems that the non-diffusable chromatin may, when conditions

warrant, change chemically so as to become able to pass through the nuclear membrane. When moisture is again available the reverse process could take place and new chromatic material be built up from cytoplasmic substance. This certainly would fit in with the changes which one is able to observe in the different stages under the microscope.

The changes in the chromatin just mentioned are not unlike those which Heidenhain ('94) has described. His oxychromatin and basichromatin could be demonstrated by staining reactions. In the rotifer cells, however, the staining is not nearly so precise and delicate in the dried tissues so that it would be difficult to say whether or not the chromatin exists in the condition just mentioned. Certain it is, however, that there is a change in the chemical nature of some of the chromatin at the time of drying and a return to normal conditions when moisture is added.

D. *Animals Recovering from Desiccation.*—The condition of the organs in rotifers recovering from desiccation is shown in Fig. 15, Plate IV. This section was made from an animal which after drying thoroughly had been put into water and then killed four hours subsequent to the addition of water. This perhaps represents a case where recovery was slower than usual, but the condition of the tissues shows that it certainly would have recovered completely.

In this same section the vitellaria are seen to be resuming their normal condition. The cytoplasm is not different from that of the normal tissue. One of the nuclei has completely recovered while the other two which appear in the section are rapidly assuming typical structure. One of the first changes noticeable in the nuclei of cells of dried animals subjected to moisture is the increase in thickness in the chromatic ring in the nucleus and the greater affinity for stains exhibited by it. This is well shown in these vitellarium nuclei. The two conditions of the nuclei shown are therefore two stages in the process of recovery. Fig. 23 represents a longitudinal section through the ovary-vitellarium of an animal which had been kept in an evacuated desiccator for fifteen days, then placed in water and killed one hour and fifteen minutes after the addition of water. The recovery of this animal was more rapid than was that of the one

represented in Fig. 15 for it had already partially unfolded at the time it was killed. The nuclei here show several chromatic patterns not figured in the other sections but they are all stages incidental to the resumption of the typical nuclear form.

Fig. 22 shows a transverse section of the vitellarium of an animal dried at room temperature, then placed in an evacuated desiccator for six days, then in water for one hour and finally killed and sectioned. The nuclei here show almost complete recovery, while the cytoplasmic part of the structure has not regained the characteristic regular pattern.

Another condition found frequently in the cytoplasmic portion of the vitellaria of rotifers recovering from desiccation is that represented in Fig. 20. The vitellarium nuclei are found in various stages of recovery, while entirely outside of the nuclear membrane and indistinguishable in appearance from ovarian nuclei are round chromatic particles. This condition is important for I believe it shows what we have not been able to demonstrate visually before, namely, that the withdrawal of water causes the chromatin in the vitellarium nucleus to diffuse into the cytoplasm, but the very withdrawal of water initiates a chemical change which causes the chromatin to lose its staining power. The addition of water causes this same chromatic material, scattered about through the cytoplasm to resume its normal staining reaction and in that way its actual presence is for the first time visually demonstrated. Gradually this extranuclear chromatin in the vitellarium disappears and it is probable that its disappearance is due to some chemical change which again causes it to assume the appearance or become a part of the regular cytoplasmic structure.

In Fig. 23 the cytoplasm of the ovary shows that it has regained its clear, homogeneous appearance. The number of ovarian nuclei is also greater than in sections of that organ which have not been recently dried. I have found this condition repeatedly in sections of animals recovering from drying. This is clearly one of the steps incident to increased reproductive activity. There are several factors involved in this process. It will be recalled that in drying the cytoplasm of the ovary becomes dense and takes chromatid stains more readily than do

sections of the ovary of animals in the free-swimming condition. Since, as we have seen, chromatic material passes from the nuclei of the vitellarium and of other tissues into the cytoplasm of the same structures it is not improbable that a similar movement would occur in the ovary. In the latter case, however, the elements are so small that one detects minute changes with difficulty even under the highest powers of the microscope.

This condition in the ovary of the recovering rotifer seems to indicate the time and manner of the increase of the ovarian nuclei.

It is well known that periods of great reproductive activity always follow periods of desiccation. Reproductive activity under normal conditions in most animals would involve an increase in the number of the sex cells. Now in the rotifer this increase might be looked for just before drying, for, if the cells of the other tissues prepare themselves for the new conditions by rearranging their elements it is not inconceivable that the sexual elements might likewise prepare for a season of unusual activity. Such, however, is not the case. By counting the nuclei in several hundreds of sections of ovaries in normal, dried, and recovering animals, I find that the average number in the first two conditions is about the same. Likewise in sections of ovaries of animals to which water had just been added there is no noticeable increase. But in sections of animals killed several hours after the addition of water one is struck by the increase in the number of the ovarian nuclei.

It would be desirable to make a definite statement as to the exact time which must elapse before the multiplication takes place but this is impossible since the time varies among different individuals. In animals dried several weeks the increase does not become apparent in any case before one and one half to two hours has elapsed subsequent to adding water to the dried animals. This time may be too short for animals whose processes go on at a much slower rate.

In connection with the observation regarding the position of chromatic bodies in the cytoplasm of the vitellarium it is interesting to note that Janson ('93) comments upon, but gives no figures of similar cases. He says: "Only twice in *P. roseola*

and *Callidina* I saw the nuclei of the ovary surrounding the large nuclei of the vitellarium but I could not distinguish whether the peculiarity was connected with egg formation or was due to pressure." In this connection I would call attention to the position of three small nuclei in the section of the vitellarium of the rotifer recovering from desiccation shown in Fig. 15. This is one of the many cases I have observed where small numbers of these nuclei are found in the vitellarium. I am certain from observations on hundreds of similar sections that this condition is due to normal causes and cannot be laid to accidental pressure as Janson suggests. I have not been able to follow all the stages in the early development of the egg, but I believe that this condition is a stage in the development of the egg and is not to be confused with those cases where chromatin arising from the nucleus of the vitellarium is found in the cytoplasm of that organ.

The condition of the different gland cells in animals recovering from desiccation is shown in Figs. 15, 18*b* and 26. Fig. 18*b* is a section of foot gland cells of a rotifer which was kept for fourteen days in an evacuated desiccator and then placed in water one and one fourth hours previous to killing. The nuclei are seen to be rapidly assuming the typical condition which is represented in Fig. 18*a*. The cytoplasmic changes in these glands are perhaps less marked than in any of the other tissues considered. This may be due in part to the nature of their contents, for being impregnated with their gelatinous secretion they would show fewer changes than would other cells whose plasma is less viscous.

The salivary glands in animals recovering from desiccation show the presence of recently acquired water by the vacuoles scattered at regular intervals throughout their cytoplasm. As recovery becomes complete the cytoplasm assumes the condition shown in Fig. 24 and a typical half-recovered condition is well shown in Fig. 26. In this figure it will be noticed that the karyosomes in the nuclei are of smaller size than in the normal tissue. They have resumed the normal condition, however, in all respects except size.

In the stomach tissue of the rotifer recovering from desiccation

the absence of the food granules is again noticed. The objection might have been offered in the case of the dried stomach that the failure of the food granules to become apparent was due not to their actual absence but to a chemical change incidental to the drying process which made them entirely lose their affinity for stains. If this were the case it seems that upon the addition of water the granules would again resume their normal staining reaction. That this possibility is not realized in fact is apparent from an examination of the stomach tissue shown in Fig. 15. The nuclei have resumed their normal appearance. The cytoplasmic syncytium of the stomach tube compares favorably with that in the normal animal, while scattered about through it are vacuoles which probably mark the previous positions of the food particles which have disappeared. In any event, the food particles have been used up and the stomach tissue has the same appearance as is found in cases where the animal is not dried, but starved.

The trochal cilia in animals recovering from desiccation under the highest magnification cannot be seen to be different from those in the normal or even in the dried animal. Absolutely no change is observable.

Upon the addition of water the hypodermal layer of the integument rapidly swells and assumes its normal thickness. It might be mentioned here again that if there were any sign of a protecting gelatinous capsule it should be observed at this point when the different tissues swell and draw apart. But here, as before, there is absolutely no sign of such a gelatinous envelope.

The cells of the peripheral layer of the brain of rotifers recovering from desiccation show the characteristic chromatic ring in their nuclei (Fig. 29, Pl. V.). This, at first sight, is remarkable because the nuclei of similar cells in the dried animals do not particularly show such a disposition of the chromatic material. However, the cells in the peripheral layer of the brain are among the smallest in the rotifer body and their nuclei are correspondingly minute. The small amount of chromatin in each nucleus could migrate toward the nuclear membrane without being especially apparent. But, as was pointed out in

an earlier paragraph, the addition of water causes a swelling together with an increase in staining capacity in the nuclear material with the result that the position of the chromatin is most plainly seen not in the dry period but just subsequent to that and before a sufficient time has elapsed to permit a normal arrangement to be brought about.

The observations upon the tissues of the dried animals recorded in the preceding paragraphs show that they react similarly, with very few exceptions, to the drying stimulus. The cytoplasm becomes denser. This is of course to be expected. The nuclei, all of which have a very definite and similar chromatic pattern, undergo a chromatic rearrangement which is striking and uniform. That this chromatic movement is the visible expression of an adaptation to a new sort of environment can hardly be doubted. The resumption of normal conditions in nucleus and cytoplasm is likewise uniform in the elements of the different tissues. If any doubt existed as to the nature of the adaptive response in the desiccated condition that uncertainty would be removed upon observing the return to normal conditions as shown in sections of recovering rotifers. The significance of the chromatic movement and its interaction with the cytoplasm in the different tissues will be discussed fully in a later section.

IV. Review and Discussion.

The literature relating to the cytology of desiccation is, in the case of animals, very sparse, and, relating to rotifers, almost nothing. While rotifers have been studied since the invention of the microscope, the chief interest has been along anatomical lines. Along with the anatomical studies, the Bdelloideæ have received consideration in discussions as to whether they actually dry up and recover from the desiccation, but apparently no one thought to examine the cytological changes underlying the process.

Pfeffer, in his "Physiology of Plants," makes numerous observations upon the subject of desiccation among members of the plant kingdom. His point of view is, of course, physiological rather than cytological.

Pfeffer suggests that where death occurs as a result of drying it may be due in part to the removal of the last traces of absorbed or combined water. He says: "Since full turgor is restored in mosses and other plants immediately after moistening them, it is evident that the osmotic materials remain as solids in the central vacuole. Evidently, therefore, the protoplasm is not injured by the concentrated cell sap. If, however, the latter is responsible for the death of certain plants on drying, we have revealed to us in such cases the immediate cause of the fatal action of desiccation. It is, however, hardly likely that the death of plants killed by the removal of more or less of the imbibed water, as well as that of those only killed by the removal of the last traces of absorbed or even combined water, is alike produced in the same way."

I believe that the work of McDougal, Long and Brown, mentioned in the next paragraph shows that plants may have their cell sap concentrated in the fashion described by Pfeffer and still recover from the process. My own observations upon *Philodina* lead me to think that death at the time of drying cannot be attributed to such a cause but rather to a lack of a complete adaptive rearrangement of cytoplasmic and nuclear material.

Among the botanists the subject of desiccation is now being studied by McDougal and his associates at the Desert Laboratory at Tucson, Ariz. A recent paper from that laboratory deals with cytological phenomena in the desiccation of *Echinocactus*. J. G. Brown, who did the cytological part of the work, examined four kinds of cells in the tissues of *Echinocactus wislizeni*, viz., integument, palisade, outer cortex and deep cortex. Starting with a description of these cells in sections of a plant grown under normal conditions he compares similar cells from sections of (1) a plant which had been desiccated six years; (2) one which had been desiccated ten months; and (3) one which had been desiccated forty-two months and then allowed to grow under normal conditions for twenty-two months.

In the palisade cells the results of desiccation were most apparent. These cells are rectangular in section, having a peripheral layer of protoplasm embedded in which are the nucleus and plastids. A corresponding cell from a plant desiccated six

years showed the cytoplasm gathered in one corner of the cell. The nucleus, which normally is of lenticular form and contains vacuoles, was still embedded in the cytoplasmic mass. It had decreased to one fourth or less of the size of the normal nucleus. The vacuoles had disappeared and the chromatic elements had for the most part become aggregated in a ring in the region of the nuclear wall.

In the palisade cells of a cactus which had been desiccated only ten months the cytoplasm did not deviate from the normal condition as in the first case mentioned, at least in so far as its position was concerned. The nuclei decreased almost as much in size and assumed practically the same form as those in the palisade cells desiccated six years. In the specimen which was desiccated forty-two months and then returned to normal conditions for twenty-two months, the cytoplasm was found to be normal as to quantity and position, while the nuclei, although increased slightly in diameter, still retained the ring form characteristic of the dried condition.

In the other tissues the results were quite similar to those recorded for the palisade cells. In the integument the cuticle thickened slightly in the specimen desiccated six years. The outer epidermal cells were thinner in the plant dried six years than in that dried only ten months, while in the one desiccated forty-two months and returned to normal surroundings for twenty-two months the epidermal walls were of about the same thickness as in the normal tissue.

The nuclei of the epidermal cells decreased to about one half normal size in *Echinocactus* No. 7. The characteristic peripheral chromatic ring appeared. In specimen No. 6 which after desiccation was returned to a normal environment, the epidermal nuclei resumed their normal condition.

In the cells of the outer cortex below the palisade layer, the cell walls increased perceptibly in thickness as desiccation proceeded and they recovered somewhat their normal thickness as moisture was again admitted. The nuclei shrunk to about half size and assumed the ring form. In the deeper cortex practically the same things happened as were just described for the outer cortex. The cytoplasm decreased almost to the point of disappearance in the cactus dried six years and gradually increased

in volume in the plants subjected to less severe conditions. The nuclei decreased to about half size. The cell-walls thickened enormously in the extreme cases of drying and in many cases never recovered normal thickness.

The results of McDougal and Brown are most interesting when compared with my own. The observations upon the cytoplasmic and nuclear changes in the dried tissues show that they are similar in many respects with those in corn embryos and rotifer tissues described in my earlier paper.

The length of time during which an *Echinocactus* may survive in the open at the expense of its surplus food material and water was found to be no more than two years while similar plants in diffuse light were sound after six years of starvation. The cactus, then, which normally lives in an environment containing little moisture is less able to adapt itself to extreme dry conditions than is the rotifer which normally swims about in water. The extreme length of time during which a *Philodina* may remain dry is not known but well authenticated records show that they have been known to withstand a period of dryness for as much as twenty-seven years. To date no experimental data are recorded to show the effects of varying intensities of light upon the time during which rotifers may live in a desiccated condition. This point I hope to treat in a future publication.

