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Effect of Chloral Hydrate
On the Plant Cell

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"The Effect of Chloral Hydrate on the Plant Cell"

BY

WILLIAM CROCKER, A. B. '02.

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

William Crocker

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EFFECT OF CHLORAL HYDRATE ON THE PLANT CELL.

1. General.

From his study of mitosis in the epithelial cells of the salamander, Rabl concluded that chromosomes never lose their individuality but remain distinct even in the reticulum of the resting nucleus. He thought that the reticulum is formed by the anastamosing of projecting bridges of the chromosomes and that these bridges are later drawn in and the chromosomes hold their former position in the newly organized spireme.

Boveri found in his studies of the abnormalities in the ages of Ascaris that the number of chromosomes that enter into a resting nucleus is equal to the number that reappear upon the later division of that nucleus. In cases where the two egg chromosomes were in some way separated each formed a nucleus of one-half the size of a normal nucleus. At the following division each nucleus showed only one chromosome.

For a review of the literature and a list of references upon the individuality of chromosomes see Wilson (The Cell in Development and Inheritance, pp. 294-301).

Two facts brought out in establishing the individuality of chromosomes especially concern us in this paper. Any portion or any excess of the number of chromosomes that normally enter into the makeup of a nucleus (if they are in some way isolated), may form a nucleus. The nucleus thus formed varies in size with the number of chromosomes that enter into its construction; and the number of chromosomes that appear at the following division is equal to the number that entered

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into its construction.

Strasburger believes the cytoplasm to consist of a fibrillar active portion, kinoplasm, and an alveolar less active portion, trophoplasm. The cytoplasm can differentiate an additional amount of kinoplasm. The kinoplasm gives rise to the fibrillae of the achromatic figure and muscle fibers; constitutes the centrosomes, peripheral cell layer (or dermoplast), and the middle piece of spermatozoa; and forms the contractile portion of cilia and flagella. Kinoplasm brings about, in the main, the more active or motor processes of the cell, while trophoplasm is concerned with nutrition. They are distinguished morphologically and by staining reaction: kinoplasm is fibrillar and generally stained deeply by gentian violet and irom haemotoxylon; while trophoplasm is alvoelar and colored but slightly by these stains.

II. Method of Experimentation.

The root tip of Vicia Faba was used in these experiments. Considerable time was spent in ascertaining the concentration of the solution and the duration of exposure that brought about abnormalities in the cell in greatest abundance, and yet left the cells and the entire root in a condition that they would continue growth when brought back into normal surroundings. A two per cent. solution acting for one hour kills the root, while a one-eighth per cent. solution acting for twelve hours brings about scarcely any abnormalities. After considerable experimenting I found that a one-half per cent. solution acting for one and one-half to three and one-half hours produces nearly all abnormalities appearing in any of the cultures and produces

and produces them in relatively great abundance.

The experiments from which the results are recorded were conducted as follows: The seeds were soaked in water twenty-four hours, and then planted in moist saw dust. After the roots had attained a length of 5-10 cm. the beans were placed on a screen with the roots dipping into water, and left for several hours in order that the roots might adapt themselves to a water medium. At 10:50 A. M. five roots were killed and the others transferred to a one-half per cent. chloral hydrate solution where they remained for one and one-half hours. At this time five more were killed and the others transferred to water to recover from the effects of the narcotic. At the expiration of every successive five hours (up to forty-five hours) five more roots were killed. We have then in each experiment a series of preparations as shown in the following table:

No. 1 Fixed from water--normal.
No. 2 " immediately after the action of chloral hydrate.
No. 3 " 5 hours " " " " " "
No. 4 " 10 " " " " " " "
No. 5 " 15 " " " " " " "
No. 6 " 20 " " " " " " "
No. 7 " 25 " " " " " " "
No. 8 " 30 " " " " " " "
No. 9 " 30 " " " " " " "
No. 9 " 35 " " " " " " "
No.10 " 40 " " " " " " "
No.11 " 45 " " " " " " "

The water and the solution were kept at 24 C. The roots were killed in weak chromo-acetic. The safranin gentian-violet orange G combination were used for staining.

III. Effects on Cytoplasm.

In many ways the effects of chloral hydrate upon the cytoplasm and nucleus are so interrelated that the discussion of one almost necessitates the discussion of the other in the same connection. For convenience and order, however, the effects upon the cytoplasm will be discussed first and those upon the nucleus later.

In many sets of the preparations, tho not in all, peculiar masses appear in the cytoplasm. These masses are more or less spherical and non-granular. They stain with safranin, but far less deeply than chromatin does. Three such masses are shown in the right of Fig. 1 and one in the left. The mass is often surrounded by a ring of varying width which stains less deeply than the mass. The outer boundary of this less intensely stained ring is always clearly marked by a line of deeper red, as is shown in Fig. 1 to the right. In the figure the masses as well as the surrounding ring appear granular. They are really homogenous, but could not be so represented with the pen.

