Uroleptus Halseyi Calkins, III. The Kinetic Elements and the Micronucleus.

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(With 4 figures in the text and plate 3—6.)

In two earlier papers [1]) I have described *Uroleptus Halseyi* and have given the history of the chromatin of the macronucleus. In the present paper I wish to extend the description of this interesting organism to include the kinetic elements and the history of the chromatin of the micronucleus. The latter involves the changes which the chromatin undergoes during the processes of division and conjugation which, in their main features at least, do not differ essentially from these phenomena which have been described repeatedly in connection with various species of ciliates. I shall not take these matters up therefore, from the standpoint of sexual phenomena but will treat the latter as incidental phases in the history of a substance, chromatin, which is regarded as essential in matters pertaining to metabolism and inheritance.

Material and Methods.

The material was collected from a fresh water pond in the vicinity of New York in October 1929 and has been maintained in mass cultures made up in hay infusion to which a grain of wheat is added. The organisms thrive in this medium and all stages in the life history are obtainable.

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[1]) *Uroleptus Halseyi* n. sp. Biological Bulletin. Vol. 57, 1929. *Uroleptus Halseyi* Calkins, II. The Origin and Fate of the Macronuclear Chromatin. Arch. f. Protistenk. Bd. 69.

For ordinary preparations I have used the method which I have employed for many years with Protozoa. A few individuals are drawn up in a capillary pipette with a minimum of water and spurted onto a cover glass which is lightly smeared with egg albumen. The killing fluid is then dropped on them and allowed to stand until it crystallizes. The cover glass is then immersed in a staining jar filled with the killing fluid and left for sufficient time to assure good fixation. The initial drop of killing fluid coagulates the albumen which holds the organisms fast and the cover-glass can then be treated as an ordinary smeared slide. For manipulation of these cover glasses we use small staining jars made like COPLIN jars but only on inch in height. These are known as the Columbia cover glass staining jars.

In fixing and staining dividing and conjugating forms better results are obtained by treating them individually to ensure the exact degree of differentiation required for different stages. The same is true for specimens designed for sectioning, and uniformly good results are obtained with single individuals whereas if sectioned in mass the results are uniformly poor. If the cell is stained with eosin prior to embedding it is easily visible on the white paraffine and can be cut in any plane desired.

A saturated solution of bichloride of mercury in 95 % alcohol has been used exclusively for fixation. This has the advantage of evaporating quickly and of penetrating rapidly as well as that of permitting the subsequent use of any kind of stain. The stains used have been mainly the FEULGEN nucleal reaction, the BORREL mixture and the iron haematoxylin stain the first either with or without a counterstain. If the latter is desired an excellent contrast is obtained with a dilute solution of acid green in water. The manner in which the FEULGEN method was used is fully given in the second of these Studies.

The illustrations accompanying the present paper were all drawn with the aid of a camera lucida but the magnifications are not uniform. These are stated in the descriptions of the figures.

The Kinetic Elemente.

There are no complex motile organs in *Uroleptus Halseyi* and the movement is correspondingly simple. Cilia, membranelles and cirri are present as in *Uroleptus mobilis* but the individual cilia are longer and more powerful. They are arranged in five longitudinal rows running from anterior to posterior ends. Two of these rows are

lateral, one is ventral, and two are intermediate and the most powerful cilia are in the lateral rows. In this respect the present organism is exactly the same as *Uroleptus mobilis.*

The adoral zone of membranelles sweeps from the ventral surface and towards the dorsal surface on the left side thus describing an incomplete spiral (Pl. 3 Figs. 1, 2). The membranelles are largest at the anterior end and desrease in size gradually towards the mouth around which they form an arc on the right side. Each membranelle is perceptibly swollen at the base thus forming a relatively thick and conspicuous basal plate. From this it tapers, cone-like, to a fine point at the tip. Below the basal plate there is a very shallow basal lamella from the inner angle of which a fibril connects with a kinetic element lying deeper in the endoplasm. These endoplasmic granules are connected in a chain which runs the entire length of the adoral zone giving the appearance of a thread with knots at regular intervals in it (Pl. 3 Figs. 1, 2, 5). Except for short stretches I have never seen this chain of granules forming a straight line the displacement being due probably to protoplasmic contraction at the time of fixation. Posteriorly the fiber of this chain ends in a considerable mass of substance the motorium (Pl. 3 Fig. 2) from which other fibrils pass out in different directions. One of these runs to the right margin of the peristome where its course could not be followed. Another runs to the proximal end of the unulating membrane which occupies the posterior third of the peristome and attached to its right border. Two other fibrils are quickly lost in the endoplasm. The motorium thus is a comparatively simple structure lying on the right side of the gullet.

The three frontal cirri are long conical structures each with a distinct basal plate. The basal plate is connected with a basal granule by two curved fibrils and the basal granule is similarly connected with the chain of membranelle granules (Pl. 3 Figs. 1, 3 and 4).

The Micronucleus.

The micronucleus of *Uroleptus Halseyi* is the most characteristic feature distinguishing this species from *Uroleptus mobilis.* Typically there are never more than two in the cell whereas in *Uroleptus mobilis* there are from two to six. It is the largest micronucleus that I am familiar with and is frequently quite as large as one of the several macronuclei (Pl. 4 Figs. 10, 11). Indeed were it not for its homogeneous structure it might be mistaken for one of the macronuclei. Its

position in the cell affords no help in its identification for the micronuclei often form continuous links in the chain of macronuclei (Pl. 4 Fig. 10). It appears to be extremely labile and is sometimes spherical, sometimes ellipsoidal in form (Pl. 4 Figs. 8, 11). It stains readily with iron haematoxylin and with the BORREL mixture and gives a positive reaction with the FEULGEN method.

The micronuclei in vegetation division do not conform to any one common type but in conjugation they pass through a consistent series of stages which can be readily harmonized with the usual ciliate type. It will be appropriate therefore, as well as convenient, to describe the vegetative and the conjugation phases separately.

A. The vegetative micronucleus.

The micronuclei originate with the second division of the zygote nucleus. Of the four nuclei thus formed one becomes the new macronucleus of the ex-conjugant, two of them form micronuclei and the fourth may degenerate and disappear or it may persist to give rise to the bimicronucleated forms of *Uroleptus* (Pl. 4 Fig. 10). The young micronuclei are homogeneous in composition and the chromatin is uniform throughout for neither the haematoxylin nor the BORREL mixture nor the Feulgen nucleal reaction gives any morphological evidence of chromatin granules. In older micronuclei however and in preparation for division as shown by the changes of the macronuclei, the homogeneous mass of chromatin is broken down and granular fragments of chromatin are scattered throughout the nucleus (Pl. 4 Fig. 15). In old cultures the *Uroleptus individuals* grow to giant size, the macronuclei increase in number (up to 26), and the micronuclei show morphological changes of characteristic type. The main substance is homogeneous but the chromatin may be in the form of granules distributed about the periphery or aggregated into a ball at one end of the nucleus or stretched out in band form along one side (Pl. 4 Fig. 16). The first condition is similar to the initial stages of the micronucleus at the outset of conjugation, but the latter condition is apparently an evidence of degeneration.