Another result of interest in the cactus experiments is that in extended desiccation and starvation the plasmatic colloids are eventually broken down by katabolic action. This katabolic activity includes hydrolysis of the cell-walls of the cortex. Now in *Philodina* katabolic activity undoubtedly takes place during the dry periods. This was most clearly seen in the tissue of the stomach-intestine. But while the reserve food granules disappear, no change is observable in the walls of any cell. It might be said from the nature of the two cases that the reaction in the cactus is irreversible while in the rotifer it is reversible, for the cells of the cactus perhaps never assume a perfectly normal condition after the drying process while a sufficient food supply is all that is necessary to make the rotifer quickly resume its normal structure.

McDougal found that in the cactus the loss in weight in full illumination may not greatly exceed 50 per cent. of the water

present without producing death by desiccation. Now under normal conditions about 95 per cent. of the weight of the cactus is made up of water. This would mean that a decrease of approximately one half in the weight of the plant as a result of drying, is usually fatal. In *Philodina* the minuteness of the animal, together with the fact that it normally lives in water, makes it almost impossible to determine the changes in weight during the drying period. It is easy, however, to follow the changes in volume which occur during drying and anyone can see if he will dry a *Philodina* upon a slide under a microscope that the animal decreases to from one third to one fourth of its original size. Since most of the weight of the rotifer body, certainly more than 95 per cent., is made up of water it will be seen at once that the percentage of substance lost by the rotifer during desiccation is much higher than that observed in the cactus.

The desiccation process in the cactus caused few changes in the thickness of cell-walls. In the plant treated for seventy-three months the cuticle was slightly thicker than normal while the outer walls of the epidermal cells were thinner. This result was obtained in the most severe test of the series. In my experiments on *Philodina* the most severe drying caused absolutely no thickening of the cell walls which bordered the surface, but on the contrary by a loss of water from the hypodermal layer an actual decrease in the thickness of the integument was observed in all cases.

Perhaps the most pronounced cytological effects of desiccation are those recorded for the cortex cells of *Echinocactus*. Here there was an entire disappearance of the protoplasts and a hydrolysis of the cell walls. There is nothing in my experiments upon *Philodina* which parallels this. The changes described in the stomach tissue are similar but in no case have I ever observed the disappearance of cell-walls or the formation of spaces as a result of the disintegration of cell-elements.

That the different changes during the desiccation process and subsequent recovery in *Echinocactus* are very slow is well shown by McDougal and Brown when they state that a plant which had been desiccated for forty-two months and was then placed under normal conditions in the soil for twenty-two months did

not entirely regain the normal condition. It would be interesting to know if the plant would have recovered if given more time for the process. In *Philodina* the process of recovery begins upon the addition of water and is complete in a short time, never longer than a few hours.

For an analysis of the meaning of these changes I should say that the cactus is unfavorable for rapid results. The great length of time necessary for changes to take place and the slowness with which normal conditions are resumed makes experimental work with a large number of specimens difficult. This probably accounts in part for the fact that McDougal and Brown make no attempt to suggest the significance of the cytological changes which they describe.

Metabolic Water and Its Relation to Desiccation.

For a complete discussion of the subject of metabolic water and its functions in an organism I would refer the reader to the excellent paper of Babcock ('12). For the present purpose it will be sufficient to outline the main points of the subject and relate them to the material under discussion.

Desiccation is the operation of drying or removing water from a substance. When the last trace of water is removed the desiccation is said to be absolute. Absolute desiccation of chemical crystals is brought about by heating the substance to dryness or when the material involved is readily decomposed by heat the operation is accomplished by the use of a desiccator. A condition of absolute desiccation can be maintained so long as the heat is applied in the one case or so long as the substance is kept within the drying chamber in the other. When the desiccating influence is removed moisture is absorbed.

Inorganic crystals can, as a rule, be deprived of their moisture only by a temperature of 100 degrees centigrade or higher. Not only are the crystals as such made dry but the water of crystallization, by virtue of which the crystals exist, is driven off and the substance assumes an amorphous condition.

Living organisms contain water combined with them in a fashion which for present purposes may be compared to water of crystallization. In order to remove all water by means of heat it is necessary to raise the temperature of the organic

material in question until actual charring takes place. It is evident, therefore, that the absolute desiccation of living substance cannot be accomplished by means of heat without destroying life. On the other hand, the second method of desiccation mentioned above will not remove the last trace of chemically combined water from living tissue. Since an absolute desiccation of living things without the destruction of life is impossible by the second method it seems certain that no rotifer or other living thing has ever lived after an absolute desiccation.

The recorded observations upon the desiccation of living things, whether animal or plant, have to do with a relative rather than with an absolute desiccation. If the water content is lowered much below the normal percentage the organism may be said to have undergone an actual or even a complete desiccation but it will be understood that in all cases this does not imply that the condition of dryness is absolute.

Water may be acquired by an organism in three ways: (1) It may be imbibed directly; (2) it may be taken in with solid food, and (3) it may be formed within the organism by metabolic changes in the organic constituents of the food and tissues induced by respiration and other vital processes.

Imbibed water probably makes up the greater part of the cell-sap. Its method of combination with the living substance may be, as Nägeli contended, not chemical but a mere physical attraction due to minute molecular aggregations designated as micellæ, between which the water enters by capillary attraction and forcing the micellæ apart increases the volume of the tissues. On the other hand, as Babcock points out, all of the phenomena of imbibition point directly to a molecular combination between the substance composing an organized body and water. The combination is in most cases feeble, since it is broken up by a relatively low temperature without changing the molecular structure of either the solid tissue or the water. It is however analogous to the behavior of many substances, both organic and inorganic, which crystallize with water of crystallization.

The water taken in with solid food probably has in part the same fate as imbibed water although its history is slightly different. In *Philodina* the distinction between these two

sources of water is not marked and the same can be said of any animal having a similar habitat and feeding habits.

The production of metabolic water, in certain stages of the life-histories of both plants and animals is sufficient for all purposes for considerable periods of time. In the resting periods of deciduous plants, in bulbs, in tubers and especially in seeds and spores, ample water is provided for all vital processes by the slow oxidation that takes place as a result of direct respiration. Hibernating animals receive no water from external sources for several months, although water is being constantly lost by respiration and excretion. Many varieties of insects such as clothes moths, grain weevils, dry-wood borers, etc., are able to subsist during all stages of development upon air-dried food materials containing less than 10 per cent. of water; in these cases nearly all the water required is metabolic.

Metabolic water may be formed as a result of two kinds of respiration. In *direct respiration* the organic matter comprising the food and tissues of an organism is oxidized by means of free oxygen derived from the air during respiration. Many organisms when deprived of free oxygen are capable of maintaining for a short time certain of the respiratory functions, and deriving energy from food material and from tissues by breaking up the molecular structure into new forms of a lower order. This is known as *intramolecular respiration.*

When a *Philodina* dries it loses all its free water. The spaces between the different organs are filled during the free-swimming existence of the animal, with fluid. This fluid between organs is the first to disappear. The loss of uncombined water is responsible in large measure for the decrease in size of the animal during the drying process. By examining Figs. 1 and 2 it will be seen that in their normal condition the organs are not closely packed together. It is these spaces between organs which allow the animal to decrease so appreciably in size and in such a decrease the fluid content of the spaces is lost.

In addition to the loss of free or uncombined water there is a corresponding though not extensive loss of combined or chemically bound water. This is the imbibed water mentioned in a previous paragraph. Just as water is distributed in the inter-

stices of a sponge, so water is held in every cell of the rotifer body. It is hardly necessary to emphasize again in this connection that such water is probably not held by capillary attraction as in a sponge but by a loose molecular union. The presence of this water in the rotifer tissues is demonstrable in a visual fashion, for in the sections of normal undried rotifers the cells all possess vacuoles between the granules or reticulum of the cytoplasm. These vacuoles or spaces are certainly filled with the more fluid cytoplasmic ingredients and their true fluid nature is demonstrated upon examination of a section of a dried animal where they have with few exceptions disappeared, or better still in an animal recovering from desiccation where the former position of granules of reserve food material is marked by vacuoles undoubtedly containing liquid. In a previous paragraph attention was called to the fact that in those cells whose cytoplasm is loosely granular in the normal condition, the granules were closely packed in similar cells of a dried animal. The change in space relation of these cytoplasmic granules is due to loss of imbibed water.

The amount of imbibed water which an organism may lose and still live varies with the kind of organism. Seeds which in a dormant condition contain from ten to not more than twenty per cent. of water may still retain their viability and germinating power if more than half of the water content is removed. This viability of corn with different degrees of moisture content is recorded by Babcock ('12) and I have repeated many of his experiments and find them to be correct.

In McDougal's cactus experiments it was found, as was before mentioned, that loss of weight in full illumination may not greatly exceed fifty per cent. of the water present without producing death by desiccation. In seeds, on the other hand, a loss of seventy-five per cent. of the water content need not necessarily be fatal and in some cases certainly the percentage loss may be higher without death resulting.

In *Philodina*, while exact quantitative measurements are exceedingly difficult, the water loss judged by decrease in size of the drying animal and by the nature of the dried tissues, must certainly be higher than that of seeds.

The Mechanism of Metabolism in the Dried Rotifer.

Admitting that the desiccation of *Philodina* is complete and admitting further that metabolism takes place though much retarded, in the dried condition, the mechanics of the metabolic activity is the next point to demand explanation.

It will be remembered that Jacobs ('09) showed by means of intra vitam staining with chemical indicators and subsequent tests with various gases, that the integument of the dried rotifer is at all times freely permeable to gases. Attention was called, in another paragraph of this paper, to the fact that many animals when deprived of their normal supply of moisture could still exist for a time by means of the direct action of oxygen from the air upon the complex materials of the tissues or upon inclusions of complex food materials within the latter. This action would of course not be sufficient to prolong life indefinitely for the time would come when all the available reserve food material would be exhausted or the accumulation of poisonous waste products might end life. That such a state of affairs might be realized in the case of a dried *Philodina* in the air is not impossible. Certain it is that this animal can live a long time; many years in fact, in a dried condition without food from external sources. Its lease of life, however, under these conditions, is not indefinite as is evidenced by the fact that under the most careful conditions of drying some of the rotifers always die. To explain these fatal cases it would seem that the store of reserve material became exhausted or the metabolic products accumulated in too great a quantity and death was the result. It seems reasonable to assume then, that under the conditions just outlined metabolic activity goes on through oxidations of complex substances within the tissues of the animal by means of oxygen obtained by direct respiration.

Those rotifers which were described as having been kept in an evacuated desiccator for varying periods and which survived the experiment could not have their metabolic activities explained upon the same basis as the ones which were dried in air. It will be recalled that mention was made of certain cases where metabolism might proceed in the absence of air by means of intra-molecular activity. In these cases the complex substances

which are an integral part of, or are merely inclusions in the tissues have their molecular structure broken up into newer forms of a lower order. In this way energy is derived from food material and life may be prolonged for a greater or less period of time depending upon the amount of reserve material present.

Both types of chemical reactions outlined above are hydrolytic processes. This means then that metabolic water is being evolved in small quantities during the entire dry period.

It should be understood here that the processes just outlined are hardly possible of actual demonstration for if they are sufficient to keep such a small organism as a rotifer living for years in the dried condition they must of necessity be exceedingly slow. Observations made by Babcock ('12) show that respiration and consequent oxidations in seeds and spores are practically suspended, it being possible to detect them only by observations extending over long periods of time. If this be true of an embryonic structure with its simple organization it is true to a greater degree in an adult animal in a state of retarded activity.

Death ensues in all dormant organisms at the end of a certain time. The period of dormancy is limited. In *Philodina*, since metabolism proceeds continuously but slowly as I have shown, the death of an animal can be attributed to starvation rather than dryness. Dryness is a contributing cause of death but the animal dies as a result of lack of food rather than as a result of lack of moisture.

We may briefly summarize the previously mentioned causes of death in the dried rotifer as follows: (1) Mechanical injury due to too rapid drying, (2) starvation resulting from lack of reserve food material, (3) poisonous effect of metabolic products and (4) insufficient time before drying to effect the nuclear-cytoplasmic reorganization.

The Significance of the Nuclear-Cytoplasmic Interchange During Desiccation.

The nucleus in the cells of most of the tissues of *P. roseola* consists, as was pointed out before, of a single large karyosome surrounded by a clear area and having for its boundary a definite

nuclear membrane. This karyosome is of course not a true nucleolus since it is made up of chromatic material. This type of nucleus has been given the name of "nucleoles noyau" by Carnoy and it has been described by numerous authors in the cells of several kinds of unicellular animals. Nucleoli showing apparently all grades of morphological and chemical properties between true plasmasomes and karyosomes have been described by various authors (see Montgomery, '98) so that it cannot be said that there are no intermediate conditions to be found. Montgomery says: "The existence of Carnoy's 'nucleoles mixtes' and 'nucleoles-noyaux' in cells of metazoa appears to be doubtful" yet the "pseudonucleoli" which he describes in the ova of the mollusc *Montagua pilata* have many of the properties of a chromatin nucleolus or karyosome.

The significance of the peripheral chromatic ring in the dried rotifer nucleus is a point which requires explanation and correlation with the other conditions incident to the adaptation of the rotifer to its dried condition. The chromatin ring undoubtedly represents a stage of chromatin migration. There are two possibilities regarding the extent of this migration. The chromatin may merely leave its place in the center of the nucleus and, by taking a position next to the nuclear membrane, remain within the nucleus proper during the entire dry period. On the other hand, some of the chromatic elements might be of such nature as to pass readily through the nuclear membrane while the latter might be impermeable for others. That materials may be changed chemically within the nucleus is admitted. In nuclei of cells which have just completed mitotic division the nucleus imbibes substances from the cytoplasm and changes them into nuclear material. It is also a matter of common knowledge that at the beginning of mitotic activity much material is cast out of the nucleus into the cytoplasm. However, it is not commonly contended that materials pass from the nucleus into the cytoplasm without a rupture of the nuclear membrane. That the latter condition is a possibility in the rotifer nucleus will appear shortly.