This abnormality often shows a striking resemblance to the food vacuoles of Infusoria. Perhaps not functionally, but direly in appearance, the mass corresponds to the food particle and the surrounding more lightly stained ring to the food vacuole. Frequently several concentric rings centered by a mass appear. In such cases the rings are clearly bounded by darkly stained fibrillar lines. Often these bounding lines seem to be the succeeding coils of a spiral in-

stead of a series of concentric circumferences. These masses were found in No. 3 but never in Nos. 2 or 4. No. 2 often shows a coarse granular cytoplasmic structure. The masses, then, seem to form before the roots have been out of the chloral hydrate five hours and to disappear before the roots have been out ten hours.

The cells that show these masses also show a tendency towards a coarse alveolar structure which is well illustrated in Fig. 1. It is not infrequent to find the entire cytoplasm of cells showing this character. Of course in these cases no masses are present. There is some evidence that leads one to believe that this alveolar structure is directly related in its origin to the masses that appear in the cytoplasm, and that each alveolus results from the solution or digestion of one of these masses. If this is the case, each of the several small alveoli in Fig. 1 resulted from the dissolution of a relatively small mass, and later a larger alveolus will appear as the result of the dissolution of the large mass at the right. The assumption is supported by the fact that only small alveoli appear in No. 3 while much larger ones are present in No. 4, and that the bounding walls of the alveoli resemble the bounding walls of the rings that surround the masses.

If this assumption is right, between the time of Nos. 2 and 3 occur the formation of the masses and the dissolution of the smaller ones forming small alveoli; while between the time of Nos. 3 and 4 the complete dissolution of the larger masses and the formation of larger alveoli. Perhaps far more conclusive evidence of the relation of the masses to the alveoli would be obtained by killing several in-

termediate stages between Nos. 2 and 3 and Nos. 3 and 4. I now have an experiment in progress to test this point.

It must be mentioned in this connection that the masses and peculiar vacuolization are entirely limited to merismatic tissue and that they are most abundant in the most rapidly dividing cells. This fact precludes any attempt to explain the vacuolization as a result of the differentiation of cells.

While the origin of the alveolar structure is not certainly established the source of the masses is perhaps yet more doubtful. If they originate from the nucleus one would expect to find some structural evidence of it, but in no case has any such morphological connection been found.

Dr. Hottes in his unpublished researches on the root tip of Vicia Faba found that high temperature (38 C.) causes a great acceleration in the activities of the cell--especially in the growth of the spindle fibers--and a very marked reduction in the size of the nucleoli. If, however, the roots were suddenly transferred to a low temperature and the rate of growth thereby reduced masses appeared in the cytoplasm, identical in appearance and staining characters to those found in chloral hydrate. In roots grown under pressure and thereby caused to do considerable work, he again found a great reduction in the size of the nucleoli. When these roots were released from pressure masses similar to those described above appeared in the cytoplasm. These researches are very strong evidence in favor of Strasburger's theory that the nucleolus is a food organ, and they indicate strongly that the peculiar masses in the cytoplasm are of nucleolar origin. If

If these masses are of nucleolar origin, the substance must be dissolved, carried out into the cytoplasm and then precipitated.

It would seem that, if the masses are of nucleolar origin, those cells which show the masses ought to possess nucleoli that are smaller than the nucleoli of normal cells. Such a relation is difficult to establish for two reasons: the amount of nucleolar substance required to form the masses might be very slight and the variation in the size of the nucleoli in normal conditions is great. I could detect no reduction in the size of the nucleoli. This matter might, however, be viewed in quite another way. Perhaps growth under high temperature, under pressure, and under normal conditions each has a rate at which the nucleolar substance is used up by the activities of the cell. Perhaps also that, if in the first two cases the roots are suddenly brought into normal conditions, the activities of the cells and the rate of using the nucleolar matter are lowered, and the stream of food (less readily reduced in its rate) from the nucleolus is caused to precipitate instead of being used up by the activities of the cell. Cells of roots grown in normal conditions may likewise show a reduction in their activities and a precipitation of the nucleolar food material by being subjected to the influence of chloral hydrate, a narcotic. Such a supposition, however, has very meager experimental proof at present. After these masses have been produced in a greater variety of ways we can eliminate the unlike conditions and select the like and essential conditions with far greater certainty.

These masses are probably in no way related to the nebenkern

found in spermatids by Butschli, La Valette, and others, for they certainly do not originate from remains of the spindle. It seems very probable, however, that they are similar in nature to the nebenkern found in the cells of the pancreas of Amphibia (Mathews, The Changes in Structure of the Pancreas Cell, Journal of Morphology, Vol. 15 Supplement).

As we have seen, there is evidence that this formation of masses in the cytoplasm and its vacuolization are nutritive phenomena; that is, effects upon the trophoplasm. There are also two very marked effects of chloral hydrate upon the kinoplasmic element of the cytoplasm. They are the tendency to bring about a lack of coordination in the action of the spindle fibers and finally the destruction of the spindle.