The two new micronuclei of the young ex-conjugant do not divide in preparation for the first division of the ex-conjugant but one of them passes to one daughter cell, the second to the other. The cell body divides when there are four macronuclei, two of the latter passing to each daughter cell (Pl. 3 Fig. 6). It is a matter of some significance that the relative times of division of the micro-

nucleus and of the macronucleus vary with the age of the organism after conjugation. As we have seen above the young micronuclei are already divided at the time of the first division of the macronucleus. At the second division of the individual after conjugation the preliminary steps in the transformation of the eight macronuclei into one division macronucleus, are all taken before the micronucleus shows evidence of nuclear activity. It is in full metaphase when the single macronucleus is dividing and in the anaphase stage of mitosis when the macronucleus has divided once (Textfig. 1). In young forms therefore, the micronucleus is slow to divide. In old forms on the other hand, particularly in cultures where conjugations have not taken place for long periods, it is the micronucleus that always leads the way in division and in many instances the micronucleus has divided before fusion of the macronuclei and in some cases even before the nuclear clefts have appeared (For history of

Textfig. 1. Micronucleus in mitosis and two daughter macronuclei in the second division of the ex-conjugant. 1800 : 1.

the macronucleus see Calkins loc. cit. 69). The significance of this time difference is probably bound up with the conditions of continued metabolism. The type of mitotic figure that is formed by the micronucleus is also dependent on the age of the organisms as will be seen below.

The micronucleus in division does not conform to any one sequence of activities which will permit of a common description. There are no less than five different types of mitotic figures which appear to be characteristic of different phases of the life cycle. Three of these types are confined to the conjugation stages but two of them at least are typical of vegetative forms. If definitely formed chromatin elements composing the nuclear plate at full mitosis are to be called chromosomes then the number and the form vary within wide limits while the size differences are so marked that we cannot speak with any assurance of individuality of the chromosomes.

The first division of the micronucleus in a young ex-conjugant gives an excellent picture of a typical mitotic figure. In the first

stages the nucleus swells to approximately twice the previous volume and becomes vacuolated thus making islands of chromatin which draw out into long strands. These strands in some cases at least appear to be connected thus forming a continuous spireme but in no preparation were the conditions favorable enough to determine accurately whether or not the strands are connected at both ends (Pl. 4 Fig. 12). The chromosomes which form from this stage are distinctly looped and the nuclear plate in the metaphase stage takes up about one-half the nucleus (Pl. 4 Fig. 13). These chromosomes are twentyfour in number and division is transverse. In the anaphase stage the daughter chromosomes are closely-packed together and quickly merge to form the homogeneous type of nucleus characteristic of the vegetative forms (Pl. 4 Fig. 14).

A second type of division figure is characteristic of older organisms. Here there is no formality of prophasic stages, chromosome formation or chromosome division, and the process of division comes amazingly close to direct or amitotic division (Pl. 4 Figs. 17—21). The polar chromatin apparently never enters into chromosome formation and as the daughter nuclei pull apart connecting strands of chromatin give rise to an effect of chromosomes. The thickness and the number of these strands vary in different nuclei and there is no possibility of longitudinal division. The chromatin strands become increasingly thin and the daughter nuclei simply pull apart. This second type of division recalls promitosis as shown by some forms of amoebae. The chromatin character of these strands is demonstrated by the nuclear dyes and by the FEULGEN reaction.

The history of mitosis in young and old forms of *Uroleptus Halseyi* may indicate a progressive weakening of the controlling factors, whatever they may be, of division. Definite chromosomes, typical of spindles of young forms, and a regular sequence of stages in mitosis, are replaced in older forms by the simple pulling apart of two equal masses of plastic chromatin material. This evidence of disorganization is manifested not only by the micronuclei but by the macronuclei as well. The eight macronuclei, typical of young forms, are derived from a single division nucleus by three successive divisions, or more exactly stated by four successive divisions for the original single division nucleus gives rise to sixteen daughter nuclei eight of which pass to each daughter cell. In old cells however, the number invariably exceeds eight and there is no uniformity of size or arrangement, the extra nuclei being formed by constrictions of the original nuclei rather than by equal division.

Notwithstanding these obvious signs of disorganization of the nuclear complex in old individuals they are still able to conjugate and with conjugation the usual sequence of nuclear changes is re-established. In cases where disorganization has gone so far that the micronuclei have entirely disappeared from the cell, a phenomenon which occasionally happens, the organisms are still able to conjugate but the process is a one-sided affair. Whether or not such conjugations result in labile ex-conjugants I do not know.

B. The micronucleus in conjugation.

The phenomena of conjugation conform to the usual ciliate type. The vegetative micronucleus swells and divides. This is usually called the first maturation or the first meiotic division. The two products of this division may divide again or one of the products may degenerate and take no further part in the proceedings. This second division in most ciliates is the occasion for the diminution by one half of the normal number of chromosomes and is often referred to as the reducing division. The two products of this division in *Uroleptus Halseyi* now divide for a third time thus giving rise to four pronuclei or their equivalents (Textfig. 2). If the two products of the first maturation division both divide again then the daughter nuclei of one of them degenerate and do not proceed to the third division. In this respect *Uroleptus Halseyi* differs from *Uroleptus mobilis* where from four to eight, or in rare cases, sixteen equivalent pronuclei may be formed. In *Uroleptus Halseyi* four is the normal number two of which ultimately degenerate, one migrates into the other organism and one remains to fuse with the migrating pronucleus. After fusion the zygote nucleus divides twice thus giving the four nuclei of the final stage of conjugation.

There are no less than three distinct types of mitotic figure in this series of conjugation stages, different at least insofar as the chromosomes are concerned. These are the first, second and third divisions of the micronucleus. The first division involves the most aberrant type of mitotic figure; the second and third differ mainly in the later stages of mitosis.

The first indications of activity on the part of the micronucleus during conjugation are shown by a slight swelling. The chromatin is homogenous and takes a faint stain with the usual dyes and gives a weak FEULGEN reaction. Around the periphery of the nucleus however, more intensely staining chromatin granules are

lined up as though compressed against the inner wall of the nuclear membrane by the homogeneous matrix of the nucleus (Pl. 4 Figs. 22—25). At this period the macronuclei have large x-granules which are eliminated later (Pl. 4 Fig. 23). The majority of these peripheral chromatin granules of the micronucleus collect at one pole where they are cast off and ultimately form the secound pole of the first meiotic spindle (Pl. 4 Figs. 26, 27). The rest of the nucleus is homogeneous and, so far as the nuclear dyes and the FEULGEN reaction can show, it is free from nucleic acid (Pl. 4 Figs. 22—26). In the meantime the nucleus continues to swell and results in a nucleus at the metaphase stage which has a diameter at least three times that of the same nucleus at the outset of conjugation. (The actual dimensions are about 7 μ and 22,5 μ). There is therefore, an enormous increase in volume of the micronucleus. When the nucleus has reached a size represented by about twice the original diameter minute granules appear in it usually near the periphery. These granules stain red with the BORREL and lavendar with the FEULGEN (Pl. 4 Fig. 27). They either increase in size or fuse to form relatively large and conspicuous granules on the periphery of the otherwise homogeneous matrix. The staining reaction however is not uniform and in some pairs of conjugants they appear as large definite granules as shown in Pl. 5 Fig. 28, while in other pairs they are smaller and less definite. The central matrix of the nucleus now begins to contract leaving the chromatin granules at or near the nuclear membrane (Pl 5 Fig. 29). Each granule retains its connection however, with the contracting matrix by a strand of the central substance and these strands ultimately form the spindle fibers. In the meantime the second pole of the spindle develops similar rediating strands and it becomes resolved into a homogeneous substance similar to that of the contracting matrix and finally has approximately the same size (Pl. 5 Figs. 30—35). The chromatin granules, at first peripheral in position, now begin to segregate towards the center of the nucleus (Pl. 5 Figs. 32—35) where they form the nuclear plate of the first meiotic spindle (Pl. 5 Figs. 36, 37). During this process of segregation they change from angular and irregular granules into definite short rods the number of which is too great to permit an accurate count, nor am I able to tell whether the rods represent the original single granules or whether they are formed by a combination of these granules. My impression is that each represents one of the original peripheral granules.