The karyosome of the rotifer nucleus being almost pure chromatin, the reason for its migration might be sought in a

consideration of some of the functions of chromatin in general. R. Lillie ('02) and others have shown "that in many tissues the nucleus is the chief agency in the intracellular activation of oxygen; and further that the active or atomic oxygen is in general most abundantly freed at the surface of contact between nucleus and cytoplasm." Now the parts of the nucleus which take part in these oxidations must be the chromatin and, perhaps, the nuclear membrane. It seems reasonable to suppose that the lack of the normal amount of water in the nucleus causes the chromatin to be unable to interact with the nuclear membrane in bringing about oxidations, and the nucleus adapts itself to the new conditions by rearranging its chromatic content in the manner described.

I have implied in the preceding paragraph that the chromatic material goes no further than the nuclear membrane and that there it is deposited to make the typical ring structure. While this may be and probably is true in cases of relatively incomplete desiccation, I think there is much evidence that in many cases the chromatin either does pass through the nuclear membrane or it is so changed chemically within the membrane that its presence cannot be detected by ordinary methods. A comparison of different nuclei in the figures of sections of dried animals shows that the peripheral chromatic ring is not nearly uniform in thickness in the different nuclei. I interpret this as meaning that varying amounts of chromatic material have been able to pass to the cytoplasm,—the ability or inability to do so depending perhaps for one thing upon the permeability of the nuclear membrane.

It will be remembered in this connection that immediately upon the addition of water to the dried animals the chromatic ring thickens and regains its normal staining power. This fact also is, I think, capable of two interpretations. It may indicate that chromatic material which moved in some form into the cytoplasm during the dry period immediately begins to return to its normal position when moisture is restored or it may be that the drying process caused the chromatin to lose its staining power to such an extent that its volume only seemed to be diminished. From the appearance of the sections I do

not think that there is any doubt but that the former supposition is correct. The chromatic material in some form actually does pass into the cytoplasm during the dry period.

Whatever may actually happen to the chromatin in the way of a change of position it seems certain that the purpose of all the position changes outlined are one, namely to keep the chromatin within a working radius of the material to be oxidized. The oxidations referred to in this connection are the breaking down of complex food materials in the cytoplasm with the consequent release of metabolic water during the process. The details of this process are discussed in another paragraph.

The nucleus of the gregarines is similar in many points of structure with that of the rotifer. Montgomery ('98), speaking of an unnamed gregarine from *Carinella annulata* says: "Now as the gregarine grows, at the same time both nucleus and the total mass of the nucleolar substance increase in size; but the nucleus cannot grow without the addition of a substance or substances to it which have been derived from without. Accordingly, I suppose that the substance of these granules has an extranuclear origin, a substance, *i. e.*, which having penetrated the nucleus from the cytoplasm, undergoes a chemical change in the nucleus and there becomes precipitated in the form of granules, for no such substance occurs in granular form in the cytoplasm. The growth of the nucleoli might then be explained on the assumption of the intussusception of this substance by the nucleoli." It seems probable that the rotifer nucleolus grows at the time of recovery from desiccation in the fashion outlined by Montgomery for the gregarine nucleolus. Drying then, brings about a reversal of this process and the normal condition is resumed only when moisture is restored.

In considering the movements of chromatin from the nucleus into the cytoplasm one is struck by the similarity between the phenomena here observed and those described by Woodruff and Erdmann in their paper upon periodic reorganization in *Paramœcium aurelia*.

These authors working upon Woodruff's non-conjugating line of *P. aurelia*, found that there was a periodic nuclear reorganization. "This nuclear reorganization consists of a gradual dis-

integration and absorption of the macronucleus in the cytoplasm. Simultaneously a multiplication of the micro-nuclei is in progress. Certain of the resulting micronuclei degenerate while the remaining one or two form the new macronuclear and micronuclear apparatus. This results in the reorganization of the cell without the fusion of two animals." They find not only that the reorganization process is coincident with the low point between rhythms but also that there is a causal relation between the reorganization process and the rhythms. Woodruff defines a rhythm as a minor periodic rise and fall in the division rate from which recovery is autonomous. Woodruff believes that the rhythmical changes which he describes are inherent in the phenomena of the cell. There are changes in *Paramœcium*, described by Calkins and others, involving a somewhat similar reorganization of the cell but whose cause may be laid in part to environmental conditions.

The life history of *Philodina* consists in periodic increases and decreases in activity as a result of environmental conditions and in many respects its structural responses are similar to those described by Woodruff and Erdmann in *Paramœcium*. The environmental stimulus acting upon the rotifer to produce the periodicity is dryness. The response to the stimulus is an interaction between nucleus and cytoplasm not unlike that taking place in *Paramœcium*. Normal conditions of organization are resumed as soon as the stimulus is removed and the noticeable result of the entire process is an increase in reproductive activity. Of course many of the physiological processes of the two animals are markedly different in many respects and would not admit of close comparison but this one particular phase in which there is such a marked agreement in the cytological phenomena accompanying a physiological state seems worthy of comment.

The Relation between Desiccation and Reproductive Activity.

It is hardly necessary to again call to mind the causal relation between desiccation and reproduction in *Philodina*. The numerous observations of many authors has established this as a fact. It is, however, profitable to speculate concerning the specific cause of the increased reproduction. It is insufficient

to say that drying is the cause. It is necessary to point out if possible how desiccation causes the reproductive increase.

In an earlier paragraph I have shown that the nuclear-cytoplasmic reorganization takes place in the ovary the same as in other tissues, and that just subsequent to the addition of water an increase in the number of ovarian nuclei takes place. I have not been able to observe these elements in the act of multiplication but I am sure concerning the results of the process if not of the method.

It seems, then, that we are dealing with an adaptive, structural response of a special sort. Whereas in the other tissues the reorganization of cell elements took place to satisfy a vegetative or metabolic demand, here we have a similar reorganization, the end of which is increased reproduction, and the stimulus to which is in both cases,—desiccation.

In addition to the increase in the number of sexual elements, a stimulus is also apparently given which leads to the immediate development of some of these elements.

That egg cells should be stimulated to develop as a result of loss of water is neither a strange idea nor a new one. Loeb ('06) in his experiments upon artificial parthenogenesis found that the unfertilized eggs of *Arbacia* and *Strongylocentrotus* could be induced to develop into swimming larvæ by immersing them in hypertonic sea-water and later placing them in water of normal concentration. That the initiation of development was due to the withdrawal of water he demonstrated by further experiments in which the eggs were not put back into sea-water of normal concentration. In this case only a loss but no taking up of the water occurred, yet swimming larvæ developed.

Philodina is a parthenogenetic rotifer, and as has been shown is stimulated to reproduce by removal of moisture from its tissues and consequently from the sex cells. It would seem, therefore, that we are here dealing with a process which is natural and commonly employed by this parthenogenetic animal as a result of environmental conditions but that the same stimulus can be employed to bring about parthenogenetic development among an entirely different group of animals which reproduce normally by the sexual method. The steps in the two processes

are not to be easily compared, yet the initial stimulus is similar and development is the result in both cases.

Desiccation Phenomena in Their Relation to the Subject of Adaptation.

That desiccation is but one of the unfavorable conditions to which rotifers may adapt themselves is evident from the literature. The Bdelloideæ can easily adapt themselves in other ways. Murray in his account of rotifer fauna observed during the first Shackleton south polar expedition found that *Callidina constricta* and *Adineta grandis*, both *Bdelloids*, were able to live for rather long periods in salt water although normally they are found only in fresh water. Murray in the account of his experiments says: "To test the degrees of cold which they could stand blocks of ice were cut from the lakes and exposed to the air in the coldest weather of the whole winter. By boring into the center of the blocks we found that they were as cold as the air. A temperature of − 40° F. did not kill the animals.

"Then they were alternately frozen and thawed weekly for a long period and took no harm. They were dried and frozen and thawed and moistened and still they lived. At last they were dried and the bottle containing them was immersed in boiling water, which was allowed to cool gradually and still a great many survived. Again they were put into sea water and into the brine from the bottom of Green Lake which is so salt that it only freezes at about 0° F. They were kept in these salt waters for a month, yet as soon as they were transferred to fresh water they began to crawl about as though nothing had happened.

"Such is the vitality of these little animals that they can endure being taken from ice at a minus temperature, thawed, dried and subjected to a temperature not very far short of the boiling point, all within a few hours (a range of more than 200° F.)."

It is desirable that the structural changes, if any, which accompany freezing and salinity of the surrounding medium be worked out in order to compare the resulting conditions with those in the dried animals. It is probable that when the different environmental conditions were brought about successively in varying combinations, the structural response is somewhat different in each case.

Bachmetjew ('07) showed that juices of the insect body do not completely congeal till they have been reduced to − 4.5° C., but at this temperature the insect does not yet die.

Reiff ('09) kept an adult *Actias selene* at a temperature of − 3° to − 6° C. from November 23 to January 3. Upon raising the temperature to 17° C. the insect again became active. The normal length of life of this species of *Actias* averages seven to eight days. The total number of days which the two insects experimented upon lived in an active condition was about two days less than the average.

Bachmetjew says that metabolism cannot take place in a frozen insect because it is impossible for the blood to circulate. However this may be, we have shown that metabolism takes place in the dried rotifer in the absence of a circulating fluid. Furthermore the experiments of Reiff just mentioned show that the total length of life is lessened in an insect when freezing intervenes. This strongly suggests that the life processes go on slowly here also. It seems desirable that the question of structural changes and metabolic processes during freezing among certain animals should be carefully investigated.

V. Summary.

1. The tissues and the parts of the individual cells of a desiccated *Philodina roseola* maintain their identity during the drying process.

2. No protecting membrane is secreted when drying begins or at any other time during the desiccation process. The integument of the dried rotifer is thinner than that of the undried specimen.

3. Metabolism goes on slowly in the dry condition as is evidenced by changes in the walls of the digestive tube.

4. Desiccation in *Philodina* may be complete but not absolute without fatal results.

5. The typical nucleus in all the tissues of *Philodina* consists of a single large karyosome surrounded by a clear space with a distinct nuclear membrane at the periphery of the clear space.

6. The general effect of desiccation upon the cells of the rotifer tissues is the production of a nuclear-cytoplasmic rearrangement

in which the chromatic part of the nucleus migrates to the periphery and the cytoplasm becomes more dense.

7. The movement of chromatin during desiccation phenomena takes place in order that the cell oxidations may continue during the dried condition.

8. The extent of the structural rearrangement which takes place in the rotifer cells is not directly proportional to the intensity of the stimulus.

9. In animals recovering from desiccation the elements of nucleus and cytoplasm gradually resume normal relationships.

10. The chromatic rearrangement in the nuclei of cells of drying rotifers takes place at the very beginning of the drying process.

11. The acceleration of reproductive activity just subsequent to drying is traceable to an increase in ovarian nuclei. This increase takes place while the animal is recovering.

12. The cytological changes attending a recovery from desiccation are in their nature the exact reverse of those taking place during the drying process.

13. The death of cells as a result of desiccation is probably not a result of the activity of concentrated osmotic materials upon the protoplasm as Pfeffer suggests.

14. The death of rotifers during the desiccation process may be due to one or a combination of the following causes: (1) Mechanical injury due to too rapid drying, (2) starvation resulting from a lack of reserve food material, (3) the poisonous effect of metabolic products and (4) insufficient time before drying to effect the nuclear-cytoplasmic reorganization.

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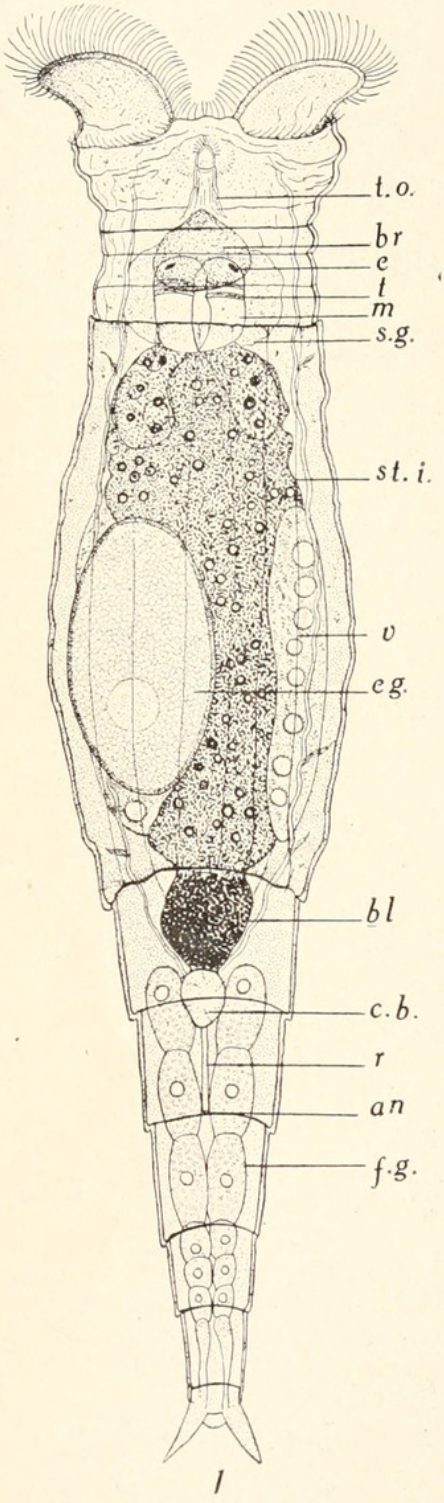
VII. EXPLANATION OF PLATES.

Plate I.

Fig. 1. Dorsal view of a living extended *Philodina roseola*. *t.o*, tactile organ; *br*, brain; *e*, eye; *t*, tooth; *m*, mastax; *s.g*, salivary glands; *st.i*, stomach intestine; *v*, vitellarium; *eg*, egg; *bl*, "blasendarm"; *c.b*, contractile bladder; *r*, rectum; *an*, anus; *f.g*, foot-gland.

Fig. 2. Ventral view of same animal. *ph*, pharynx; *s.g*, salivary gland; *f.c*, flame cell; *e.c*, excretory canal.



