METHOD OF CELL-DIVISION IN THE SEX CELLS OF TAENIA TENIAEFORMIS.¹

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EIGHT PLATES

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INTRODUCTION

Flemming ('92) in his review of the literature on amitosis from 1841 to the beginning of 1893, concludes that investigation has shown that amitosis is connected with a high specialization of the cell and may be a forerunner of degeneration. This is in harmony with the views of Ziegler and Vom Rath, that those

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cells which divide amitotically are in process of degeneration. In a discussion of the question of amitosis Vom Rath ('91) states that when a cell has once divided amitotically it never again divides mitotically. Since 1892 a number of observers have recorded the occurrence of amitosis both in the somatic cells and the sex cells, but its unquestionable occurrence has been shown in few cases.

In the present paper the division of the sex cells only will be considered. Whether the sex cells or their progenitors ever divide amitotically is a question of interest on account of its bearing on a number of theories, among which are those of heredity, the continuity of chromosomes, and the relation of the sex chromosomes to the determination of sex. A number of observers have described amitotic division in both the testes and the ovaries of many animals. Meves ('94) describes the spermatogonial cells of *Salamandra* as dividing amitotically in the autumn and mitotically in the spring. However, his description of amitosis is very different from the process as usually described. He describes and figures the nucleus as being divided by the apparent constricting power of a ring-shaped centrosphere.

Preusse ('95) describes the appearance of amitosis in the ovaries of Hemiptera. Gross ('01) also finds amitosis in the ovaries of Hemiptera, but he contends that those cells which divide amitotically are degenerating or are secretory cells and therefore do not give rise to ova. Amitosis is recorded as occurring in the spermatogonial cells of *Amphiuma* by McGregor ('99). He says: "Amitosis occurs among primary spermatogonia and is, apparently, a normal process. Secondary spermatogonia divide only by mitosis and contain the somatic number of chromosomes."

In 1904 Child gave a brief account of the occurrence of a mitosis in *Moniezia* in the early stages of segmentation. In a series of papers ('07) he described amitosis as taking place in the development of the reproductive organs, in the spermatogonial divisions, in the oogonial divisions, and in later segmentations. He also finds mitoses in all these places except in the later segmenta-

tions, but states that the mitoses are not at all frequent and that amitosis is the usual method of division. In his conclusions, in speaking of amitosis and mitosis, he says: "There can I think be little doubt that the two forms of cell division correspond to different physiological conditions of the nucleus. Judging from the visible phenomena, it also seems probable that mitosis is associated with cyclical and amitosis with acyclical processes." And again he says: "The regions where mitoses are most abundant may be regions of slowest division instead of the only regions where division is occurring."

Hargitt ('06) with reference to cell-division in *Clava leptostyla*, says: "During the early cleavage, even up to the sixteen-cell stage, no evidence of mitosis has been found." Later in the same paper he says: "The facts seem clearly to justify the general conclusion that for a time in the early history of the development of the egg, nuclear activity differs greatly from the ordinary forms of mitosis, and appears to involve direct of amitotic division."

Beckwith ('09) in working on the same form, finds no evidence whatever of amitotic division, and states that both maturation and early cleavage take place by means of mitosis and not amitosis. She explains the nuclear nests of Hargitt by the condition of the nuclear reconstruction after cleavage and the lack of the appearance of the maturation divisions and fertilization by the fact that the eggs were not found at the right time of the day. Eggs collected from 4 to 6 A.M. show typical stages of maturation and fertilization.

Richards ('09) finds no amitosis in the oogenesis of *Taenia* and concludes that his observations "on the process of oogenesis point to mitosis as the usual method of cell-division." The same author (Richards '11) in his conclusions on his work on *Moniezia*, says: "In the early stages of sex cell development mitosis unquestionably occurs (probably periodically), while amitosis is not evident in my preparations; and finally there cannot be the slightest doubt that the cleavage of the ovum takes place by mitosis."

Child ('11) in a paper in which he bases his observations on slides of *Moniezia* prepared by Richards, finds what he interprets as numerous cases of amitosis. In speaking of his work and that of Richards, he says: "The difference between us seems to me to rest now on Richards's failure to recognize, or interpret as I have done, what his material actually shows." Further in the same paper he says: "I am perfectly well aware that none of these figures and likewise none of my earlier figures constitute a real demonstration of the occurrence of amitosis, for such a demonstration is impossible in fixed material."

The occurrence of amitosis both in the oogonial and the spermatogonial cells of *Leptinotarsa signaticollis* is recorded by Wieman ('10). He says that "amitosis is merely transient and inconspicuous in the ovogonia. In the spermatogonia it is more prominent, persists longer and is involved in the formation of the cysts."

Foot and Strobell ('11) describe amitosis as taking place in the ovaries of *Protenor*. Payne ('12) has shown in *Celastocoris* that the cells which Foot and Strobell describe as dividing amitotically are food cells and therefore do not give rise to ova.