It is evident that these effects will be shown only by those cells that are influenced by chloral hydrate when they are in division. Fig. 2, which is drawn from a slide of preparation No. 5, material killed fifteen hours after the action of chloral hydrate, shows both these effects. This cell was probably in division when the material was in the solution of chloral hydrate (the evidence for this will be brought out later). One-half the daughter chromosomes have been thrown to one pole in the customary way and are organizing a regular nucleus. The other half of the daughter chromosomes have been thrown to the other pole, but in two groups connected by a bridge of one or two chromosomes; and they are organizing a very irregular nucleus consisting of two larger portions connected by a narrow bridge. The material of the spindle is very evident between the

two organizing nuclei; but the individual fibers are entirely broken up. At this stage the spindle would ordinarily have gone far towards the construction of the cell wall; but here the spindle is so badly injured that it is doubtful whether the cell wall will be constructed at all, and certainly not in the ordinary way. Fig. 3 shows another cell from preparation No. 5. The spindle here has begun the formation of the cell wall, but a central bridge of chromatin connects the two organizing nuclei. This central bridge of chromatin probably resulted from the destruction of spindle fibers at the time they had drawn the last two chromosomes only part way to the poles. Fig. 4 shows a similar condition excepting that the wall formation has not progressed so far and the bridge of chromatin is at one side instead of central. Fig. 5 shows two masses of chromatin organizing nuclei, the remains of the disorganized spindle, but no sign of a cell wall. It is probable that this cell would later have contained two resting nuclei as is the case in Fig. 6. Fig. 8 represents three almost equal organizing nuclei in the same cell without any sign of a cell wall forming to separate them. This drawing was taken from preparation No. 7, material killed twenty-five hours after the action of chloral hydrate. It seems to represent a marked case of the broken coordination of the spindle fibers and the final destruction of the spindle. Probably this cell would later have shown three resting nuclei.

Figures 2 and 8, then, show both the breaking of the coordination in the action of the spindle fibers and the final destruction of the spindle; while the other figures mentioned above show only some other

injury to or the destruction of the spindle. In many of the preparations there are mitotic figures in which the coordination is clearly broken while the spindle still seems to be in good condition. Fig. 7 from preparation No. 7, material killed twenty-five hours after the action of chloral hydrate, is a striking example of this. Here the chromosomes have been thrown in three directions and a typical tripolar figure formed. The poles are of unequal size: the lower one seems to be one pole of a regular bipolar figure, while the other two poles seem to have originated from the division of the chromosomes that would normally have formed the other pole. Such tripolar tripolar figures were not of very frequent occurence in the preparations--not more than a dozen were ever found in a single slide.

These figures were not found in material killed immediately after the action of chloral hydrate, but first appeared in material killed fifteen hours later and were most abundant in material killed twenty-five hours later.

It seems peculiar that the chromatin masses of Fig. 7, which is from material killed twenty-five hours after the action of chloral hydrate, have not yet begun to construct nuclei; while the chromatin masses of Figures 2-5, killed fifteen hours after the action of chloral hydrate, have progressed far in the organization of nuclei. Three explanations for the apparent slowness of the chromatin in the tripolar figures may be suggested. These cells may have begun division some time after the roots were transferred from chloral hydrate to water, and thereby may have been influenced in its division by a concentration of chloral hydrate much below one-half per cent. due to

its diffusion thru the water. In this case we must assume that the low percentages broke the coordination of the spindle fibers without otherwise seriously injuring the spindle. If this supposition is true lower percentages of chloral hydrate ought to produce tripolar figures with the spindles in good condition. A series of experiments with low percentages acting for different durations of time produced no such effects; hence this supposition seems invalid. It may be that cells as represented in Fig. 7 were in division when the roots were in chloral hydrate, but that the chromatin was exceptionally slow about organizing nuclei. It is peculiar, however, that a cell so sturdy in one respect—as is indicated by the condition of the spindle--is so slow in its functioning in another. It might again be supposed that the cytoplasm retained the effect of the chloral hydrate and that this retained effect was sufficient to destroy the coordination of the spindle fibers without otherwise seriously injuring the spindle. This supposition seems to agree best with the data.

Often when the figure is bipolar the chromosomes are distributed very unequally to the two poles. This is probably the explanation of the marked difference in the size of the two nuclei in each of the cells of Fig. 9.

Hertwig, Galiotti, and others have produced asymmetrical mitosis by the use of poisins and other chemical substances (quinine, chloral, nicotine, etc.). Klebs, Hausemann and Galiotti have demonstrated its frequent occurrence in abnormal growths, such as cancers and tumors. Galiotti produced asymmetrical figures in the epithelium of the salamander by the use of cocaine, antepyrin, and quinine. In all

these cases of asymmetry both bipolar figures with poles of unequal size and multipolar figures were found along with a greater number of symmetrical figures.

Lustig and Galiotti noticed that the unequal distribution of the chromatin is always accompanied by inequality of the centrosomes, which in turn produce unequal amphiasters. They concluded from this that the unequal division of the centrosome is probably the cause of the unequal distribution of the chromatin. Wilson believes that the tripolar figure is formed by one of the two centrosomes dividing and thereby forming three amphiasters instead of two and that quadripolar figures are formed by both centrosomes dividing and forming four amphiasters. Whatever value this explanation may have for cells that posess centrosomes, it certainly can not hold for forms in which there is no evidence of the existence of centrosomes, as is the case in Vicia.

