The Conjugation of Paramaecium aurelia (caudatum).

By

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(With plates XII—XVIII.)

It has been shown that the life history of a protozoon such as *Paramaecium* is characterized by a gradually waning vitality expressed by decrease in the division energy and by the loss of certain internal features. So constant is this phenomenon that we have been justified in comparing the life history of a protozoon with that of a metazoon and in finding corresponding stages that may be interpreted as youth, adolescence and old age. On the basis of this comparison we have advocated a new conception of the protozoon individual and have recommended the abolition of the single cell as the unit of species (Calkins 1906). According to this conception the protozoon individual, like a metazoon, consists of the entire cell progeny derived from a single original fertilized cell, the entire congeries of cells exhibiting the same variations in physiological activity that characterize the periods of youth, adolescence, and old age in higher animals. Our experiments on the life history of *Paramaecium* have shown that vitality, as expressed by the division energy, has more or less regular cycles, and that the race, or the individual in the new sense, dies during the depression period unless stimulated by artificial means or by conjugation. We have shown that such an artificial stimulus is afforded by the substitution of a medium rich in salts, for the

customary diet, or, in some cases, by the addition of a single simple salt like potassium phosphate, to the usual food medium (CALKINS 1904). It was observed, also, that the periods of regularly recurring depression were due to the apparent exhaustion of certain physiological activities, sufficient, indeed, to bring about death of the series, but capable of re-invigoration by the addition of artificial stimuli. Such depression periods are very different from the complete exhaustion of both physiological and germinal activities that comes with the exhaustion of the potential of division energy stored up in the recently fertilized cell, and we recognize that the effects produced by artificial stimuli are more nearly comparable with the prolongation of vegetative life than with artificial fertilization.

Conjugation between two cells results in the complete re-invigoration of all activities, both physiological and germinal. It results in the formation of a new individual protozoon no less truly than does the fertilization of a metazoon egg result in a new individual metazoon. It cannot be regarded as a mere process of rejuvenescence, or „Erfrischung" of the old protoplasm but must be interpreted as a complete reorganization and combination of new protoplasmic materials in no less a degree than we find in other kinds of living things. We know the morphological changes that are brought about when the depressed race of *Paramaecium* is stimulated to new activity by salts or diet, and in a general way we know what takes place during conjugation of two germ cells. In the former case the macronucleus and general endoplasm are visibly affected and restored to the appearance characteristic of healthy activity; in the latter case the micronucleus undergoes a number of peculiar processes, certain portions are eliminated and certain micronuclear products unite to form the fertilization nucleus. From this fertilized nucleus new macronuclei and new micronuclei are produced and thereafter these retain their original characteristics throughout the life history of the individual.

It was with the hope of finding out exactly what takes place in the micronuclei during this process of fertilization that we undertook the study of the cytology of conjugation in *Paramaecium*, using methods that have been successful in working out the maturation phenomena in higher animals and plants. Our study has resulted in the confirmation of the general course of conjugation as given in most textbooks, and in addition we have added the details of the maturation process; an interpretation of the enigmatical crescent; and the discovery that the conjugating pronuclei are differentiated

into a large and a small form which may be interpreted as male and female pronuclei.

Since WEISMANN's classical essays on the composition of the chromosomes and their importance in matters of heredity these elements of the germ cells have had an ever growing interest to biologists. In connection with MENDELian inheritance these parts of the nucleus have developed a new interest through the researches of MONTGOMERY, SUTTON, STEVENS and WILSON, and the hypothesis is becoming more and more probable that the chromosomes severally represent adult characters or groups of characters. The study of the chromosome therefore has a high theoretical interest and some further insight into its biological significance may be obtained by an examination of these elements in protozoa. Here however, in the majority of cases, we are confronted by peculiarities in the chromatin that seem to be unique, occurring only in the lower forms of life. Some of it is apparently differentiated for a purely vegetative function and some appears to be primarilly designed for reproduction and inheritance. In the form of the chromidium it may be either one or the other; thus in *Actinosphaerium* what MESNIL has called the trophochromidium, has to do mainly with metabolism according to R. HERTWIG, while in the rhizopods the distributed chromatin or idiochromidium of MESNIL, is the material mainly concerned with reproduction. In the infusoria the dimorphic nuclei show this chromatin differentiation common to all protozoa, the macronucleus representing the trophochromidium, the micronucleus the idiochromidium. In *Paramaecium* therefore our problem covers more than the mere maturation of the chromosomes for it involves the underlying factors of differentiation, the macronucleus derived from a product of the micronucleus representing a differentiation into somatic protoplasm.

Paramaecium is well adapted for a study of this character because of its world-wide distribution, the frequency with which it has been studied, the fact that it is the most common type of the unicellular animals in the class room, and mainly because of the large size of its maturation nuclei, and the ease with which the organisms may be raised and controlled in large numbers.

I. Material and Method.

Conjugating *Paramaecium* may be easily obtained in the laboratory by seeding a hay infusion with a dozen or more Paramecia from natural pond water and leaving it for some weeks in the ordinary room temperature of the laboratory. After such a period they form a thick white band immediately below the surface of the water where they get the right proportion of oxygen. With a fine pipette the white band is removed and the thousands of cells are transferred to a small clean watch glass of about 10 cm diameter containing fresh hay infusion. The watch glass is then covered and set aside for from 24 to 48 hours, when, if it is a mature culture, many conjugating pairs will be found. If the first trial is unsuccessful there is nothing to do but wait until the cells are in the physical condition when conjugation is possible, but if the right moment is seized fully 90 % of the organisms will be united in pairs.

In studying the life history of *Paramaecium* it was found that the physical condition of the cells which makes conjugation possible, comes at the time when the division energy begins to decline, and that failure to conjugate at this time leads to death during the ensuing period of depression unless the organisms are artificially stimulated (CALKINS 1902). PEARL (1906), studying conjugating *Paramaecium* biometrically, found a measurable difference in the cells that conjugate from those that do not and confirms the oft-repeated observation that *Paramaecium* during conjugation, is smaller than at other times. It appears, then, from purely external observations that the physical make up of *Paramaecium*, at periods of conjugation is different from that of ordinary seasons as indicated by plasticity or miscibility, and by smaller size.

For killing, warm bichloride of mercury with 5% glacial acetic acid, FLEMMING's osmic mixture and picro-acetic acid were used, the best results being obtained with the first. Bichloride dissolved in 10 % formalin was also tried but gave less satisfactory nuclear results on sectioning the material.

The following small point in technique may be useful in connection with other kinds of protozoa. Draw a rich culture of conjugating forms into a fine pipette with as little water as possible and place in a round bottom vial. Add the killing agent and allow the organisms to settle. After decanting off the liquid, allow the mass to stand without fluid until the organisms and zoogloea are matted into a more or less homogeneous mass, and this, by careful hand-

ling with the various liquids afterwards, retains its solidarity and may be treated as a macroscopic object. In our work such masses were passed through the regular grades of alcohol, embedded in paraffine and cut in sections not more than 5 microns thick. The sections were stained with HEIDENHAIN's haematoxylin, or with polychrome methylin blue and eosin, the former giving the best results especially when counterstained with aqueous eosin.

In addition to this mass method we tried fixing and sectioning single isolated pairs in the expectation of finding a good sequence of stages in this manner. HAMBURGER (1904) states that she obtained her best results by this method. but our experience does not conform with her's in this regard for the time element varies in different cases to such an extent that one is unable to judge from one pair the stage of fertilization in any other. Our best results were obtained by killing in mass from the same culture at different times, and in this way we obtained hundreds of pairs in the same stage of development, the main difficulty in this method being that we have no idea of the length of time required for each stage in the maturation process.

The previous observers who have studied *Paramaecium aurelia* (*caudatum*) have confined their attention to total preparations, and this is undoubtedly the reason why the maturation processes are not better known. We have found that the entire cell when stained and mounted is far too thick and too dense for study of the chromatin, and such preparations are suitable only for the general course of conjugation, and useful only as a check on the sections. HAMBURGER adopted the section method in her study of *Paramaecium bursaria*, and PRANDTL studied the conjugation of *Didinium nasutum* in the same way, and the beautiful results which they each obtained are due largely to their adoption of this method of study.

II. The Phenomena of Conjugation in Paramaecium.

The history of observations upon *Paramaecium* conjugation has been written so often that we will here pass over the main steps as hastily as possible. With BÜTSCHLI (1876) the length of time required for conjugation, and the general external features were established, and the nuclei in mitosis were described for the first

time and compared with the nuclei of metazoan cells. BÜTSCHLI also correctly interpreted the significance of conjugation, and pointed out the relation of macro- and micronuclei. The first details of the process of conjugation however were not given until 1888—89 when both MAUPAS and R. HERTWIG worked them out independently and came to approximately the same conclusions, the former for *P. caudatum*, the latter for *P. aurelia*, and our study confirms their general account. The minutiae of the maturation processes were not given by either observer, nor did HAMBURGER get much farther in connection with the maturation processes of *P. bursaria*. All of these observers noted the formation of the crescent and interpreted it as a normal phase in the maturation of the nuclei, but the origin of the first maturation spindle from the crescent has never been worked out, while some observers, notably GRUBER (1886), have regarded it as an abnormal structure.

A. General outline of the process of conjugation (Plate XV).

The two conjugating cells are of about the same size and so placed that the mouth parts are directly opposed. As many observers have noted and as PEARL has proved, the conjugating forms are smaller than those which are not united. They are not the smallest forms to be found in the life history however, this condition being characteristic of the first two generations after separation and before the young individuals have had an opportunity to take in food (figure 32). The union of the two gametes is facilitated by a characteristic physical and chemical condition which we have previously described as the miscible state (CALKINS 1902). At first the two gametes are so loosely joined that a sharp spurting from a pipette loosens the connecting protoplasm and separates the two cells, which may then continue to live apparently without any ill effects from the rude handling to which they had been subjected. In one case such a forcibly separated conjugant continued to live throughout more than 158 generations of divisions thus indicating that the conditions of the organism under which conjugation takes place are not symptoms of immediate degeneration. However after complete union, it is almost impossible to separate them and one or the other is frequently killed without separating, by the forceful ejection from the pipette. Sections show that the protoplasm is continuous from one individual to the other, a fact indicating that there may be some interchange of cytoplasmic material in the way sug-

gested by Hicksox (1902) in the case of *Dendrocometes* although the absence of characteristic granules in the connecting bridge is somewhat against this conclusion. The plasticity of the protoplasm at this period is further shown by the frequent out-bulging of the body due to the tension caused by a nucleus in the telophase of division, the bulge resembling the formation of a polar body in metazoan eggs.

The union of the two individuals is apparently the signal for the beginning of the maturation processes. In each cell the micronucleus leaves its position in the cleft of the macronucleus and begins to swell while the chromatin, which in the resting nucleus is more or less compact and homogeneous, becomes definitely granular and oriented in lines radiating away from the polar division center (plate XV figures 22 and 23). This separation of the micronucleus and fragmentation of its chromatin, is a condition not entirely confined to the maturation period. but appears occasionally in cultures under conditions which we have not been able to define.

The result of this increase in size is a long drawn out nucleus with the division center at one end and chromatin arranged in fine threads which form a reticulum or branching network throughout the nucleus. This straight and narrow condition is followed by a characteristic bending until the micronucleus assumes the form of a crescent, the horns being drawn out into sharp points (figure 24). The reason for this peculiar curvature of the nucleus is not clear but it probably has something to do with the growth in length of the chromatin lines, and with the shifting of the division center from the end to the center of the elongated nucleus, the bulk of the division center having greatly increased in the meantime. In the majority of cases the crescent lies with its long axis at right angles with the long axis of the animal but the long axis of the first maturation spindle which is derived from the crescent is always at right angles to the long axis of the crescent, and quite regardless of the animal's orientation. This first spindle is formed by threads of fine material arising from the division center while the chromosomes are divided before the spindle form is attained, so that the first evidence of a typical spindle is not found until the anaphase of the first division. The two nuclei resulting from this first division pass at once without a resting phase into the nuclear plate of the second maturation spindle, and four nuclei are formed which are apparently of the same type, although two of them soon begin to show the concentration of chromatin that marks the first step in their degeneration

(fignres 25 and 26). The other two undergo a characteristic chromatin change, and one of these (sometimes both) undergoes a third division to form the stationary and migratory pronuclei. In each of the conjugants the pronuclei thus formed are differentiated into a larger (stationary, or female), and a smaller (migratory, or male), nucleus. Both are then elongated or drawn ont into a spindle form, although this has nothing to do with a mitotic spindle as some have claimed, for the chromatin is in granule form and not in the form of chromosomes.

Interchange and fusion of the pronuclei then take place, a larger pronucleus in each cell uniting with the smaller one from the other cell, and shortly after this the two organisms separate, each the fertilized egg cell of a new *Paramaecium* individual.

The old macronucleus does not begin to fragment until after the two cells have separated, and the process of fragmentation is very slow, requiring one or two days. In the meantime the fertilization nucleus divides rapidly three consecutive times until eight micronuclei of similar character are formed. Four of these begin to swell and to metamorphose into macronuclei and these, surrounded by fragments of the old one, are well developed by the time of the first cell division which apparently never takes place before the third day after separation. The first division results in the formation of two very small cells each provided with two macro- and two micronuclei, and these grow to nearly full size before the second division takes place two or more days later (figures 28—32).

With this precocious division of the fertilization nucleus into eight micronuclei it is quite possible that some abnormal distribution may occur in the subsequent cell division. It is in this way that we would interpret that condition of *Paramaecium aurelia* with two nuclei which has been erroneously regarded as sufficient evidence for distinguishing it from *P. caudatum* with one micronucleus. That this distinction is unjustified has been already shown, the physiological differences between the two being due to the abnormal relations of nucleus to cytoplasm (CALKINS 1906).

As to the fertility of conjugation in *Paramaecium* we have previously shown that both ex-conjugants are not equally vigorons (CALKINS 1902, CULL 1907), and that in about 70% of the pairings this discrepancy holds.

B. Cytological Details of the Phenomena of Conjugation.

1. The first maturation spindle, and its origin (Plates XII and XVI).

The micronucleus of *Paramaecium aurelia* (*caudatum*), as in other species of the genus, lies normally in a small cleft in the macronucleus. It stains readily with most nuclear dyes and takes such an intense color that it is not infrequently described as possessing homogeneous and densely packed chromatin. When carefully extracted after staining however, the granular character of the chromatin can be made out, and, if counterstained with eosin, the granules may be seen aggregated about a definite, though small, division center situated at one pole of the nucleus. From a denser mass of granules at this pole of the nucleus, the chromatin extends towards the opposite pole in constantly decreasing density, so that both poles have a lighter appearance than the central part.

Although usually partly embedded in the substance of the macronucleus, the micronucleus on occasions may emerge from its customary position and be carried about in the endoplasmic cyclosis. This condition was frequently observed during the study of the life cycle and was particularly noticeable during periods of depression. At such times too, the ordinary structure was lost and the micronucleus appeared abnormally large and the chromatin loosely granular (Calkins 1904).

While this separation of the micronucleus is undoubtedly an abnormal feature during the ordinary vegetative activities of the cell, due possibly to the disturbance in metabolism from one cause or another, it is a normal feature of conjugation. Shortly after the cells unite, the micronucleus emerges from its nest in the macronucleus and begins to swell. The division center becomes more conspicuous and has a definite outline (figures 1, 22, 35). The chromatin accumulates in a band of large granules about the division center while the opposite end of the nucleus appears quite empty. It frequently happens that the nucleus seems to be in the metaphase of division, the division center forming one pole, the empty end of the nucleus forming the other, and the granular chromatin band forming the apparent nuclear plate. Such a stage however is at best only temporary for the coarser grains of chromatin soon begin to disintegrate into smaller granules which radiate out from the division center in lines (figures 2, 36). These lines of chromatin are all oriented towards the division center, the proximal ends for

a long period being heaviest. The nucleus at this stage resembles that of Noctiluca where the chromosomes are similarly directed towards the division center and where the growing chromosomes obtain their material from the disintegration of the large chromatin reservoirs. It differs from *Noctiluca* however, in the fact that in the latter the division center is outside of the nucleus and divides before the chromosomes are formed.