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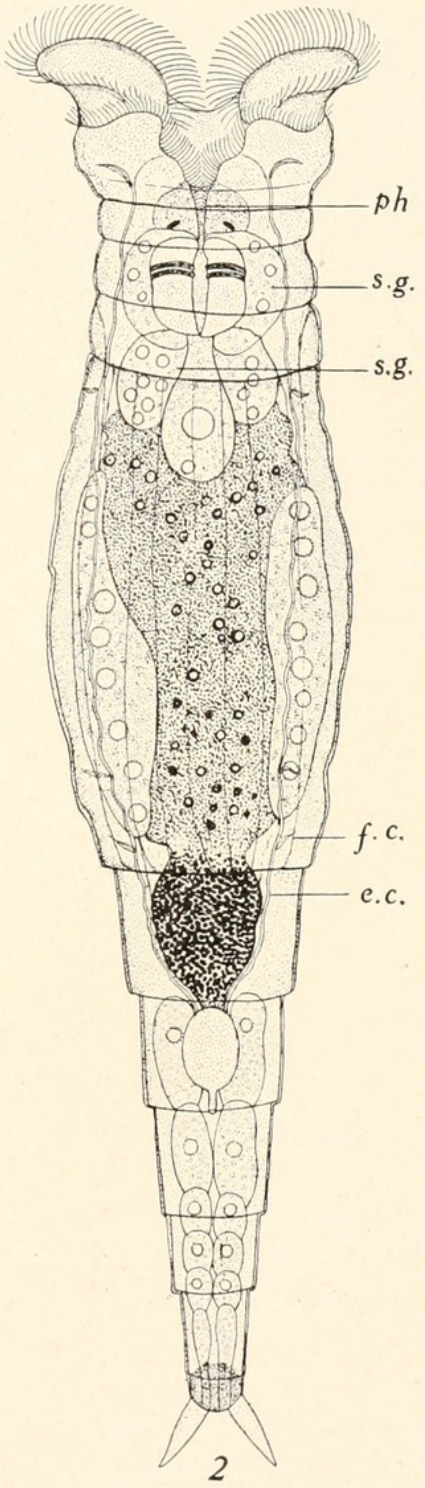

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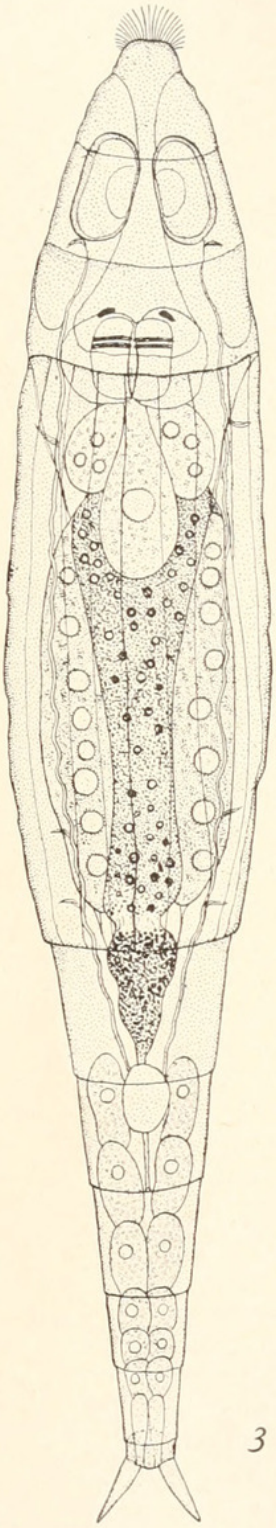
PLATE II.

FIG. 3. Ventral view of *Philodina roseola* with trochal discs retracted and proboscis extended.

FIG. 4. *Philodina roseola* in contracted condition.

FIG. 5. *P. roseola* in dried contracted condition.

FIG. 6. Side view of foot of *P. roseola* showing toes.



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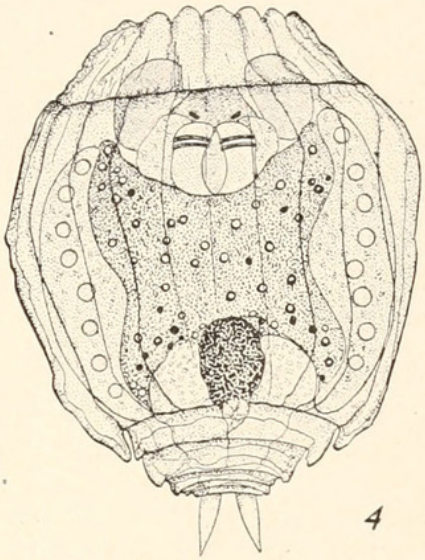

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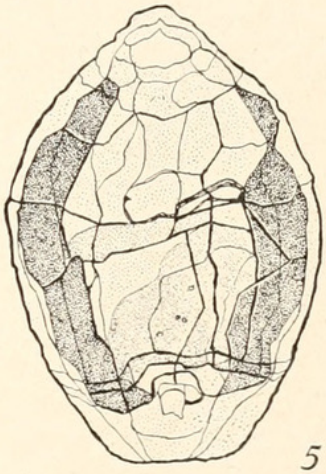

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Plate III.

Fig. 7. Section through brain and posterior part of pharynx of normal extended animal. Zeiss. Oc. 6, Obj. 2 mm.

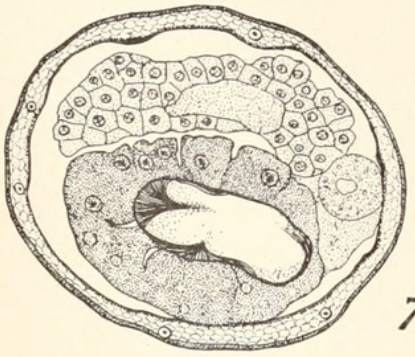
Fig. 8. Section just posterior to the mastax and through salivary glands and oesophagus. Zeiss Oc. 6, Obj. 2 mm.

Fig. 9. Section through mid-body region. Leitz Oc. 4, Obj. 2 mm.

Fig. 10. Section through mid-body region of animal containing egg. Zeiss Oc. 6, Obj. 2 mm.

Fig. 11. Section through point of juncture of stomach-intestine and "blasendarm." Zeiss Oc. 6, Obj. 2 mm.

Fig. 12. Section through contractile bladder and foot-glands. Zeiss Oc. 6, Obj. 2 mm.



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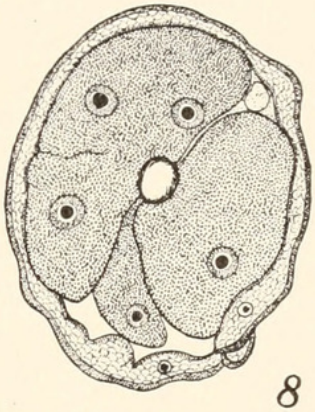

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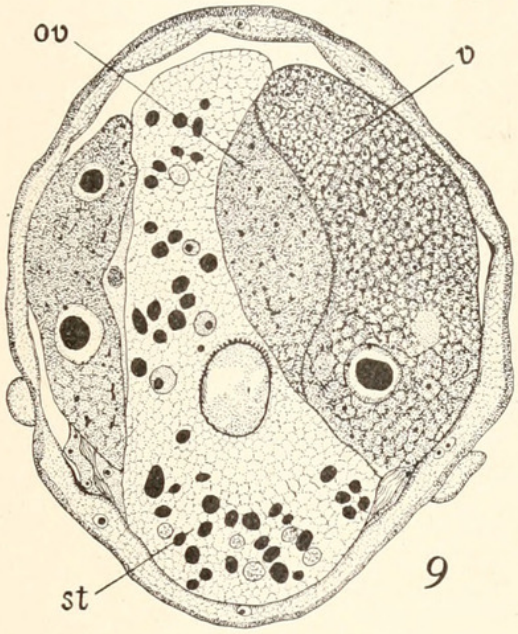

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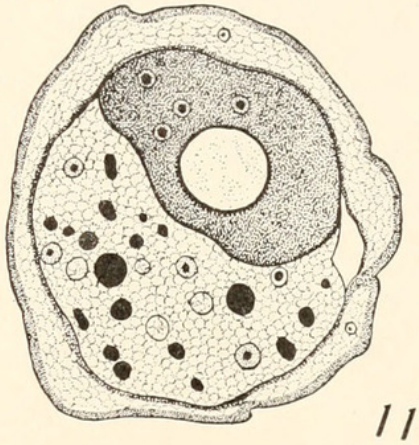

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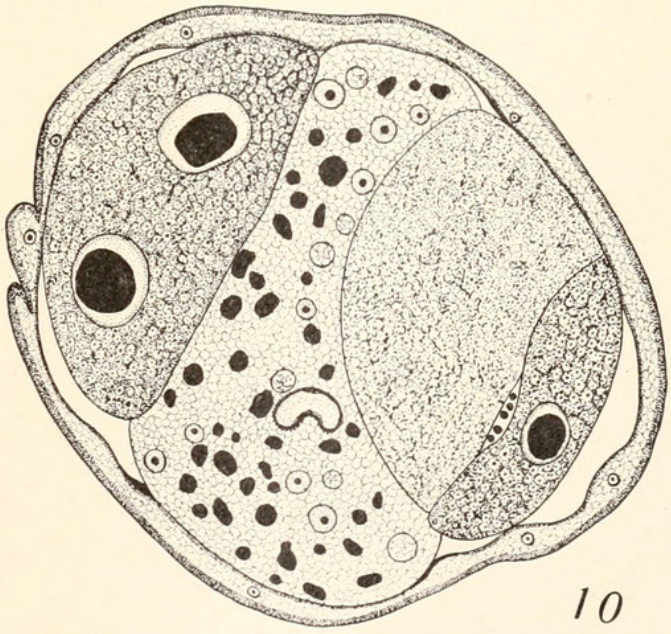

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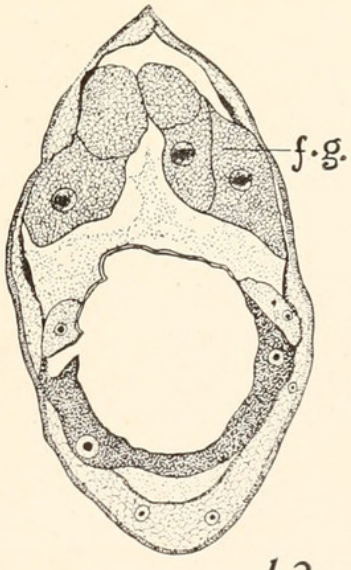

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Plate IV.

Fig. 13. Frontal section through middle of contracted *P. roseola*, not dried. Zeiss Oc. 6, Obj. 2 mm.

Fig. 14. Slightly oblique section through a contracted animal, not dried. Leitz Oc. 4, Obj. 2 mm.

Fig. 15. Frontal section through middle of a rotifer recovering from desiccation. Animal was killed four hours subsequent to the addition of water. Zeiss Oc. 6, Obj. 2 mm.

Fig. 16. Frontal section through rotifer kept in an evacuated calcium chloride desiccator for eighteen days previous to fixation. Leitz Oc. 4, Obj. 2 mm.

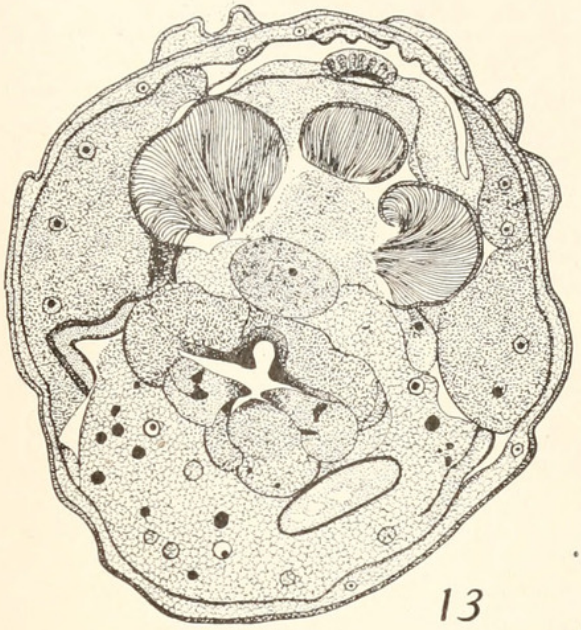
Fig. 17. Section of foot-gland cells of a rotifer which was kept in an evacuated desiccator for fourteen days previous to time of fixation. Leitz. Oc. 12, Obj. 2 mm.

Fig. 18*a*. Section of foot-gland cells of a normal active animal. Leitz Oc. 12, Obj. 2 mm.

Fig. 18*b*. Section of foot-gland cells of an animal kept for fourteen days in an evacuated desiccator and then placed in water for one and one-fourth hours previous to fixation. Leitz Oc. 12, Obj. 2 mm.

Fig. 19. Section of foot-gland cells from animal dried one week at room temperature. Zeiss Oc. 6, Obj. 2 mm.

Fig. 20. Section of vitellarium from animal dried five days at room temperature, placed in water four hours, then killed and sectioned.



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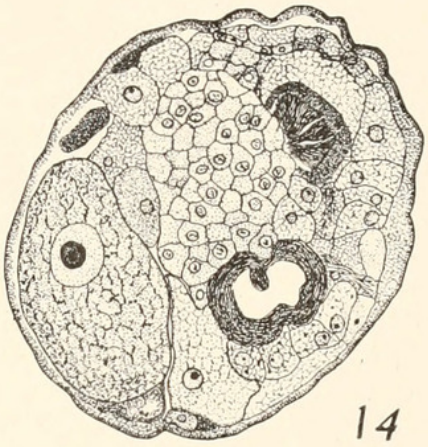

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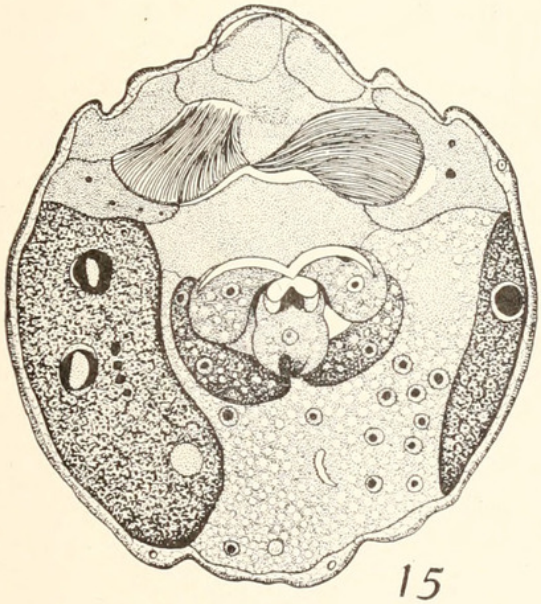

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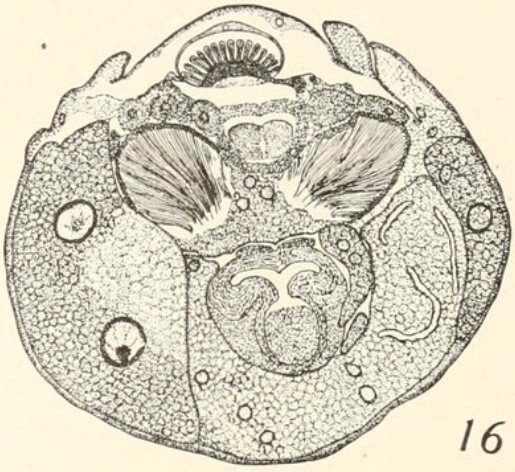

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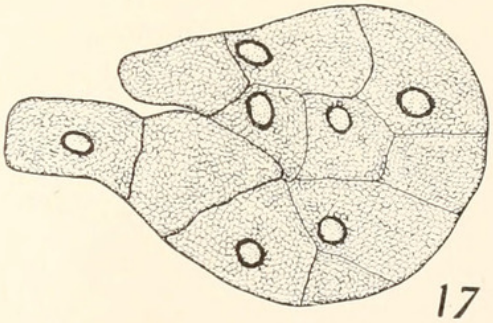

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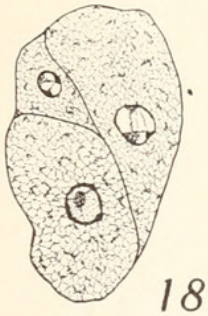

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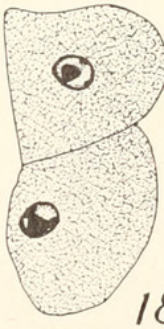

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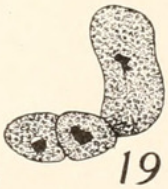

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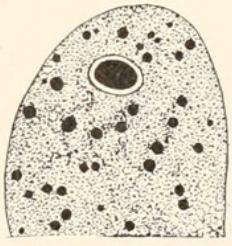

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Plate V.