These rods forming the nuclear plate are the chromosomes of the first meiotic division (see Pl. 3 Fig. 7). In attempting to count them I have repeatedly counted up to forty eight but they are too confusing for accuracy. Such counts are always made with the camera lucida a point being noted for each rod in sharp focus, but the difficulty is obvious. It will be well within the limits of accuracy to state that the number of chromosomes is about fifty.

There is no evidence at all that these rods divide either longitudinally or transversely, they merely sort out into two plates of approximately twenty four chromosomes each (Pl. 5 Figs. 38—40).

The later telophase stages and the actual division of the nucleus were not found in any preparation showing this first division. As in *Uroleptus mobilis* these stages are probably of very short duration and their demonstration would be only the result of a fortunate chance. Second division figures, however, are relatively frequent in the preparations (Pl. 6 Figs. 41—43). Here the short rods of the first division apparently retain their individuality, but now they begin to lengthen and at what may be termed the metaphase of division, they are in the form of twentyfour long rows of chromatin granules (Pl. 6 Fig. 42). The staining capacity at this period is somewhat reduced and the Feulgen reaction indicates that the nucleic acid is relatively weak. Division is transverse and the daughter plates of chromosomes are made up of twelve rows of chromatin granules which are so closely set that the lines appear to be continuous (Pl. 6 Fig. 43).

Immediately after this second division the most surprising phenomenon of the entire series of conjugation processes takes place. The nucleic acid of the chromosomes shows a remarkable increase; the chromatin granules coalesce to form long homogeneous rods twelve in number (Pl. 6 Fig. 46), and these are the chromosomes of the metaphase stage of the third division figure. The loosely constructed chromosomes of the second meiotic spindle condense and fuse to the smaller number immediately after division and in the third division figure for the first time in the maturation phenomena we find a spindle with all of the usual features of a ciliate micronucleus in meiosis (Pl. 6 Figs. 44, 45).

The twelve chromosomes of the third spindle divide transversely despite the fact that they frequently appear to be connected at one end thus forming loops (Pl. 6 Figs. 45—47). Similar loops may or may not be present in the daughter chromosome groups

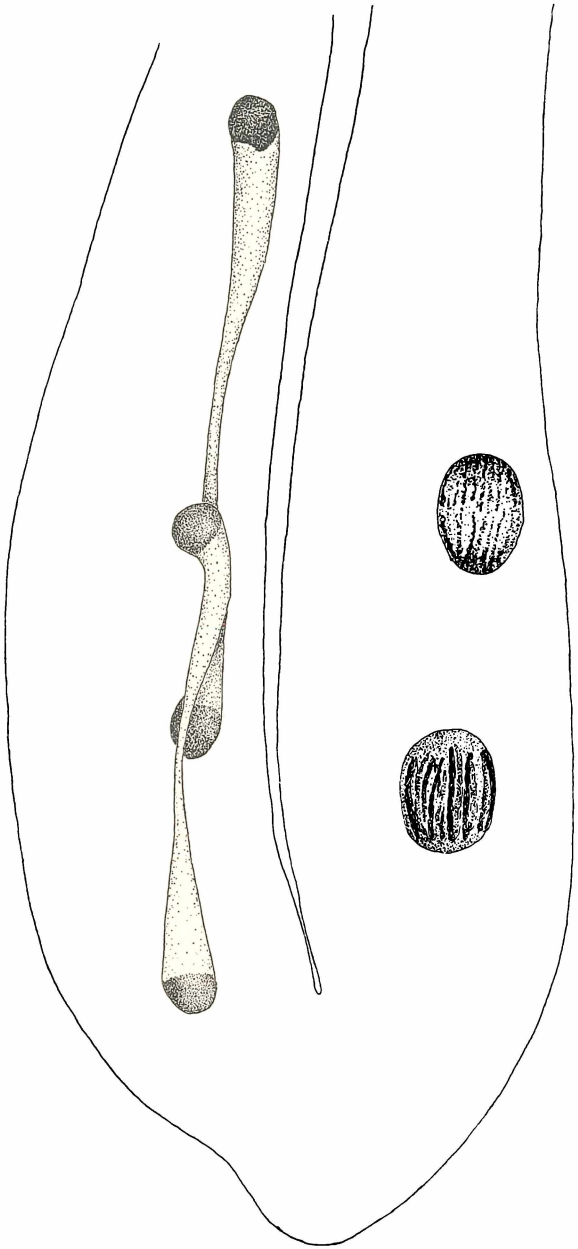
(Pl. 6 Fig. 47). These daughter chromosomes soon lose their clean-cut rod-like appearance and the bulk of their chromatin collects at the two poles to form the characteristic and homogeneous pronuclei (Pl. 6 Fig. 48). Division of the nucleus is not sharp and direct as in the first two divisions but follows the typical history of the ciliate third division. The two poles separate with a long connecting strand in which there still remain a few irregular granules of chromatin close to the poles (Textfig. 2, Pl. 6 Fig. 49). When the attenuated strand is finally broken the two pronuclei are separated by a distance equal to fully one half the length of the organism. There are always two sets of these pronuclei but never more than this whereas in *Uroleptus mobilis* there may be as many as eight pronuclei and in rare cases sixteen.

The homogeneous pronuclei now begin to swell and a cone of denser protoplasm precedes each pronucleus as it moves into the other organism of the pair (Textfig. 3 u. 4). It is the same type of attraction sphere that we described in the case of *Uroleptus mobilis* but in the present form the pronuclei are so large that the attraction spheres appear relatively small. The pronuclei are very much swollen at the time of fusion which occurs in the anterior third of the cell (Pl. 6 Fig. 50).

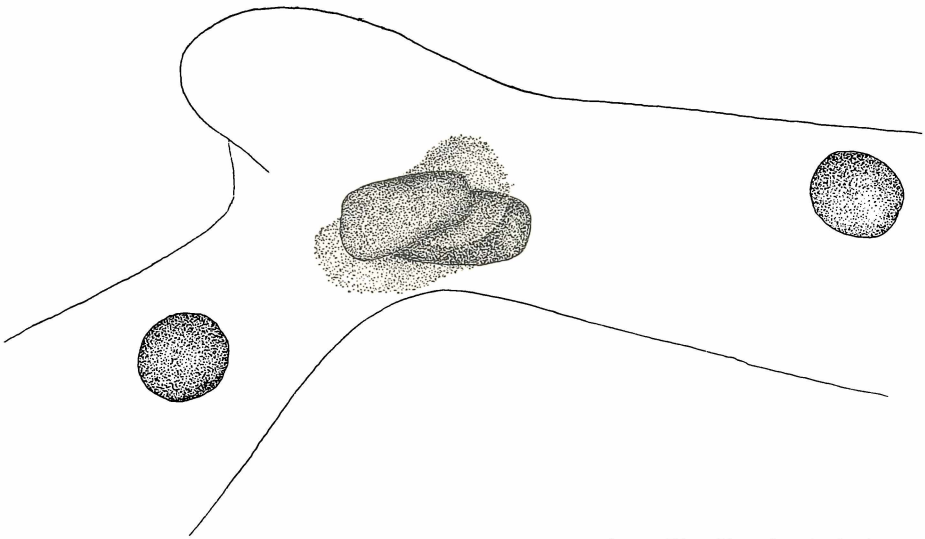
The actual fusion of the pronuclei has not been observed with certainty although some preparations give the appearance of this stage, these are too indefinite however to justify a drawing. This is the only serious gap in the continuity of the observations and I hope to obtain further conjugation material which will show this stage. It is evidently of short duration in time and this is also true of the prophase stages of the first division of the amphinucleus so that I can give no history of the origin of the chromosomes in this spindle. The first definite stage at hand is the metaphase of the first amphinuclear spindle (Pl. 6 Fig. 51). Here the chromosomes are small, twentyfour in number entirely different from those of the third maturation division. The spindle is again different from any other spindle in the life history but is of the same general type as the first meiotic spindle in conjugation (cf. Pl. 5 Fig. 37 and Pl. 6 Fig. 51). It is more elongate however and has a smaller number of chromosomes in the nuclear plate.