Elongation of the micronucleus continues with the growth of the chromatin lines which become more and more reduced in thickness as they elongate. The division center also increases much in size, elongating as the nucleus elongates, and is always distinctly marked off from the chromatin and from the nuclear membrane. The latter must have a considerable power of distension for it stretches from a size covering a nucleus of 8 μ to one of 65 μ and is never broken throughout the entire process of maturation.

The increase in size of the micronucleus continues until it is quite as long or even longer than the macronucleus, and has a very different appearance from the old micronucleus being many times longer, provided with rounded ends and with its chromatin in the form of a fine meshed reticulum. This reticulum has arisen from the lateral branching or lateral union of the lines of chromatin which finally become extremely delicate and of uniform diameter throughout. At this time also, the nucleus assumes the form of a crescent which is brought about by the ends of the nucleus approaching, or even passing one another. The mechanism of this crescent formation is difficult to make out and it seems to be in no way connected with the division center for this, at the time of crescent formation, is still small and distinctly outlined near one end of the nucleus (figures 3, 4, 5, 37, 38, 39, 40). It may be due possibly to the resistance of the nuclear membrane against the elongating lines of chromatin, the crescent form being a natural result of such conditions. This is the more probable because the lines of chromatin do not fill the entire space within the crescentic nuclear membrane, but only the concave aspect of the crescent, while a considerable space remains between the convex wall and the chromatin. This space is later filled in by the substance of the division center. The nucleus in the crescent form is regarded by BÜTSCHLI 1888—89 as the „Knäuel" stadium or spireme stage, and this view is adopted by HAMBURGER, who gives proof of it in her excellent description of this stage in *P. bursaria*.

The crescent stage is the most important period in the entire maturation process of *Paramaecium* for it is here that the division

center divides and forms the spindle fibres, and it is in this stage that we find the counterpart of those processes which, in the higher animals, we call synapsis.

The actual divison of the division center we have not seen and we imagine it is more a flow of substance than a clear-cut division such as we find in a metazoan centrosome for example. The material of the division center angments in volume after it leaves the end of the nucleus to take a position in the clear space of the crescent (figures 5, 6, 39, 40) and traces of delicate lines are found in it, the beginnings of the spindle fibres. These at first run from end to end of the crescent, but as the division center divides these fibres are drawn into the spindle which forms at right angles to the crescent, and thus the horns of the crescent do not form the poles of the spindle but opposite points on the nuclear plate. That is to say, the division center does not divide in the long axis of the crescent hut at right angles to that axis, and as the halves separate the spindle fibres are drawn out into a typical spindle figure.

While this change is taking place in the division center the chromosomes are forming in the crescentic nucleus. They arise as double rods from the fine network of chromatin running from end to end of the crescent, and are formed by the transverse division of the fine lines of chromatin so characteristic of the elongated micronucleus. In cases where the nucleus is still in the crescent form, these short wavy threads or loose ends as they appear, are best seen in their full number (figures 6, 7, 8, 41—44).

It is in this crescent stage of the nucleus that the chromosomes divide longitudinally, and this stage therefore represents the nuclear plate phase of the ordinary mitosis. This division of the chromosomes actually takes place long before the spindle is formed, and all stages in the process may be found from the first indication of a split at the end of a chromosome through Y and V forms to the complete separation of the daughter chromosomes after the spindle is completed. We cannot tell for a certainty whether the division of these elements represents a longitudinal division of the original single rod of chromatin, or whether it represents a separation of two previously adjacent rods that have united in synapsis. The latter hypothesis seems to us the more probable because 1) if they represent the original single rods the number of chromosomal elements should be much larger than it is for we would have to have all of the long lines of chromatin fragmenting into pieces of similar short length, and 2), the dividing chromosomes are much thicker than the

lines of chromatin from which they arose, and 3) the lines of chromatin may be traced into a regular network of fusion in the elongated nucleus. From these considerations therefore it appears that the chromosomes of the first maturation spindle represent double elements which have probably arisen by the lateral union of chromatin lines, or what is known as parasynapsis, and in a stage of nuclear activity which in higher animals and plants is known as the spireme, this stage being characterized by the crescent, a structure which seems to be peculiar to the infusoria.

The figures and photographs will give a much clearer idea of this first maturation division than pages of description, the most important point being the change in axis during the division, a feature which appears to be constant in the division of these nuclei in *Paramaecium*, for it is characteristic of the second maturation division and of the early somatic mitoses. The nucleus appears to be a highly plastic structure and the material of the division center a soft permeable mass which penetrates all parts of the nucleus. The nuclear membrane also is flexible and readily takes form from any change in inner configuration. Figure 35 of plate XVII represents a micronucleus at the beginning of conjugation, with the chromatin granules aggregated about the divison center (cf. photograph 1) figure 36 represents an early stage in the elongation of the nucleus and the formation of the lines of chromatin, processes which are further advanced in the nuclei represented in figures 23, 37 and 38 (cf. photographs 2 and 3 plate XII). In all of these early stages the division center remains at the extremity of the nucleus, growing in size as the nucleus grows, and retaining its terminal position until the crescent is formed as shown in figures 24, and 39. Here the network of chromatin is distinctly visible as also in figures 36 to 38 and in photographs 3 and 4, and the division center is represented as sub-terminal in position and partly surrounded by the chromatin. (In the photograph 4, the division center is not clear but its position is indicated by the light spot near the right horn of the crescent). The division center becomes more clearly defined as this crescent stage passes into that of the first spindle, it becomes much larger and moves around until it occupies the central part of the convex side of the crescent as shown in figures 40 and 41, and in photograph 5. At this period of development the chromatin is occasionally condensed into a mass on the concave side of the crescent and with irregular lines of chromatin radiating out from it in all directions (figure 40 and photograph 5). This undoubtedly represents the

post-synaptic or concentration stage which Mc CLUNG (1905) has named the stage of synizesis, and which many cytologist regard as a normal step in the process of maturation. Others however are inclined to interpret this as an artifact, and this would seem to be the more reasonable interpretation in *Paramecium*, for it is very rarely found and then in cases where the fixation and general appearance of the cell are not above suspicion.

Figure 41 and photograph 6 represent the transverse division of the chromatin lines into chromosomes far too numerous to count; the division center is now extended along one side of the nucleus which is still in the crescent form, the section showing only one of the horns. The last chromosomes to be formed are those derived from the chromatin lines at the poles of the crescent, and this tardiness is carried through the entire process of division, the chromosomes derived from this region being the last to divide (cf. figures 44 to 48 and photograph 9 where some undivided chromosomes may still be seen at the edges of the nuclear plate). This figure 41 represents a section of the division center which has now spread over on one side, the section therefore being a horizontal section through the convex side of the crescent. Figure 43 and photographs 7 and 8 represent a more advanced stage in the development of the division center in a section in the same plane as that of figure 41. The division center now extends from one end to the other of the nucleus and the spindle fibres for the first time begin to show. Photograph 8 pictures a similar nucleus in which the division center is shown in section on both sides of the mass of chromosomes, that is, the division center has divided and the two masses now represent the poles of the first maturation spindle, with the chromosomes in the nuclear plate between them, and the spindle fibres extending throughout the nucleus. This division and this arrangement of the substance of the division center explains the characteristic change of axes from the crescent into the first maturation spindle.

Returning now to the chromosomes in the nuclear plate stage, figure 41 represents them as short and somewhat crinkly rods, with here and there an indication of their double nature. The division of the chromosomes takes place at this early period and throughout the nuclear plate chromosomes may be seen in various stages of division. Some of them appear as Y forms, others as Vs, others as rings, and still others as twisted figure 8 forms (figures 42—46). Division therefore is longitudinal and is almost completed by the time the spindle is fully formed, the daughter chromosomes

then appearing as short rods lying end to end, or with a last terminal attachment, such attached forms being the last chromosomes to be formed from the ends of the crescent. Even in the fully formed spindle, these horns of the crescent still appear as points on the equator, a last reminiscence of the change in axes of the nucleus (figures 42–48). A convincing argument that the divisions of the chromosomes is longitudinal and not transverse as previous observers have stated, lies in the fact that chromosomes, still undivided, are found in both the early and late anaphase stages of division. Here they may be in the form of loops, Vs or Ys (figure 48–51) or with the last terminal attachment in the late anaphase (figure 51 and photograph 10).

There seems to be a considerable growth of the chromosomes in length during the process of division, but whether or not this is actually the case is difficult to say. It may be due merely to the straightening of the twisted chromosomes after division, but in later divisions there is actually an elongation as may be seen by comparing the photographs and the drawings of later stages. In some cases the daughter chromosomes are turned over at the ends so as to form loops of considerable length (figure 48), a fact that would seem to indicate the absence of a terminal attachment of spindle fibres to chromosomes, and, indeed, the most plausible interpretation would seem to be that the chromosomes are not attached but simply move along the paths of the fibres. That these lines are actually fibres and not lines or paths of tension in the substance of the division center is indicated by the telophase stages in the subsequent maturation divisions.

The final stage in this first division must take place quickly for in the hundreds of cases that we have examined only twice have we found it. It is characterized by a sharp cleavage through the center of the nucleus which does not draw out into a long telophase stage with interzonal fibers as in the subsequent mitoses. After the division the daughter nuclei remain for a time end to end and their peculiar structure permits of their identification wherever seen. The daughter plate of chromosomes is always nearer one pole than the other (plate XVI figure 52 and photograph 11).

It has been impossible to count the chromosomes in this first division figure, the number being too great and the elements too closely packed together. An approximate enumeration however, made from a cross section of the nuclear plate in the anaphase of the second division, gave 165 chromosomes, but whether or not this represents a reduction in the number characteristic of the vege-

tative nuclei, cannot be stated with certainty, for the fertilized nucleus and the first cleavages of this nucleus are much smaller than the maturation nuclei and the chromosomes are much more densely packed, while the amount of chromatin, notwithstanding the union of the two pronuclei, is less than in the maturation nuclei, a loss, as we shall show in a subsequent section, due to the quantities of chromatin eliminated with the connecting strands of the second and third divisions. The number 165 is at best only approximate, but the number in the early somatic divisions as nearly as we can make it out, is greater than this, and our conclusion is that reduction in number takes place during the synaptic processes of the first maturation nucleus and while in the crescent stage.

Our description of this first maturation division in *Paramaecium* is obviously entirely different from any previously given, and is so unique as to justify a certain amount of scepticism, but we trust that the photographs and the camera-drawn figures will carry conviction. That the process of division is far more complicated than that which R. Hertwig (1889) gives is beyond any question of doubt. He clearly made out the transformation into the crescent or „sickle" form, but is very indefinite in his descriptions of the later change into the spindle, from which we gather that he made out no definite chromosomes but only mere accumulations or „heaps" of chromatin granules which gather in lines in the nuclear plate and along the course of the spindle fibers. He infers that these heaps of granules divide transversely and so form the daughter plates, an inference in no way supported by our observations. Subsequent workers have not added any important observations to clear up the obscurity of Hertwig's and Maupas' accounts. Hamburger 1905 does away with the matter of chromatin granules by clearly showing the origin of short chromosomes in the late crescent, and from the network or spireme of chromatin, but there is a gap in her account from this stage to the anaphase stage of the first division, nor does she give any account of the history of the division center. Nor is Prandtl's account of the first maturation spindle in *Didinium nasutum* entirely satisfactory, the origin of the small chromosomes from the original grains of chromatin being most unsatisfactory, for he says: Alles Chromatin wird in feinen Körnchen wieder auf einem Punkt in der Nähe der Kernwand konzentriert, und es strecken sich von hier erst wenige, dann immer mehr Fäden des Liningerüstes nach der gegenüberliegenden Membranseite, hier einen Pol ausbildend. (1906 p. 233). We cannot find any reason

for this preliminary heaping of chromatin grannles from his account of the later stages and we must infer that a number of intermediate stages have been overlooked. R. HERTWIG's estimate of the number of chromosomes in *Paramaecium* is unquestionably wrong, the four or five spindle fibers in the anaphase of the second division upon which he based his estimate, being a concentration of many fibers after division. His mistake was undoubtedly due to the failure to recognize the real chromosomes, and to his misinterpretation of the heaps of granules.

2. The second maturation spindle (Plates XIII and XVII).

Of the 18 to 24 hours required for the process of conjugation the greater part of the time is taken up in the prophase of the first division and in the separation of the chromosomes, although a large number of stages of the nuclei in the anaphase of division (cf. figures 9, 49 and 50) are found. The second division apparently requires mnch less time, and in this the greater number of stages are those of the late anaphase and of the telophase. As stated above the nuclei of the second maturation period may always be distinguished in the early stages by the asymmetrical position of the nuclear plate (figures 52, 53, 54 and photograph 11). For a long time it was our impression that such nuclei represent the metaphase of the second division, and it was extremely difficult to interpret the constantly recnrring division figures like those represented in figures 55, 57, 58 and 59 and in the photograph 12. These stages give nnmistakeable evidence that the chromosomes are divided again longitudinally for the small shelf-like turn in the center of the chromosomes in the early anaphase as shown in figures 55 and in photograph 12 indicate such an origin of the danghter chromosomes. It was found that this supposedly metaphase stage is in reality only the prophase of the second divisiou, and that the chromosomes arising from the first maturation division are single elements. at first, but later become double by a longitudinal split running from end to end of each chromosome (figure 54). It was also fonnd that whereas the supposed metaphase is characterized by an asymmetrical nuclear plate, the anaphase is perfectly symmetrical and we were led to the conviction that the anaphase of the second maturation division, like that of the first, is formed by a right-angled change of axes. The proof of this is shown in figures 55 and 56 and in photographs 12 and 13 where the original axis of the second

maturation nucleus is indicated by the short shelf-like projections in the centers of the chromosomes. The nearly divided chromosomes such as those shown in figure 55 arise from V formed elements as shown in figure 56 and in photographs 12 and 13. The nucleus shown in figures 55 and 56 and in photographs 12 and 13 is particularly illuminating in this connection, for, figure 55 and photograph 12 represent one section of the nucleus in the anaphase, while figure 56 and photograph 13 represent the next section of the same nucleus. In the latter section the chromosomes lie on the periphery of the nuclear plate and are not as far advanced in division as the more central chromosomes shown in the former figure and photograph. They also show a differently turned long axis, in fact an axis turned in the same direction as the small shelves in the center of the chromosomes in figure 55. They are not exactly at right angles to the long axis of the divided chromosomes, but they indicate how the divided chromosomes in the anaphase stage have arisen from the longitudinally split chromosomes of the prophase, and this evidence, taken with that of the re-established symmetry of the spindle figure, is sufficient to warrant the assumption that division occurs through a change in axes.

We have not been able to trace the actual division of the division center, but have reason to believe that, as in the first maturation nucleus, the second maturation nuclei are highly plastic, and that division is in reality only a flow of substance in opposite directions, this flow carrying with it the loose ends of the longitudinally split chromosomes.

As figures 26, 57 and 60, clearly show, both nuclei of the second maturation stage divide, and are without evidence of degeneration until after the second division. Immediately after this division, however, both resulting nuclei of one of them begin to degenerate, and degeneration is sometimes well advanced before division of the other nucleus is accomplished (figure 55). In some cases again, four long drawn out nuclei in the telophase stage may be found in the two cells and this apparently, is the normal process. The elongated telophase is distinctive of the second and third divisions, and marks these mitoses sharply off from the first maturation division. The connecting strand consists of innumerable fibers interspersed with chromatin (figures 60 and 61 and photographs 14 and 15). This stage, apparently, takes some time for it is frequently found and cannot be mistaken for anything else. The resultant daughter nuclei (figures 61 and 62) have a different fate, one degenerating, the other

dividing again to form the pronuclei. In both however, there is the same characteristic change in the chromosomes from compact and apparently homogeneous elements, to lines of granules evidently strung along on spindle fibers and running from pole to pole of the nucleus, and, with this change in structure, the micronucleus is ready to form the pronuclei by a third division.