To the above observations might be added those of Glaser, Johnson, Young and others. In most cases, however, where amitosis has been described as occurring in the sex cells, subsequent observers have shown that a different interpretation is possible and that it yet remains to be proved that the sex cells may divide amitotically and afterward give rise to new individuals.

In September, 1910, at the suggestion of Dr. Payne,² I began my work on cell-division in the sex cells of the tape worm. On account of the theoretical interest of the question, I have confined my work to the sex cells. If amitosis can be proved to take place in the cells which afterward go to form new individuals, the occurrence of it in the somatic cells will not be questioned.

² I wish to thank Dr. Fernandus Payne for many helpful suggestions during the course of my work and to express my indebtedness to B. H. Ransom for identifying my material and to C. E. Wilson for assistance in securing material.

MATERIAL AND METHODS

The form which I have selected for my studies is *Taenia teniaeformis* (Bloch, 1780) (Stiles and Stevenson, '05), a cestode which is a common parasite in the small intestine of the domestic cat. Both fixed and live material have been used.

1. Fixed material

In order to ascertain whether or not the time of the year has any influence upon the character of cell-division or the apparent frequency of cell-division, cats were killed every month in the year and often three or four times during the month. As soon as the host was dead the intestine was opened and the tape worms were put into killing fluid. Also to determine whether or not the time of the day has any influence upon cell-division, material was fixed at all hours during the day from five o'clock in the morning until eleven o'clock at night. Some of the cats were fed all they would eat for three or four days previous to killing, others were starved for the same length of time, and still others were killed as soon as they were obtained. This was done in order to determine whether or not the food supply has any effect on the character of the cell-division. Most often the host was chloroformed, but to make sure that the chloroform has no effect upon the character of cell-division some cats were stunned by a blow upon the head and then bled to death. The character of cell-division or the apparent frequency of cell-division did not seem to be influenced by the time of the year, the time of the day, the amount of food material, or the use of chloroform.

The following killing and fixing agents were used; sublimate-acetic (a saturated aqueous solution of corrosive sublimate, one-hundred parts and ten parts glacial acetic acid); picro-acetic (a saturated aqueous solution of picric acid one part, distilled water two parts, and 1 per cent glacial acetic acid); alcohol-acetic (equal parts absolute alcohol and glacial acetic acid); Flemming's strong solution; Bouin's fluid; Gilson's mercuric-nitric mixture; Carnoy's fluid; and a mixture of Gilson's fluid and 4 per cent chromic acid

in the proportion of ninety parts Gilson to ten parts chromic acid. When a host contained more than one tape worm usually two or three killing fluids were used.

The following stains were used: Heidenhain's iron-alum-hematoxylin with a counter stain of eosin and without counter stain; safranin and gentian violet; safranin, gentian violet, and lichtgrün; Auerbach's fluid; alum-carmin and osmic acid; Delafield's hematoxylin; and Conklin's hematoxylin. Although all the above stains show nuclear structure, the combination which shows centrosomes and spindle fibers best is Heidenhain's iron-alum-hematoxylin without counter stain, following a fixation in Flemming's strong solution. These structures were more clearly visible when, before being stained, the sections were bleached twenty-four to forty-eight hours in turpentine at a slightly elevated temperature.

After fixing, the tape worms were cut into pieces, each containing from two to twenty-five proglottids, depending upon the size of the tape worm and the size of the proglottids. Two entire tape worms, a small one indicating that perhaps it was young and a large one probably older, were sectioned. Of the others, pieces at intervals of four to twenty-five proglottids were sectioned. The sections were cut $3\ \mu$ thick. Some sections were cut cross and others sagittal. The pieces not sectioned were preserved in 85 per cent alcohol and saved for further reference. The character of cell-division was found to be the same both in the large and the small worms.

2. Living material

At two different times I made observations on living material, believing with Richards that "for amitosis there is but one absolutely certain criterion, the observation of living material and subsequent study of fixed material under observation" and with Child that a real demonstration of the occurrence of amitosis is impossible in fixed material.

I found by experiment that *Taenia teniaeformis*, when placed in Ringer's solution and kept at a temperature of $39^{\circ}\text{C}.$, will live outside the body of the host forty-eight hours or more.

The Ringer's solution was put into an open vessel in an open paraffin oven. By means of a Bunsen burner the oven was kept at such a temperature that the Ringer's solution was 39°C. As soon as the host was killed, the tape worms were taken from the intestine and transferred to the Ringer's solution. Immediately a proglottid from that portion of the worm which, in accordance with my former observations, contained segmenting ova, was transferred to a slide on a constant temperature stage which had previously been heated to 39°C. Here the proglottid was opened and some of the ova were teased out in the Ringer's solution and covered with a cover-glass. To prevent rapid evaporation the cover-glass was sealed with a ring of vaseline. Besides preventing evaporation the vaseline supported the cover-glass. The first preparation was observed two hours with a 4 mm. objective and a Zeiss 12 compensating ocular. Other slides were prepared in a similar manner and observed for different lengths of time, varying from ten minutes to three hours. Typical resting nuclei were seen, but in no case could there be determined any indication of cell-division or even a slight constriction in the form of the nucleus. Frequently the cells collected in groups, usually of twos but sometimes more than two cells were in a group. At first sight these groups of cells might have been mistaken for constricting cells but closer observation revealed that they were not cells in the process of constriction but merely cell groups, or more exactly speaking, groups of ova, for it was possible to distinguish two and even three nuclei in a single ovum.