Fig. 21. Longitudinal section through ovary-vitellarium of a rotifer dried in an evacuated desiccator for fourteen days previous to time of fixation. Leitz Oc. 8, Obj. 2 mm.

Fig. 22. Cross-section of vitellarium of animal recovering from desiccation. Animal was kept in an evacuated desiccator for six days, then placed in water for one hour at the end of which time it was killed. Leitz Oc. 8, Obj. 2 mm.

Fig. 23. Longitudinal section of ovary-vitellarium of animal recovering from desiccation. The rotifer was kept in an evacuated desiccator for fifteen days, then placed in water for one hour and fifteen minutes at the end of which time it was fixed. Leitz Oc. 8, Obj. 2 mm.

Fig. 24. Cross-section of mastax and salivary gland of a normal undried animal. Zeiss Oc. 6, Obj. 2 mm.

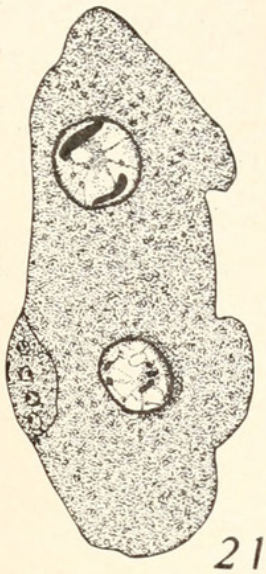
Fig. 25. Section of mastax and salivary gland of an animal dried in an oven at 40° Centigrade for four days. Zeiss Oc. 6, Obj. 2 mm.

Fig. 26. Section of salivary gland cells from an animal desiccated, kept four hours in water, and then killed. Zeiss Oc. 6, Obj. 2 mm.

Fig. 27. Cross-section of brain of *P. roseola*. Normal active animal. Zeiss Oc. 6, Obj. 2 mm.

Fig. 28. Cross-section of brain of a rotifer kept in an evacuated desiccator for fourteen days previous to time of fixation. Leitz Oc. 8, Obj. 2 mm.

Fig. 29. Cross-section of brain of *P. roseola* dried twenty-four hours, put into water for one hour and then killed. Leitz Oc. 8, Obj. 2 mm.



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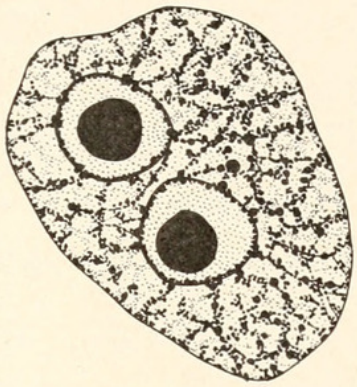

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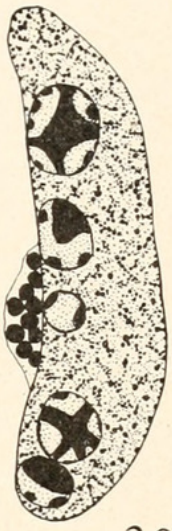

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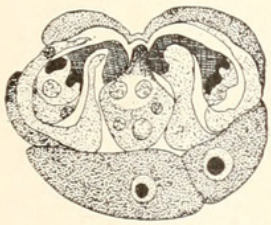

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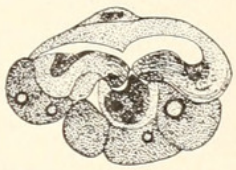

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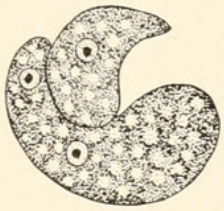

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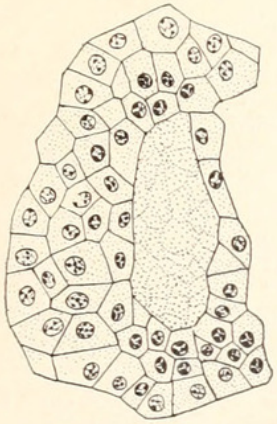

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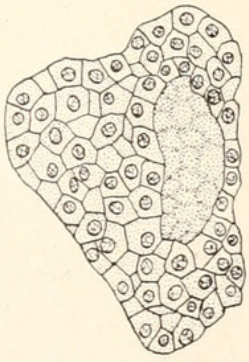

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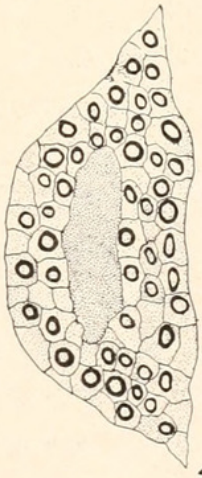

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MITOCHONDRIA AND OTHER CYTOPLASMIC STRUCTURES IN THE SPERMATOGENESIS OF PASSALUS CORNUTUS.

E. L. SHAFFER,

PRINCETON UNIVERSITY, DEPARTMENT OF BIOLOGY.

A. INTRODUCTION.

In course of my study on the spermatogenesis of *Passalus cornutus* (one of the Lacunid beetles), the mitochondria and other cytoplasmic inclusions came to my attention. Although I was primarily interested in the study of the nuclear changes with especial reference to synapsis, it seemed desirable to make a study of the cytoplasmic structures above mentioned, since they showed so clearly in my preparations. I shall therefore reserve for a later report a description of the nuclear changes involved in the course of spermatogenesis.

I am glad to acknowledge my indebtedness to Prof. E. G. Conklin, of Princeton University, under whose guidance and criticism this work has been pursued. I also wish to thank Mr. Milton P. Hunter, of Westtown, Pa., from whom I received the material used in this study.

B. MATERIAL AND TECHNIQUE.

There are two pairs of more or less bulb-shaped testes situated in the posterior part of the abdomen. These were dissected out in Ringer's solution and immediately fixed in one of the following fixation fluids: Hermann's, Flemming's strong solution, and Benda's modification of Flemming's fluid. Iron hæmatoxylin followed by either Lichtgrün, erythrosin or Bordeaux red was the stain most used, especially when the fixations were with Hermann's or Flemming's fluids. The Benda crystal violet-alizarin staining method was employed on the material fixed in the Benda fluid. On the whole, material fixed in strong Flemming's fluid (three or four hours) and then stained in iron hæmatoxylin followed by one of the counterstains previously men-

tioned, was the most satisfactory. In Hermann and Benda material there is a tendency, especially in the spermatocytes, for the mitochondria to agglutinate in dense irregular masses.

C. Observations.

(*a*) *Chromosomes.*

Although not primarily concerned with the chromosomes in the present report, it may be of some interest to at least mention these in a general way. I have as yet been unable to find spermatogonial plates of sufficient clearness to make accurate drawings, but all counts indicate that the chromosome number is 26, including an unequal pair. This has been confirmed by a study of the ovaries in which all dividing follicle cells show 26 chromosomes and these can be arranged in equal pairs (Fig. 3). The metaphase plate of the first spermatocyte (Figs. 12, 33) shows 13 bivalent chromosomes, one of which represents the unequal (sex) pair. The elements of the sex pair separate in the first maturation division and divide equationally in the second division.

(*b*) *Mitochondria.*

I shall not attempt to review the vast literature which has grown up bearing on the subject of the mitochondria. Duesberg ('11) has given a rather complete review of the literature, so that it will not be necessary to do so here. I shall, however, discuss the works of others insofar as these may be related to my observations on *Passalus*.

1. *Spermatogonia.*—In the primary spermatogonia, which are situated at the blind end of the testes, I have been unable to find conclusive evidence of the presence of mitochondria (Fig. 1). The cytoplasm is usually of a homogeneous, non-reticular and finely granular structure. These granules do not stain in anything but the plasma stain, and therefore cannot be considered as mitochondria. There are usually present, in the cytoplasm, several deeply staining bodies (hæmatoxylin) which are perhaps similar to the chromatoid corpuscles of other workers. It is possible that these may really be of a mitochondrial nature, but their subsequent fate cannot in any way be related to the mitochondria of the later stages. Lewis and Robertson (1916) in

their study of the living cells of *Chorthippus* (Orthoptera) found that mitochondria were present in the primary spermatogonia in the form of granular threads; but there is no evidence for such structures in *Passalus*.

The secondary spermatogonia are arranged in cysts in the form of a rosette, and are generally pyramidal in shape. Both nuclear and cytoplasmic volume is noticeably smaller in these than in the primary gonia. The cytoplasm always shows a marked affinity for the hæmatoxylin and this is due to the presence of numerous mitochondrial granules (Figs. 4, 23) which are scattered diffusely throughout the cytoplasm. Payne (1917) found granular mitochondria in the spermatogonia of *Gryllotalpa*, while Duesberg (1910) figures similar structures in *Blaps*. On the contrary Duesberg describes the mitochondria of the gonia of *Blatta* as being present in the form of threads (chondrioconts). Payne, Schäfer ('07) and others have found that the mitochondria of the spermatogonia are localized at the inner ends of the cells (*i. e.*, the end bordering on the cyst cavity). As I have before stated, in *Passalus* the mitochondria are diffusely spread and often are actually absent from the inner ends of the cells. Montgomery (1911) was unable to find evidence of "indubitable mitochondria" in the spermatogonia of *Euschistus*, although he found granules which he considered to be disintegrated idiozome material.

Fig. 5 shows a degenerating spermatogonium. It is noticed that the cytoplasm is much more deeply staining and the mitochondrial granules are much larger. The chromatin of the nucleus is concentrated into one or two karyosomes. The increase in the size of the mitochondrial granules is probably due to an agglutination of the smaller normal ones. Cowdry (1916) in his excellent review on the functional significance of mitochondria, has called attention to the relations of mitochondria in pathological tissues. He mentions the work of Scott who found that in fatty degeneration of the pancreas there was an agglutination of the mitochondria. It is quite possible that the degeneration of cells which is so common in insect spermatogenesis is of the fatty degeneration type. A study of the behavior of the mitochondria in degenerating sperm cells may throw some light

on their rôle in normal processes. This is, however, beyond the scope of the present work.

The spermatogonial cysts which are in mitotic activity, stand out very clearly in contrast with the resting cysts. This is because of their lighter staining capacity; whether this is in turn due to the partial disappearance of the mitochondria, could not be ascertained (Figs. 2, 24). Buchner (1909) found that in *Gryllotalpa vulgaris* the mitochondria disappear during or just before cell-division. There are three possible explanations for the partial loss of mitochondrial structure during mitotic activity; (*a*) at this time the cytoplasmic volume is much greater and hence the mitochondria are more diffusely spread; (*b*) the dissolution of the nuclear membrane sets free a large amount of karyolymph which perhaps dilutes the cytoplasm and obscures the mitochondria; (*c*) the mitochondria may dissolve or become chemically changed so that they are no longer recognizable as such. At any rate, they soon appear in great numbers after cell-division, so that their partial disappearance was apparent and not real.

2. *Spermatocytes.*—In the spermatocytes at the beginning of the growth period, the mitochondria are still in the form of diffusely spread granules. There is a noticeable increase in their numbers as the growth period progresses, which plainly indicates that new ones are being formed (Fig. 6). Usually a denser perinuclear zone of cytoplasm can be seen during the growth period; the significance of this zone will be discussed later.

As the late prophase approaches, the cytoplasm becomes filled with numerous delicate threads deeply staining in hæmatoxylin. These are the filar mitochondria (chondrioconts) and they first appear at this time, although there is a slight indication of delicate granular threads in some of the earlier stages. The origin of these threads could not be traced, but it is quite likely that they are genetically related to the granules of the preceding stages. In the first spermatocyte the mitochondria appear diffusely spread when material has been fixed in Flemming's fluid (Figs. 7, 8, 25, 25*a*). In Benda and Hermann material the mitochondria agglomerate in dense irregular masses, still showing their filar nature (Figs. 9, 26). The threads tend to

lie with their length extended in the direction of the main axis of the cell. Sections of the cell taken at right angles to its longitudinal axis show the mitochondria on end view (Fig. 25*a*). As the nuclear membrane dissolves and the first maturation spindle is forming (Figs. 10, 29), the mitochondria are usually localized at one side of the spindle. When the spindle has fully formed, the mitochondria begin to envelop it at first from one side (Fig. 11) and later entirely surround the outer spindle fibers, being equally distributed along their lengths (Figs. 13, 30, 31). By the time the metaphase has been reached, the spindle has been entirely surrounded by mitochondria. Figures 12, 32, 33, 34 represent cross-sections at different levels of the first maturation spindle at metaphase, showing clearly the relations of spindle and mitochondria. As is best seen in the anaphase (Figs. 13, 35) they do not entirely reach to the two poles of the spindle, so that there is a short uncovered part near the centrosomes.