For a review and list of the literature on asymmetrical and pathological mitosis see Wilson (The Cell in Development and Inheritance, pp. 97-99).

IV. Effects on the Nucleus.

It is evident that the two effects upon the kinoplasm which were just discussed bring out peculiar modifications of the nucleus and naturally lead up to the discussion of the effects on the latter. Before discussing the effects upon the nucleus, however, we must review an article recently published by Waldemar V. Wasielewski (Theoritische und Experimentalle Beitrage zur Kenntniss der Amitosis, Jahrbücher f. wiss. Botan., Bd. XXXVIII, Heft 3, pp. 377-420) in which he claims to

have produced amitosis in the root tip of Vicia Faba by the use of chloral hydrate. At the time his paper appeared I had almost completed my researches and had arrived at essentially my present conclusions so far as the features discussed by Wasielewski are concerned.

After a rather extended review of the historical and theoretical features of the knowledge of amitosis he takes up the discussion of his researches. He mentions that he started out in his experiments with very definite aims; first to find a means of producing, in abundance and with certainty, amitosis in the embryonic tissue of flowering plants (root tip of Vicia Faba); then to study the behavior of the nucleus and cytoplasm in this process.

Following the suggestion of Nathansohn's work on Spirogyra (Physiologische Untersuchungen über amitotische Kerntheilung, Jahrb. f. wiss. Botan. Bd. 35) he sought to accomplish his aims by the use of ether, but after considerable variation in the concentration of the solution and duration of exposure he was unsuccessful. Still believing that the results of Nathansohn were due to narcotic action, he turned to chlorals and here met with success. After considerable experimenting he found that a one-half per cent. chloral hydrate solution acting for one hour produced abundant amiotic divisions, if the roots were then washed in flowing water for one hour and afterwards grown in sawdust for twenty-four hours.

He says that the first sign of amitosis is the doubling of the nucleolus. He describes this doubling as taking place in a typical manner: the nucleolus becomes considerably elongated, is later restricted at the equator, and finally cut into two daughter nucleoli.

Before the division of the nucleus is completed the daughter nucleoli often begin a second division. In only one case did he find the second division taking place simultaneously with the first. By what he took to be an unbiased count he found that 12.9 per cent. of the cells in the tip of a normally grown root showed double nucleoli; while 25.9 per cent. of the cells of roots exposed to one-half per. cent. chloral hydrate for one hour and then put in flowing water for one hour showed double nucleoli. He describes the doubling of the nucleolus as half completing the amitotic division of the nucleus.

Aside from the doubling of the nucleolus and a slight elongation of the nucleus an amitotically dividing nucleus is not at first unlike an ordinary resting nucleus. After these two initiative steps the reticulum begins to divide, generally into two equal parts, tho sometimes into parts of very different size. In this connection he names and describes two sorts of direct nuclear division. One he terms diaspase (distraction) of the nucleus. This, he points out, is found in Saccharomycetes and Valonia and begins, after the division of the nucleolus, with the nuclear substance traveling to opposite poles and forming a dumb-bell-shaped nucleus which finally forms two nuclei by the gradual narrowing of the restricted boundary. He emphasizes the fact that the nucleolus and nuclear reticulum are active in this process, while the nuclear membrane is comparatively inactive. He terms the other sort of direct division diatmese (dissection) of the nucleus and cites as an example the internodal cells of Chara. He characterizes it as being marked by great activity of the nucleolus and the nuclear membrane, which cuts the nucleus in two, and compara-

tive inactivity of the nuclear reticulum. In the direct division of Spirogyra Nathansohn noticed that the nucleus is divided by a deepening restriction that extends entirely around it. In his researches Wasielewski observed that the restriction is a half ring. He describes it as a modification of diatmese of the nucleus and thinks it the only form of direct division existing in his preparations. He mentions the difficulty of explaining why the nucleolus and nuclear membrane, which are normally inactive during division, become very active under the influence of chloral hydrate; while the nuclear reticulum, normally active in division, become inactive under the same influence.

Schmitz and Fairchild described a peculiar sort of mitosis in Valonia. The nucleolus dissolves; and chromosomes are formed but they do not split longitudinally. The nuclear substance, enclosed in the sac-like nuclear membrane, now moves towards opposite poles and forms a dumb-bell-shaped mass which finally separates into daughter nuclei. Wasielewski terms this hemimitosis. Beginning with a sort of division that shows great activity of the nucleolus and nuclear membrane but inactivity of the nuclear reticulum, he believes we can pass step by step to a sort of division that shows inactivity of the nucleolus and nuclear membrane but great activity of the nuclear reticulum. He mentions the stages: diatmese, diaspase, hemimitosis, mitosis. He claims to find forms which resemble the peculiar mitosis in Valonia and which are intermediate between his diatmese and mitosis.