To make sure that cell-division was taking place in the worms, this experiment was repeated about a week later and pieces of the worm upon which the experiment was being made were killed in Flemming's strong solution. The results of the observations on the live material were the same as in the first experiment but when the fixed material was sectioned it showed mitotic division.

Soon after the above experiments were made there appeared an article by Morse ('11) in which he reports similar results. In his experiments, which were upon *Calliobothrium* and *Crossobothrium*, he used the plasma of the host as a medium. Although

he does not state definitely how long a time a single observation was extended, evidently it extended over a much longer time than mine, for he says:

I am under the impression that cells which are cultured in this way do not undergo cell-division at all. I made charts of my slides which I had under observation for a week at a time and checked the behavior of all the cells in each slide with camera lucida drawings, comparing those made one day with those made on the day previous. If any increase in number of cells had occurred, I should have noticed it, of course.

Since the cells in Morse's experiments behaved in every way similar to the cells in my experiments, the comparatively short time that my experiments were under observation can make no difference. The only conclusion that can be drawn is that, under the conditions of the experiment, the nuclei do not divide.

OBSERVATIONS

Observations have been made upon the spermatogonial divisions and spermatogenesis, oogonial divisions, synapsis and the growth period, maturation divisions, and the cleavages. More attention has been given to the cleavages because it is here that the character of cell-division is most uncertain.

1. Spermatogonial divisions and spermatogenesis

My observations on the spermatogonial cells show nothing that I can in any way interpret as amitosis. It is true that I find few cells which are in the characteristic stages of mitosis. Most of the spermatogonial cells are in the so-called resting stage. I find no constricting nuclei and no cells which contain nuclei in contact. In early post synapsis the chromatin assumes the form of a more or less connected spireme, usually in contact with a large nucleolus which lies to one side of the nucleus (fig. A, plate 1). A group of cells in the anaphase of the first spermatocyte division is shown in figure B. The spindle fibers are very delicate, and, at the poles, there are small but very distinct, deeply staining centrosomes. No aster and no definite cell-wall is distinguishable. The second spermatocyte division follows

the first without any intervening resting stage. In figure D are shown two cells in the metaphase of the second spermatocyte division while in figure C are shown two cells in the metaphase and two in the anaphase. In the late anaphase the chromatin is collected in a mass at the poles. Occasionally there is a small portion of chromatin which lags behind the mass. This is illustrated in 1 and 2 of figure E and 1 of figure F. The spindles are even more delicate than in the earlier anaphases and the metaphase. I have seen no division of the centrosome.

2. Oogonial divisions

In the oogonial divisions mitoses are very frequent and there are no constricting nuclei nor nuclei in close contact. Plate 2 shows oogonial cells in different stages of mitotic division. The spindle is very similar to the spindle in the spermatocyte divisions but the centrosome is different. Here the centrosome is globular in form (showing a circle in section), comparatively large and stains a little more deeply than the spindle fibers. No aster is present. Some parts of the cytoplasm stains a little more deeply than others, thus giving it a mottled appearance. In the so-called resting stage the nucleus is very large and contains a large nucleolus which usually lies to one side of the nucleus and stains like chromatin. The chromatin is more or less scattered through the nucleus as a finely granular reticulum. The nuclear membrane stains like the chromatin and chromatin granules are distributed around the periphery of the nucleus. Figure E, plate 2, shows two oogonial cells in the resting stage and figure A, plate 3, shows one in the resting stage and the other in the telophase. The nuclear membrane has not yet formed, nor has the cell completely constricted.

3. Synapsis and the growth period

At the beginning of the growth period a marked change takes place in the appearance of the cells. There is a slight increase in the amount of the cytoplasm and a great increase in the mass of the chromatin. The chromatin takes the stain very readily

and forms into an indistinct spireme which becomes a greatly tangled mesh, lying to one side of the nucleus (fig. E, plate 3), The nucleus is large and lies to one side of the cell.

Figure C, plate 3, shows a group of cells in the early post-synaptic stage. There is not much change in the cytoplasm but the chromatin forms a definite, coarsely granular spireme which is in contact with a large nucleolus. In synapsis no nucleolus could be distinguished. This change in the chromatin goes on until it forms a finely granular reticulum which is in contact with the nucleolus. The chromatin remains in this condition during the remainder of the growth period.

4. Maturation and fertilization

Since both Child and Richards agree that maturation takes place by mitotic division, I have done no more upon this than figure a few maturation divisions. Figure F, plate 3, shows the metaphase of the first maturation division. The mitotic figure in maturation differs from the mitotic figure in segmentation, in having a smaller centrosome, and in the form of the chromosomes. The chromosomes in maturation are irregular, while in segmentation they are more definitely limited. The maturation spindle is always very long and one pole lies near the periphery of the cell. The segmentation spindle may be long or short and may lie in almost any position.