There is no resting stage between the first and secont amphinuclear division. The chromosomes become more difficult to stain with any nuclear dye and are irregularly distributed in the nucleus (Pl. 6 Fig. 52). The second amphinuclear spindle thus has the

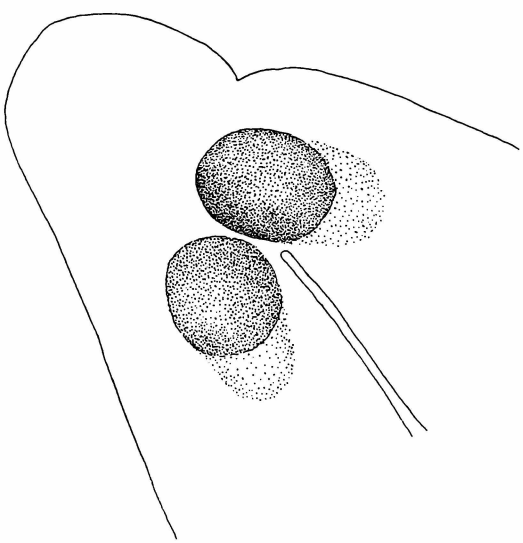
general character of the second meiotic spindle from which it may be distinguished by the stage of degeneration of the macronucleus



Textfig. 2. Third maturation division. One individual of the two conjugants has two nuclei in the metaphase stage, the other nuclei are in the telophase stage of pronuclei formation. 950:1.



Textfig. 3. Crossing of the two pronuclei in conjugation. The direction is indicated by the attraction sphere which is always in advance. 1400 : 1.



Textfig. 4. Pronuclei after passing each other and now migrating towards the stationary nuclei in each cell. 1800 : 1.

and by the fact that the conjugants have either separated or are in the final phases of separation.

With the completion of this second amphinuclear division we come back to our starting point in the history of the micronucleus. The resulting four daughter nuclei are small and homogeneous in make-up. Two of them form micronuclei of the ex-conjugant, one degenerates and one forms the new macronucleus.

The relative duration of the successive stages of the micronucleus in conjugation are roughly indicated by the number of

times such stages are found in a given number of selected pairs. Thus in one hundred selected pairs in which the condition of the micronucleus is clearly indicated there were 70 cells in which the micronucleus is in some stage of the first meiotic division or about 35 %; of these 56 were in the prophase stages, 10 in the metaphase stage and four in the anaphase. None was found in the telophase stage. In 12 individuals the micronucleus was in the second meiotic spindle stage, 52 were in the third division spindle stages, 20 in the pronucleus or interchange stages, none in the fusion stage, 16 were in the first amphinuclear division stages and 30 were in the second amphinuclear division stages. From these figures it appears that, relatively, the first meiotic division requires the longest time for preparation of the chromatin and the spindle and that the third division requires the next longest time and it is significant that these periods involve the most characteristic and profound changes in the chromatin and in the chromosomes.

The entire history of the micronucleus in the life cycle is a record of changes which are manifested not only by the chemical make-up but by the forms assumed by the chromatin elements in division. There is an undeniable wax and wane of the nucleic acid component of the chromatin which underlie the variations in the staining capacity of the nucleus. At some periods the change is rapid as shown by the weakness, the intensity and again the weakness of the Feulgen reaction prior to, during and immediately after the third maturation division. The relative weakness of the nucleic acid after this division persists throughout the remainder of the conjugation processes and it begins to strengthen only with the advent of the vegetative activities of the ex-conjugant.

The up-building of the nucleic acid is a more leisurely process than is its break down. In number of these studies (Calkins l. c.). I have traced the origin of the chromatin in the development of the new macronucleus of the ex-conjugant. The young macronucleus ("placenta") does not stain and is visible as a clear vesicle during life. Then exquisitely minute granules which stain red with the Borrel mixture and violet after the Feulgen reaction may be seen throughout the matrix substance of the nucleus. These granules are the first morphological evidence of the nucleic acid or chromatin of the nucleus. They become progressively larger and ultimately entirely replace the original matrix giving rise to a large macronucleus with its characteristic massive type of structure. In the meantime it is no longer visible in life but now stains intensely with all

nuclear dyes. That there is a gradual manufacture of nucleic acid is undeniable and the process in *Uroleptus Halseyi* requires from two to three days.

An essentially similar process of nucleic acid formation occurs during the meiotic divisions of the micronucleus in conjugation the peak of nucleic acid intensity being reached only in the third nuclear division. As shown above, the micronucleus is homogeneous in structure at the beginning of conjugation and stains only feebly with the nuclear dyes. In this it resembles the young macronucleus of the ex-conjugant. There is a difference however for in the micronucleus at the commencement of conjugation chromatin granules of minute size may be found about the periphery and just within the nuclear membrane or attached to it (Pl. 4 Figs. 23—25). Some of these become metamorphosed into the second pole of the first meiotic spindle while the matrix of the nucleus condenses to form the first pole leaving strands of its substance connected with the ramaining chromatin granules of the periphery. As the granules are drawn into the nuclear plate they increase in size, become rod-shape, and increase in staining capacity. This increment in the total amount of chromatin is relatively small compared with the great increase after the second meiotic division where, by growth, coalescensce and condensation the enormous chromosomes of the third division spindle are formed.

These changes in the nucleic acid content of the nucleus find a further expression in the forms assumed by the nucleus in mitosis. Generally speaking, the greater the intensity of the nucleic acid the more metazoa-like are the mitotic figures and the more perfect the chromosomes. The first vegetative division for example of an ex-conjugant is characterized by a mitotic figure which conforms to the scheme of division of a typical metazoan cell (Pl. 4 Figs. 12, 13). With age of the culture and after an unknown number of divisions, the staining reaction of the chromatin is decreased, no chromosomes are formed and the nuclei divide by a peculiar promitotic process that is neither mitosis nor amitosis (Pl. 4 Figs. 17—21). Similarly with the second meiotic division and the early divisions of the amphinucleus. Here the staining capacity is weak and the chromosomes are reduced to small and irregular elements (Pl. 6 Figs. 42, 52).

What now, can be said about the chromosomes of *Uroleptus Halseyi* from a morphological point of view? Is the unit chromosome one of the 50 chromatin elements which make up the nuclear plate of the first meiotic spindle or is it one of the 12 great chrom-

atin rods of the third maturation division? or is it one of the 24 rods of the early vegetative division? If it is one of these what becomes of it in the vegetative divisions of old individuals? If there is a continuity of chromosomes it is assuredly difficult to follow.

Nevertheless and despite the fact of the periodic manufacture of nucleic acid I think there is a continuity of nucleic acid in the micronuclear history of *Uroleptus Halseyi.* The peripheral granules of the transforming micronucleus prior to the first meiotic spindle, minute and inconspicuous no doubt, are nervertheless the centers of nucleic acid formation and effect the continuity of chromatin and of chromosomes from the old into the new generation. There is a complete absence of anything like the spireme stage so characteristic of metazoan meiosis. The number of these chromatin elements in the nuclear plate of the first meiotic spindle is approximately 48. Are they chromosomes? If so then there is a loss of 36 chromosomes in the later history of the micronucleus. As a matter of fact I do not think they are chromosomes; I believe that they are chromioles and that this first meiotic division is a preliminary division of the micronucleus prior to the formation of the real chromosomes and that the first and second so-called meiotic divisions in *Uroleptus* are together, the equivalent of the spireme stage and first meiotic division of forms other than the ciliates. In other words the conditions that are brought about by the first meiotic spindle in the vast majority of organisms are brought about in *Uroleptus* and other ciliates probably, by the first two divisions which follow one another in quick succession. The chromosomes here are retarded in development and are formed as distinct morphological units only in the anaphase stage of the second division.