The question arises as to the method of movement of the mitochondria in order that they establish this relation with the spindle; is it an active or a passive movement? Many observers on living cells have noted that the mitochondria are generally of a vibratile or motile nature. According to Lewis and Lewis (1914) the mitochondria are never at rest; "a single mitochondrium sometimes twists and turns rapidly as though attached at one end, like the lashing of a flagellum, then suddenly moves off to another position in the cytoplasm as though some tension had been released" (p. 332). If it is true that they possess the power of active movement, it may be possible in this way to explain their movement to the spindle. Lewis and Robertson (1916) state that "the mitochondria migrate from the two masses of mitochondrial granules and elongate towards the poles of the spindle," etc. (p. 108). Whether they mean by this an active or a passive migration is not clear from their account. It seems highly improbable that the movement is active. Some observers have supposed that their movement is influenced by the centrosomes, but there is no conclusive evidence that this is the case. It is more likely that the movement of the mitochondria to their position on the spindle is due to the cytoplasmic

movements during metakinesis which Conklin has shown, plays such an important rôle in the localization of cytoplasmic substances in the egg.

There is no evidence from my observations on *Passalus* that the mitochondria divide autonomously, as has been maintained by some workers. As the cell constriction continues through the equator of the spindle (Figs. 14, 35), the mitochondria are divided by this constriction and the daughter cells (second spermatocytes) receive approximately equal amounts of mitochondria. The mitochondrial masses then move a short distance toward the poles; this is evidently caused by a further elongation of the spindle. Montgomery (1911) has described a similar method of division in *Euschistus*. The fact that mitochondria are found in equal amounts in daughter cells is supposed by some to favor the view that they divide autonomously; but it is well known that yolk and similar substances are often found in equal amounts in daughter cells, yet no one maintains that yolk granules divide autonomously. Fauré-Fremiet (1911) describes autonomous division in the mitochondria of the Protozoa, and Wilke (1912) does likewise for the spermatocytes of *Hydrometra*. Payne (1917) does not incline strongly to the view that the mitochondria are divided by the cell constriction, while Lewis and Robertson (1916) do not clearly state whether their division in *Chorthippus* is autonomous or passive.

Following the first maturation division, there is a short interkinesis during which the second maturation spindle is formed (Figs. 16, 36). At this time the mitochondria are lying at one side in a rather dense mass which shows a lighter central portion through which the spindle of the previous division passed. The behavior of the mitochondria during the second maturation division is precisely the same as in the first division (Figs. 17, 37). They again surround the spindle peripherally and are divided by the equatorial constriction. As a result of this division both spermatids receive approximately equal amounts of mitochondria (Fig. 18).

The mitochondrial mass contained in the spermatid gradually becomes more compact and henceforth may be designated as the Nebenkern. It stains intensely with the basic stains and

often shows a granular structure. It often shows a lighter central portion which represents the position the spindle had occupied (Fig. 20*d*). Soon after the Nebenkern has become a spherical body, it begins to show a peripheral lighter area which is of a vacuolar nature (Fig. 19). As the spermatid elongates the Nebenkern becomes elongated and the vacuolization of the peripheral layer becomes more marked (Fig. 20, *a*, *b*). It becomes divided into two halves and is later pierced by the axial filament (Figs. 21, 39). As the axial filament grows out, the Nebenkern continues to elongate forming a sheath about it. The material of the Nebenkern thus extends from the centrosome for a considerable distance along the axial filament (Fig. 22). Fig. 20, *c* is that of a cross-section through the tail of a spermatid showing the two halves of the Nebenkern on each side of the axial filament. Whether or not the Nebenkern sheath extends to the free end of the axial filament could not be determined. As the transformation continues, the Nebenkern becomes more lightly staining, and all evidences of its mitochondrial nature are lost.

3. *Discussion.*—I have been unable to obtain any definite and conclusive evidence as to the manner in which the mitochondria arise. There can be no doubt that they increase in numbers during the growth period, and the question arises: to what influence do the mitochondria owe their origin? This has been and still is a much-debated cytological question, and much depends on the final solution of the problem.

Meves, Bouin, Duesberg and others have always maintained that the mitochondria are persistent and self-perpetuating structures, much as the chromosomes of the nucleus are at present regarded. On the other hand, Goldschmidt and his pupils, Buchner, Jordan, Wildman and others have derived the mitochondria from the nucleus, and have thus maintained that they are akin to the chromidia of Hertwig. Vejdovský has traced their origin from the sphere material, while Montgomery in *Euschistus* concludes that they are probably derived from the idiozome or the nucleus, or by a "joint action" of both. In their studies on the living sperm cells of *Chorthippus*, Lewis and Robertson are unable to find any evidence that the mitochondria

are derived from the nucleus. It is thus evident that the facts are conflicting on all sides. If it is true, as I have previously stated (p. 408), that the mitochondria are not present in the primary spermatogonia of *Passalus*, then we cannot maintain that they are persistent and self-perpetuating. I am, however, not entirely convinced that they are absent in the primary spermatogonia and shall await fresh material for a study of the living cells.

It is possible that the increase in number of the mitochondria during the growth period is due to a simple growth and division of those already present. I cannot find any evidence for this in my material. In nearly all the growth stages of the first spermatocytes, there is present a denser and more deeply staining perinuclear zone. Schäfer (1907) has figured similar conditions in *Dytiscus*, while Voivnov (1903) shows it more strikingly in *Cybister* and calls it the "zona interna." During the maturation divisions, the "zona interna" surrounds the spindle peripherally and forms the Nebenkern of the spermatid. As Duesberg (1910) has pointed out, it is quite evident from this behavior that the "zona interna" is really of a mitochondrial nature. Giardina (1904) has studied the oöcytes of a number of forms (*Periplaneta*, *Stenobothrus*, *Gryllus*, *Mantis*, etc.) and figures well-defined perinuclear zones usually of a granular nature. Whether this zone is formed by a diffusion of chromatin in the form of a solution out of the nucleus into the surrounding cytoplasm or whether it arises in situ in the cytoplasm by interaction with the nucleus, is a question which Giardina discusses at considerable length. He concludes in favor of the latter view. Payne (1917) has described a similar perinuclear zone in the oöcytes of *Gryllotalpa*, but he definitely states that this consists of mitochondria. As the oöcyte grows, the mitochondria migrate centrifugally into the cytoplasm. Vejdovský (1911–12) shows the mitochondria arranged in a definite perinuclear zone in the spermatocytes of *Diestramena*. It thus seems quite probable that the perinuclear zones described by Giardina are really of a mitochondrial nature. The occurrence of this zone in such close connection with the nucleus and especially during a period when the mitochondria are certainly increasing in number, is

highly indicative of an interaction between nucleus and cytoplasm. The relation of this zone to the mitochondria seems to be a strong argument, at least, that it is the locus of mitochondria formation.[1]

The association of the spindle and the mitochondria has led to some confusion in regard to the origin of the Nebenkern, so that some workers have ascribed its origin to the spindle remains of the second maturation division. Arnold (1908) comes to this conclusion in his study of *Hydrophilus*, as does Baumgartner in *Gryllus*. Voivnov, as previously mentioned, derived the Nebenkern from his "zona interna" and the peripheral spindle fibers. Munson (1906) in his work on *Papilio* also derives the Nebenkern from the spindle remains, but his observations are interesting since they clearly show that he has confused these with the mitochondria. According to his view, it is only the "outer, granular" mantle fibers of the spindle which take part in the formation of the Nebenkern. It is evident, in light of the recent work, that the "outer, granular" mantle fibers are really mitochondria. That he actually saw the mitochondria and misinterpreted them, is evident from the following quotation: "Often a few scattered chromatin segments are found scattered along the spindle fibers, or else drawn out into stainable threads parallel with the spindle fibers" (p. 91).

(*c*) *Spindle Derivatives.*

When one attempts to review the literature on the subject of spindle derivatives and their histories, one is immediately confronted with a maze of conflicting observations and interpretations, to say nothing of a nomenclature which is almost hopelessly confused. The names mitosome, idiozome, attraction-sphere, centrosphere, astrosphere, Nebenkern (of older workers), etc., are all examples of the existing confusion, and should caution us against hasty interpretations of such structures. Meves (1899) states that he first applied the term "idiozome" to those compact bodies in the spermatogonia and spermatocytes which

[1] It is interesting to note in this connection that the material of the yellow crescent in the ascidian egg has been shown by Conklin to be found at various times in a perinuclear position; and Duesberg (1913) has shown that this zone is extremely rich in mitochondria.

surround the central corpuscles (centrosomes). By a disintegration of the idiozome, the centrosomes are set free and then take part in the next cell-division. In the rat, however, Meves found that there is no disintegration of the idiozome, but that the centrosomes wander out, leaving the idiozome intact. As division progresses, the latter dissolves and disappears. There is no essential difference between the "attraction-sphere" of Van Beneden, the "centrosphere" of Strasburger and the "astrosphere" of Fol and Boveri; and so far as I have been able to ascertain, there is no fundamental difference between these last named structures and the "idiozome" of Meves. One thing is clear,—that these structures all refer to the achromatic substance of the spindle situated at the poles and usually enclosing the central corpuscles. To avoid any possible misuse of these terms I shall employ the non-committal term "sphere" to denote this portion of the spindle. With regard to the remains of the spindle proper, there is less confusion of terms, and I shall use the term "mitosome" or "spindle remains" to designate this structure.

1. *Spermatogonia.*—The spermatogonia in *Passalus* in mitotic activity stand out very sharply in contrast with those in the resting condition not only because of their lighter staining capacity (as mentioned on p. 410), but also because they tend to become round in outline and the cell walls become more sharply defined. This is indicative of an internal pressure which Reinke (1900) calls the "mitotic pressure." As the gonial anaphase progresses, a well-defined cell-plate makes its appearance and stains deeply in hæmatoxylin (Figs. 2, 24). As the telophase advances, the spindle and cell-plate become more compact, the former taking the acid stains and the latter taking the basic stains. Fig. 4 shows several resting cells from a spermatogonial cyst with the spindle remains (mitosome) running from cell to cell. Very often the spindle remains from each division become so connected that they form sort of bond between all the cells in a cyst. Hegner (1914) has described a similar condition in the spermatogonial cysts of *Leptinotarsa decemlineata.* In this case, however, he found that material fixed in Carnoy's fluid always showed the spindle remains taking the basic stain.

Günthert (1910) has described similar results in the differentiation of nurse cells and oöcytes in *Dytiscus* and Govaerts (1913) has done likewise in *Carabus*. In *Passalus*, it is only in the region of the cell-plate that the spindle takes the basic stain. In cross-section the spindle remains appear as plasmasome-like bodies in the cytoplasm. Often there may be seen deeply staining granules adhering to the surface of such bodies; these I take to be portions of the deeply-staining cell-plate.

2. *Spermatocytes.*—The spindle remains of the last spermatogonial division (together with the cell-plate) persist into the spermatocyte and remain very conspicuous throughout the entire growth period (Figs. 6, 8, 27). Every spermatocyte exhibits these spindle remains, either in their original positions connecting cells or else in the form of plasmasome-like bodies lying free in the cytoplasm. Similar conditions have been shown by Voivnov in *Cybister* and by Munson in *Papilio*. In *Gryllotalpa*, Payne states that "there is no indication that a sphere or the spindle and astral fibers persist after cell-division." In his figure B, plate 1, he shows two plasmasome-like bodies present in the cytoplasm of a spermatocyte during the early growth period. As to the origin of these bodies, he is uncertain, but he is not ready to admit that they may be idiozome material. I agree that it would be rather speculative to assign the term "idiozome" to these bodies, but in *Passalus* it is quite clear that similar bodies are derived from the spindle. I am not able to establish the presence of a definite "idiozome" or sphere material in the spermatocytes of *Passalus* and just where the centrosomes lie hidden during the growth period is a difficult matter to determine. Since the cells are so filled with mitochondria at this time, it seems almost impossible to definitely locate them. The centrosomes are first discernible when the first maturation spindle makes its appearance. From the long persistence of the spindle remains of the last spermatogonial division, it may be concluded that its substance must be of some inert, resistant material. Munson (1906, p. 90) makes the following remarks on *Papilio:*

"The resistance of the maturation spindles to reagents is remarkable. In studying the living dividing cells on the slide,

I have seen the cytoplasm gradually disintegrate, become vacuolated, and disappear while the spindles remain as perfect as ever. In testing the effects of reagents, too, I have been able to dissolve practically the whole of the cytoplasm of all the cells of the cyst, while the spindles remained, showing a system of connected spindles throughout the whole cyst."

There are other structures related to the spermatocytes which are of a more or less problematical nature. In Figs. 8 and 28 there will be noticed a large vacuole in each of the spermatocytes; these are to be found in nearly every spermatocyte during the late prophases and thus far I have been unable to find them in the earlier stages. The vacuoles are filled with a homogeneous fluid (perhaps a gel) which takes the plasma stain but lightly. Sometimes there may be several vacuoles in a single cell. In cysts of spermatocytes having such vacuoles, one often finds in the cyst cavities round or oval bodies of a granular nature (Fig. 8) resembling very much the cytoplasm of the spermatocytes. Furthermore, in such cysts the sides of the spermatocytes bordering on the cyst cavity are of an irregular outline and often distinct pseudopod-like projections of the cytoplasm are given off. It is therefore highly probable that the small bodies found free in the cyst cavity have arisen from the pseudopod-like projections or buddings from the cytoplasm of the spermatocytes. What the significance of either the vacuoles mentioned above, or of the casting off of portions of the cytoplasm may be, I am unprepared to state. The presence of vacuoles and pseudopods at the same time may indicate that the vacuoles are cast out by means of setting free the pseudopods. But this view is at present untenable, for I have never actually seen vacuoles within the pseudopods; furthermore the contents of the vacuoles are always of a homogeneous appearance, while the cast off parts of the cytoplasm are granular as described above. Voivnov has found in *Cybister* that vacuoles make their appearance in the late prophases of the spermatocytes. These differ in appearances, at least, from those found in *Passalus* in that they contain definite bodies as inclusions. Voivnov also finds that they are later cast out into the cyst cavity. He considers these to be portions of the sphere (idiozome) which are under-

going disintegration. In support of this view, he finds that they appear at a time when the centrosomes are first set free and often they may be found in close spatial relations to the centrosomes.

Whether or not the vacuoles and the cast-off portions of the cytoplasm in *Passalus* correspond to the conditions described by Voivnov in *Cybister* I am at present unable to say. The origin of the vacuoles and their relation to the cytoplasmic buddings can be best studied in the living material, and I shall leave the question undecided here for lack of evidence.