Child states that in no case has he observed asters at any stage in maturation in *Moniezia* but thinks that they probably do occur. Richards finds faint astral radiations in the same form. In *Taenia* I find asters, but in some cases they are very faint, as also are the achromatic spindle fibers. However, after being fixed with Flemming's strong solution, followed by a slow bleaching in turpentine and being stained with Heidenhain's iron-alum-hematoxylin, the asters and spindles are more plainly visible. Figure H, plate 3, illustrates the more plainly visible astral rays.

Inasmuch as fertilization does not bear directly upon the character of cell-division I have not included that in my observations.

5. *Cleavages*

Since fertilization has not been considered the relation of the two pronuclei previous to the first cleavage spindle has not been observed. In *Taenia*, Richards describes cleavage as taking place by mitotic division. In *Moniezia*, he finds frequent mitoses and no facts which he is able to interpret as amitosis. Child says, in his earlier paper, that in *Moniezia* the first cleavage is usually or always mitotic but cases of mitotic divisions are rarely seen after the first cleavage. He finds what he interprets as frequent cases of amitosis. In his later paper he says that mitosis occurs much later than the first cleavage but that the prevailing method of segmentation is by amitotic division.

a. Character of the cleavage. In *Taenia*, I find frequent cases of mitosis, not only in first segmentation but also in later segmentations. In fact, there are very few sections in my material where segmentation is taking place which do not have evidences of mitotic figures. The figures of mitosis that I have shown in plates 4, 5 and 7 might be duplicated many times. In *Taenia teniaeformis* it is not a question of whether mitosis occurs in some stages of segmentation and not in others, nor is it a question as to the frequency of its occurrence. The question is, whether or not mitosis is the only method of segmentation. The figures of plate 6 are essentially similar to Child's figures of amitotic division. I find these figures about as numerous as those which are unquestionably mitosis. These conditions occur, not only in later segmentations, but in early segmentation and many that I have figured are taken from first and second segmentations. The question is, do these admit of any interpretation other than that the nucleus has divided amitotically? I will discuss this question later.

In cleavage, nuclear division takes place very much in advance of cytoplasmic division. In the early divisions it is the exception and not the rule to find even a constriction in the cytoplasm. This gives rise to a syncytial condition. This syncytium persists until very late cleavage. Richards says that, in properly fixed material, he has never seen an egg syncytium. It cannot be the lack of proper fixation or stain which gives this syncytial

condition, for I find it with all the different fixations and stains. There is no indication whatever of poor fixation in those slides from which I have taken my drawings.³

Judging from the comparative size of the nuclei, the first division is an equal one. This is what would be expected if the division is mitotic. Soon after the first division there is a difference in the rate of nuclear division. I cannot say at just which division this difference in rate occurs. All the figures in plate 7 show nuclei of different sizes. I have never found two nuclei differing appreciably in size in which I could find evidence with any degree of certainty that they came from the same mother nucleus. For this reason I account for the difference in size of the nuclei by a difference in the rate of nuclear division.

b. Length and position of the spindle. As I have said before, the segmentation spindle may be long or short and may lie in almost any position with reference to the periphery of the cell. It may lie near the center of the cell with the cytoplasm almost equally distributed around it, as in figures A and B, plate 4, or near the periphery, as in figures E and F of the same plate. In these cases the spindle is comparatively short. Figures C and D, plate 4, show the position of the spindle intermediate between those shown in figures A and F of this plate, but of the same relative length. In figure H of the same plate the spindle is very long, extending from one side of the cell to the other. Figures I and J, plate 4, show cells in very late anaphase of first segmentation. The spindle fibers are not visible, but the centrosome and the masses of chromatin are very plainly visible. There is no doubt that in these cases the spindle was long. Figure G of the same plate shows a cell which has a spindle comparatively longer than those shown in figures A to F inclusive, and shorter than those shown in figures H to J inclusive, of this plate. All these are examples of the first segmentation spindles.

The same variation of the relative length and position of the spindle is found in later segmentations. However, here the length may depend somewhat upon the size of the nucleus, which is,

³ Dr. Richards has seen some of my slides and says there is no question but that the fixation is good. However, he did not examine them with reference to the question of a syncytium.

perhaps, determined by the rate of division as described before. Therefore, the comparisons of length are by no means so certain as the position. Figure A, plate 7, shows a spindle, one pole of which is far from the periphery of the cell and the other lies in contact with a nucleus in the prophase. In figure C of the same plate is shown a spindle which has cytoplasm distributed almost equally around it, while figure B shows a spindle, neither pole of which lies near the periphery, yet one pole is very much farther from the periphery than the other. In figures D, E, G and I of the same plate are shown metaphase plates lying in different positions with reference to the other nuclei. In figure D are two metaphase plates which lie rather near each other, one of which is in close proximity to a small nucleus in which the chromatin is in a more or less perfect spireme. In figure I is a metaphase plate, surrounded by nuclei, the chromatin of which is in a reticulum or broken spireme. These nuclei are of different sizes. In figure N, plate 5, the metaphase plate is somewhat removed from the other nucleus in the cell. From these facts it may be concluded that the mitotic figure may lie in almost any position with reference to the periphery of the cell and the other nuclei in the cell.