According to this interpretation of the second maturation spindle it appears that both first and second divisions of the chromosomes are longitudinal and that no transverce division, or reduction, in WEISMANN's sense, occurs here. The evidence, however, is so convincing that this interpretation is correct that no other possibility is suggested, and since it falls in line with some of the more recent work on the maturation chromosomes in higher animals and plants we adhere to it.

Turning now to this stage in the maturation in other protozoa we find again, that our description differs entirely from the published accounts of others. For *Didinium*, PRANDTL gives nothing but the stereotyped scheme of metazoan maturation, and HAMBURGER overlooks again the stages intermediate between our prophase and telophase. Her figures representing what she interprets as the metaphase of the second division show the same asymmetrical arrangement of the chromosomes, while her telophases are quite symmetrical, and as she does not go into the matter of chromosome division in detail, we infer that she regards this division like the first, as transverse. Neither MAUPAS nor HERTWIG went into the details of this second maturation mitosis, and as the maturation process in other infusoria has not been worked out, we are forced to find our analogies in groups other than the protozoa.

A word as to the degeneration phenomena before passing to the curious third division of the micronucleus. This has been so often and so well described by our predecessors that we need not add to the length of the present paper by going over it again. An interesting and significant feature is the loss of the material of the division center. In the functional nuclei this is an important part of the nuclear material, but in the degenerating nuclei it is the first to disappear, and with its disappearance comes the increasing concentration of the chromatin into, finally, a homogeneous spherical mass which persists in the cytoplasm until after the union of the pronuclei.

3. The third maturation spindle (Plates XIII and XVIII).

It is perhaps, a misnomer to call the division figure of the third division a spindle, and we would probably come nearer the truth if we described this as direct division of the nucleus instead of mitosis.

This third division is different from both of the others and has a more interesting theoretical importance. There is no change in axes and no longitudinal division of the chromosomes, and there are no definite spindle fibers. The chromosomes resulting from the second maturation division, as we have seen, break apart into granules, these granules lying apparently along the course of the spindle fibers. The third division consists in the separation of one group of granules from another group derived from the same original chromosome. That is, the third division is transverse separating different elements of the original homogeneous chromosome that had twice divided longitudinally.

Another important and significant feature of this third division lies in the fact that the dividing nucleus is heteropolar. One pole is measurably smaller than the other, and the smaller portion forms the wandering, or male, pronuclens, while the larger portion forms the stationary or female, pronucleus. This difference in poles shows very clearly in figures 64 and 65 and in photographs 16 and 17, and the same difference is maintained throughout the division. The smaller nuclens is not always the one nearer the point of migration into the other cell, and we cannot confirm MAUPAS' statement that the wandering nuclens is a chance nucleus that may happen to be in the vicinity of the protoplasmic bridge at the time of interchange. The significance of this sexual differentiation will be considered under the general discussion.

One of the most important features of this third division is the elimination of chromatin which takes place here. The division figure is characterized by a very long and curved connecting piece which is made up almost entirely of chromatin condensed in a homogeneons rod (figures 66, 67 and 68 and photograph 18). With the loss of chromatin the nuclei lose their deep staining capacity, but we do not interpret this as due to any change in the chemistry of the staining reaction, but rather to the diminution in quantity of material to be stained, and the two pronuclei appear to be the same in finer structure althongh differing in size. The material that is lost here, plus that lost in the large connecting strand of the second maturation

division, sums up a good loss of the nuclear material, and the pronuclei, when ready for union, have a much less dense appearance than do any of the preceding nuclei.

The details of this third division can be made out almost as well with total preparations as with sections and our account, therefore, does not differ in essentials from that of our predecessors. The difference in size of the pronuclei, indicating as it does a possible differentiation into female and male nnclei, has not been noted before in the case of any species of *Paramaecium*, but PRANDTL describes such a difference in the pronuclei of *Didinium*, and calls attention to the fact that in MAUPAS' figures of the pronuclei a distinct difference can be made out, and he points out the same difference in HOYER's figures of the pronuclei of *Colpidium colpoda*, in neither case however did the author speak of the difference. The difference in the *Didinium* nuclei is about the same as that in *Paramaecium* so far as the measurements go, but the pronuclei in *Paramaecium* are not surrounded by definite radiations such as PRANDTL describes in the cytoplasm of *Didinium*. That the difference is real and not due to pressure etc. is shown by the measurements of a number of cases which I subjoin together with measurements of the micronuclei given by PRANDTL, as follows:

	Paramecium aurelia (caudatum)				*Didinium nasutum*			
	Length in μ		Width in μ		Length in μ		Width in μ	
	Male	Female	Male	Female	Male	Female	Male	Female
1st pair	17	19	11	11	7.8	9	5.2	7.2
2nd "	15	16	11	12	7.8	9	7.8	9
3rd "	13	15	9	12	7.8	9	5.2	7.2
4th "	12	18	8	10	9.8	10.4	5.2	8.5
5th "	13	15	10	12	7.8	7.8	5.1	6.5
6th "	13	17	7	9	6.5	9	4	7.8

4. Union of the pronuclei (Plates XIV and XVIII).

There is nothing in the union of the pronuclei of *Paramaecium* that has not been noted by previous observers. We agree with HAMBURGER that the union is first at one pole of the pronuclei, and that the fusion from this time on makes slow progress. The membrane separating the two bodies dissolves slowly and the beginnings of the first vegetative division are seen even before union is completed (figures 27 and 70, and photograph 19). This union takes place close

to the point of union of the two organisms, and the chromatin is in the characteristic granule form of the pronuclei. At this period there is the same measureable difference in the fusing pronuclei that we have seen in the formation of the pronuclei, the measurements of the case represented in photograph 19 being 18 μ : 10 μ and 15 μ : 6.5 μ respectively.

5. Reorganization of the nuclear apparatus (Plates XIV, XV and XVIII).

The main features of this process were accurately described by Maupas and by R. Hertwig, the latter especially giving an excellent account of the disintegration of the macronucleus and formation of the new ones.

The first division of the fertilized nucleus takes place before the chromosomes are organized and, as stated above, even before the nuclei are completely united. We have not been able to follow this division as completely as the others and it is evident that it takes place very quickly. Hamburger obtained much more satisfactory results with this stage than we have, and there is reason to believe that the process is the same in the two cases. She found that the individuality of the pronuclei continues even into the telophase, and that complete union is accomplished only in the reorganization following the first vegetative division. Certainly in the second division the chromosomes are completely re-formed and appear in the nuclear plate of the second division in the same form as in the maturation nuclei (figures 71 and 72 and photograph 20). The nuclei are all much smaller however in the vegetative mitoses and the history of the chromosomes much more difficult to make out. Divisions follow one another rapidly until eight nuclei are present, four at each end of the degenerating macronucleus. The distribution of these two sets of four nuclei is very interesting for it does not take place until the third division, the first two divisions ending without long connecting strands (figures 72 and 73). The third division differs from the first two in having long connecting strands in the telophase, the great length of the strands resulting in the separation of the two groups of nuclei by the length of the macronucleus (figures 74 28 and 29).

Disintegration of the old macronucleus does not begin until the second division of the vegetative nucleus, and the first indication, as Maupas and Hertwig early pointed out, is the peculiar vermiform

structure that it assumes (figures 72—74, 28—29 and photograph 20). The material of the old nucleus then arranges itself into a tightly wound and later a loosely-wound thread or skein, the meshes of which become thin in spots (figure 28). Later the strands fragment at these thin spots and the old macronucleus is thus dissolved into a group of at first ellipsoidal, and later into spherical granules, which gradually lose their staining capacity and ultimately disappear, although evidences still persist in the third and fourth division of the cell body some days later (figures 30—32).

The new macronuclei are formed from four of the eight micronuclei. The first step is the loss of the chromosome structure and distribution of the chromatin in the form of granules throughout the nucleus. Then the chromatin metamorphoses into a more fluid-like substance which fills out the distended nuclear membrane, while residual chromatin granules are collected in the center of the nucleus. When submitted to the action of the hardening fluids this more liquid substance of the nucleus contracts away from the nuclear membrane just as it contracts upon fixation in the later stages. This material is very finely granular and appears perfectly homogeneous while the larger granules of chromatin in the center gradually lose in size and definition (figures 33, 34 and photograph 21).

In the meantime the micronuclei dwindle in size, lose their linear appearance which characterizes the first two or three divisions of vegetative life, and become much more homogeneous and granular in appearance. They are distributed unevenly throughout the cell, and are often difficult to distinguish from the degenerating fragments of the old macronucleus. The division center is localized in one pole of the nucleus and the granules are arranged about it. In this stage, before the young macronuclei are fully developed, the cell divides once and then once again, before it is re-established with the normal nuclear relations and ready to start another cycle of activities as a new *Paramaecium* individual.

General Discussion.

In the remarkable series of events which we have described in the preceding pages lies the secret of that unfathomable marvel, the beginning of a new individual. In the life history of such an

individual *Paramecium* it has been shown that there are well marked variations in vitality accompanied by definite structural changes, variations which lead finally to pecnliar periods of depression and physiological death, which however may be averted by artificial stimnli. It has been shown further, that this artificial stimulation is not successful an indefinite number of times and that a period finally comes when all attempts to revive the flagging energies fail, and the individual dies of old age no less surely than does the higher animal and plant. And now, in this chain of remarkable processes taking place in the conjngating Paramecium we have seen as much perhaps as can be made ont at the present time, of the phenomenon of revitalization through fertilization. We have seen that the quantity of chromatin increases to many times its original volume, the relatively large mass of chromatin in the solid chromosomes of the first maturation nucleus being a striking contrast to the few scattered granules in the original nucleus; we have seen that these chromosomes divide twice by ongitudinal division, and a third time by transverse division, and we have seen finally, that the greater bnlk of the chromatin is again thrown off during the second and third divisions, to degenerate in the cytoplasm. The most important elements of the new individual are formed from the fusion of two of these purified (?) micronuclei and this combination nucleus, since the cytoplasm remains for the most part the same, must hold the potential of vitality of the new individual. Strictly speaking the cytoplasm does not remain the same for it is enriched by the addition of material dissolved into it from the old macronucleus, from the disintegration of three of the micronuclei of the maturation divisions, and by the slight interchange of cytoplasmic materials which takes place while the two cells are united, but these additions to the cytoplasm with the exception of the last perhaps, are all micronnclear derivatives in the long rnn, the disintegrating macronucleus itself, coming from a previously fertilized micronucleus.

The details of the processes of chromosome division and maturation of the nuclei in *Paramaecium* are strikingly similar to those of the germ cells of higher animals and plants in the underlying features, and in the singular adherence to the form of the operation in the maturation of eggs and spermatozoa. And the marvel in *Paramaecium* is all the greater that here the new individnal arises Phoenix-like from the ruins of the old. The similarity of the phenomena is so great that interpretation of the one must apply equally to the

other, for protozoon and metazoon present, in these processes, only modifications of the same mechanism in Nature. The modifications however, are important and may throw some light on the final interpretation of the phenomena, which, all will agree, is far from being established at the present time.

The first and most important of these modifications has to do with the theory of the individuality of the chromosomes, a favorite hypothesis amongst biologists ever since WEISMANN's classical essays on the nature of the germ plasm, and one which has been strengthened and supported by experiments on MENDELIAN inheritance on the one side and observations touching on the specificity of chromosomes on the other. The one, experimental, tending to show that the germ cells in respect to a given specific character are pure, the other, morphological, tending to show that such characters, or complexes of characters, are held in certain chromosomes of the maturation period. The former maintained by BATESON and other experimenters of the MENDELIAN school, the latter, by BOVERI, WILSON, MC CLUNG, MONTGOMERY, SUTTON, STEVENS and other cytologists. A number of observers have shown that the chromosomes after mitosis pass into the reticulum of the resting nucleus without losing their identity, and emerge again at the time of the next mitosis from the reticulum passing apparently by the same routes into the new chromosomes to be equally divided. Such evidence in favor of the individuality of the chromosomes in higher forms of life cannot be found in *Paramaecium*, for here, in the vegetative divisions at least, the chromosomes are very different from those of the maturation divisions, both in size and in number, while the huge number of chromosomes in the first maturation nucleus arises from only 12 or 15 chromatin granules at the outset of conjugation. In *Paramaecium* therefore, if the chromosomes, at maturation, represent specific potentials of the later individual, they must fuse, in the vegetative periods to form chromatin granules which represent not specific, but aggregates of specific characters. We would interpret the maturation chromosome in *Paramaecium* therefore, as a much more simple element than the maturation chromosome in higher forms of life, representing, possibly, only one specific character of the later organism, whereas in metazoa the single chromosome must represent a group or complex of characters. The large number of chromosomes in *Paramaecium* is difficult to interpret on any other hypothesis, and if they represent specific characters the large number is necessary for it must never be forgotten that the single celled

organism has the same number of functions to perform as the higher animal or plant.

In other types of protozoa the nuclei and chromosomes are for the most part different from these in *Paramaecium*. In the flagellates for example, there are usually a few granules of chromatin which at the time of maturation are equally divided and reduced to one half the number, sometimes with, again without, the formation of tetrads (*Trypanosoma*, *Herpetomonas*, *Trypanoplasma* etc.). These we would interpret as chromosomes of a higher type than those of *Paramaecium*, and more like the chromosomes in metazoa. In *Noctiluca* (CALKINS 1898), they are more like *Paramecium* again, the 9 to 11 chromatin reservoirs representing the aggregates of chromosomal elements comparable with the few granules in the resting nucleus of *Paramaecium*.

In other words, we interpret the maturation chromosome of *Paramaecium* as the simplest known type of chromosome, representing, ex hypothesi, the unit character of the later organism, the multitude of these single elements taking the place of the complex chromosomes characteristic of higher forms. We believe that with the evolution of the animal and plant types has gone the evolution of the nuclear structures, and that the chromosome has evolved no less surely than the nervous system, and from a relatively "simple homogeneity to a complicated heterogeneity", the maturation chromosomes of the mammal representing the highest type of development of these structures.

This view of the *Paramaecium* chromosome is in no way opposed to the trend of current opinion regarding the nature of the reducing divisions in germ cells, an opinion which seems to be gradually taking definite form despite the varied and apparently contradictory results. Briefly reviewing the history of this opinion, we find that biologists go back to WEISMANN's essays on development and inheritance in 1887 when he made the remarkable prophecy that two kinds of division would be found, the one involving a longitudinal split of the chromosome, the other a transverse division. The former had already been recognized and WEISMANN called it an equal or equations division, since it affected only the quantity of the chromatin, while the latter, a matter of pure speculation on his part, for it had never been observed, he conceived as taking place in such a way that each daughter nucleus would contain only half the number of germ plasmic elements contained in the mother nucleus, and he ventured the opinion that it might be transverse. He called

this second division a reducing division, holding that different qualities or characteristics were resident in definite portions of the thread or chromosome and that such a division would affect the quality of the nucleus. This reducing division, he surmised, would take place in the formation of the polar bodies and in the analogous stages in spermatogenesis.