This interpretation is in no wise inconsistent with the usual history of maturation in other animals. In the Metazoa for example, as well as in some Protozoa, the chromatin is distributed as chromioles or chromomeres throughout the nucleus. In maiosis they become arranged in a split spireme which, by segmentation and condensation, gives rise to the double chromosomes. In *Uroleptus* there is no spireme stage comparable with that of Metazoa but there are equivalents of the chromomeres. Fusion of these chromomeres and condensation into chromosomes are brought about at a relatively late period in maturation.

With this interpretation the matter of reduction in the number of chromosomes offers no great difficulty. The 48 more or less

chromatin elements of the first meiotic division are separated into two groups of 24 each; these string out as fibers in the second division and are apparently divided transversely, 12 passing to each pole. They never reach the pole however for in the anaphase stage they fuse and condense into 12 characteristic and typical chromosomes of the third division figure. If these constituent chromomeres are qualitatively different then we are confronted by a real problem. The first division may be a reduction division in this sense, but the second and the third are equally so for in both the division is transverse. I see no way out of the difficulty except by questioning the fact of qualitative difference. Each chromomere may be regarded as a center of nucleic acid formation, specific for *Uroleptus* indeed but not necessarilly qualitatively different from other chromomeres. *Uroleptus*, morphologically, is no more than a single cell and the imagination balks at a conception of organization by genes whereby the different parts of a single cell are developed as a result of stimulation by specific centers located in the chromatin. What argument can be given for a theory that postulates 48 qualitatively different chromomeres in *Uroleptus*, or a fortiori, upwards of 150 chromosomes in *Paramecium caudatum*? The theory is not elastic enough to cover cases in which the number of chromosomes reaches into the hundreds (e. g. *Noctiluca*) or into thousands (e. g. *Aulacantha scolymantha*). I suspect that we have been too subservient to tradition and to formula in our dealings with Protozoa and have been too prone to harmonize the maturation phenomena in Protozoa with the exact meiotic processes in Metazoa.

Further evidence against the theory of qualitative differences is afforded by the normal happenings in the ciliates. In all careful studies of the maturation phenomena in many different ciliates a reduction in number of chromosomes is described as taking place in either the first or the second division. But in practically all cases a third or pronuclei-forming division occurs and here the division is transverse and frequently heteropolar. On the theory of qualitative differences in the chromomeres this would have the effect of another reduction division and the theory would have to be modified to account for it. Still further evidence against the theory is afforded by merotomy experiments on conjugating ciliates. *Uroleptus mobilis* for example, was cut while in conjugation. Scores of pairs in different stages of conjugation were operated and amongst them were cases in which the two pronuclei were passing each other in the protoplasmic bridge connecting the two organisms. The

anterior ends containing these pronuclei were removed, stained and mounted and the position of the pronuclei established beyond doubt. The two remaining fragments of the pair in each case were then cultivated in isolation cultures and these resulted in tpyical life cycles which were no different from the cycles of normal ex-conjugants [1]). The two fragments were cultivated independently and in each of them presumably there was only one functional pronucleus. Supporters of the theory of a qualitative difference in the chromosomes might argue that a full complement of necessary qualities was present in the half nucleus; with this argument I am in full agreement but the difficulty comes from the fact that when these operated cells reached the stage of sexual maturity and conjugated the number of chromosomes was found to be exactly the same as in a normal ex-conjugant series, and reduction in number followed the usual procedure. The probable interpretation is that the chromosomes were regenerated as are other organoids of the cell after mutilation.

The results of the earlier studies on *Uroleptus mobilis* and of the present studies on *Uroleptus Halseyi* lead me to the conclusion finally that the theory of multiple genes in the chromosomes is untenable for these ciliates. Nucleic acid however is essential for the continuation of metabolic activities and the present observations show that it is sometimes abundant at other times much less abundant in the cell. There is evidently a remarkable power of nucleic acid formation or, in other words, of chromatin regeneration. This is clearly indicated at certain stages of meiosis, but is much more noteworthy in the formation of the new macronucleus of the ex-conjugant when a relatively vast store of nucleic acid is manufactured and continues to be manufactured to supply the eight to twentysix massive macronuclei of the vegetative individuals.

It is quite probable that thet nucleo-protein of *Uroleptus Halseyi* is specific for this organism and specifically different from that of all other organisms. As a part of the fundamental organization it takes part in the metabolic activities of the general protoplasm as a result of which the cellular structures of the derived organization of the adult are formed. The fully formed chromosomes as they appear in the third maturation nuclear division may be interpreted as similar derived structures of the chromatin with a function to perform in fertilization and with a limited activity. Their chromatin

[1]) Calkins, 1921, Journ. Exp. Zool. Vol 34 No. 3.

is enormously amplified in the new macronucleus to subserve the functions of vegetative metabolism, and condensed in the new micronucleus where it apparently plays a passive role in metabolism.

The theory of the gene in modern genetics does not postulate any visible or known thing. In the hands of MORGAN and his school however, evidence has been gained which amounts almost to proof that some physical entity in the nucleus in some way controls the development of a specific character in the adult, that certain entities have a definite spatial relation with each other in a given chromosome, and that each such entity has its mate derived from the other parent. The constancy in number of chromosomes of a species, the established facts of meiosis involving the orderly association of chromosomes two by two, together with the segregation of characteristics in the germ cells as demonstrated by genetics, form the basis of facts upon which the theory of the gene has been established. In many ways it resembles the particulate theory of WEISMANN but is a more perfect and a more durable fabric in which the warp is constituted by the facts of cytology, the woof by the science of genetics.

We do not homologize the digestive system of a ciliate with that of a metazoon, yet we are prone to believe that chromosomes have the same significance and follow the same course throughout all nature. We interpret stages in meiosis of a ciliate with the same formula of words that we use for metazoa and if there is a discrepancy we are more apt to believe that our interpretation of the facts is at fault than we are to accept the facts as different. The third meiotic division in ciliates has always been a stumbling block to cytologists and no solution being possible along the lines of traditional reduction processes it has been ignored for the most part by cytologists. If we are comitted to the gene hypothesis the assumption of an equational division at this stage is imperative provided there are two or more dissimilar genes. If on the other hand there is only one type of gene then it would be immaterial whether the chromosome division is equational or transverse, quantity of chromatin would be the only essential factor. This appears to be the case with *Uroleptus Halseyi* and other ciliates in which the chromosomes of the amphinucleus do not appear again in the same forms until the next following third division in meiosis, and where all divide transversely at each division.

WEISMANN'S famous prediction of a reducing division was later confirmed not indeed in the exact form of the prediction of a

transverse division of chromosomes at some stage in the life history, but by separation of entire chromosomes. Should an actual transverse division distinct from crossing-over phenomena, of the chromosomes of metazoa be proved the whole fabric of the theory of the gene would be loosened and mutilated. The entire absence of chromosomes in the adult *Uroleptus Halseyi*; the recognized transverse division in the third meiotic division of all ciliates; the transverse division at each stage of meiosis in *Uroleptus Halseyi*, all indicate that here, if we are to include ciliates in the theory of the gene, we are not dealing with any carefully organized aggregate of genes comparable with metazoen chromosomes, but with a single type of gene in each chromosome. If this is true there would be not more than twelve types of gene in *Uroleptus Halseyi*.