c. Reconstruction of the nucleus. In the reconstruction of the nucleus the first mitotic structure to disappear is the spindle. Soon after its disappearance, the chromosomes become somewhat scattered and the centrosome becomes less clearly visible. The chromatin becomes more or less ragged and a light area appears around it. With the further breaking up of the chromatin the definite boundary between the cytoplasm and the nucleus appears. Some of the chromatin is distributed around the periphery of the nucleus. The reconstruction, or the rearrangement of the chromatin material may begin some time before the chromosomes have reached the poles, or it may not begin until they are very near the poles. Figures A to H inclusive, plate 5, show nuclei in different stages of the process of reconstruction, while figures I to J of plate 4, show the chromatin near the poles, but the rearrangement of the chromatin has scarcely begun. In figure F, plate 5, the chromosomes have begun to assume the ragged ap-

pearance, showing that they are in the process of reconstruction. The light area is appearing around the mass of chromatin, but the centrosomes are still faintly visible. Judging from the position of the centrosomes, this spindle was evidently a long one, and evidently reconstruction began when the chromatin was some distance from the poles. If these daughter nuclei should completely reconstruct and should reach the usual size of nuclei of the resting stage of cell-division, there is no doubt that they would lie in close contact if not even press against each other. It can easily be seen that such nuclei could give rise to nuclei having the relative positions of the nuclei shown in figures H to K inclusive of plate 6, or to daughter nuclei which would be in as close contact as those shown in figures F and G of the same plate. On the other hand, it would be pretty hard to imagine how the daughter nuclei, arising from the reconstruction of the chromatin masses as shown in figures I and J, plate 4, could lie in close contact. They would undoubtedly give rise to daughter nuclei with a relative position similar to that of those shown in figure L of plate 6.

Figure G, plate 5, shows the process of reconstruction of the nucleus more nearly completed than is shown in figure F of the same plate. The centrosomes have disappeared and the chromatin is in a more finely divided state. Although the reconstruction is by no means completed and the nuclei have not reached the usual size, the two nuclear areas lie against each other. If reconstruction should be completed, it is entirely possible that it would give rise to a condition such as is shown in figure E of plate 6. The reconstruction of the chromatin in a division like the one shown in figure D of plate 5, could very easily give rise to daughter nuclei having the relative position of the nuclei shown in figure J of plate 6. Here the nuclei lie in contact with the periphery of the cell on the opposite sides and yet they touch each other.

The reconstructions just described are reconstructions after the first segmentation spindle. Reconstructions of later segmentations are shown in figures G and H of plate 7. The nuclei shown in the process of reconstruction in figure H, are evidently

the result of the division of a large nucleus. If reconstruction should be completed, one daughter nucleus would lie very near to, if not in actual contact with, two other nuclei of the same ovum. The other daughter nucleus would lie very near the periphery of the ovum. In figure G of the same plate is a reconstruction which is the result of the division of a smaller nucleus than that in figure H. If reconstruction were to be completed in this case, one daughter nucleus would lie in contact with one other nucleus of the same ovum, if not with two. These figures show, at least, that nuclei may lie in contact without having arisen from the same mother nucleus. If this be true, then the fact that two nuclei lie in contact is no evidence that they have arisen by amitotic division.

d. The condition of the chromatin in the nucleus. Wilson says: "Amitosis, or direct division, differs in two essential respects from mitosis. First, the nucleus remains in the resting state (reticulum), and there is no formation of a spireme or of chromosomes. Second, division occurs without the formation of an amphiaster." In my preparations many of the nuclei which lie in close contact have the chromatin in a more or less perfect spireme. This is true, even in those nuclei which lie in such close contact that a definite boundary between them is not visible. The nuclei in the figures of plates 6 and 7 show this condition. Figure I, plate 5, shows four smaller nuclei in which the chromatin is in a more or less finely granular reticulum, and a large nucleus in which the chromatin is in a connected spireme.

Child has suggested that it is possible that the smaller cells are the result of amitotic division and the larger ones of mitotic division. His reason for this conclusion is that he has observed mitotic figures more often in large cells than in small ones. He also says that the cells are small because they have divided more often than the large ones. But he further assumes that the process of division by amitosis is a more rapid process than by mitosis. I think that this assumption is hardly justified. It is true that there are more changes taking place in mitotic division; that is, that it is a more complicated process than amitotic division, but the length of time required for a cell to pass completely through

a process of division may be determined only by observation. If the time had been determined for a cell to divide mitotically and for a cell of the same material under the same conditions to divide amitotically, Professor Child's statement would have been more nearly justified.

