AMITOSIS IN CELLS GROWING IN VITRO.

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(3 PLATES, 27 FIGURES.)

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INTRODUCTION.

During recent years the conviction among cytologists has become more and more strongly intrenched that the problem of amitosis can not be satisfactorily solved by investigations based alone upon the study of non-living tissue, but that its successful conquest must rely principally upon the correct interpretation of the succession of morphological and physiological changes revealed by prolonged observation of the living cell, under normal and artificially varied conditions. Yet until the advent of the tissue culture method, workers in biology might well have despaired of ever being able to attack the question in this way. It is fortunate, therefore, that the technique of tissue cultivation has been so perfected that even the minute structural details of the living cell may be readily observed, and that the inspection may be continued for hours at a time; fortunate, too, that configurations which may be interpreted as stages in the process of direct division, such as dumb-bell-shaped nuclei apparently undergoing constriction, and bipartite nuclei, are not infrequently found in tissue cultures, and, furthermore, that fixed and stained preparations, to assist in the interpretation of the appearances

presented by the living cells, are easily made from such cultures.

These favorable circumstances plainly indicated the course to be followed in an attempt to gain some knowledge of direct cell division, and accordingly continuous observations of living cells, and supplemental studies of fixed and stained cells of the same type, were carried out.[1]

Method

The cells upon which observations were made were growing at a temperature of 39° to 40° Cent. from the tissue of embryo chicks from two to ten days old. Cultures were prepared by the method of Lewis and Lewis ('15), Locke solution being used as a medium. Activation of the growth was accomplished by the addition to the medium of a small quantity of autogenous embryo extract or bouillon. Thus the medium contained, besides the various salts, small quantities of dextrose (.25 to 1 per cent.) and protein. To offset the concentration due to evaporation during planting and in the moist chamber it was found to be of advantage to dilute the medium by adding 20 to 25 per cent. of freshly distilled sterile water. Heart tissue was most frequently used, and gave growths which were most serviceable for observation during the second twenty-four hour period.

By arranging the microscope within the incubator where the tissues were cultivated it was not necessary to expose them to a changed temperature during observation. A ray screen of copper sulphate solution was found to be advantageous when artificial light was used. Evaporation of the drop, with condensation about the walls of the moist chamber, was lessened by placing a small drop of distilled water in the cavity of the depressed slide, and by eliminating air currents from the vicinity of the culture.

Light seemed to have a deleterious effect upon the living cultures, so that lengthy continuous observation was not found to be practicable. Accordingly inspections were made as short

[1] The procedure of studying amitosis by the tissue culture method was suggested by M. R. and W. H. Lewis, and I desire to record my appreciation of their kind assistance and the use of their large collection of fixed and stained cultures, which was utilized in the investigation. To Prof. F. R. Lillie I also am indebted for his courtesy in placing a room at the Marine Biological Laboratory at my disposal, where some of the work was carried on.

as possible, and the light immediately turned off. The difficulties attending direct and prolonged observation of the living cell are not inconsiderable, for the eye must become accustomed to distinguish minute structures through the contrast afforded by varying grades of refractivity. At first only the most refractive bodies are discernible, standing out as bright points or lines, but gradually the less obvious structures, as mitochondria, and amœboid cell processes, come into view. Migration, in many of the cells, is quite active, and hence it is necessary to make observations frequently to prevent the cell from wandering away from the field of vision and becoming lost. The extreme sensitivity of the cell to light and heat, and to changes in osmotic pressure of the media from evaporation within the moist chamber, is responsible for the untimely termination of many observations, and this difficulty becomes the more important when it is realized that the study of a single cell must cover many hours to be complete.