With this conception of the germ plasm of protozoa we strengthen the conclusion that there has been an evolution of the chromosome no less than an evolution of the digestive, nervous or excretory system. The chromosomes of man qualitatively, are probably as different from those of *Drosophila* or of *Uroleptus* in respect to the characteristics represented, as the human organism is different from the fly or the ciliate, and all intermediate grades of complexity might be expected in chromosomes of animals between the ciliates and man. It is possible that there has been an evolution of the gene itself but there has also been an evolution of the combination of genes. The single type of gene of *Uroleptus* if a minute bit of specific nucleo-protein, is probably as complex chemically as the specific bit of nucleo-protein of any single type of gene of a metazoan but the former works alone, so to speak, and its activity is less conditioned or modified by the activity of associated genes than in metazoa.

With a single type of gene in each chromosome it is immaterial whether the chromosome divides longitudinally or transversely for division would be equational in either case. If a single chromosome is made up of a single type of gene there is no a priori reason why different chromosomes should not be made up of the same type of gene. On this supposition it would not be difficult nor inconsistent to account for the enormous numbers of chromosomes in radiolaria, in *Noctiluca* and the dinoflagellates generally. Morgan states that there are upwards of two hundred genes in the X-chromosome of *Drosophila* [1]). Single genes here cannot be interpreted as

[1]) Morgan, The Theorie of the Gene, Yale Press 1926, p. 52.

single specific entities for, on the theory, they are represented by two single specific entities in every cell of the body of the fly all of which have been derived by division of the supposedly independent single genes of the gamates. It is more correct therefore, to speak of a single type of gene rather than a single gene if we conceive the gene as a physical entity. In the same way I picture a single type of gene of *Uroleptus* as a specific entity which by self-multiplication gives rise to a multitude of duplicates in the huge chromosome of the third maturation division spindle. There are twelve of these chromosomes of types of gene in each gamete nucleus, hence twelve pairs of allellomorphs in the amphinucleus distributed in twentyfour chromosomes. If each of these twenty-four is represented by two identical units formedby division, there would be fortyeight or twelve sets of four identical genes. Two of these four are separated from two leaving twentyfour after the first meiotic division, and one is separated from one with the second meiotic division, leaving twelve. These twelve divide transversely at the third division but this is immaterial if each chromosome represents a single type of gene. It would be quite different however, if a chromosome here were conceived as made up of two or more genes arranged in linear series as postulated for *Drosophila*. On the theory of the gene as formulated by MORGAN and his school, I conclude therefore, that there is only one type of gene per chromosome in *Uroleptus Halseyi*.

Explanation of Plates.

Plate 3—6.

Plate 3. *Uroleptus Halseyi*.

Fig. 1. Section through the anterior end showing part of the adoral zone and the three cirri. The row of basal granules of membranelles and of the cirri is broken. 1400 : 1.

Fig. 2. Section through the anterior end showing the greater part of the adoral zone, the motorium, and coordinating fibrils running to the membranes and to the row of basal granules of the membranelles. 1400 : 1.

Fig. 3. Section showing one frontal cirrus, basal plate, and fibrils to basal granule and to membranelle fibril. 1400 : 1.

Fig. 4. Similar section at another angle. 1400 : 1.

Fig. 5. Transverse section through adoral zone showing one membranelle with basal lamella and fibrils to the basal granule. 1700 : 1.

Fig. 6. Dividing *Uroleptus Halseyi.* Two dividing macronuclei and one micronucleus in each daughter cell. 950 : 1.

Fig. 7. *Uroleptus Halseyi* in conjugation. The micronuclei are in the full metaphase of the first meiotic division. 650 : 1.

Plate 4. *Uroleptus Halseyi.*

Fig. 8. Group of macronuclei and four products of division of the amphinucleus of a young ex-conjugant. 950 : 1.

Fig. 9. Macronucleus ready to divide and two micronuclei derived from the second division of the amphinucleus. 950 : 1.

Fig. 10. Chain of macronuclei and two micronuclei in a young vegetative stage. 950 : 1.

Fig. 11. A group of macronuclei and two ellipsoidal micronuclei of an older form. 950 : 1.

Fig. 12. Micronucleus of young ex-conjugant in spireme stage of early vegetative division. Feulgen reaction. 1750 : 1.

Fig. 13. Micronucleus of young ex-conjugant in metaphase stage of early vegetative division. Borrel stain. 1750 : 1.

Fig. 14. Micronucleus of young ex-conjugant in anaphase stage of early vegetative division. Feulgen reaction. 1750 : 1.

Fig. 15. Reticulate micranuclei from old vegetative forms. Borrel stain. 1750 : 1.

Fig. 16. Micronuclei of old vegetative forms with condensed spherical or band form chromatin. Borrel stain. 1750 : 1.

Fig. 17. One of the macronuclei with nuclear cleft and micronucleus in telophase of division, from an older from. Feulgen reaction. 1750 : 1.

Fig. 18, 19, 20 and 21. Micronuclei of old individuals in various stages of division without chromosomes. Feulgen reaction and iron haematoxylin (20). 1750 : 1.

Fig. 22. Micronuclei from a pair in early conjugation. Peripheral chromatin granules and central matrix. Feulgen reaction. 1750 : 1.

Fig. 23. Two macronuclei with X-granules and two Micronuclei from a pair in early conjugation. Iron haematoxylin. 1750 : 1.

Fig. 24 and 25. Two pairs of micronuclei with segregation of chromatin granules which will form the second pole of the first meiotic spindle. Feulgen teaction. 1750 : 1.

Fig. 26. Two macronuclei with X-granules and two micronuclei forming the second pole of the first spindle. Iron haematoxylin. 1750 : 1.

Fig. 27. Later stage in conjugation. The micronuclei are much swollen and have minute chromatin granules on the periphery of the central matrix. Feulgen reaction. 1750 : 1.

Plate 5. Conjugation.

Fig. 28. Unusual, probably pathologic, micronuclei from a conjugating pair with chromatin in form of large peripheral granules. Iron haematoxylin. 1750 : 1.

Fig. 29. Early stage in the condensation of the central matrix with connecting strands to the peripheral granules and to the second pole of the spindle. Feulgen reaktion. 1750 : 1.

Fig. 30—35. Stages in the formation of the first meiotic spindle; the peripheral granules are apparently drawn into the nuclear plate. Some of these stages recall the "parachute", nuclei of other ciliates (33). All iron haematoxylin. 1750:1.

Fig. 36—37. Metaphase of the first meiotic division with about 48 small rod-like chromosomes derived from the peripheral granules. (This stage of conjugation is represented in Plate 3 Fig. 7.) FEULGEN reaction. 1750 : 1.

Fig. 38—40. Early anaphase of first meiotic division whereby the chromosomes are separated into two groups of twentyfour each. Feulgen reaction (38—39) and iron haematoxylin (40). 1750 : 1.

Plate 6.

Fig. 41—42. Second meiotic spindles in which the chromosomes of the first division are drawn out into long rods. In each case represented here the micronuclei of the conjugating mate are in the stage of the third division. FEULGEN and iron haem. 1750 : 1.

Fig. 43. Anaphase of the second meiotic division: the chromosomes are undoubtedly dividing transversely. Iron haem. 1750 : 1.

Fig. 44. Two micronuclei from individuals of the same pair. One nucleus is in the stage of the second meiotic spindle, the other in the third division stage. FEULGEN. 1750 : 1.

Fig. 45. Two nuclei in the metaphase of the third division each with twelve chromosomes. FEULGEN. 1750 : 1.