The fulfillment, in essence, of WEISMANN's prediction was one of the spectacular events in biology and within five years it had been practically demonstrated in a variety of forms, but the results of the investigations which his speculations called forth have led to the most contradictory interpretations of the maturation divisions and to such a tangle of contrary statements that biologists are still unable to see the light. The divergent views may be brought together under two heads, first, those maintaining that the one division is transverse and the other longitudinal, and second, those holding that both maturation divisions are longitudinal. Amongst those holding to the former view are KORSCHELT (1895), GRIFFIN (1899), VEJDOWSKÝ (1903), SCHOECKERT (1902), and FOOT and STROBELL (1905), all of whom worked on annelids; HENKING (1891), RÜCKERT (1894), VOM RATH (1892 and 1895), HAECKER (1892 and 1895), PAULMIER (1899), MC CLUNG (1900), SUTTON (1902), MONTGOMERY (1901 and 1905) all working on arthropods, and, in addition, PROWAZEK (1901 and 1902) on *Helix*, LILLIE on *Unio* (1901), and DUBLIN (1905) on *Pedicellina*. For plant organisms SCHAFFNER (1897), GREGORY (1904), STRASBURGER (1904), and FARMER and MOORE (1905) concluded that the first division of the spore and pollen mother cell is transverse.

On the other hand, many observers both zoologists and botanists have found that both of the maturation divisions are longitudinal. BOVERI (1887) in his paper on *Ascaris* was the first to observe this, and HERTWIG (1890), BRAUER (1893) and TRETJAKOFF (1904) confirmed him. The same fact was ascertained by FLEMMING (1887) in his work on the salamander, BOVERI (1890) on molluscs and *Sagitta*, MEVES (1897) on the salamander, and (1902) on molluscs, MC GREGOR (1899) on *Amphiuma*, VON LENHOSSÉK (1898) BRYCE (1902) on echinoderms, SCHREINER and SCHREINER (1904) on *Myxine* and (1905) on *Tomopteris*, and by the botanists SARGANT (1895), STRASBURGER (1895), GUIGNARD (1899), BERGHS (1904 and 1905) GRÉGOIRE (1899) and ALLEN (1904 and 1905). The difficulty with the double longitudinal divison is to ascertain which, if either, is the reducing division in the WEISMANN sense.

Gregoire (1905) has made an effort to bring all of the results thus far obtained into conformity with his so-called heterohomotypical scheme. According to this the first division separates the two constituent chromosomes from one another, and at the end of the metaphase, or during the anaphase of the first division, the daughter chromosomes undergo a longitudinal division. In the second maturation mitosis these longitudinal halves separate from each other. In such a case the process would be a pre-reduction. There are not a few observers however, who seem to have found a post reduction in various animals, a fact making no real difference theoretically in the result, vom Rath (1895), Rückert (1894 and 1895), Griffin (1899) Vejdowský and Mrazek (1903), working on animal oogenesis and McClung (1900 and 1902) and Sutton (1902) on spermatogenesis comprise a few of those who have obtained this result. According to Grégoire's heterohomotypical scheme there could be reduction only in the first division, since it separates the original chromosomes from each other, and he ungenerously rejects all accounts which disagree with his interpretation as due to inaccurate or to superficial observations. It is quite obvious that in any case an interpretation is valueless unless the origin of the double chromosome is known, and this leads us to the problem of synapsis.

Moore (1895) in his work on the spermatogenesis of elasmobranchs gave the name synapsis to that phase in the development of the sex cells in which the chromatin, while concentrated into a dense mass at one side of the nucleus, unites to form the double chromosomes. This contraction with synapsis, he thought, takes place before the chromosomes of the first maturation division are formed, and he believed that the reduction in the number of chromosomes takes place during this stage since only half the somatic number of chromosomes emerge from it, and these are markedly double. But there is still doubt in regard to the nature of this contraction phase, which has not been found to occur universally. McClung (1902), Schreiner and Schreiner (1905) and Schaffner (1906) believe that it is merely an artifact, while many other zoologists and botanists declare it to be a constant and important feature of maturation. Sargant (1897) found it in the living cells of *Lilium*, but they had first to be teased from the anthers and manipulated under the microscope, so the conditions were hardly normal. Cardiff (1906) considers this contraction a constant morphological feature of the mother cell and makes the interesting suggestion that here with the accompanying synapsis, is the „critical stage in the history of the organism, the

end-result of fertilization" for it is only at this time that the real mingling of ancestral nuclear elements takes place. Since its original use by MOORE a loose application of the term synapsis has been made, and gradually it has come to refer not only to the union of chromosomes, but to the entire contraction phase as well, that being wanting in some animals and plants. MCCLUNG now (1905) restricts the term to the actual fusion-stage of the chromosomes, and gives to the contraction phase, which occurs after synapsis, the name syuizesis.

The union of chromosomes occurs, according to MONTGOMERY, DUBLIN, STEVENS, and SUTTON, during the telophase of the last spermatogonial or oogonial division and just preceding the growth period, but it does not become apparent until much later. MONTGOMERY (1900) was the first to point out that in this union of two chromosomes in synapsis, one probably comes from the male, and the other from the female parent. SUTTON confirmed the observation in the case of the lubber grass hopper *Brachystola*, and was the first to clearly establish the union of like chromosome with like, and subsequent observers have abundantly confirmed him.

The union of chromosomes in this way brings about a reduction in the number, and the bivalents thus formed may, according to the species in which they occur, remain simple double rods, or give rise to all of the various shapes which chromosomes may assume, Vs, Ys, twisted forms rings or double crosses, all of which are reducible to the double rod. Such chromosomes may be concentrated into smaller four-sided tetrads, or they may go into the maturation spindle in their original forms.

The manner in which the like chromosomes unite is highly important, for a knowledge of this point in any case is absolutely necessary as a basis for a correct interpretation of the maturation divisions. Here, however, observations differ as to the details. According to one group of observers, headed by MONTGOMERY, who has described the mode of chromosome union in *Peripatus* (1900), Hemiptera (1901), and in amphibia (1903) the chromosomes unite end to end, and SUTTON observed the same method of union in *Brachystola*, BLACKMANN (1907) in *Lithobius*, DUBLIN (1905) in *Pedicellina*, and FOOT and STROBELL (1905) in *Allolobophora*, while the botanists FARMER and MOORE (1905), GREGORY (1904) and STRASBURGER (1904) found it in different plants.

Another set of observers however, holding just as tenaciously to their conclusions, maintain that the chromosomes unite side by

side. WINIWARTER (1900) was the first to note this in his observations on the oogenesis of the rabbit and of man, and SCHOENFELD (1901) noted a like union in the spermatocytes of the ox, MARÉCHAL (1904) in elasmobranchs, MOORE and EMBLETON (1906) in Amphibia, SCHREINER and SCHREINER (1904) in *Myxine* and (1905) in *Tomopteris*, TRETJAKOFF (1904) in *Ascaris* and STEVENS (1905) in *Aphis*. In plants GRÉGOIRE (1904), BERGHS (1904 and 1905), ALLEN (1904), ROSENBERG (1904) and OVERTON (1905) have found a similar conjugation of the chromosomes side by side.

It has been observed that in those cases where two longitudinal divisions take place, the synapsis preceding the first division has been by union side by side or by parasynapsis. In general, investigators have interpreted the first division as a reducing one, being merely a re-opening of the synaptic space, and the second as an equal division. TRETJAKOFF (1904) holds, however, that the second is the reducing division. Some workers, chiefly early ones who presupposed end to end union or telosynapsis, believe that both divisions are equal and that there is, consequently, no reduction in WEISMANN'S sense. BONNEVIE (1905) is the latest one to set forth this view in her paper on *Enteroxenos*.

In those species where one maturation division is transverse and the other longitudinal, the union of chromosomes appears to be end to end. The transverse division is, doubtless, a reducing division separating as it does the two entire chromosomes constituting the bivalent, and the longitudinal division is, therefore an equation division. May it not be then, that these apparently conflicting results may be reconciled in the possibility that two longitudinal divisions always follow parasynapsis, while one transverse and one longitudinal division always follow telosynapsis?

Turning again to *Paramaecium* the maturation divisions of the chromosomes are both longitudinal and if our rule is to work both ways the first of these divisions should be preceded by parasynapsis. We have seen that the chromosomes are formed by the transverse fragmentation of the long lines of chromatin in the crescentic nuclens, and that they are double when they emerge from the crescent into the nuclear plate. The long narrow, drawn out nuclens, and the linear arrangement of the chromatin make it almost a demonstration that telosynapsis is impossible, and that the chromosomes, if they unite at all, do so by typical parasynapsis. We assume, therefore, that in *Paramaecium* the unit-characters, or at least simple chromosomes, unite side by side in parasynapsis, and that as in

higher animals the union is probably between male and female chromosomes representing the same character. The second maturation division is an equation division and halves the simple chromosomes, the split occurring as we have seen, in the primary nuclear plate phase of the second maturation division (figure 54).

The third division of the nucleus in *Paramaecium* has nothing to do with the problem of reduction in WEISMANN's sense, but it offers a very pretty problem in protozoan cytology, and throws some additional light on the question of male and female chromatin.

We have seen that after the second division of the maturation nucleus the chromosomes lose their homogeneous character and disintegrate into lines of chromatin granules, this occurring sometimes in the telophase of the second division. In some cases furthermore, the disintegration occurs in one of the daughter nuclei while the other retains the characteristic homogeneous structure of the chromosomes (photograph 15 and figures 61 and 62). The former indicates the nucleus of the second spindle that is destined to form the two fertilization nuclei, while the latter degenerates as one of the polar body equivalents.

The linear arrangement of the chromatin granules in the third division of the nucleus is significant for it shows that each chromosome provides some of the material of the wandering pronucleus, but that the chromosomes are not divided in the middle is shown by the diverse size of the two daughter nuclei. The significance of this peculiar division lies in the fact that it must have something to do with the male pronucleus and with the biological phenomenon of the double or mutual fertilization.

If WILSON's (1905, 1906) interpretation of the idiochromosomes in hemiptera as sex determining chromosomes is correct, then we have in the metazoa a possible analogy with what takes place here in *Paramaecium*. In some cases he finds one more chromosome in the somatic cells of the female than in the male, and in other cases where the number is the same, one of the idiochromosomes is larger in the female than in the male. In metazoa the chromosome stands for an aggregate of characters and it is not improbable that such chromosomes represent complexes of characters that are either male or female. In *Paramaecium* there is a like difference in the quantity of chromatin in the two pronulei, and, in mutual fertilization, the one with the smaller quantity migrates to fuse with the larger nucleus of the other cell. But in *Paramaecium* there is no obvious difference in the conjugating individuals that can be interpreted as sexual

differentiation and the significance of a male nucleus is difficult to understand for that reason. It may be a case where the female characters are always dominant. We believe that we have found a rudimentary sex-differentiation in the difference in vitality of the two ex-conjugants, a difference suspected by CALKINS in his experiments on the life history of *Paramaecium* (1902) and proved by Cull in her observations upon the vitality of ex-conjugants (1907).

In other protozoa there are, here and there, instances of the elimination of chromatin in sexually differentiated forms. The best instance is that of the flagellates belonging to the genus *Trypanosoma* and allied forms in which SCHAUDINN (1904), PROWAZEK (1905), and KEYSSELITZ (1906) for different species, have shown that there is an elimination of a certain part of the nuclear contents (blepharoplast material) in the development of the female organism, and an elimination of the whole or part of another portion (the trophonuclens) in the development of the male. In these cases there seems to be an unmistakeable connection between the male characters and the specific part of the fertilization nucleus to which WOODCOCK (1906) gives the name kinetonucleus, and a similar connection between the trophonucleus and the female characters. In these cases therefore we find a dimorphism in the nuclei of the vegetative forms, and a dimorphism of the same type as that between the pronuclei of *Paramaecium*. In *Trypanosoma*, however, the kinetonuclens or male nucleus gives rise to daughter elements which become the nuclei of eight microgametes, and the chromatin of this kinetonucleus differs from that of the male nucleus in *Paramaecium* in holding the specific characteristics of a male organism. How are we to correlate the two types?

There is little evidence to indicate the lines of evolution that have been followed in the development of the participants in conjugation. The view that is usually adopted, without supporting evidence, is that the isogamous type like that of *Paramaecium*, was primitive and has developed into an anisogamous type with sexually differentiated gametes (e. g. HARTOG 1906). It is our belief that the reverse has been the case and that the *Paramaecium* type of conjugation has arisen from a type with sexually differentiated gametes, with intermediate stages in forms like the Vorticellidae, where the size difference is great in *Lagenophrys ampulla*, less marked in *Epistylis*, and still less in *Vorticella;* and in Trachylinidae, where in *Lionotus fasciatus* the two organisms are alike save for a slight difference in size (CALKINS 1902). In the

Vorticellidae the microgamete fnses with the macrogamete and there is no mutual fertilization, but in *Paramaecium* and probably in *Lionotus*, mutual fertilization takes place. The case of *Lionotus* is to be interpreted as a reminiscence of anisogamy, and we would expect in this case, that the smaller conjugant, if fertilized, would have a reduced vitality. In *Paramaecium*, finally, there is no morphological evidence of tbe relation to an earlier anisogamous condition, but there is well-marked physiological evidence in the lesser vitality of one of the ex-conjugants, apparent in 72 % of all conjugations in which the history of both was followed (CULL 1907).

It appears to us therefore that the specific male chromatin which in *Trypanosoma* is early separated from tbe tropbonucleus and the female chromatin, is retained in the micronucleus of *Paramaecium* until the third division, and that here it does not lead to the formation of microgametes but fuses directly with the female pronucleus of the other organism, a mutual fertilization taking place as a result of the equal potency of the conjugating individuals, but in one of which we still find a reminiscence of an original male gamete in the relatively weaker vitality.

At the present time biologists are devoting much time and attention to the factors of development in higher animals, and the general tendency is towards the view that differentiation, whether normal or regenerative, is brought about by the action of specific protoplasmic stuffs. Some have located such determining elements in the so-called formative stuffs of the egg cell, and some have carried the analysis stil further by tracing the origin of snch stuffs back to tbe chromatin of the egg nucleus (e. g. LILLIE 1906). In *Paramaecium* we have noted that certain functions are intimately connected with the macronucleus while others, apparently, have nothing to do with it (CALKINS 1904). Thus the ordinary vegetative activities do not appear to have anything to do with the micronucleus but seem to vary with the conditions of the macronncleus. Here, then, in the single cell, we find differentiation into at least two kinds of protoplasm which have been distinguished as somatic and germinal respectively, the former having to do with the individual (in the wider sense), the latter with the race. We have been unable to tell from morphological data which of the first eight somatic nuclei are destined to form four macronuclei; so far as we have seen there is no difference in the chromatin of these nuclei until that swelling begins which characterises the young macronucleus. In such forms the chromatin is concentrated in a central granular

mass from which a more fluid and a more homogeneous chromatoid material exudes and this condenses, on hardening, into a more solid mass with grannles in the center (Fig. 33 and 34 photograph 21). Here, differentiation takes place, therefore, first by nuclear division, and second, by chromatin metamorphosis, and once transformed, the diffuse chromatoid substance cannot return to the concentrated form from which it arose, and the macronucleus is ever after a macronucleus until its ultimate dissolution. Here then, is an early differentiation of the chromatin itself, which can scarcely be laid to a „function of position", nor to the effect of action of the surrounding parts or environment upon the elements of the cell. We are absolutely at a loss to understand why, from external sources at least, the third division of the fertilization nucleus, gives rise to four macro- and four micronuclei, but by exclusion of all external factors which must operate equally on all eight nuclei, we are led to the conviction that the determining factors are within the chromatin, and that they find their expression at the time of the third division for the same reason, probably, that at a given time the egg of a metazoon gastrulates, or differentiation of the various organs begins. In other words, we have to do here with a fundamental biological problem, the solution of which would carry us well along towards the solution of the great problem of development.

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Descriptions of Photographs and Figures.

Plate XII. Photographs.

Figure 1. Photograph of micronucleus and macronnclens at the commencement of the matnration processes. (Cf. figures 35.) × 1200 diam.

Figures 2 and 3. Photographs of elongation stages of the micronucleus showing the division center at the right end in each case, and the elongating lines of chromatin forming a black network. (Cf. fignres 36, 37, and 38.) × 1200 diam.