He noticed that amitotically dividing cells are very slow about building their walls. Considerable time elapses between the completed

division of the nucleus and the beginning of the cell wall. This he believes explains the existence in the preparations of many binuclear cells such as seen in Fig. 9. Indeed the wall formation is often delayed so long that the two nuclei begin a second division (mitotic, however, now) without any sign of a wall separating them (see Fig.10). He describes this as daughter division beginning before the mother division has completed itself, and believes that the delayed wall will finally be built. If this wall were not built the inner poles of the two mitotic figures in Fig. 10 would form a binuclear cell. This would reappear after every successive division and persist thruout. He found no binuclear cells in material killed fifty-five hours after the action of chloral hydrate.

The cell wall, he found, is generally built between the two nuclei. Occasionally, however, it is started at the same side of both nuclei, but in such cases is never completed. In older cells which do not contain sufficient cytoplasm to fill the entire lumen of a cell, a bridge of cytoplasm stretches entirely across the cell between the nuclei. The wall is built along this bridge of cytoplasm: sometimes at one border of the brdige; sometimes centrally thru its mass. Often the nuclei are near the place of wall construction, tho not always; and the wall is often placed in a cleft of the cytoplasm, tho frequently not. He notes that the wall begins on one border of the cell and builds across as a growing half ring; and puts great stress upon the fact that it resembles the method in lowly organized plants where the wall begins as a ring on the entire periphery and continues its growth by additions to the inner edge of this ring.

Both amitosis and this peculiar wall formation he believes to be atavic characters brought on by the influence of the narcotic. He thinks: the narcotic pushes the cell back myriads of generations in a few minutes; effaces those characters latest acquired, such as mitosis; and brings into appearance the primitive ones. He compares this to the effect of an anasthetic on man. Under its influence those characters latest acquired, such as consciousness, fade away first; but the deeper-founded constitutional characters are the last to succumb.

He then asks the question: Can a nucleus that has divided amitotically later divide mitotically? and answers it in the affirmative. After working out two almost metaphysical reasons for this answer, he then gives what he considers conclusive evidence. The amitotically divided nuclei can, he believes, be distinguished from those of mitotic formation by the delay in building the wall. He finds the nuclei, which are thus distinguished as of amitotic origin, later dividing mitotically (see Fig. 10). He was in no case able to totally suppress mitotic division and have present only amitosis.

In his cultures he used one-half or three-fourths per cent. solution, acting for one hour. His list of cultures are:

No. 1 killed immediately after the action of chloral hydrate.

No. 2	"	1-1/2 hours	"	"	"	"	"	"	
No. 3	"	3	"	"	"	"	"	"	"
No. 4	"	7	"	"	"	"	"	"	"
No. 5	"	25	"	"	"	"	"	"	"
No. 6	"	31	"	"	"	"	"	"	"

No. 7 killed 48 hours after the action of chloral hydrate.

No. 8 " 55 " " " " " " "

He found amitosis (diatmese) first appearing in No. 4; most abundant in No. 5; and last present in No. 7. The abundance of amitosis, he notes, forms a very regular curve with the maximum in material killed twenty-four hours after the action of chloral hydrate.

While he found binuclear cells in abundance he in no case found cells containing more than two nuclei.

There is one other point brought out in his paper, which we must mention, before beginning a discussion of the article. He noticed many nuclei of very irregular form (see Figures 11 and 12). These nuclei as well as the cells that contain them are much larger than the surrounding ones. Often, too, there are three or four nucleoli in each nucleus. He was unable to give the significance of the large size of the nucleus and cell or of the numerous nucleoli. He believes, however, that in part, at least, the irregularities of the nucleus are due to amoeboid movements. He mentions this tendency towards amoeboid movements as another effect of chloral hydrate upon the nucleus.

Nearly all abnormalities of the nucleus mentioned in Wasielewski's article were found in preparations similarly treated; but there were many forms found in mine which he makes no mention of and which lead me to quite a different interpretation of structures he explains as amitotic. Many of these structures appear in preparations corresponding to which he has none, for example, material killed fifteen hours after the action of chloral hydrate.

In my original cultures I had only three preparations: material killed immediately after the action of chloral hydrate, material killed five hours later, and material killed twenty-four hours later. In material killed twenty-four hours after the action of chloral hydrate I was struck by the abundance of forms resembling Fig. 13--Wasielewski's typical diatmese of the nucleus. I at first interpreted these as amitosis, but sought to make this sure by finding the genesis and fate of the forms. To this end I began conducting my experiments as shown in the table given early in this paper. I found in material killed five and ten hours after the action of chloral hydrate many forms like Fig. 14, and in material killed fifteen hours after this action numerous forms like Figures 3, 4 and 5. It is evident that when the chromatin mass of Fig. 14 organizes a resting nucleus, this nucleus will show a considerable indentation on one side not unlike that shown by the resting nucleus of Fig. 13. The same is true of the chromatin mass of Fig. 4 which is already well started with the nuclear formation. Again I find, occasionally in preparations killed twenty-five hours after the action of chloral hydrate and frequently in preparations killed twenty hours after this action, forms like Fig. 16. This is clearly one of the nuclei which Wasielewski describes as dividing amitotically (by diatmese) in the spireme stage of mitotic division. When compared with the nuclei of the small cell of Fig. 18 or the mononuclear cell of Fig. 17, we see that the nuclei of Figures 13 and 16 are of about double size.