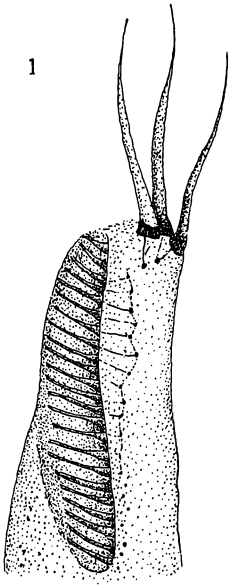
Fig. 46. Products of the second meiotic division. These nuclei were in the mate of the individual represented in Fig. 42. Compare also Fig. 43 and note the heavy condensation of the chromosomes shown in Fig. 46. Iron haem. 1750 : 1.

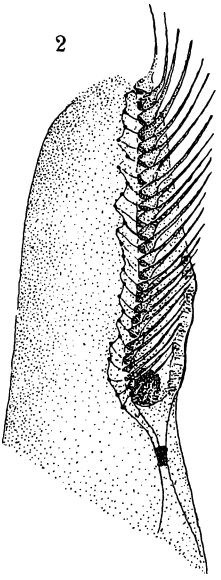
Fig. 47—49. Stages in the third, or pronuclei-forming division. Iron haem. Cf. Textfig. B. 1750 : 1.

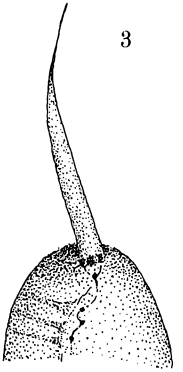
Fig. 50. Pronuclei just before fusion. Iron haem. 1750 : 1.

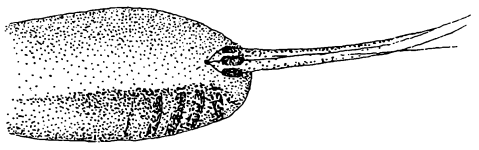
Fig. 51. First division of the amphinucleus. The chromosomes are again small and twentyfour in number. FEULGEN. 1750 : 1.

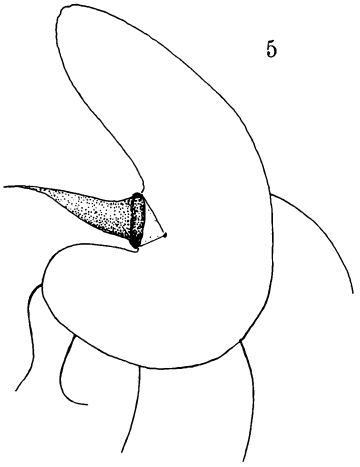
Fig. 52. Preparation for the second division of the amphinucleus one of the nuclei shown in polar view. The two conjugating individuals are now separating. Iron haem. 1750 : 1.

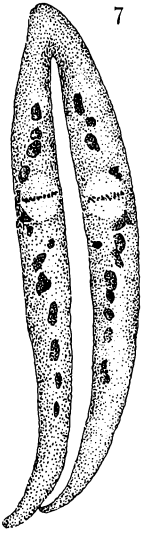


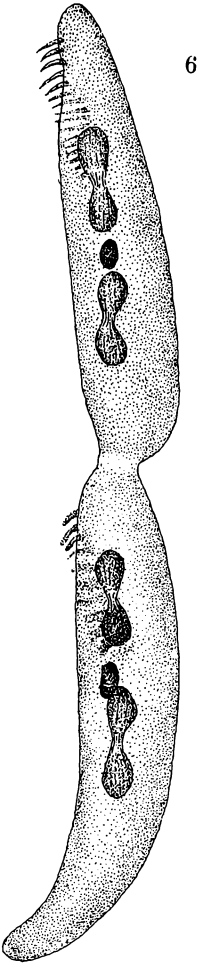






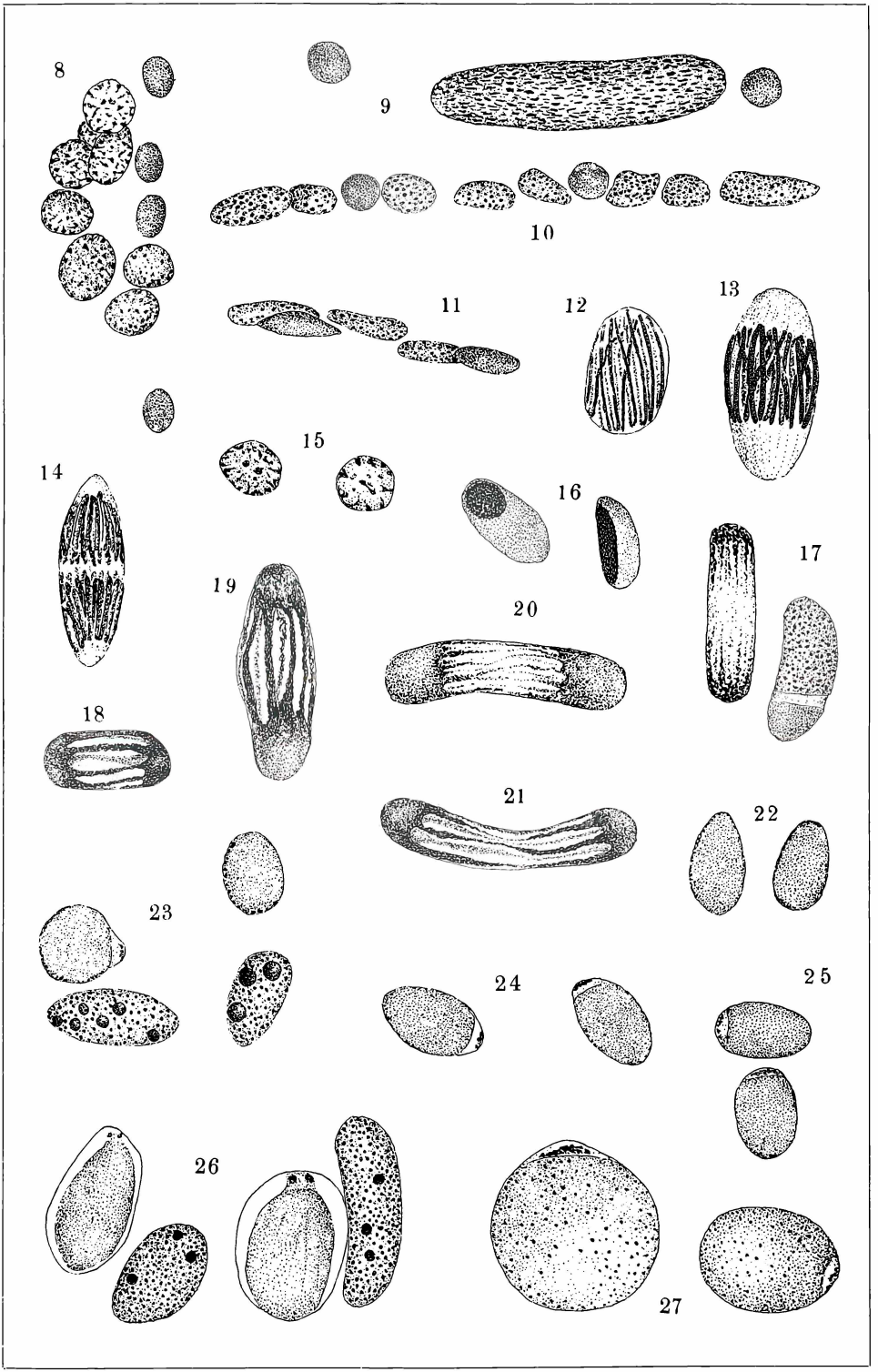






Calkins.