Figures 4 and 5. Photographs of the nnclens in the crescent phase to show the division center on the right convex side and the chromatin in 4, in characteristic net form, while in 5 it is in the so-called synizesis stage. (Cf. fignres 39 and 40.) × 1200 diam.

Figure 6. Micronncleus immediately after the crescent phase showing the short chromosomes and the division center drawn out on one side of the former crescent × 1800 diam.

Fignres 7 and 8. Photographs of the micronuclens in the nuclear plate stage showing the concentration of the material of the division center at the two poles at right angles to the former long axis of the crescent. The fine striations indicated are the spindle fibers. The chromosomes in each figure are in characteristic division forms. (Cf. figures 41 to 46.) × 1800 diam.

Fignre 9. Photograph showing two micronnclei in the early anaphase of the first maturation division. Details of chromosome structure can he seen in each. (Cf. figures 47 to 50.) × 1800 diam.

Figure 10. Photograph of the micronucleus in a later anaphase stage. A few lagging chromosomes in the center are not yet divided, there is no elimination of chromatin at this division. (Cf. Fig. 51). × 1800 diam.

Plate XIII. Photographs.

Figure 11. Photograph of the micronucleus at the end of the first maturation division. The asymmetrical position of the chromosomes is characteristic of the nncleus immediately after the first division. The chromosomes are not yet divided preliminary to the second maturation division. The nnclens shown in the cell on the left is a cross section of the second maturation spindle in the telophase and is the eqnivalent of the larger nncleus after division. (Cf. figures 52, 53, and 54) × 1800 diam., the relatively enormous size of this nuclens is due to flattening.

Figure 12. Photograph of micronnclens in the early anaphase of the second maturation division. In the center the short shelves at right angles to the long axis indicate the last point of attachment of the longitudinally divided chromosomes. (Cf. figures 55, 57 and 58.) × 1200 diam.

Figure 13. Photograph of another section of the same nnclens as that shown in figure 12 and is a tangential section with only a few chromosomes. Some of these are in V form and indicate the axis in which the chromosomes originally lay, at right angles to the long axis of the dividing nncleus. (Cf. figures 56.) × 1800 diam.

Figures 14. Photograph of a late anaphase stage of the second maturation division. The interzonal spindle fibers are thickened by fusion, and are beginning to take the stain. (Cf. fignres 60, 61 and 62). × 1800 diam.

Figure 15. Photograph of micronucleus in the telophase of the second maturation division showing the greater concentration of the interzonal fibers and disintegration of the chromosomes into granules. The apparent difference in size of the two ends is due to the fact that one is slightly out of focus and beginning to degenerate. × 1800 diameters.

Figure 16. Photograph of the micronucleus at the outset of the third division. The difference in the two poles is already noticeable and the chromatin of the male and female pronuclei can be distingnished. The nucleus shown on the right is in a similar stage but is cut in cross section. (Cf. figure 63.) × 1800 diameters.

Figure 17. Photograph of the micronucleus in the early anaphase of the third division showing clearly the heteropolar condition leading to the differentiated pronuclei. The nucleus on the right is in a slightly earlier stage than that of figure 16 left. (Cf. figures 64 and 65.) × 1800 diam.

Plate XIV. Photographs.

Figure 18. Photograph of the micronucleus in the late telophase of the third division showing clearly the difference in size of the two pronuclei; the granular chromatin and the heavy connecting strand packed with concentrated chromatin. (Cf. figures 66 and 67.) × 1800 diam.

Figure 19. Photograph of the pronuclei uniting. In the lower cell both pronuclei are in focus and the difference in size can be made out although not so clearly as in the object itself owing to a slight inclination in the section. In the other cell the larger, or female pronucleus, is in focus while the smaller lies below. (Cf. figure 70.) × 1200 diam.

Figure 20. Photograph of the macronucleus and micronucleus; the latter in full mitosis for the second somatic division. The macronucleus shows the vermiform structure characteristic of the beginning of disintegration. The micronucleus shows the densely packed chromosomes and their asymmetrical distribution in the nucleus recalling the early phases of the second maturation division. (Cf. figure 71.) × 1800 diam. (Note the small size as compared with the maturation nuclei.)

Figure 21. Photograph of the cell during metamorphosis of the micronuclei into macronuclei. Three young macronuclei and two micronuclei are in focus. In the former the denser central chromatin masses are clearly marked off from the lighter and more fluid peripheral substance. The micronuclei are in two stages of condensation, the upper one (to the right) showing the membrane. (Cf. figures 30 and 31.) × 1800 diam.

Plate XV. Camera drawings.

Figures 23 to 27. Drawings from total mounts to show the general course of maturation and nuclear interchange. All stages are not given, the third division of the micronucleus for example, being omitted. For details see figures of plates 5, 6 and 7.

Figure 28. Camera drawing of the macro- and micronuclei at the period of reconstruction. The former is in the loosely wound skein stage which follows the tightly wound stage shown in photograph 20. The eight micronuclei show no signs of metamorphosis, their characteristic arrangement results from the long connecting strands shown in figure 74. × 750 diam.

Figure 29. Camera drawing of macro- and micronucleus at a somewhat later period of reconstruction showing the fragmentation of the macronuclear skein at the thin points shown in figure 28. The micronuclear structure is the same as in figure 28. × 420 diam.

Figure 30. Camera drawing of cell at the time of reconstruction of macro- and micronuclei, and ready for the first cell division. The macronuclei still have traces of the central chromatin granules; the micronuclei are characteristically dense; while the cell body still retains fragments of the degenerated macronucleus.

Figures 31 and 32. Camera drawings of the first somatic division and one cell of the second generation. Two macro- and two micronuclei go to each of the daughter cells which are smaller than at any other time during the Paramecium cycle.

Figures 33 and 34. Camera drawings of two stages in the metamorphosis of the micronucleus into macronucleus. In 33 there is no finely granular material, the nucleus appearing like the micronuclei with the exception of the membrane which is swollen; in figure 34 the nucleus is much larger and the fiuely granular material is concentrated by the hardening agents about the chromatin granules in the center. × 1350.

Plate XVI. Camera drawings of the micronucleus, preceding and through, the first maturation division.

Figure 35. Camera drawing of the micronucleus and macronucleus at the onset of maturation. The chromatin granules of the micronucleus are gathered about the division center at one pole; the number of these granules having nothing to do with the number of chromosomes later to appear. × 1100 diam.

Figures 36, 37 and 38. Camera drawings of three stages in the elongation of the micronucleus. The division center is at one end while the elongating lines of chromatin leave but little opportunity for anything but lateral union, or parasynapsis. (Cf. photographs 2 and 3.) × 1350.

Figures 39 and 40. Camera drawings of the micronucleus in the crescent stage. The division center begins to migrate from the end towards the center of the convex side. In figure 40 the chromatin is in a contraction stage which may be an artifact. (Cf. photographs 4 and 5.) × 1500 diam.

Figure 41. Camera drawing of the micronucleus at the time of chromosome formation. The lines of chromatin have divided by cross division into many double chromosomes. The division center has migrated towards the center of the convex side of the crescent and is ready to divide. (Cf. photographs 6, 7 and 8.) × 1500 diam.

Figures 42, 43, 44, 45 and 46. Camera drawings of the micronucleus during the period of the formation of the first maturation spindle and division of the chromosomes. Many of the chromosomes are in the form of rings, or Y or V, all of which may be traced back to the split chromosome. In figures 45 and 46 the substance of the division center has concentrated at the poles of the future spindle, while the chromosomes now form the nuclear plate. (Cf. photographs 7 and 8.) × 1500 diam.

Figures 47, 48, 49 and 50. Camera drawings of the micronucleus during the anaphase of the first maturation division. The chromosomes have nearly finished their division and now stretch out as straight rods along the spindle

fibers; a few are still in the V or Y form as shown in figure 49. The sharp points in figures 47 and 48 are the last reminiscence of the sharp ends of the crescent the complete history being shown in figures 42 to 48. (Cf. photograph 9.) × 1500 diam.

Figure 51. Camera drawing of the micronucleus in the late anaphase of the first maturation division. Some of the lagging chromosomes indicate the longitudinal division. (Cf. photograph 10.) × 1500 diam.

Figure 52. Camera drawing of the macronucleus and micronucleus-immediately after the first maturation division. There is no connecting strand between the two nuclei. The daughter chromosomes are asymmetrically placed, the shorter pole indicating the point of attachment. × 1500 diam.

Plate XVII. Camera drawings of the micronucleus during the second maturation division.

Figures 53 and 54. Camera drawings of two nuclei shortly after the first maturation division. In 53 the asymmetry is clear and the chromosomes are distinctly single (cf. photograph 11). In 54 the chromosomes are distinctly split and indicate the next longitudinal divison. × 1200 diam.

Figure 55. Camera drawing of the micronucleus in the early anaphase of the second maturation division. The chromosomes are not yet separated and are connected at one end in such a way as to indicate that division was logitudinal, the small shelves that are left, lie in the plane of the chromosomes as shown in figure 53. The smaller nucleus to the left is a degenerating nucleus of the first division a very unusual thing for both products of the first division usually divide again. (Cf. figure 12, a photograph of the same micronucleus as that shown here in which, for the sake of clearness, only a few of the chromosomes ar represented). × 950 diam.

Figure 56. Camera drawing of the section next to that shown in figure 55. The chromosomes here represented lie on the periphery of the micronucleus and are not as far advanced in division as those shown in figure 55. Some are still in the V form; in others the arms of the V are widely separated an all indicate that division was accomplished through a change in axes. (Cf. photograph 13.) × 1800 diam.

Figure 57. Camera drawing of two cells at the period of the second maturation division. In the upper one both nuclear products of the first division are in the anaphase of the second mitosis while in the lower one each of the nuclei has divided the products of one are already beginning to degenerate, while of the other, one will degenerate, the other will form the pronuclei. × 950 diam.

Figures 58 and 59. Camera drawings of two micronuclei in the same stage as that shown in figure 55, the chromosomes in the last stages of division. × 1200 diam.

Figures 60, 61 and 62. Camera drawings of micronuclei in the early telophase of the second maturation division. In 61 and 62 the heteropolar character of the daughter nuclei is due to the precocious degeneration of one nucleus while the other shows the fragmentation of the chromosomes characteristic of the third micronuclear division. (Cf. photograph 15.) × 1350 diam.

Plate XVIII. Camera drawings of the third division; of the pronuclei; and of reorganization.

Figures 63. Camera drawing of the micronnclens at the ontset of the third division. The chromatin is in characteristic grannle form and there is evidence of heteropolar asymmetry. A degenerating micronncleus is pictured to the right. (Cf. photograph 16) × 1000.

Fignres 64 and 65. Camera drawings of the micronncleus in the anaphase of the third division. The heteropolar character of the division fignre is clearly apparent. (Cf. photograph 17.) × 1600.

Figures 66 and 67. Camera drawings of the micronnclens in the early telophase of the third division. The chromatin is finely granular and arranged in straight lines continnons with the material of the connecting strand. One of the nnclei (the wandering, or male nnclens) is distinctly smaller than the other. (Cf. photograph 18.) × 1300 diam.

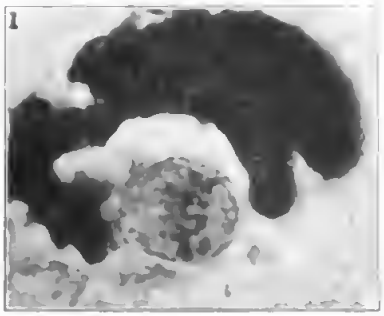
Figure 68. An abnormal division figure showing pronnclei and straight connecting strand with mnch chromatin. × 1000 diam.

Fignre 69. Camera drawing of one of the pronnclei (the larger) after the third division. × 1650 diam.

Figure 70. Camera drawing of the pronnclei dnring fnsion. The difference in size is clearly seen in the pair on the right, while in the pair on the left the male pronuclens is nnderneath. (Cf. photograph 19.) × 1350 diam.

Fignre 71. Camera drawing of the micronnclens at the period of the second somatic division. (Cf. photograph 20). × 1350 diam.

Figures 72, 73 and 74. Camera drawings of the macro- and micronnclei dnring the second and third somatic nuclear divisions. In 73 the macronncleus is assnming the skein form which is shown broken up in 74. In the latter figure the long connecting strands by which the eight micronnclei are separated into two gronps of fonr each, are clearly shown. (For further stages in re-organization see Plate XVI).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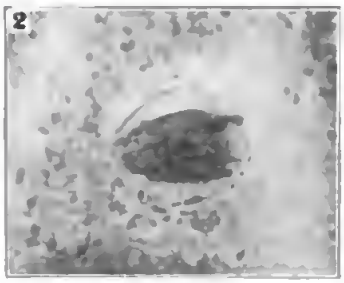
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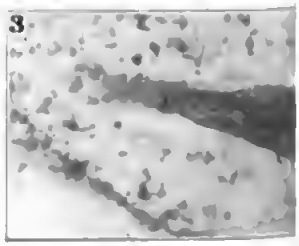
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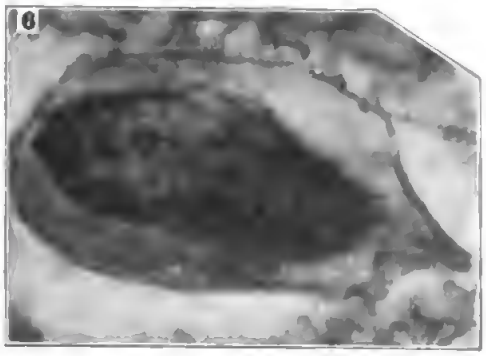
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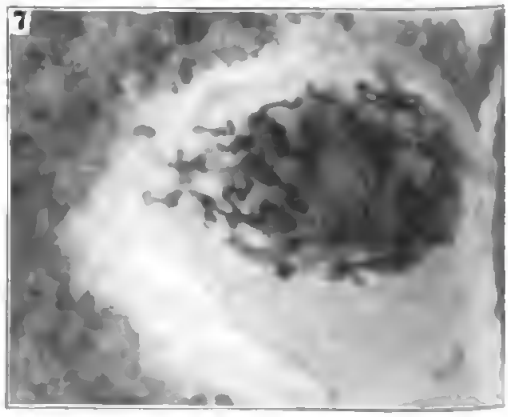
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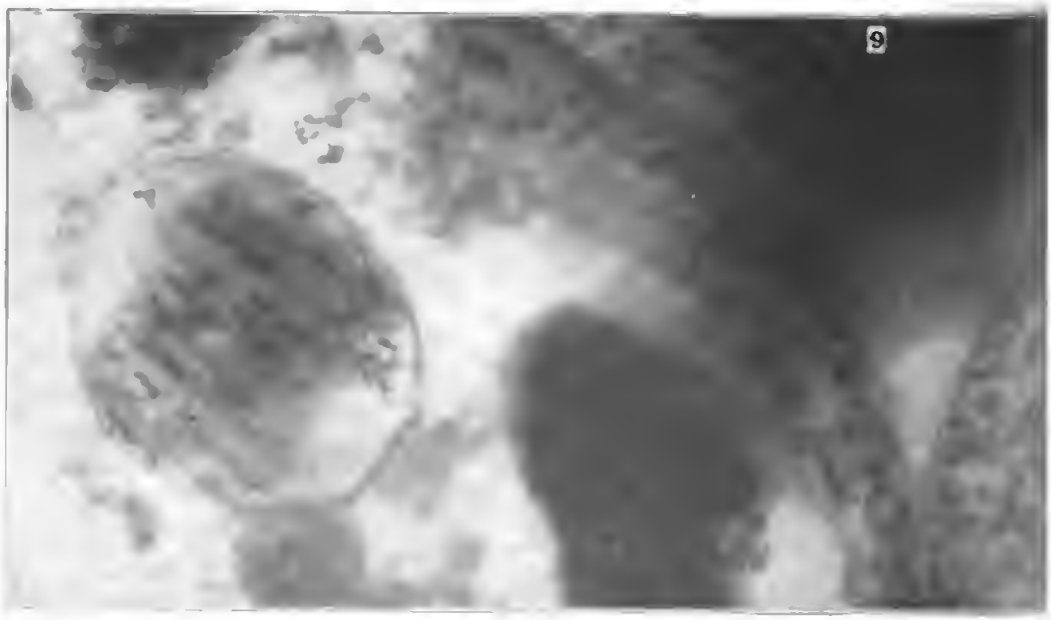
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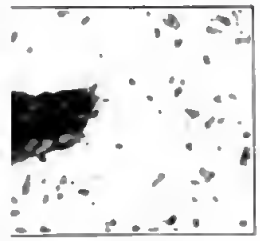