3. *Maturation Divisions and the Spermatid.*—The spindles developed for both maturation divisions are relatively very large, both in actual dimensions and in number of spindle fibers. Correlated with the large size of the spindles is the relatively large nucleus, containing little chromatin but a large amount of karyolymph. The view of Conklin and others, that the spindle grows at the expense of the karyolymph is certainly substantiated in the case of *Passalus*. In both maturation divisions cell-plates are developed which are very much smaller than those found in the spermatogonia (Figs. 14, 8), and the remains of the spindles persist for a considerable time after division. In the anaphase of the second maturation division, the centrosomes are still distinguishable lying close to the chromatin masses at the poles. In the telophase of this division they are found lying on the nuclear membrane. It is because of this position on the nuclear membrane that they are not easily detected, but careful searching and focusing will show their undoubted presence (Fig. 18). Thus in the spermatid, the centrosome is still to be seen closely attached to the nuclear membrane; it gradually shifts its position until it comes to lie between the nucleus and the Nebenkern. Usually there is a precocious growth of the axial filament (Fig. 19) before the centrosome arrives at its ultimate position. At this time, the centrosome appears double, while the axial filament grows out between the two halves of the Nebenkern and becomes associated with the latter in the formation of the tail, as shown before (p. 413). As transformation continues (Figs. 21, 22), the centrosomes become more and more closely associated with the nucleus, until in the older stages they are scarcely

distinguishable from the head of the spermatozoön, and hence there is no well-defined middle-piece.

In the youngest spermatid there is always present a cytoplasmic body of a refringent nature which takes the plasma stain (Figs. 19, 38). One portion of it is more compact, while the other part is in the form of a vacuole containing a deeply staining (hæmatoxylin) body. This structure is undoubtedly a spindle derivative, but whether it represents the sphere material or the mitosome I am unable to state. As to the origin of the deeply staining body contained within it, I am equally unable to explain. It may possibly represent a portion of the cell-plate which has become detached from the cell wall and has become encompassed in the remains of the spindle.[1] In older spermatids a compact portion of the spindle derivative is found lying near the nucleus in that part of the spermatid which is destined to give rise to the head end of the spermatozoön (Fig. 21). In later stages, as the nucleus becomes laterally compressed, this structure is transformed into the acrosome. The remainder of the spindle derivative is sloughed off into the tail of the spermatozoön and gradually disappears.

4. *Discussion.*—As has been previously stated, the axial filament in *Passalus* arises in connection with the centrosome. Munson (1906) has expressed an entirely different view concerning its origin in *Papilio*. His view is that the axial filament represents a much compressed portion of the cytoreticulum and has no relation to the centrosome. Accordingly, he finds that in the stages of development of the axial filament occasionally three or four filaments may be present in a single spermatid, but these finally unite into a single thread. Paulmier (1899) has described double and quadruple spermatids in which two or four axial filaments were present, but each was connected to a centrosome. Munson's view arises from the fact that he has assigned a wholly different function to the centrosome. His figures of the spermatids of *Papilio* show spindle derivatives each containing a deeply staining body which he interprets as

[1] Duesberg (1908) figures a similar spindle derivative in the spermatid of the rat which he designates as the "idiozome." It also consists of a vacuolar portion which contains a deeply-staining granule which is not a centrosome, just as in *Passalus*.

the centrosome. The conditions here are very much like those in *Passalus* (Figs. 19, 38), but in the latter case the deeply staining body is certainly not a centrosome. The sphere (?) of the spermatid of *Passalus* has no connection with the centrosome. This is in agreement with Montgomery's conclusions in *Euschistus*. According to Munson, the centrosome gives rise to the acrosome of the spermatozoön.

Payne (1917) has approached the subject of spermatid transformation with more or less scepticism as to the generally accepted origins of the structures present here. I am in hearty accord with this point of view, but it seems that Payne has carried matters too far. I agree that to call the structure from which the axial filament grows a centrosome without tracing its history from the second spermatocyte, is highly speculative. But to say, as Payne does, that at a certain stage of the spermatid "there is nothing in the cytoplasm but mitochondria" (p. 309) is equally as dogmatic and unwarranted in light of the observations of many other workers. In *Passalus* there can be no doubt that the centrosome of the spermatid has actually been carried over from the preceding cell-division. Furthermore, in the youngest spermatid there can always be found the refringent cytoplasmic body which is undoubtedly a spindle derivative. In *Gryllotalpa*, Payne finds in the stages succeeding the young spermatids which contain no other cytoplasmic structures but mitochondria, the sudden appearance of two deeply staining bodies, one of which later forms the acrosome, while the other is pushed off into the tail. Since these were not present in the earlier stages, they have apparently arisen "de novo," that is, they are newly differentiated parts of the cytoplasm. One might then expect to find developmental stages of such structures, but Payne does not indicate such. However, it seems to me that the building-up of the spermatozoön from the spermatid is a process involving no differentiation, but a *transformation* of differentiations already present. All the structures needed in the building up of the spermatozoön are at hand, and there is no further elaboration of new ones. Munson (1906, page 96) has clearly expressed a similar view:

"The comparative inertness of the nucleus at the close of the

last maturation division and ever afterward would not justify us in assuming that this is a growth period in the history of the spermatozoön; but rather that it is a transformation period of those organs that are already present and fully-grown in the spermatid stage. This transformation in all the organs of the cell is merely an elongation such as could be brought about, doubtless, by prolonged lateral pressure."

D. General Considerations.

The importance of the mitochondria as bearers of hereditary units rests on their mode of origin and maintenance through the cell cycle, their behavior in fertilization and their rôle in differentiation. If, as has been previously discussed, the view is correct that the mitochondria owe their origin to materials derived from the nucleus or by the activity of the nucleus, then their importance in heredity can only be secondary.

Just how much of the spermatozoön enters the egg is a matter of importance in ascertaining the rôle of the mitochondria. In *Nereis*, Lillie (1912) has found that the middle piece and the tail of the spermatozoön do not enter the egg. On the other hand, Meves (1911) has shown that the entire spermatozoön enters the egg of *Ascaris*, and Van der Stricht (1909) has shown similar results in the bat. It therefore seems impossible to make any generalizations on this subject until more work on the details of fertilization has been done. In the case of *Peripatus*, Montgomery (1912) has shown that the mitochondria are entirely lost in the spermatozoön, being thrown off within certain cytoplasmic lobes. Here, at least, the mitochondria of the spermatozoön can play no part in the transmission of hereditary characters.

The mitochondria have been most exhaustively studied in somatic cells where they are present in a variety of forms. Certain workers (Meves, Duesberg, Hoven and others) have maintained that the mitochondria give rise to myofibrils, neurofibrils and other somatic differentiations; but these views have not been strongly substantiated. The work of Cowdry (1914) is strong evidence that the mitochondria of nerve cells are not transformed into neurofibrils.

The researches on the chemistry of mitochondria are practically all in agreement that they are phospholipins or lecithin-albumins. No one has attempted to show that yolk is a bearer of hereditary units; yet yolk is chemically allied to mitochondria. In fact Fauré-Fremiet has shown that mitochondria actually transform into yolk. The work on the chemistry of mitochondria indicates that they are of great importance in the metabolic activity of cells, but our knowledge of their relation to heredity is negative. To say that the mitochondria are not the bearers of hereditary units is not denying that they may *influence* heredity in some cases, just as we know that heredity can sometimes be influenced by environmental conditions of food, temperature, etc.

One fact which has come clearly to light from this study is that beginning with the spermatogonia and continuing up to the spermatid, there is a progressive elaboration of mitochondria. They are then transformed into a definite structural part of the spermatozoön, the sheath of the axial filament. This progressive increase in the amount of mitochondria seems to indicate that they are differentiation products. Hence if there is any genetic continuity between the mitochondria of successive cell generations, it is only of a limited sort. The conception that the mitochondria present in the somatic cells are the direct descendants of those of the germ cells from which they have arisen, certainly has very little evidence in its favor. It seems more probable that mitochondria are in the nature of cytoplasmic differentiations, akin to metaplasm (yolk, etc.) and without a definite relation to the development of hereditary characters, but with the capabilities of influencing development insofar as they may be related to the metabolic activity of cells. It is possible that in the spermatozoa, the mitochondria merely function as locomotory organs.

Summary.

1. Although mitochondria can not be definitely demonstrated in the primary spermatogonia of *Passalus cornutus*, they are present in the secondary spermatogonia in the form of numerous and diffusely spread granules.

2. The mitochondria increase in number during the growth

period, and in the later stages are found in the form of threads (chondrioconts) which lie in the direction of the chief axis of the cell.

3. During the maturation divisions, the mitochondria envelop the spindle peripherally and are divided by the cell constrictions, so that daughter cells receive approximately equal amounts.

4. The mitochondria of the spermatid form the Nebenkern, which later is pierced by the axial filament. As the latter grows, the Nebenkern elongates, forming a sheath about it.

5. Spindle remains are found forming connections between the spermatogonia. The spindle remains of the last spermatogonial division persist throughout the entire growth period of the spermatocyte.

6. A spindle derivative is found in the spermatid, a portion of which gives rise to the acrosome of the spermatozoön.

7. The centrosome of the second maturation division is carried into the spermatid and gives rise to the axial filament of the spermatozoön. The centrosome becomes so closely associated with the nucleus that there is no well-defined middle-piece in the spermatozoön.

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ABBREVIATIONS.

c., centrosome; *m.*, mitochondria; *N.*, Nebenkern; *s.*, spindle derivative.

All drawings were made with the aid of a camera lucida at table level using a Zeiss 12 ocular and a 2 mm. oil objective. The reproductions have been reduced one-third.

EXPLANATION OF PLATE I.

FIG. 1. Primary spermatogonium. No clear indication of the presence of mitochondria. Note small mass of chromatoid substance in cytoplnsm.

FIG. 2. Anaphase of secondary spermatogonium. Note lightly-staining cytoplasm, well-defined cell-plate, and the tendency of the cell to become round.

FIG. 3. Metaphase plate of ovarian follicle cell, showing thirteen equal pairs of chromosomes.

FIG. 4. Resting stages of secondary spermatogonia. Mitochondria granular and diffusely spread. Note persistence of spindle and cell-plate.

FIG. 5. Degenerating spermatogonium. Mitochondria larger in size; chromatin concentrated in two large karyosomes.

FIG. 6. Pachytene stage of first spermatocyte. Mitochondria still granular, with only a slight indication of threads forming; note denser perinuclear zone and the persistence of the spindle remains of the last spermatogonial division.

FIG. 7. Late prophase of first spermatocyte, Flemming fixation. Nucleus uncut. Mitochondria filar, with their lengths in the direction of the chief cell axis.

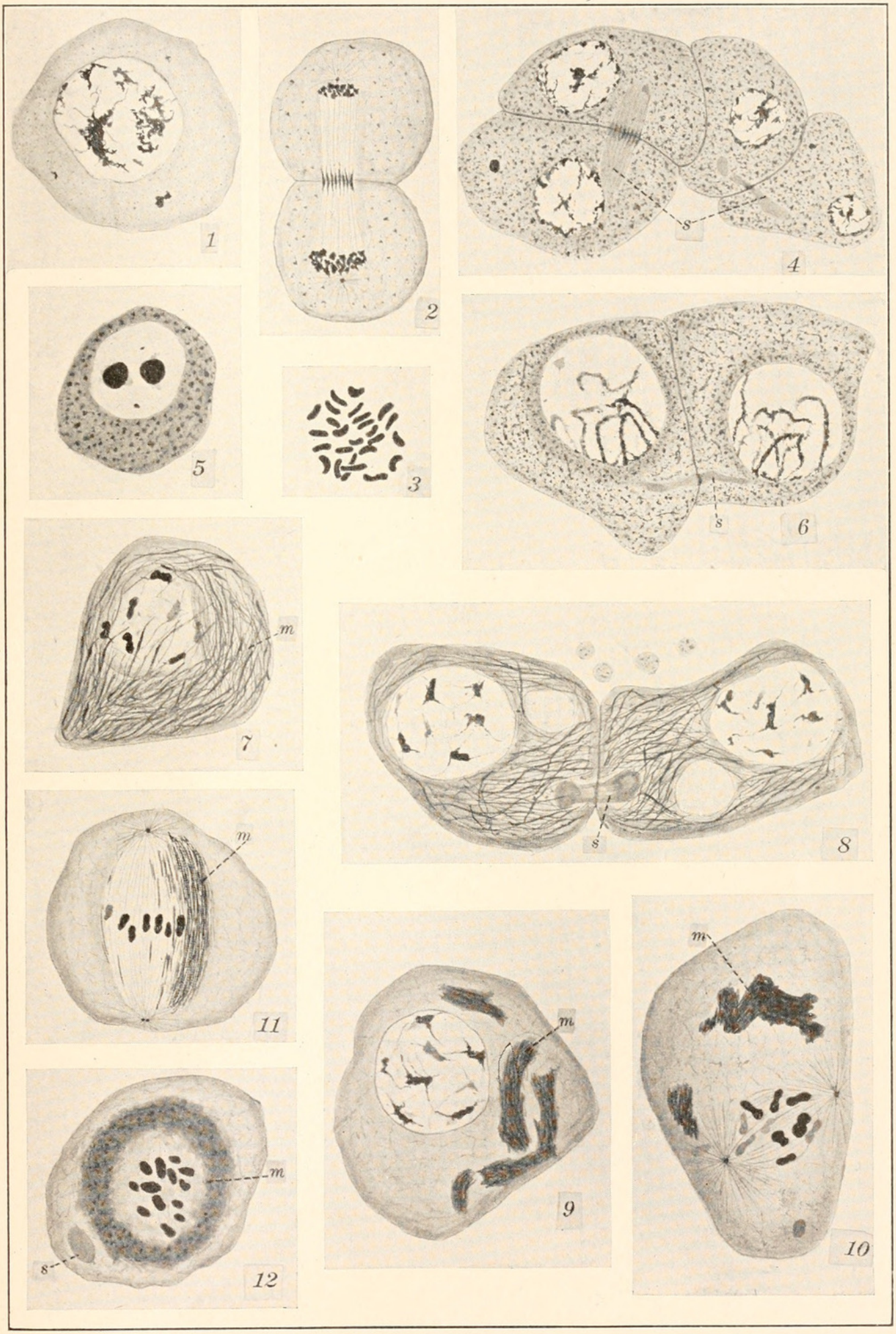
FIG. 8. Similar stage as above, showing remains of spindle still persisting, two large cytoplasmic vacuoles of problematic origin, and small bodies in the cyst cavity which have been budded off from the cytoplasm of the spermatocytes.