It was at first planned to select a single living normal cell and observe it at frequent intervals over a long period in the hope that eventually a cell would be found which would divide by amitosis. This course, however, did not prove to be practicable on account of the infrequency with which amitosis, even of the nucleus alone, is met with. In a series of 20 fixed and stained growths from the heart of the embryo chick, in which there was a total of 41,725 cells,[1] only 50 constricted nuclei, which could be regarded as directly dividing, were found (a ratio of 1 to 835), and thus the chances of the occurrence of amitotic nuclear division in a cell selected at random were but little better than one tenth of one per cent. To avoid loss of time, therefore, the plan was adopted of selecting a cell in which the amitotic process

[1] The method adopted in making counts was as follows: 20 good preparations from cultures of chick heart of various ages and stages of growth which had been fixed and stained were selected. A small square was ruled with a diamond upon a piece of glass after the method of Isaacs ('15), and this ruled glass was inserted in the ocular so that a definitely outlined field was marked off upon the tissue culture preparation on the stage. By manipulating the mechanical stage successive fields could easily be brought into view, and the cells contained in them counted; in this way all the cells in the entire new growth were counted except imperfect cells and those near the original piece, which were several layers deep and were indistinct.

appeared to have already commenced, viz., in which the nucleus showed elongation and equatorial constriction, and observing in detail the subsequent changes which it underwent.

Such a cell presents an appearance of the following general type. The nucleus is somewhat lengthened, and, in the zone equidistant from its poles there is to be seen, on one or both sides, an indentation. In this concavity is situated characteristically a body, the centrosphere (fig. 1, *c*) whose refractivity is somewhat greater than that of the surrounding cytoplasm. Its outline is indistinct, but the edge seems to be irregular with short toothlike processes. These change their shape slowly, and give the impression of being pushed out and drawn in very gradually. They are intimately related to definite refractive bodies—the mitochondria—which, often rodlike and sometimes threadlike in form, radiate from the periphery of the centrosphere. Indeed the movements of the latter (described by Lewis and Lewis '15) may be responsible for the apparent movement in the periphery of the centrosphere.

Preparations fixed with osmic acid vapor and stained with Heidenhain's iron hematoxylin (which was the method generally employed) disclosed a minute granular body, generally paired, within the centrosphere, which is recognized as the centrosome or centriole (Fig. 24).

The position of the centrosphere within the nuclear concavity or cleft has been noted by various authors, including Maximow ('08), who found the centriole-pair thus situated in cells, the nuclei of which appeared to be dividing directly, in the mesenchyme of the embryo rabbit. In the cells of tissue cultures examined the centrosphere was absent from the cleft in only two per cent. of cases, and in these exceptions it may have been originally situated as in the others.

Observations.

A number of extended observations were made upon living cells containing such elongated and constricted nuclei, and, as a general rule, the nucleus rounded out again, assuming the usual form. Thus it was demonstrated that constriction alone does not indicate that the nucleus will divide directly. Finally,

however, a cell was found in which the nucleus divided directly while being watched, and the following paragraph, extracted from the protocol written at the time, is a brief description of the process as observed. The drawings (Plate I.) were sketched free-hand from observation at fifteen-minute intervals, and afterward retouched by reference to fixed and stained cells of similar morphology.

8.45 P.M. A cell (Fig. 1), growing from a 57-hour culture of 5-day chick heart, presented an elongated nucleus with a concavity upon one side. The outline of this concavity was indistinct on account of the fact that the centrosphere (*c*) was situated very close to it.

9.00 P.M. (Fig. 2). The nucleus is now straight and the indentation is almost obliterated.

9.15 P.M. (Fig. 3). The general outline of the cell has changed, and this has been followed by change in shape of the nucleus. At first there were two nucleoli within the nucleus, the lowermost being paired, but at 9.30 (Fig. 4) the latter appears as a dumb-bell-shaped body. The nucleus is now rounded.

9.45 P.M. (Fig. 5). The nucleus is still rounded, and the nucleolar substance consists of two masses close together.

10.00 P.M. (Fig. 6). The nucleus has become elongated again. There is no apparent cleft, but the left side, against which the centrosphere is resting is not so distinct as the right. The central nucleolus now appears as a single structure and some refractive substance, resembling another nucleolus, is seen in the lowermost pole of the nucleus.

During the next two fifteen-minute intervals (Figs. 7 and 8) a shortening of the nucleus occurred, a shallow concavity to the left being noted. This concavity is deeper at 10.45 (Fig. 9) its outlines being somewhat indistinct and the nucleus has increased in length; across the middle of the nucleus is a refractive line.