These facts suggest, at least, an explanation--differing radically from Wasielewski's both as to origin and fate of the structures--

for nuclear forms like Fig. 13. The spindle of Fig. 14 was probably in the act of pulling the chromosomes to the poles when affected by the chloral hydrate. The narcotic broke up the coordination of the spindle fibers and the simultaneous movement of the chromosomes to the poles, and finally lead to the destruction of the spindle. As a result of the broken coordination and final destruction of the spindle a lateral bridge of chromatin connects the two larger masses that have been drawn to the poles. Such forms as Fig. 13, then, do not result directly from the effect of chloral hydrate upon the nucleus but indirectly from the effects of chloral hydrate upon the kinoplasmic element (spindle) of the cytoplasm. These effects we have discussed in the early part of the paper. It is evident that the number of chromosomes entering into the construction of such nuclei as that of Fig. 13 is the number that would normally construct two nuclei. In accordance with the features of the individuality of chromosomes discussed in the early part of this paper these nuclei are about double the size of normal nuclei. For convenience they will be termed giant nuclei.

My preparations lead me to believe that binuclear cells, also, originate from the destruction of the spindle. In preparations killed five and ten hours after the action of chloral hydrate many cells are found in which the chromatin masses are thrown to opposite poles with the disorganized spindle mass between them, but no sign of a cell wall. In preparations killed fifteen hours after the action of chloral hydrate many forms like Fig. 5 appear. In these the chromatin masses have already begun the organization of nuclei. Here, too, the disor-

ganized spindle mass is still present. In Fig. 6, material killed twenty-five hours after the action of chloral hydrate, the resting nuclei are formed and the spindle has almost entirely disappeared. Fig. 10, material killed thirty-five hours after the action of chloral hydrate, shows the two nuclei of a binuclear cell in division.

Very often such binuclear cells show the wall partly built as is the case in the cells of Fig. 9. These, I believe, are identical with the partly built walls reported by Wasielewski, but of course he could not interpret them as being built by the spindle and yet maintain the amitotic origin of the two nuclei. My preparations have led me to believe, however, that all partially built walls that Wasielewski reports are built by the spindle in the ordinary way before its destruction, and that they are never completed. Wasielewski fails to see the connection of these partially built walls with the spindle, because he takes only end products, that is, observes the forms after the resting nuclei are fully organized and the remnants of the spindle have disappeared. In Fig. 3 we find that, altho the chromosomes were not all pulled apart, the wall had progressed far in its construction before the spindle was destroyed. Here, too, contrary to Wasielewski's idea, the cell wall is first constructed in the central part of the cell. I found a number of binuclear cells already in mitotic division which showed the cell wall between them only partially built. Wasielewski has little if any evidence that such a wall will ever be entirely built and he has still less evidence that his "incomplete mother division" will ever be completed by the building of the lacking wall. Contrary to his observations, I found binuclear

cells in the old specialized tissue of the root in material killed sixty-five hours after the action of chloral hydrate. This indicates that his "incomplete mother division" has not yet been completed. It is my belief that binuclear cells originate from the destruction of the spindle before the wall is even partly constructed.

(It must be stated in this connection that peculiar wall formations, not at all to be connected with these just discussed, are found in many of my preparations. I am at present unable to explain their significance. I have material in preparation to clear this point.)

My preparations indicate again that Wasielewski's large irregular nuclei (Figures 11 and 12) are produced by the broken coordination of the spindle fibers and the final destruction of the spindle. In such cases only a few of the chromosomes are thrown out from the mother aster in a scattered manner, when the destruction of the spindle occurs. Since such nuclei contain the chromosomes that ordinarily form two nuclei they are double normal size. If the spindle is destroyed before any chromosomes have been drawn from the mother aster a regular giant nuclei will be formed. I believe the large nucleus of Fig. 18 has originated thus.

Wasielewski mentions that he failed to produce similar abnormalities of the nucleus by the use of ether. Dr. Hottes in his unpublished researches reports similar forms produced by a ten per cent. ether solution.

Contrary to Wasielewski's results I found many trinuclear and a very few quadrinuclear cells (Figures 8 and 17). We have already ex-

plained that these originate by the spindle fibers throwing the chromosomes in three or four different directions, forming as many distinct groups of chromatin. The average size of such nuclei is, too, it must be noted, approximately inversely as the number. This agrees with the features of the individuality of chromosomes already discussed. Notice that the nuclei of the large cell of Fig. 17 average about half the size of normal nuclei.

Wasielewski has emphasized the doubling of the nucleoli as evidence of amitosis. Such a relation is hard to establish, because of the great variation in this respect even in normal material. I connect the fragmentation of the nucleoli with nutritive processes (agreeing with Strasburger's theory of the function of the nucleolus), rather than with division.