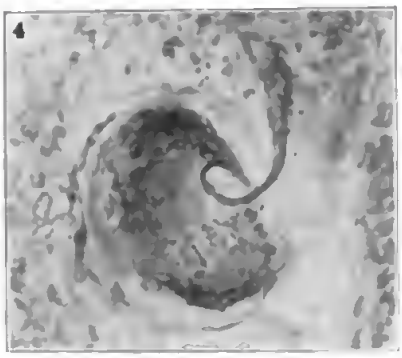

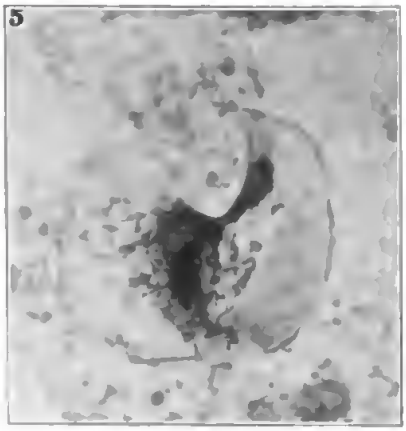

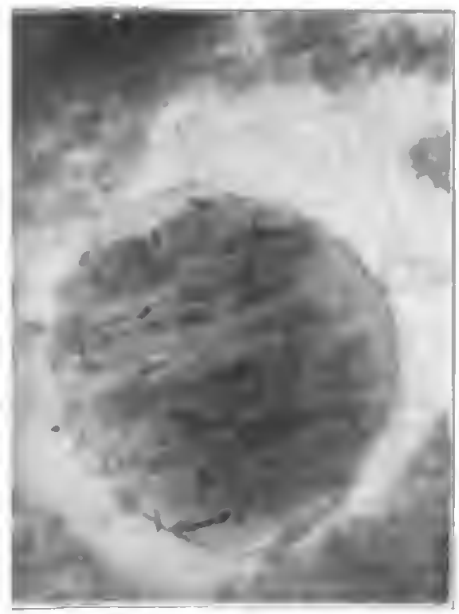

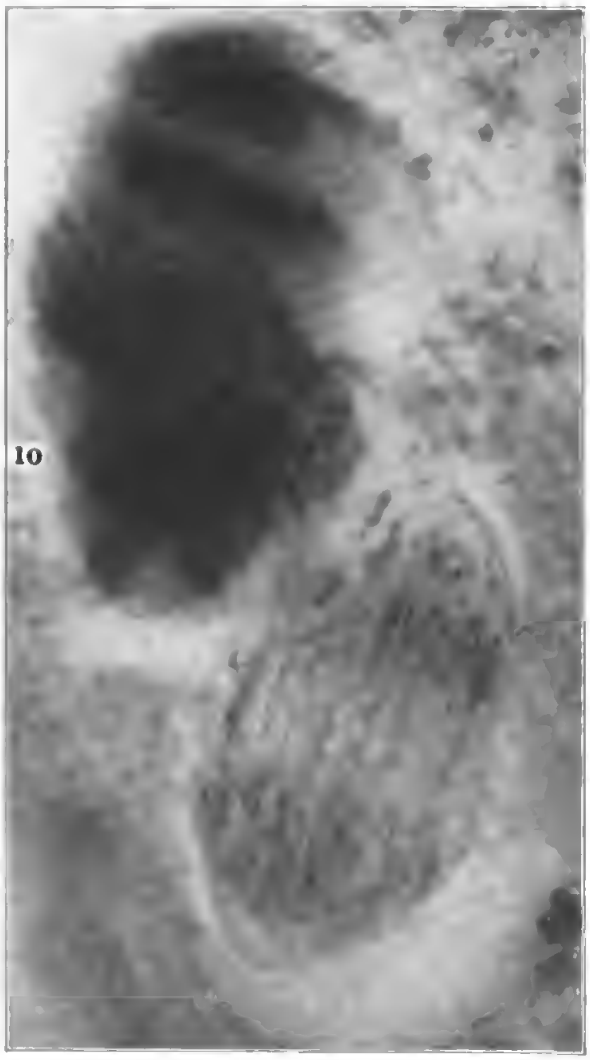

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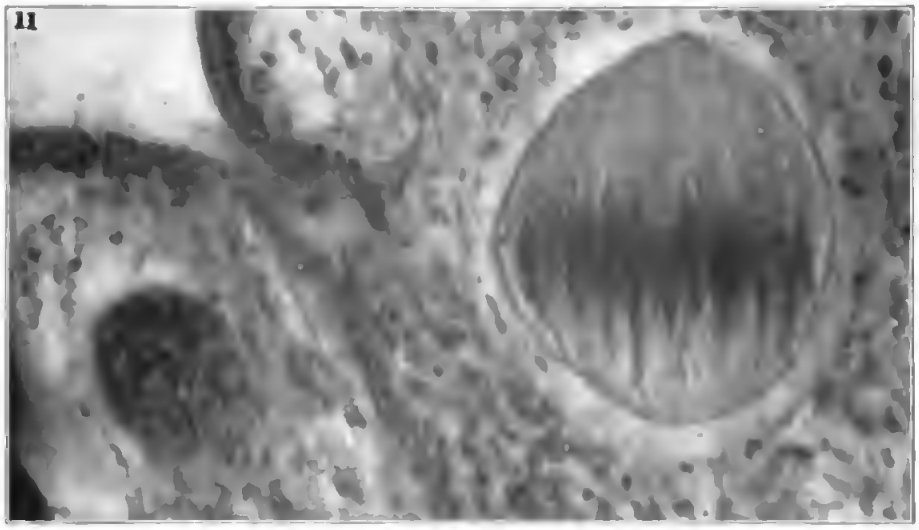

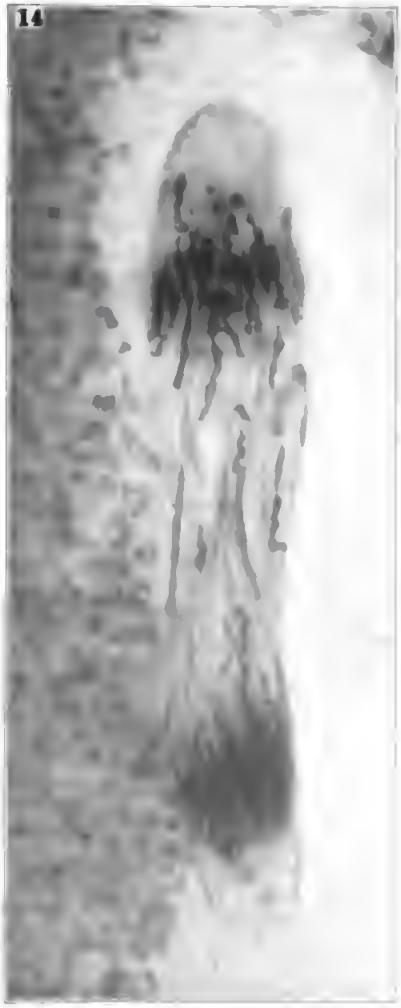

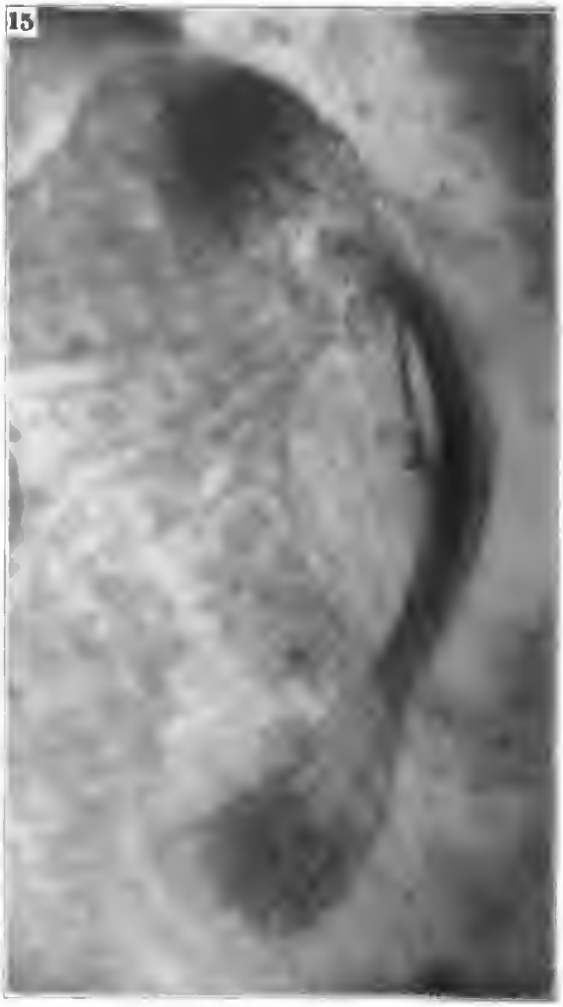

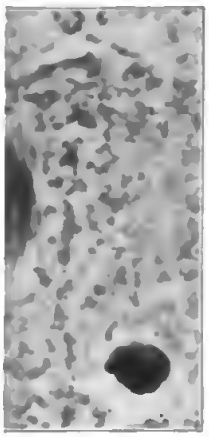

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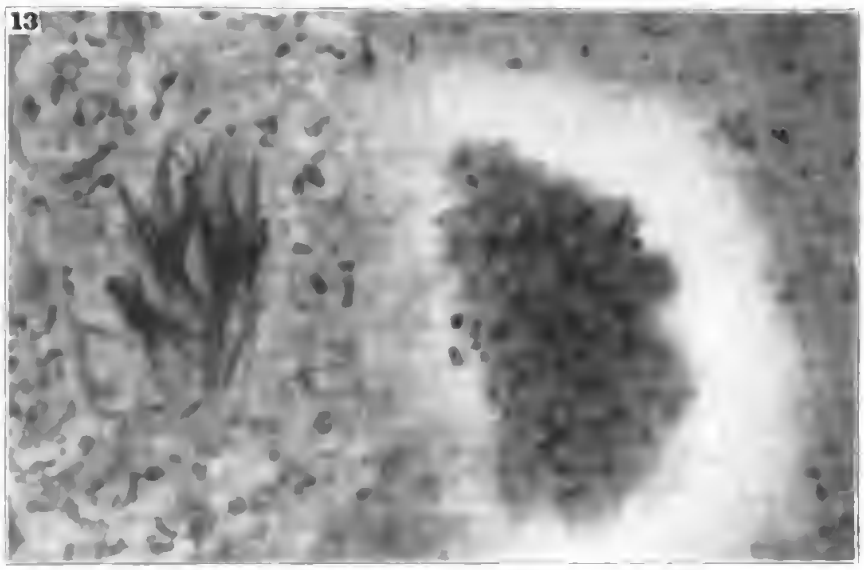

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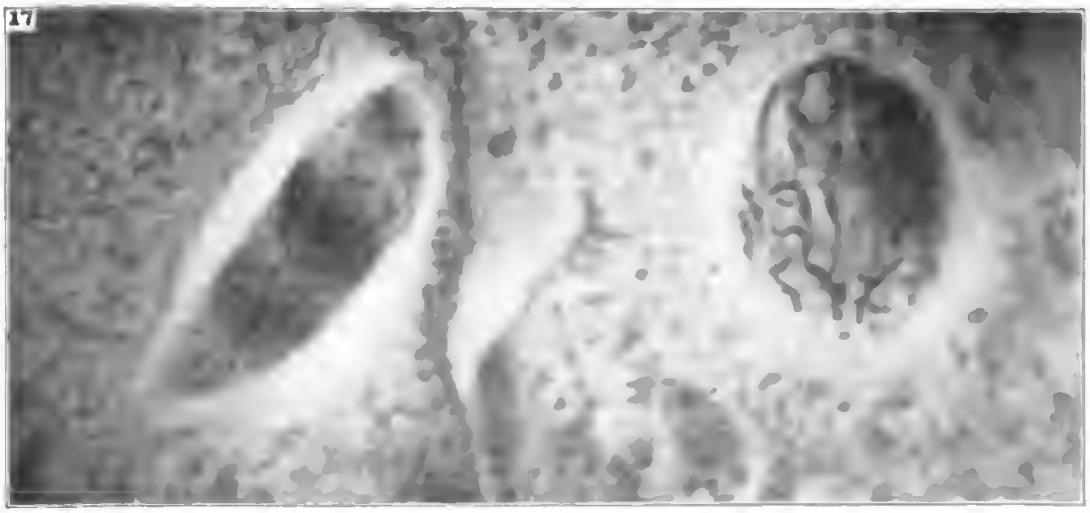