11.00 P.M. (Fig. 10). The concavity is seen with difficulty, and the line across the nucleus persists. Fifteen minutes after this (Fig. 11) the elongated nucleus shows an indentation upon the right side, opposite the one upon the left, and it appears to be undergoing constriction into two parts of equal size. Between the two nuclear portions the refractive line is seen as before, and, from the appearance shown in cells fixed and stained with iron hematoxylin (Fig. 24) this is evidently a strand of mitochondria.

11.30 P.M. (Fig. 12). The nuclear sacs are apparently quite separate, and between them is the strand of mitochondria, and also part of the centrosphere, this body, still undivided, having retained its position with reference to the nuclear cleft.

Judging from the behavior of this and other elongated, bent and dumb-bell shaped nuclei it would seem that the nucleus may return to its original rounded form provided the constriction has not gone too far, but, if the degree of constriction passes a certain critical point the nuclear sacs become completely separated.

Furthermore, after the critical point is passed the division occurs very rapidly.

The study of fixed and stained specimens served to throw considerable light upon the process of direct division, for, by searching the field, transitional forms were encountered, suggesting stages in the history of the living nucleus just described. In the terminal stage of direct nuclear division, such as that shown in Fig. 24, mitochondria were found characteristically lying across the slender strand of nuclear membrane which was all that remained of the connection between the two portions of the nucleus, and the centrosphere (which is undivided in these transitional forms, and also in the end product of nuclear amitosis, the binucleate cells) is situated in the cleft dividing these parts.

This process involves a more or less equal mass division of the nucleus without chromosome formation. It results in the production of a binucleate cell, and, if the process is repeated, of a trinucleate or multinucleate cell. Direct nuclear division was not observed beyond the bipartite stage, but it seems rational to suppose that (excluding the foreign body giant cells of Lambert (1912 *a* and *b*) the giant cells of tissue cultures are formed from the successive direct splitting of the nuclear fragments which become larger by normal growth. With this latter there is apparently associated an increase in the cytoplasm also.

It was not practicable to make a complete study of amitosis upon the same cell, and the latter stages of this process, succeeding direct division of the nucleus, were studied by selecting living binucleate cells and making prolonged observations upon them. A typical case, from a 24-hour culture of 8-day chick heart, is given as follows:

11.50 A.M. The cell, which was of the characteristic connective tissue type, showed two separate nuclear sacs, whose adjacent surfaces were in close contact. One sac contained three nucleolar fragments, the other one. Fat granules were fairly abundant, and were principally congregated at the nuclear poles. Mitochondria, long and threadlike, stretched between these granules and, where the latter were abundant, the strands of mitochondria tended to arrange them in rows. Mitochondria also radiated from the single centrosphere, situated opposite the area of contact of the two nuclear sacs. The triangularly shaped cell body was connected with adjacent cells by three main processes.

12.20 P.M. A narrow interval can be seen between the nuclear parts, showing that the latter have moved apart and are quite separate. Their position also has

changed. Variation in the nucleoli is noted, there being now three in one nuclear sac and four in the other. The outline of the cell body is now quadrilateral, and this shifting of form has perhaps accounted for the rearrangement in relative position and relationship of the nuclear parts. These, at 2.30, were again in contact, but subsequently repeated the process of moving apart and coming together three times during the observation, which ended at 11.15 p.m. During this time (11½ hours) the cell was observed continuously, and underwent constant minor changes, such as that of the outline, shifting about of cytoplasmic structures, and breaking up, recombination and variation in size and shape of the nucleolar fragments. After almost twelve hours the cytoplasm showed no indication of dividing.

These observations brought out the fact that what might be mistaken for a single nucleus divided by a membrane across its equator is really two nuclear sacs pressed close together, the equal tension in the two bodies resulting in a flat membrane between them, made up of the surfaces of contact. Child (1911) describes a type of amitosis which is characterized apparently by the growth through the nucleus of a membrane or plate, the subsequent splitting of which leads to the production of two nuclear sacs quite separate one from the other. Nuclear fission of this type was not found, and appearances suggesting a process of this kind probably result from the close relationship of separate nuclear parts, similar to the condition found in the cells of tissue cultures. Partitions within the nucleus are also simulated, in these flattened cells, by long nucleoli, mitochondria or folds in the nuclear membrane.