VERLAG VON GUSTAV FISCHER IN JENA



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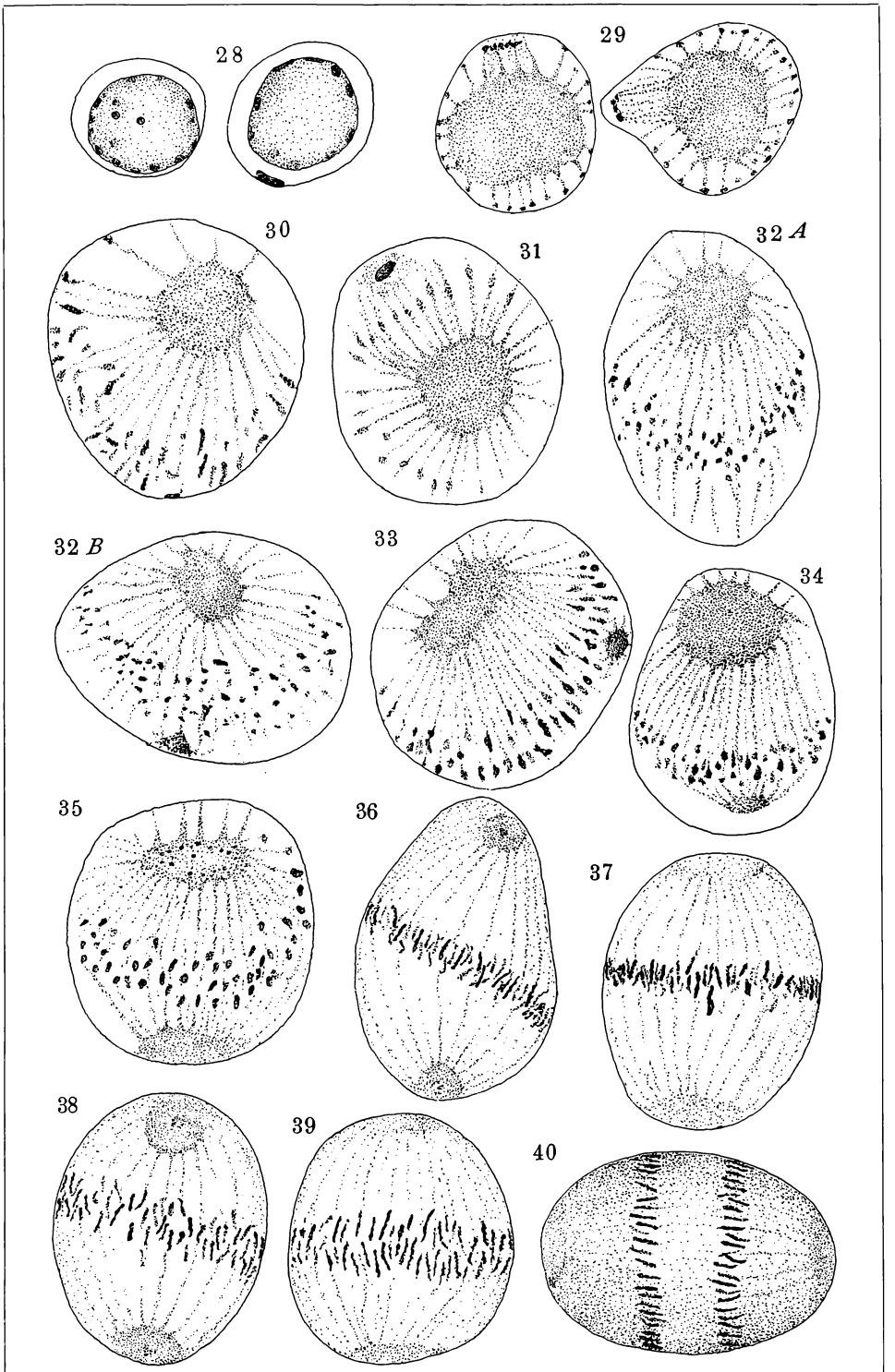

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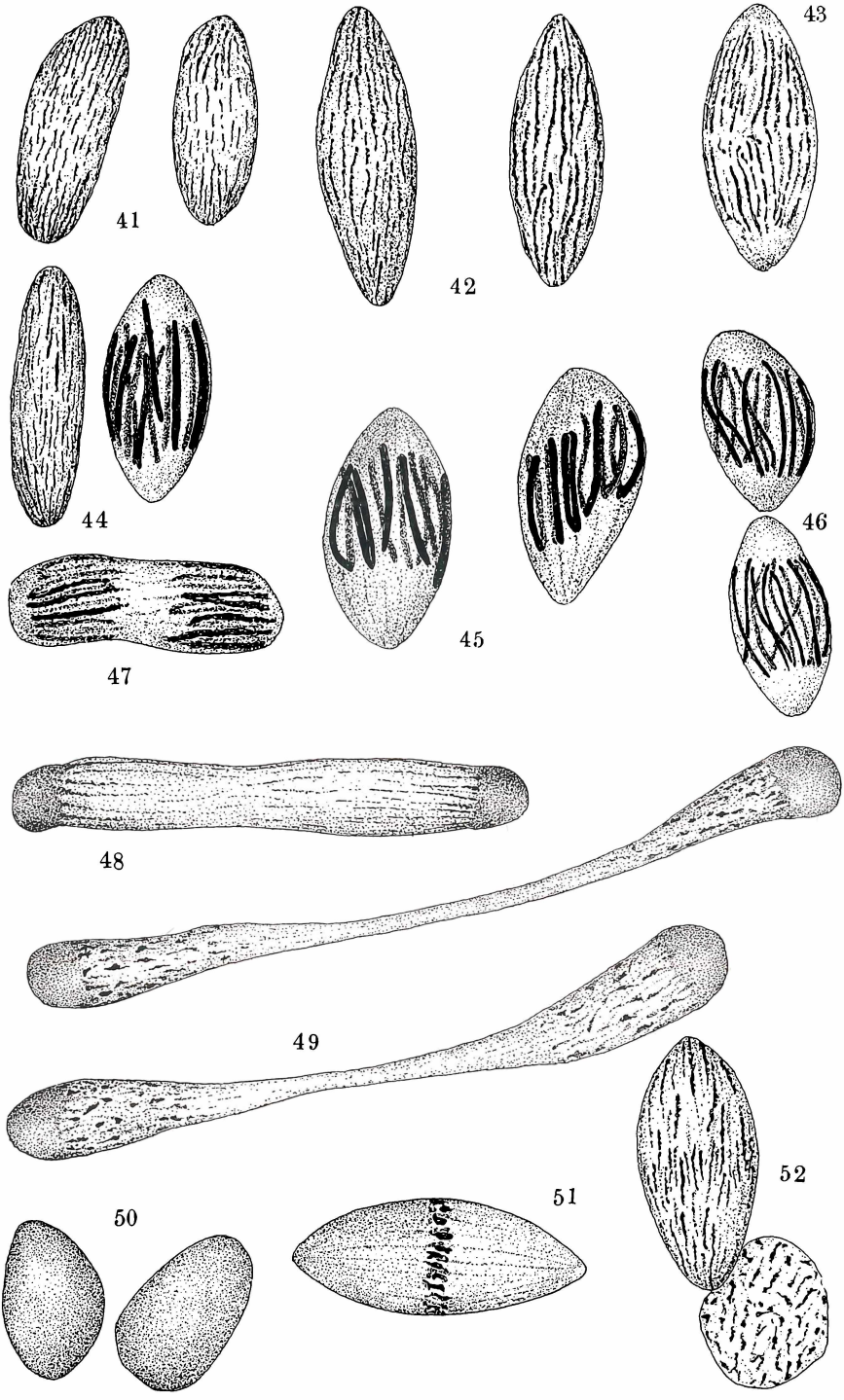

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