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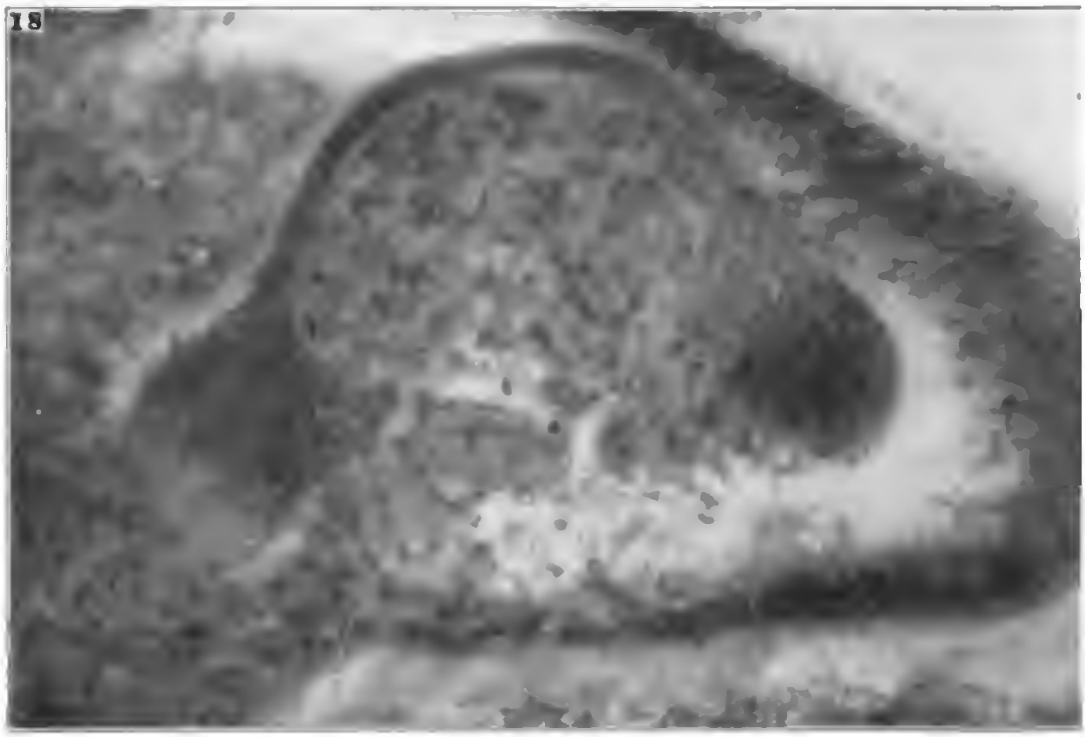
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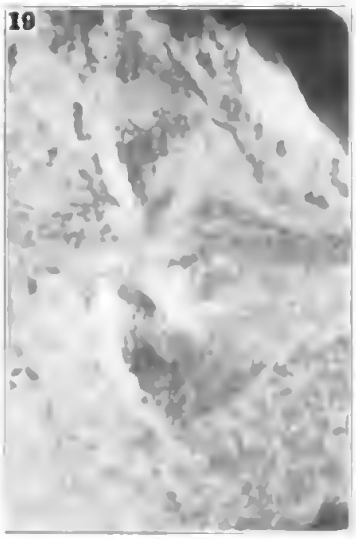
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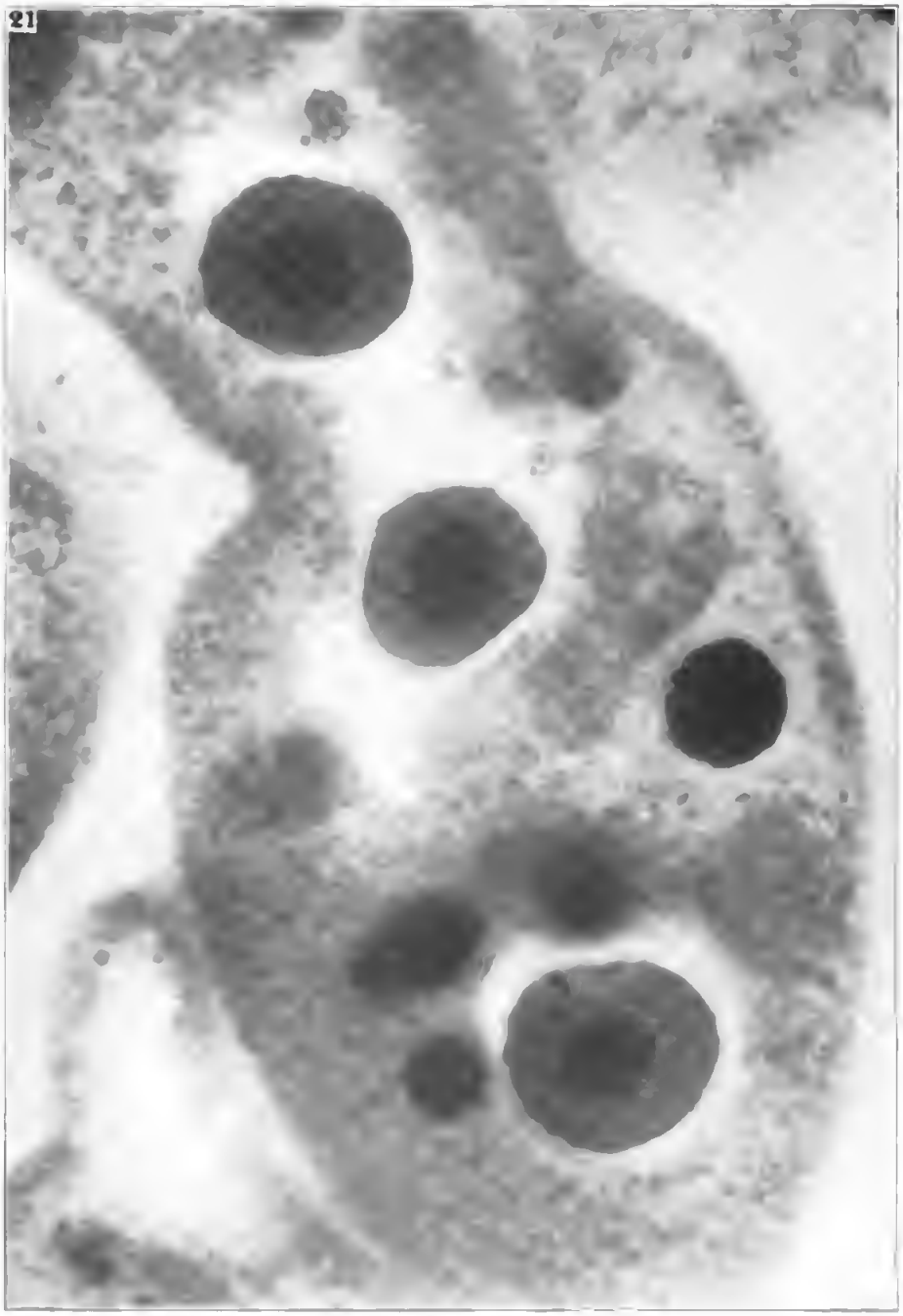

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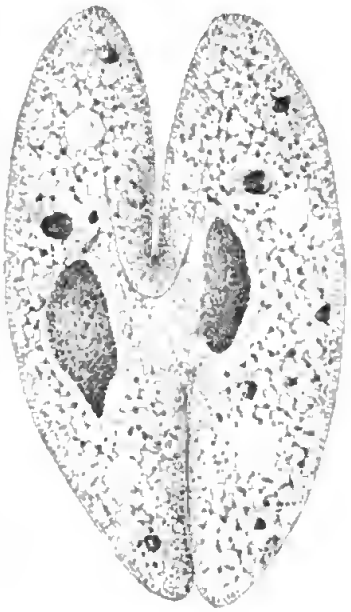

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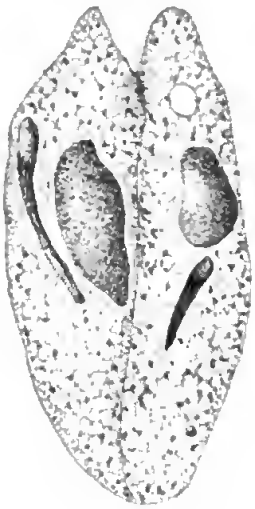

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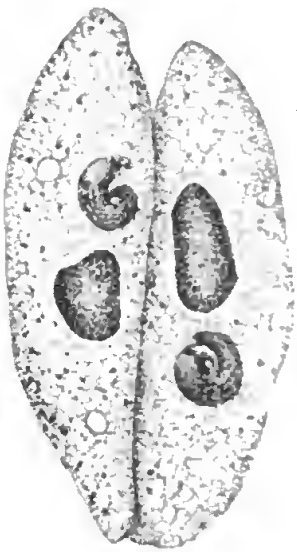

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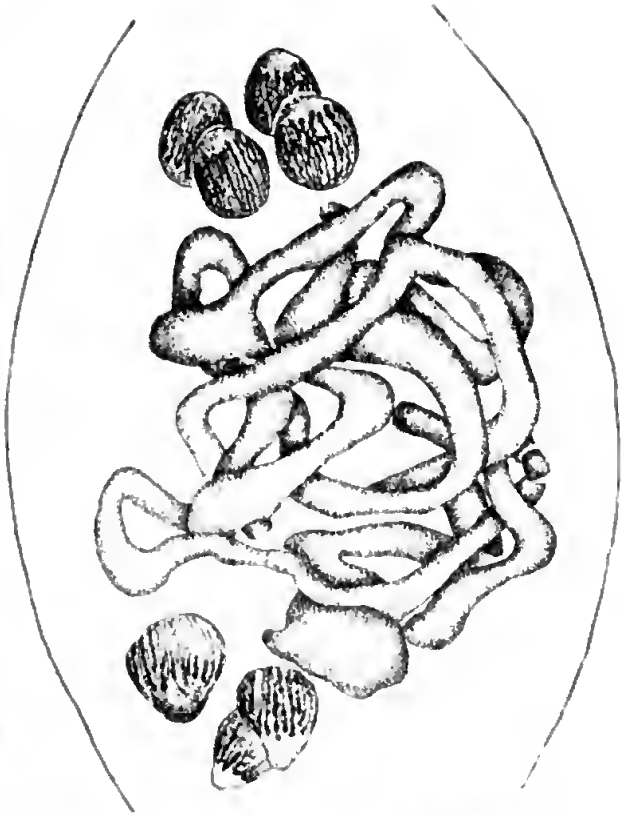

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Calkins and Cull.

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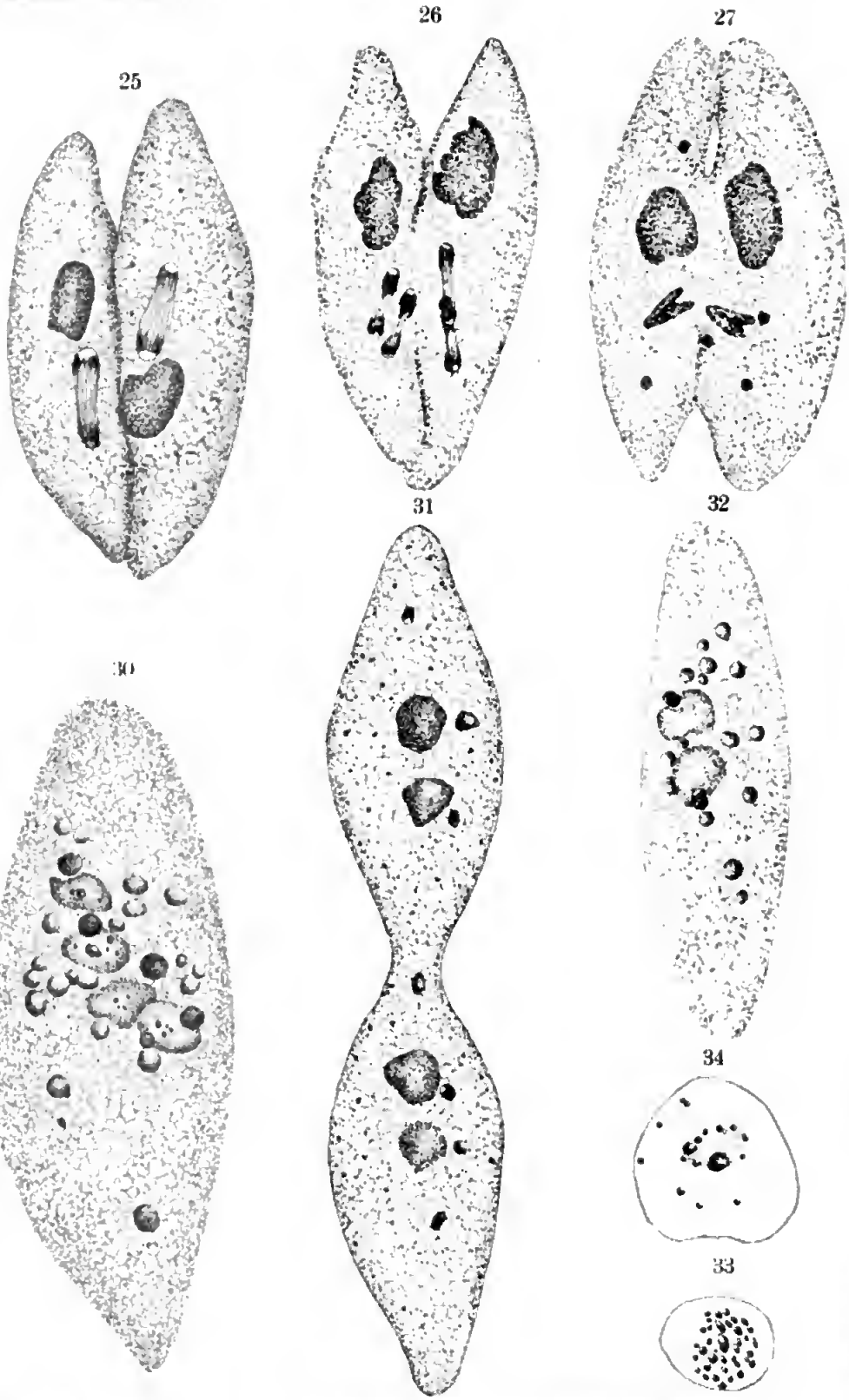

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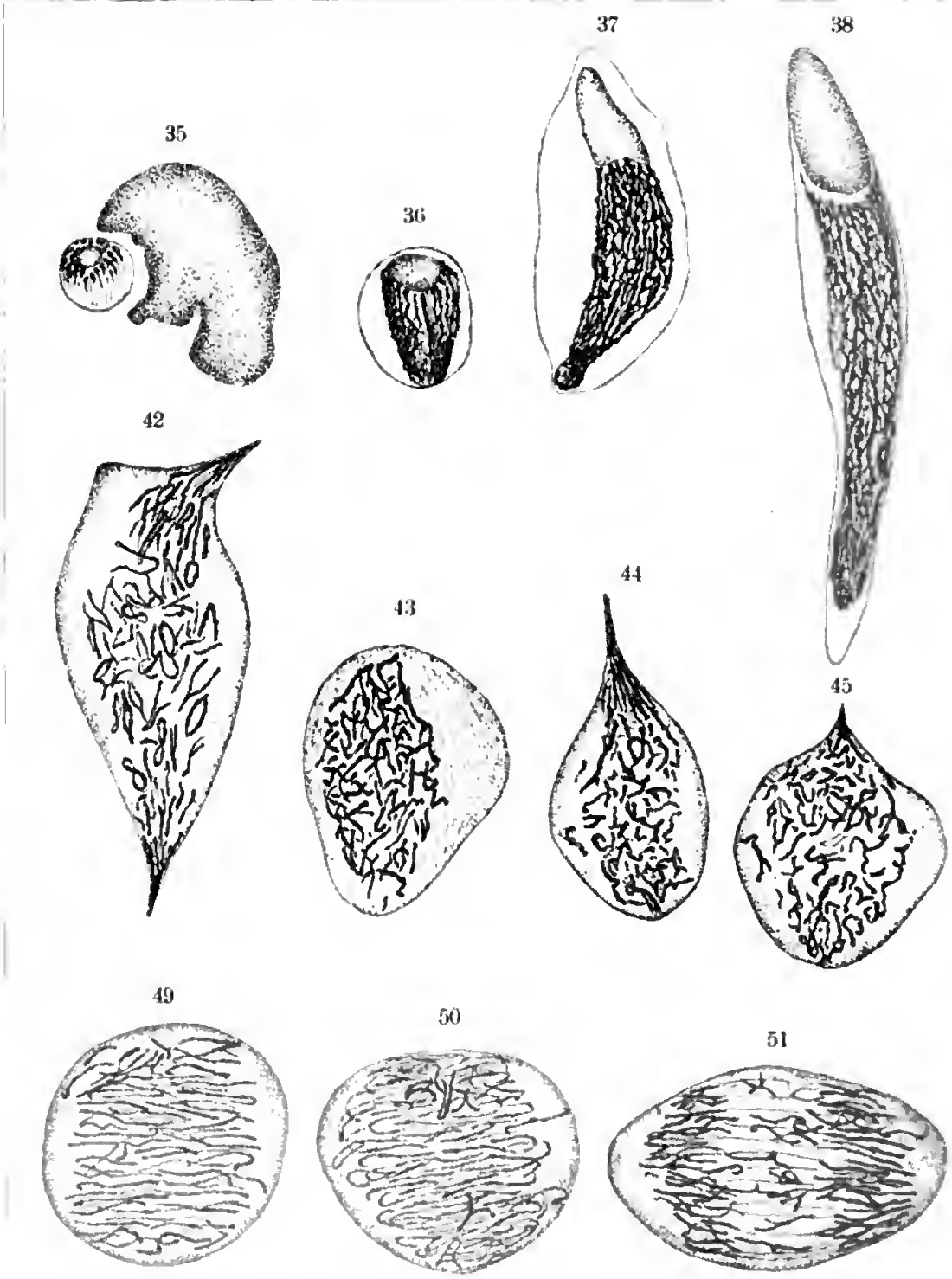

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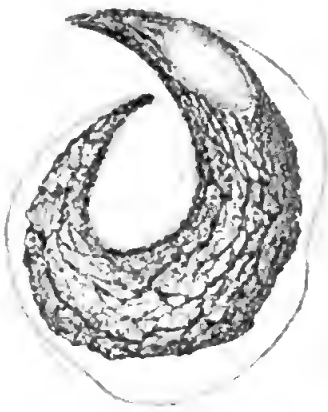