If the fact that the chromatin is in the form of a spireme be an indication that cell-division is taking place by mitosis, in figure I, plate 5, we might interpret the large nucleus as dividing mitotically and the smaller nuclei amitotically. However, figures H and I, plate 7, show both large and small nuclei in a connected spireme. It seems that it is perfectly possible that the difference in the character of the chromatin in the nuclei shown in figure I, plate 5, might be interpreted as different stages of the prophase. Mitosis unquestionably occurs in the smaller nuclei as well as in the larger ones. Figure C of plate 7, shows a spindle in the metaphase of mitotic division. Since the segmenting ovum is a syncytium, the size of the cell cannot be determined, but the nucleus giving rise to the spindle was undoubtedly smaller than the largest nucleus in the ovum. The nuclei in the process of reconstruction shown in figure G of the same plate, are undoubtedly smaller than the large nucleus of the same ovum and could not greatly exceed in size any nucleus shown in the figure. The mitotic figures shown in figures A and B of this plate could not produce daughter nuclei which would exceed in size the other nucleus of the respective ova.

Granted that nuclei which lie in close contact afford evidence that nuclear division may have taken place amitotically and that the imperfect spireme is no indication that mitosis is taking place, how could the position of the nuclei forming a triangle in figure L, plate 7, be explained? The nuclei are of nearly the same size and lie in very close contact, although the boundaries between them are clearly visible. If they have come about by the process of amitosis, which nuclei were the first to constrict off? It seems that it would be necessary to assume an unequal division and that one division has followed the other very closely before the nuclei have moved apart. Without these assumptions, I see no possible explanation for this arrangement of the

nuclei if they have arisen by the process of amitosis. The same statement will apply to the nuclei shown in figure K of the same plate. If we assume on the other hand that nuclear division has taken place by mitosis the condition shown in figure A offers an explanation for the condition figured in K and L. In the process of reconstruction the nuclei have come to lie in close contact. The sister nucleus of at least one of these nuclei is in another section of the same ovum.

COMPARISON WITH MONIEZIA

When I had almost concluded my work on *Taenia*, I received some of Dr. Richard's slides of *Moniezia*.⁴ The examination of these slides shows that the character of cell-division in *Moniezia* is for the most part similar to that of *Taenia*. The figures of plate 8 have been taken from *Moniezia*. They show late segmentation and the cell boundaries are not visible. Early cleavage takes place by mitosis and the blastomeres are distinct. Later, as Child ('11) states, the cell boundaries become less distinct and entirely disappear, giving rise to a syncytial condition. When the cell boundaries are distinct (in the early cleavages) in *Moniezia*, the nuclei do not lie in contact nor is there any condition which indicates that cell-division has taken place by amitosis. Later, when the cell boundaries have disappeared, numerous cases of nuclei which lie in contact are found, and sometimes they are so close together that the surfaces between them are flattened. The unquestionable cases of mitosis are fewer in these regions, but that mitosis does take place here is shown in figures B, D, and E. In *Taenia*, where, in the early cleavages, the ovum is a syncytium, nuclei may lie in close contact any time after the first cleavage, as has already been shown.

Since, among the slides of *Moniezia*, that I have examined, there is only one of the late segmentations, the comparison of the length and position of the spindle has not been made, but the reconstruction of the nucleus is similar to that of *Taenia*. The nuclei in the process of reconstruction shown in figure E, plate

⁴ I take this opportunity of expressing my indebtedness to Dr. Richards for his kindness in loaning me these slides.

8, are essentially like those shown in figure G, plate 5. In figure E the section passes through the middle of only one nucleus, but it shows a small portion of the other. However, the light areas appearing around the chromatin lie almost in contact, and reconstruction is far from being completed. The nuclear membrane has not yet appeared and the chromosomes have not lost their identity although they have the ragged appearance described above in the reconstruction of the nucleus in *Taenia*. If reconstruction should be completed, the nuclei would undoubtedly lie in as close contact as the two nuclei shown at 1 of the same figure, if not closer.

There is also the same variation in the condition of the chromatin in *Moniezia* as there is in *Taenia*. Some of the nuclei show the chromatin in a finely granular reticulum, typical of a resting stage, others a more coarsely granular reticulum, bordering on the formation of the spireme, and still others show the spireme in almost all degrees of perfection. No one of these conditions is confined to any particular sized nucleus. The reticulum is found in the large as well as the small nuclei. The same is true of the spireme. In figure D are shown three nuclei in a row and in close contact. The chromatin of the middle nucleus is in a spireme, while the chromatin of the other two nuclei is in a finely granular reticulum. In figure C two nuclei lie so close together that the surfaces of contact are flattened and yet the chromatin in each nucleus forms a perfect spireme which is in contact with a large nucleolus. The two large nuclei of figure A are in as close contact as those described in figure C, but the spireme is much less perfect, while the chromatin in the two smaller nuclei of the same figure forms an almost perfect spireme. The same variation is shown in all the figures of this plate. If the fact that the chromatin is in the form of a spireme, be an indication of mitosis, the two small nuclei of figure A are in the prophase of mitotic division.