These observations also illustrate the characteristic behavior of the nucleolar bodies, which undergo constant changes in size, shape, number and position. These bodies stain well with gentian violet when applied to the living culture. They appear as dark masses after iron hematoxylin, but if differentiation with iron alum is carried too far what was before a homogeneous mass becomes a collection of granules (Fig. 27). The nucleolus appears to be a concentrated gel of varying density, the granules representing the denser areas.

Similar observations upon other living cells were carried out, and in no case did any direct fission of the cytoplasm occur; this finding was supported by the study of fixed preparations. On the contrary the history of these binucleate cells was the same as that of mononucleate cells of the same type. It was even found that mitosis occurred in these cells, for, during the obser-

vation of a binucleate cell in a young culture the good fortune was encountered of witnessing this entire process in it. A graphic register of the successive changes is afforded by the drawings (Plate II.) which were made with the aid of a camera lucida at the times stated. The following is extracted from the protocol written at the time of examination:

11.55 A.M. A typical connective tissue binucleate cell (Fig. 13) from a 19-hour culture of 7-day chick heart was selected for observation. The two approximately equal, sharply outlined nuclear sacs are in close contact, causing a flattening of the apposed surfaces. Each nucleus contains a single, somewhat irregular nucleolus. The cell is long and narrow, and the long axis of the nucleus is parallel with that of the cell in which it is contained. A single centrosphere (*c*) is found opposite the area of contact of the two nuclear parts. Numerous fat globules and mitochondria, the latter showing characteristic movements, are seen.

12.40 P.M. (Fig. 14). The nucleus is still double, and the principal change noted is the appearance of an additional nucleolus in the lower nuclear part.

1.20 P.M. (Fig. 15). The two parts of the nucleus are distinct. Only one nucleolus is now seen in each nuclear sac.

1.50 P.M. (Fig. 16). The cell outline has become modified, the cell body being shorter, and the long axis of the nucleus has changed so that it is now almost at right angles to its former direction. The nuclear surfaces are in close contact and the upper end of the membrane formed by their approximation is indefinite in outline.

3.05 P.M. (Fig. 17). The cell body has become more fusiform, and the long axis of the nucleus has rotated through 90°. The double membrane (formed by the areas of nuclear wall in contact) dividing the nuclear portions cannot be made out except at the left side, and there indistinctly. An indefinite, refractive substance, granular in character, is scattered along the line where this membrane had been; the nucleoli are indefinite, and the uppermost one has been joined by an additional, very small, mass of the same character. The upper part of the nuclear membrane is not so distinct as heretofore.

It would appear that the change which has occurred in the part of the nuclear membrane dividing the two nuclear sacs is a part of the general change affecting the entire nuclear wall and leading to its gradual disappearance, and the process is similar to that which occurs in the early stages of mitosis.

5.05 P.M. (Fig. 18). The refractive material in the zone formerly separating the two nuclear sacs is more prominent than before; it seems to be chromatin. The nuclear membrane is faintly marked.

Half an hour later this cell is seen to become gradually smaller, and to draw in its processes. At the same time the nuclear parts become smaller. The nucleoli also undergo diminution in size. Finally the cell takes a rounded and thickened form—6.00 P.M. (Fig. 19)—and is much more refractive than the cells surrounding it: in fact it resembles a cell in the prophase of mitosis. The fat globules and mitochondria assume a wreath-like appearance about the central clear space, in which the nucleoli soon disappear. Though the main mass of the cell is almost circular there are narrow processes attached to each pole. The cell remains apparently unchanged for some time, though undoubtedly important readjustments are going on within it.

6.50 P.M. (Fig. 20). The cell is even smaller than before, and more rounded. In the clear area within it is a refractive bar, which proves to be the equatorial plate of chromosomes. Individual chromosomes cannot be distinguished, so that it is impossible to count them, but their ends may be seen, as they project towards the poles of the spindle. The chromosomes show a slow, oscillatory type of movement, slight in extent. The spindle is represented by a fairly clear area, shaped like two cones base to base, at the extremities of which the centrosomes are situated. Astral rays cannot be seen. Mitochondria and fat globules encase this central area containing the spindle like a shell. This becomes evident by focusing up and down. Though the uppermost part of the spherical cell is on a level with the flat resting cells, which are to be found about it, the lowermost part is much below this, due to the fact that the cell is thickened, and projects into the medium.