Nathansohn, in an article already cited, claims to have produced amitosis in Spirogyra. His arguments are ably answered in an article by Häcker (Mitosen im Gefolge amitosenähnlicher Vorgänge, Anatomischer Anzeiger 1 Januar 1900 Bd XVII), and by forthcoming works of Dr. Hottes. Häcker terms the distorted mitosis which we have described as pseudoamitosis and maintains that it has no other meaning than a distortion of the karyokenetic process by interference with its delicate machinery.

V. Methods Used by Wasielewski.

Some very serious faults may be urged against Wasielewski's method of research--faults which undoubtedly go far in leading him to misinterpret these abnormalities of the nucleus.

1. He started out with the intention of bringing about a certain

result--amitosis. Under the influence of this aim anything that had the appearance of amitosis seemed to be interpreted as such without a scrutinizing test to see if it could be interpreted otherwise.

2. He took, almost entirely, end products--material killed twenty-four hours after the action of chloral hydrate.

3. He failed to make preparations that would show the genesis of these end products--material killed fifteen hours after the action of chloral hydrate.

4. Finally he ignored or failed to see forms that would clearly show the fate of these products (especially his diatmese of the nucleus)--spireme stages as shown in Figures 16 and 20.

VI. Weakness of the Hypothesis of Amitosis.

The hypothesis--that these abnormalities of the nucleus are due to amitosis--shows some very weak points:

1. It assumes a thing which Wasielewski acknowledged is peculiar and which, it seems, is highly improbable: "Wie es kommt, dass die Chloralbehandlung bei einem Theil des Kernes, dem Gerüst, eine inactivirende, lähmende Wirkung hat (indem die hier sonst eintretenden Substanzsonderungen und-verschiebungen unterbleiben), bei anderen Theilen dagegen (Nucleolus und Kemmembran) eine activirende, erregende Wirkung (indem wir hier sonst nicht auftretende Bewegung sich vollziehen sehen), wissen wir nicht."

2. It gives no explanation for forms like those shown in Figures 2, 3, 4, 5, 7, 8, and 14.

3. It again breaks down in the presence of forms like Figures 16 and 20.

4. It is an attempt, in short, to explain, in a very hard way and in a way disagreeing with many of the facts available, a matter that can be explained much more simply and in a way according with all facts at hand.

VII. Conclusions.

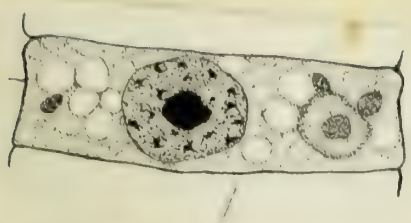
1. Chloral hydrate brings about the deposition of certain masses in the cytoplasm.

2. These masses may be of nucleolar origin and they may be caused by the chloral hydrate lowering the cytoplasmic activities without greatly reducing the food current from the nucleolus.

3. The chloral hydrate breaks up the coordination in the action of the spindle fibers and finally destroys the spindle.

4. Many abnormal nuclear structures--polynuclear cells, irregular giant nuclei, regular giant nuclei, and nuclei which Wasielewski describes as dividing by diatmese--result from the effects mentioned in (3).

5. The retained effects of the chloral hydrate often break up the coordination of the spindle fibers without destroying the spindle, thereby producing tripolar figures with the spindle in good condition.



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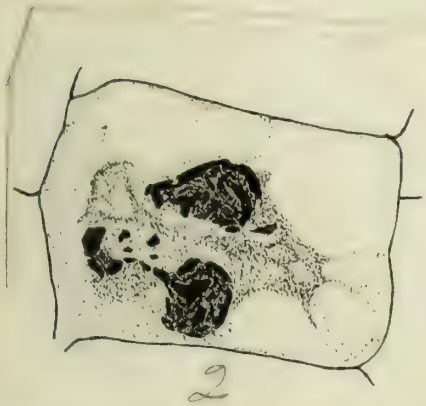

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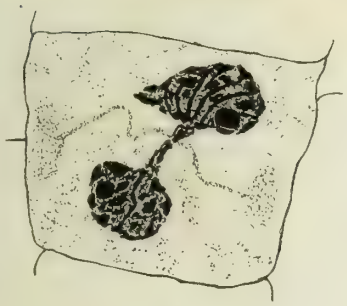

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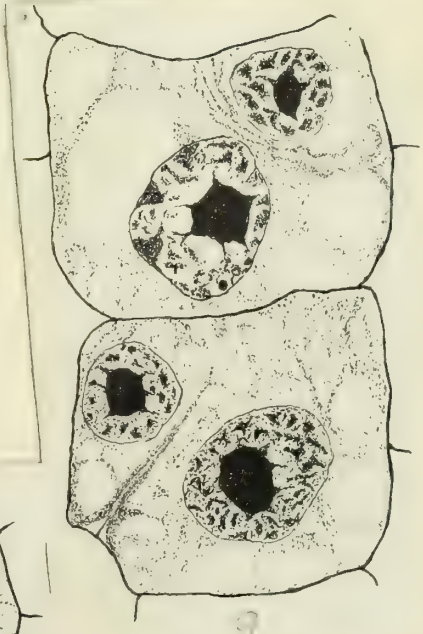