The character of the cleavage, the reconstruction of the nucleus, and the character of the chromatin in the nucleus in *Moniezia* offer no more indication of amitotic division than they

do in *Taenia*. In fact, the indication of amitosis in *Taenia* is greater than in *Moniezia*, for, as has been said before, in *Taenia* nuclei are found lying in contact, even in the first segmentation, while in *Moniezia* this condition is not found until late segmentations.

DISCUSSION

My observations have not shown that amitosis does not take place in *Taenia* or *Moniezia*, but they have shown no condition which cannot be as readily explained as the result of mitotic, as of amitotic division. Since all those who have worked on cell-division in the cestodes record the occurrence of mitotic division, at least occasionally, those conditions which would be difficult to explain, if amitosis were the only method of cell-division, need not be discussed again. The absence alone of unquestionable cases of amitosis is, of course, no absolute proof that it does not take place. Whether a cell divide mitotically or amitotically is, in itself, of no significance. From the standpoint of the mere increase in the number of cells, it matters not what the character of the cell-division is. The question becomes of interest beyond the mere fact of its occurrence, when its bearing upon other biological questions is considered. Among these questions are the theories of heredity, the continuity of chromosomes, and the relation of the sex chromosomes to the determination of sex.

The fact that the germ cell is a single cell which gives rise to a new individual, however simple or complex that individual may be, must be the foundation for a discussion of any theory of heredity. This single cell may be the entire individual as in some protozoa; it may be a cell similar to the somatic cells, as in reproduction by budding; it may be the unfertilized ovum, as in parthenogenetic reproduction; or it may be the fertilized ovum, as in bisexual reproduction. This cell, whatever it is, contains in it the sum-total of the heritage of the species. The characteristics of the species are transmitted to the next generation by the division of this one cell. No matter what the character of the cell-divi-

sion is, it must be such that at least those cells which give rise to the new germ cells will contain the bearers of the characteristics of the species, or the characteristics themselves in potentia.

Strasburger, Weismann, Kölliker, and Oscar Hertwig, independently and almost at the same time, identified the nucleus as the bearer of the hereditary qualities. This view, while held by many, is by no means universally accepted. If it be true that the nucleus is the bearer of the hereditary qualities, and if division be amitotic, it must be assumed that the nucleus as a whole is the bearer of the characteristics, and, so far as the hereditary qualities are concerned, there can be no differentiation of the nuclear material.

The theory of the continuity of chromosomes is far from being absolutely proved or universally accepted. However, Rabl, Zur Strassen, Boveri, Van Beneden, Morgan, Wilson, Payne and others have made observations that give some very strong evidence in favor of it. If it should be proved that amitosis does not take place in cells which are the progenitors of new individuals, this would give no direct proof of the theory. On the other hand, if it should be proved that amitosis does occur in cells that are the progenitors of new individuals, it would offer very strong evidence against it. If the nucleus divide amitotically, the chromatin which goes to one daughter nucleus gets into that particular daughter nucleus rather than into the other, by chance. Such a condition would be one of the strongest evidences against the continuity of chromosomes.

If there be a mass division of the chromatin, and consequently no continuity of chromosomes, the accessory chromosome and the idiochromosomes and their relation to the determination of sex have no significance unless as Wilson ('06) and Morgan ('09) have suggested, sex is determined by the quantity of the chromatin. Their regular occurrence and their uniform behavior in a species would be difficult to harmonize with amitotic nuclear division.

SUMMARY

In conclusion, the evidence presented in these observations show that:

1. Neither the character of cell-division nor the apparent frequency of cell-division is influenced by the time of the year, the time of the day, the amount of food material, or the use of chloroform.

2. Under the conditions of my experiment the ova do not segment outside the body of the host.

3. Division in the spermatogonial cells is unquestionably mitotic. I find no condition that might be interpreted as amitotic division.

4. The spermatocyte divisions are mitotic.

5. The second spermatocyte division follows the first without an intervening resting stage.

6. In oogonial divisions mitosis is very frequent and there is no evidence of amitosis.

7. The maturation of the ovum takes place by mitotic division.

8. Mitotic division occurs both in late and early cleavages.

9. In the cleavages nuclear division takes place very much in advance of cytoplasmic division which results in a syncytial condition of the ovum.

10. The mitotic figure in the cleavages may lie in any position with reference to the periphery of the ovum and to the other nuclei of the ovum.

11. The cleavage spindle may be long or short and the reconstruction of the nucleus may begin when the chromatin is some distance from the poles of the spindle.

12. By the time reconstruction is completed the daughter nuclei become very large and consequently may lie in close contact.

13. The close contact of nuclei is no indication of the character of the cell-division.

14. In *Taenia teniaeformis* I have found no condition that cannot be as readily explained as the result of mitotic division as of amitotic division.

15. The character of the cleavage, the reconstruction of the nucleus, and the character of the chromatin in the nucleus in *Moniezia* offer no more indication of amitotic division than they do in *Taenia*.