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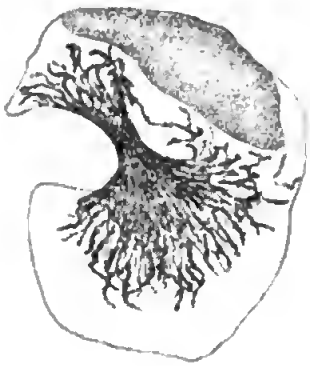

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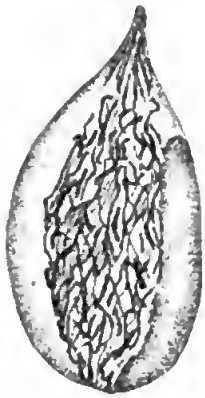

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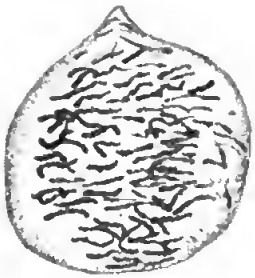

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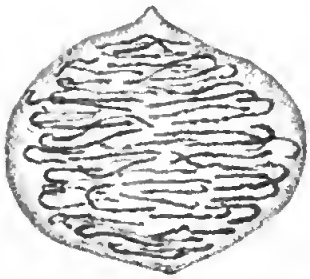

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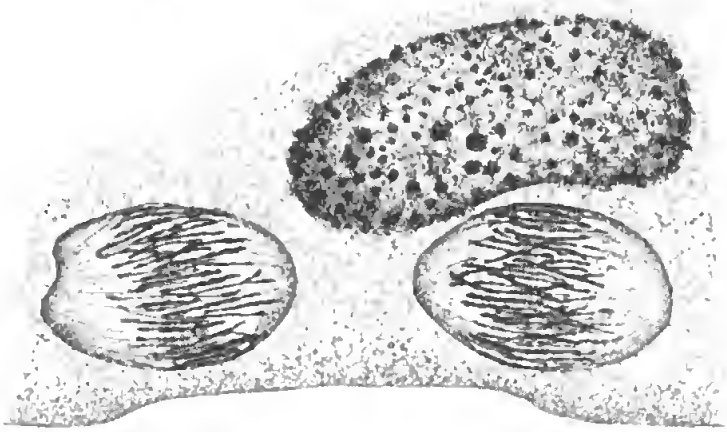

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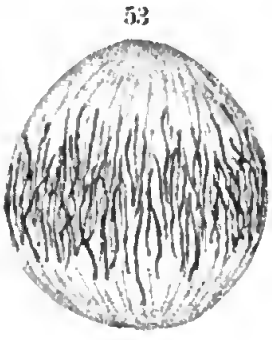
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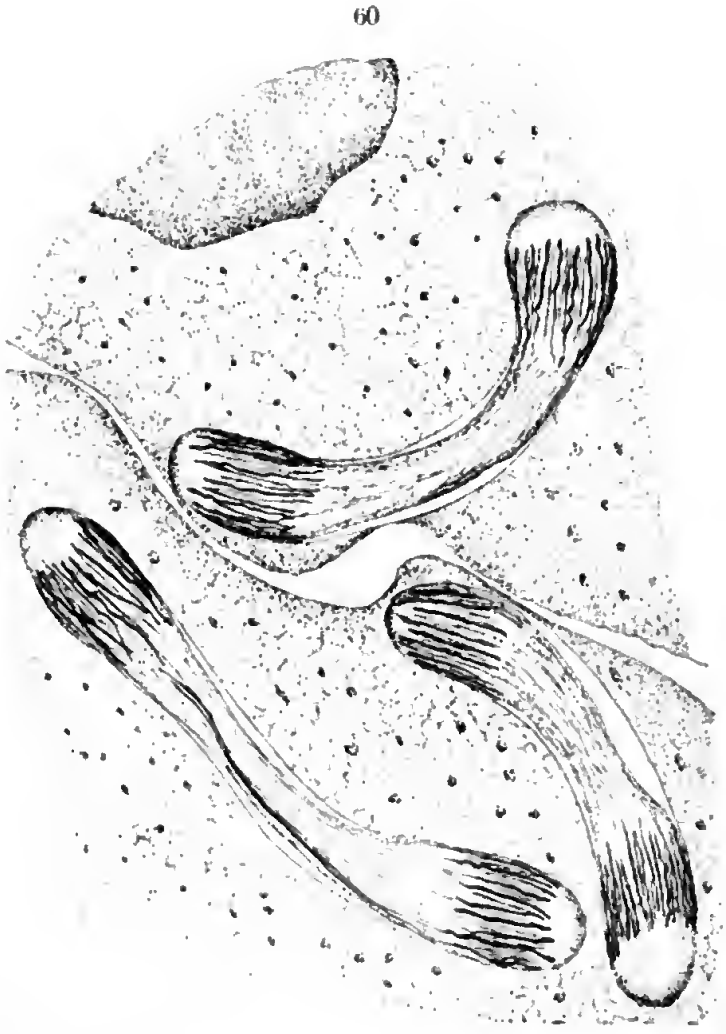
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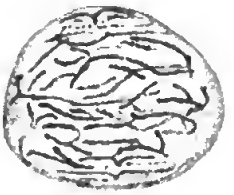

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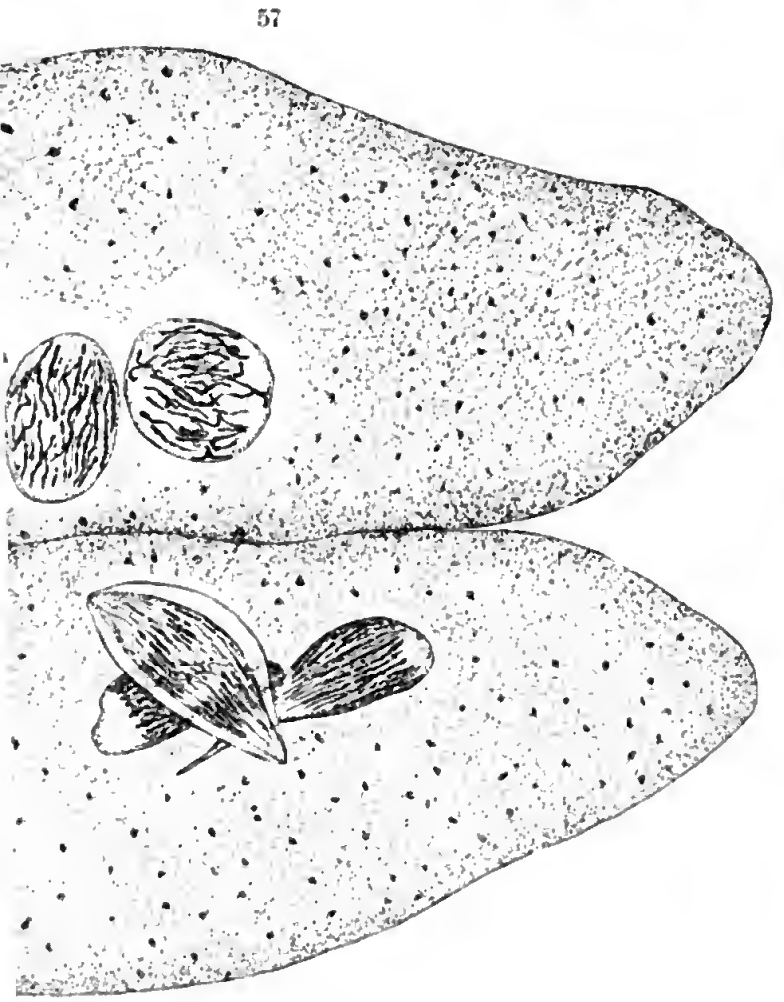

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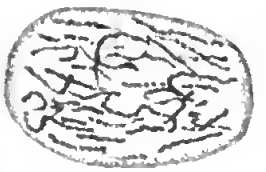

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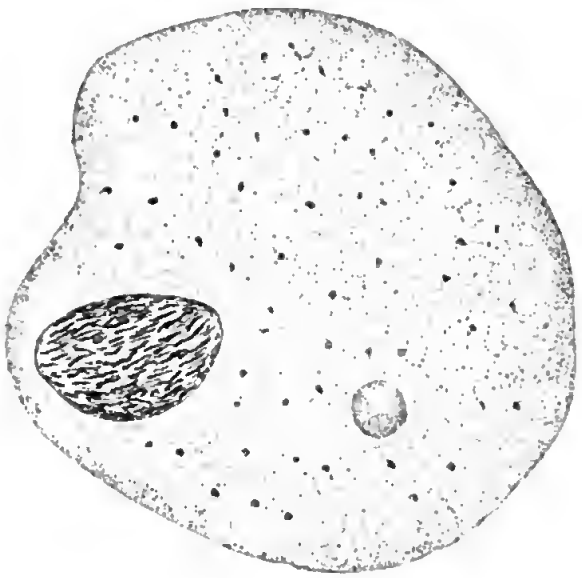

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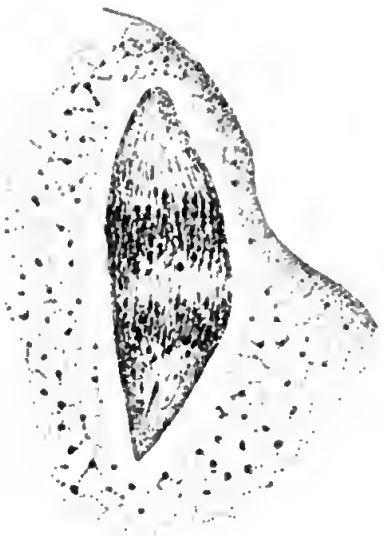

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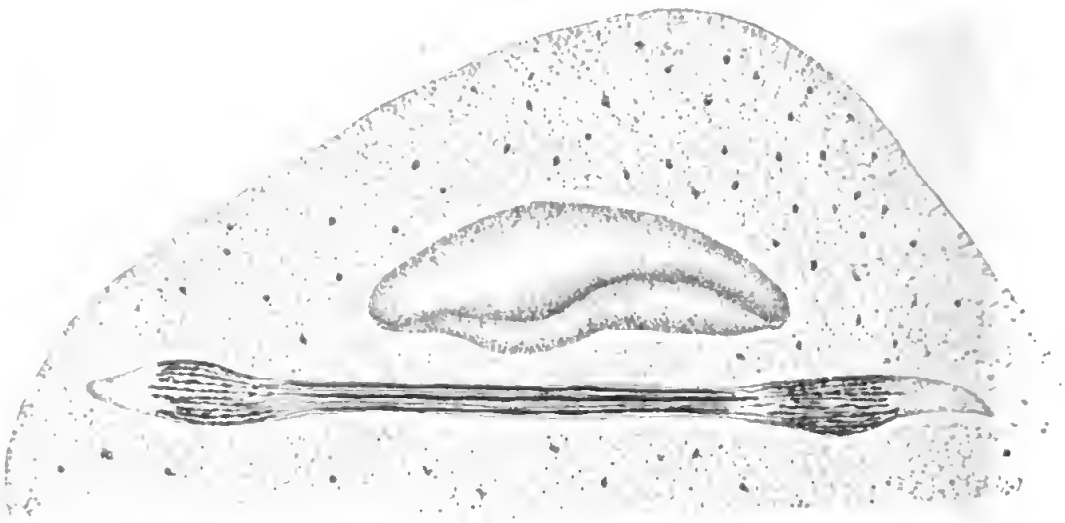

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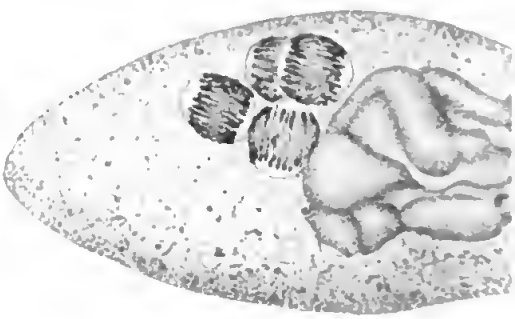

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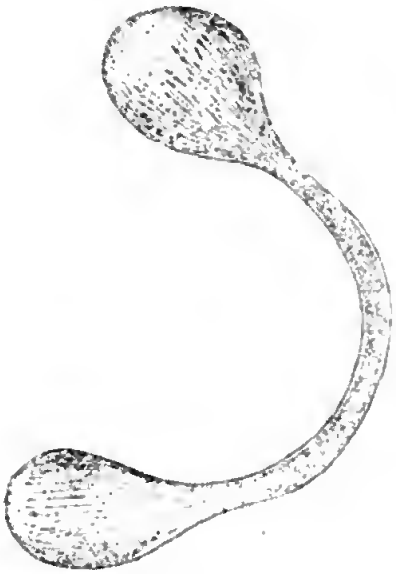

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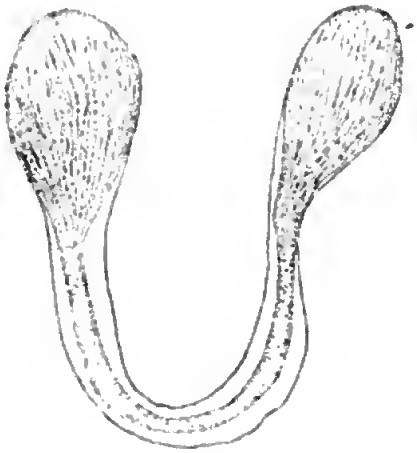

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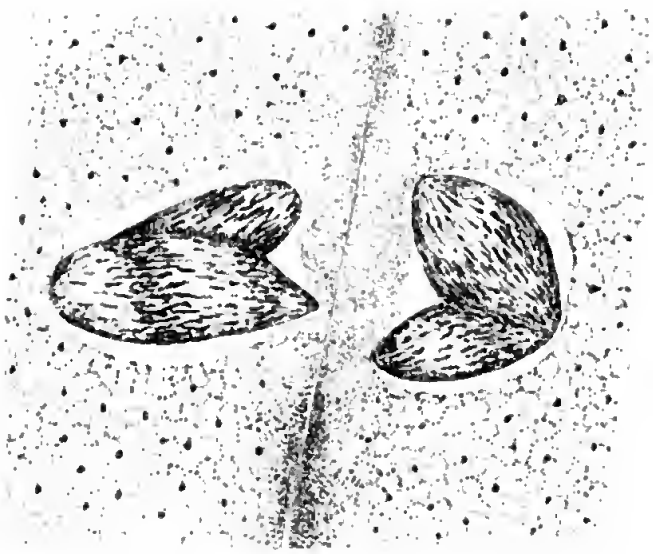

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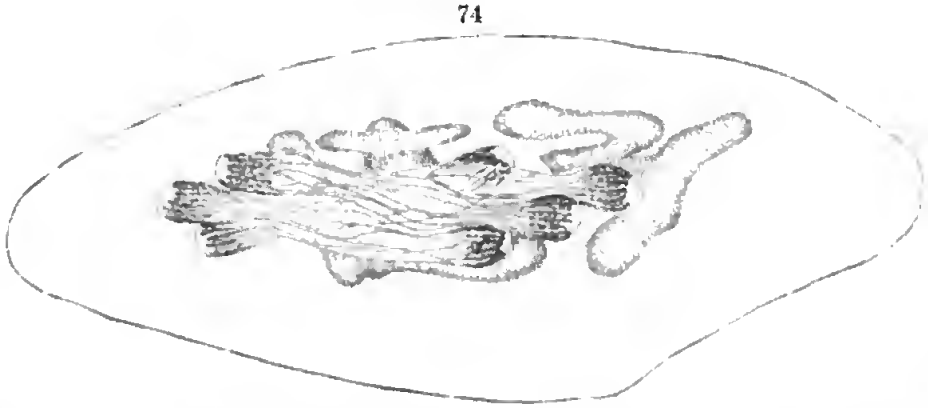

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