FIG. 9. Prophase of first spermatocyte, Hermann fixation; showing agglomeration of the mitochondria.

FIG. 10. Formation of the first maturation spindle; mitochondria at one side.

FIG. 11. Mitochondria beginning to envelop first maturation spindle.

FIG. 12. Cross-section of the metaphase plate of first spermatocyte, showing mitochondria completely surrounding the spindle. Thirteen bivalent chromosomes.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EXPLANATION OF PLATE II.

FIG. 13. Anaphase of first spermatocyte, showing relation of spindle and mitochondria.

FIG. 14. Late anaphase of first spermatocyte. Cell constriction has divided mitochondria so that the daughter cells contain approximately equal amounts.

FIG. 15. Cross-section of first spermatocyte near pole of spindle, showing absence of mitochondria here.

FIG. 16. Second spermatocytes (interkinesis). Mitochondria present in daughter cells in compact masses, also showing the persistence of the spindle and cell-plate.

FIG. 17. Metaphase of second spermatocyte. Mitochondria again surrounding the spindle peripherally.

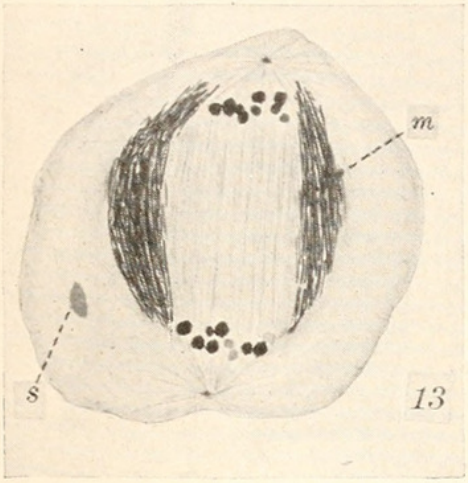
FIG. 18. Telophase of second maturation. Mitochondria again divided by the cell constriction into two equal masses. Note position of the centrosome (*c*) lying on the nuclear membrane.

FIG. 19. Spermatid. Nebenkern (*N*) derived from the mitochondria, with a peripheral vacuolated portion. Persistence of centrosome (*c*) with a precocious growth of the axial filament; spindle derivative (*s*) enclosing a deeply-staining body.

FIG. 20. Changes in the Nebenkern during transformation of spermatid. *c*, cross-section of the tail showing the Nebenkern lying on each side of the axial filament. *d*, Nebenkern with lighter central portion where spindle of previous division had passed through.

FIG. 21. Stage in the transformation of the spermatid. A portion of the spindle derivative (*s*) occupies a position at the head end and a portion passes into the tail. Nebenkern elongating, and the axial filament growing out of the centrosome (*c*) between the two halves of the Nebenkern.

FIG. 22. Later stages in the transformation of the spermatid. *b*, giant spermatid.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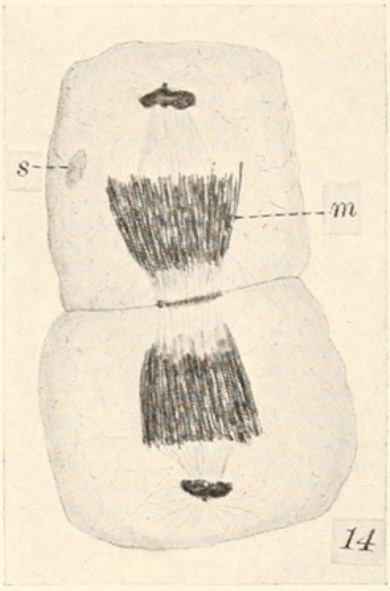
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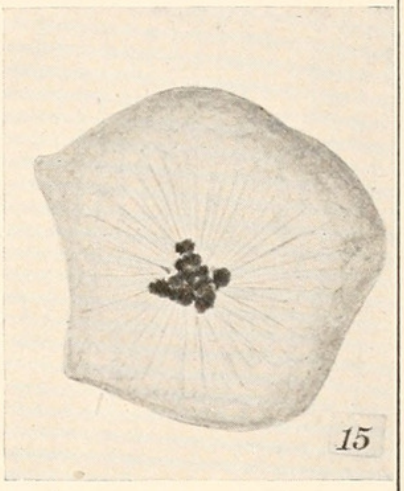
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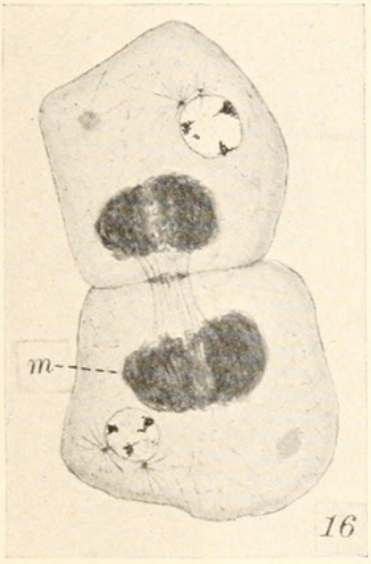
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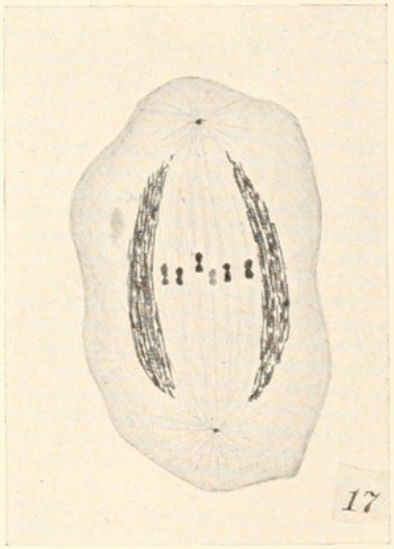
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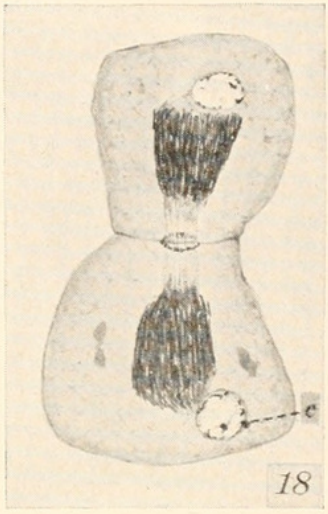
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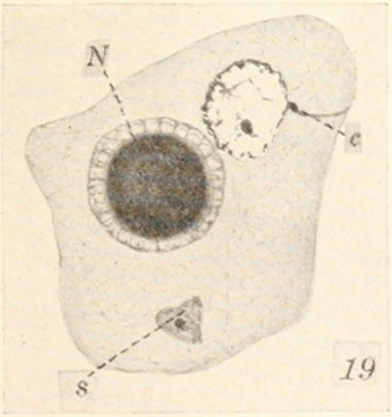
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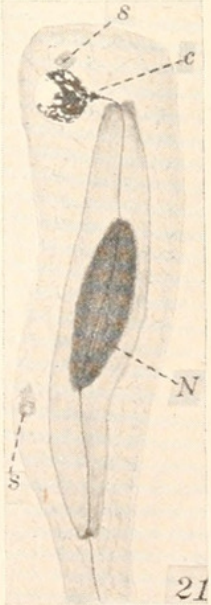
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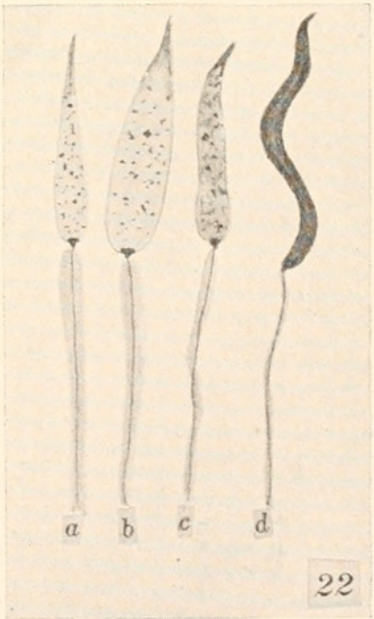
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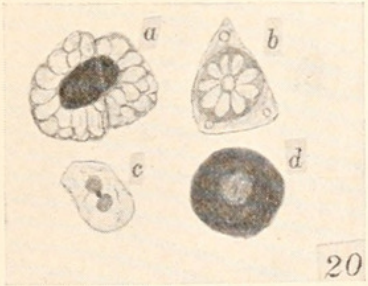
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EXPLANATION OF PLATE III.

Photomicrographs taken at a magnification of about 1,500 diameters.

FIG. 23. Resting secondary spermatogonia, showing diffuse granular mitochondria deeply staining in iron-hæmatoxylin.

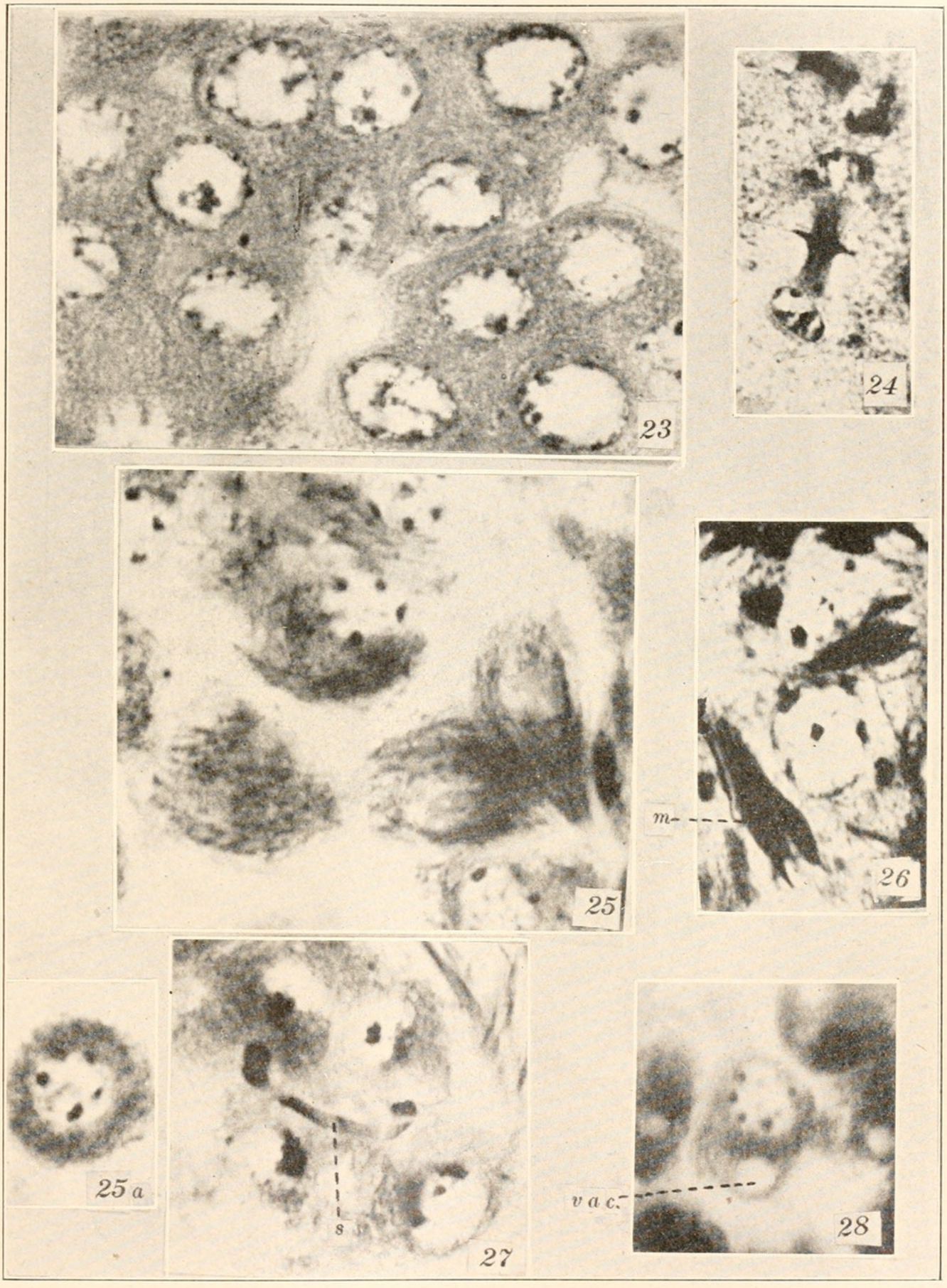
FIG. 24. Telophase of a secondary spermatogonium, showing the deeply staining cell-plate. The rest of the spindle here shows somewhat darker than it really is; it always takes the plasma stain.

FIG. 25. Late prophases of first spermatocyte; Flemming fixation. The mitochondria are filar, with their lengths extended in the direction of the chief axis of the cell; the nuclei here are uncut and somewhat out of focus. Fig. 25*a* is a section at right angles to the chief axis of the cell and shows the mitochondria on end view.

FIG. 26. First spermatocyte from a Hermann fixation. The mitochondria are agglomerated in dense masses (*m*).

FIG. 27. Part of a cyst of first spermatocytes to show the spindle remains (*s*) forming a connection between several cells; parts of it stain deeply with iron-hæmatoxylin.

FIG. 28. First spermatocyte greatly destained to show the cytoplasmic vacuole of unknown significance.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EXPLANATION OF PLATE IV.

FIG. 29. Formation of first maturation spindle; the mitochondria are at one side and are beginning to envelop the spindle.

FIG. 30. Metaphases of first maturation division; the mitochondria completely surround the spindle.

FIGS. 32, 33, 34. Cross-sections of the first maturation spindle, showing its relation to the mitochondria.

FIG. 35. Late anaphases of the first maturation division; the mitochondria are densely staining and closely applied to the spindle. The mitochondria do not reach to the poles.

FIG. 36. Second spermatocyte; interkinesis. Mitochondria in a dense mass awaiting the formation of the second maturation spindle.

FIG. 37. Second maturation spindle forming with the mitochondria at one side.

FIG. 38. Young spermatid with deeply staining Nebenkern (*N*) lying close to the nucleus, and the spindle derivative (*s*) which contains a deeply staining corpuscle. Centrosome not clearly in focus.

FIG. 39. Later stage in spermatid transformation, showing the elongation of the Nebenkern after the axial filament has grown out.


m
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