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EXPLANATION OF PLATES

All figures were drawn with a Zeiss compensating ocular no. 6 and a 1.5 mm. objective at table level with the aid of a camera lucida and then enlarged two-and-a-half diameters. The figures on plates 1, 2, 3, and 8 were reduced one-third and those on plates 4, 5, 6, and 7 were reduced one-half. The figures on plates 1 to 7 inclusive were made from slides of *Taenia teniafermis* and those on plate 8 were made from slides of *Moniezia*.

PLATE 1

EXPLANATION OF FIGURES

- A Early post synapsis spermatogonial cells.
- B Anaphase of first spermatocyte division.
- C Metaphase and anaphase of second spermatocyte division.
- D Metaphase of second spermatocyte division.
- E Late anaphase of second spermatocyte division; 1 and 2, a portion of chromatin which lags behind the mass of chromatin.
- F Late anaphase of second spermatocyte division; 1, a portion of chromatin which lags behind the mass of chromatin.

PLATE 2

EXPLANATION OF FIGURES

- A, B and C Metaphase of oogonial cells.
- D Metaphase and late anaphase of oogonial cells.
- E Resting stage of oogonia.
- F and G Metaphase and early anaphase of oogonial division.

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PLATE 3

EXPLANATION OF FIGURES

- A Telophase and resting stage of oogonial cells.
- B Post-synapsis of oogonial cells.
- C Early post-synapsis of oogonial cells.
- D Metaphase of second maturation division.
- E Synapsis of oogonial cells.
- F, G and H Metaphase of first maturation division.

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PLATE 4

EXPLANATION OF FIGURES

- A Metaphase of first segmentation.
- B and C Early anaphase of first segmentation.
- D Metaphase of first segmentation.
- E Anaphase of first segmentation.
- F Metaphase of first segmentation.
- G and H Anaphase of first segmentation.
- I and J Late anaphase of first segmentation.
- K and L Later segmentations, showing resting nucleus and spindle.

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PLATE 5

EXPLANATION OF FIGURES

A, B, C and D Anaphase of early segmentation.

E, F, G and H Telophase of early segmentation.

I Later segmentation, showing a metaphase plate, a large nucleus with the chromatin in a spireme, and smaller nuclei with the chromatin in a reticulum.

J and K Early segmentation showing one nucleus with chromosomes and the other with the chromatin in a spireme.

L Early segmentation with a nucleus apparently constricted and the chromatin in a spireme.

M Metaphase plate of early segmentation.

N Early segmentation showing a metaphase plate and the other nucleus with the chromatin in a spireme.

O Early segmentation with a nucleus apparently constricted and the chromatin in a spireme.

PLATE 6

EXPLANATION OF FIGURES

All the figures on this plate show early segmentation and the chromatin in a more or less perfect spireme.

A and B Nuclei between which no definite boundary is visible.

C, D and E Nuclei in which a more or less definite plate marks the boundary.

F and G Nuclei close together; the sides which are almost in contact are flattened.

H, I, J and K Nuclei in contact.

L Nuclei some distance apart.

PLATE 7

EXPLANATION OF FIGURES

A Late segmentation with one nucleus in metaphase one pole of the spindle in contact with the other nucleus which is in prophase.

B Segmentation, showing one nucleus in prophase and one in metaphase; the nuclei are some distance apart.

C Late segmentation with one nucleus in metaphase and the others in different stages of prophase.

D Two metaphase plates and other nuclei in prophase.

E Segmentation, showing one large nucleus and two smaller nuclei in prophase, and one metaphase plate.

F Late segmentation, showing chromatin in the process of reconstruction and nuclei in prophase.

G Late segmentation, showing metaphase plate, early telophase and prophase.

H Late segmentation, showing nuclei in early telophase and both large and small nuclei in prophase.

I Segmentation, showing metaphase plate and nuclei in prophase.

J Late segmentation, showing different sized nuclei in contact in which the chromatin is in prophase.

K Segmentation, showing the nucleus apparently being constricted into three parts.

L Late segmentation, showing the nuclei in contact; the chromatin is in a fine spireme.

PLATE 8

EXPLANATION OF FIGURES

The figures on this plate are from slides of *Moniezia* showing segmentation.

A Two large nuclei in contact, in which the chromatin is in a fine spireme, three smaller nuclei in which the chromatin is in the same form, and two still smaller nuclei in contact in which the chromatin is in a coarse spireme.

B A metaphase plate, two nuclei in contact, in which the chromatin is in a fine spireme, a large nucleus in contact with the periphery of the cell and the chromatin in a fine spireme, and a large nucleus in which the chromatin is in a coarse spireme.

C Three nuclei with the chromatin in a coarse spireme and three in which the chromatin is in a fine spireme.

D Part of a segmentation spindle, three nuclei in a reticulum and one with a coarse spireme.

E Two nuclei with chromatin in telophase, two in reticulum, and two in a coarse spireme, 1, a small nucleus in contact with a large nucleus.

F Four nuclei of different sizes and of different relative positions with the chromatin in a fine spireme and two smaller nuclei with the chromatin in a coarse spireme.