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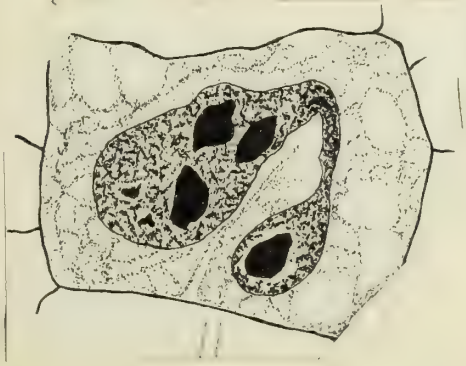

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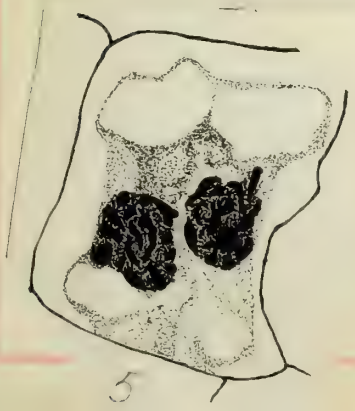

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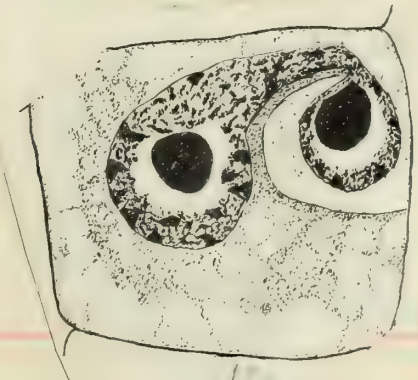

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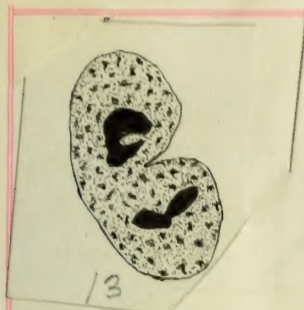
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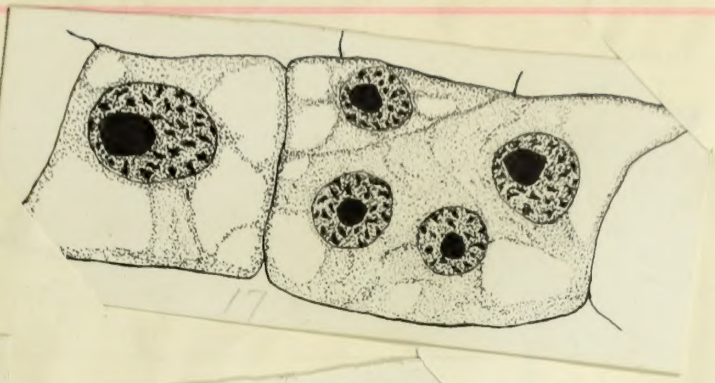


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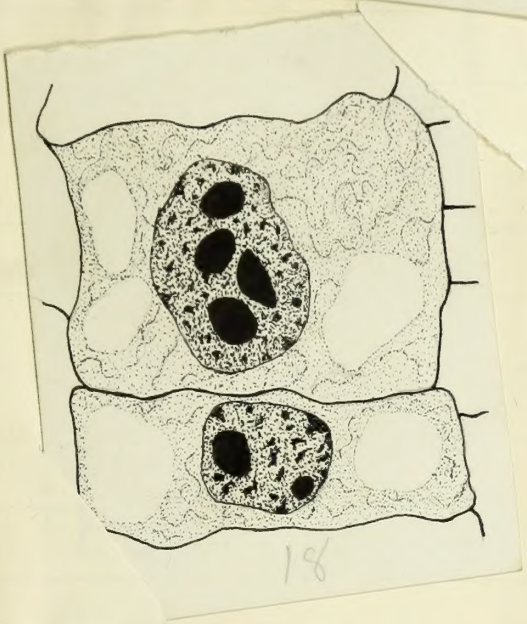
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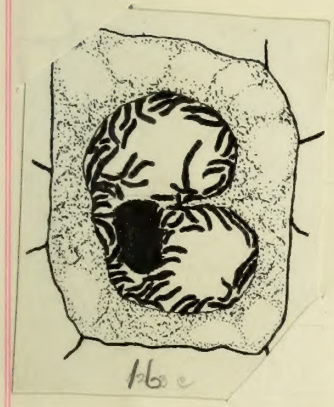
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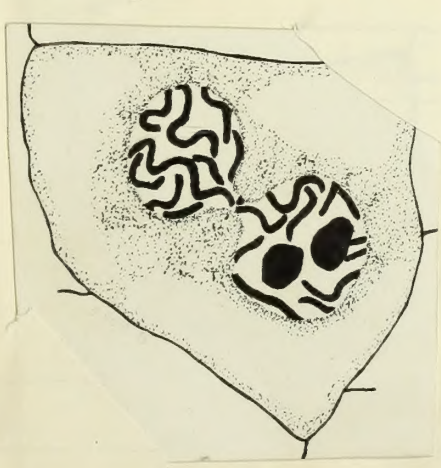

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