7.05 P.M. (Fig. 21). The cell has suddenly become elongated and constricted at the equator; the plate of chromosomes has evidently split, and the anaphase of mitosis is being witnessed. The constriction about the middle of the cell can be seen to be increasing, causing streaming of globules toward its extremities. At the same time small, bubble-like processes emerge from the borders of the cell, seeming to be forced out by the internal pressure of the cell body. Into these protuberances granules flow but later return into the main mass of the cytoplasm. These processes soon become flattened and extended, forming pseudopodia possessed of hyaline borders with amœboid movement. The individual chromosomes of the two masses in the expanded extremities gradually lose their distinctness and become dispersed.

7.25 P.M. (Fig. 22). The constriction is more marked and the cells are almost entirely separated. They are also becoming flattened out. In the upper daughter cell a clear space for the nucleus is appearing. The constricted zone is somewhat more highly refractive than the surrounding tissue and resembles a short thread. This probably contains part of the remains of the spindle. Nuclear details are not yet visible.

As the cell is watched nucleoli become manifest, at 7.35 P.M. two of these being seen in each daughter nucleus in a clear space, surrounded by a distinct nuclear membrane.

8.00 P.M. (Fig. 23). The daughter cells are now almost of normal size. Each nucleus has a concave side, as is usual, and in each concavity is the new centrosphere, from which the mitochondria radiate. Two nucleoli appear in each nucleus. The cells are spread out and flattened, and the fat granules are disposed as in the ordinary cell.

The entire observation covered eight hours. The more active part of mitotic division occupied about two hours, but if the initial nuclear changes be included the duration of mitosis is much longer.

The sequence of changes followed in the above cell are in almost all respects similar to those of mitosis many times observed in the mononucleate cell. The only difference is that in the cell described there were to begin with two separate nuclear sacs instead of one. From the presence, in fixed preparations, of bipartite nuclei each portion of which is in a condition of spireme, and the absence, from such preparations, of bipartite nuclei in

which one portion only contains a skein, it is gathered that the prophase in these cells is characterized by the spireme appearing coincidently in the separate nuclear sacs (Fig. 25); later there is formed from the double spireme a single equatorial plate of chromosomes. Though these could not be counted there is no reason for believing that their number was more than that normal for the mononucleate cell.

Not only has mitosis been demonstrated by observation, both on living and fixed preparations, to occur in binucleate cells, but it has been found that it occurs relatively as frequently in these cells as in those with a unipartite nucleus. By making cell counts in the aforementioned 20 preparations from chick heart 375 binucleate cells were found in a total cell count of 41,725. Thus binucleate cells made up a percentage of the total of 0.9, or a ratio of 1 in 111. Among these binucleate cells 2 were found which were in the prophase of mitosis,[1] a percentage of 0.53. Of the mononucleate cells, which numbered 41,106, 47 were in the prophase, or 0.114 per cent. By comparison of these ratios it is found that mitosis occurs 4.65 times as frequently among the binucleate as among the mononucleate cells; allowing for the limited scope of the observation it seems reasonable to conclude that mitosis is as frequent a phase of the life of binucleate cells as of mononucleate, and it would seem that this is their normal method of proliferation. If, in addition, they be considered as dividing by direct fission (for which there is no evidence either from living or fixed material) they would then be possessed of an ability to multiply in excess of that of the mononucleate cells, and there is no reason for supposing this to be the case.

No evidence was brought to light that the separate parts of the bipartite or multipartite nucleus ever combine except during mitosis.

Another interesting observation was made in a fixed preparation, viz., that the early changes in the chromatin which presage the onset of mitosis (*i. e.*, the clumping of the chromatin and its

[1] Prophases alone were counted, in estimating cells in mitosis, since in this stage alone is it possible to distinguish the bipartite from the monopartite nucleus, on account of nuclear fusion in stages later than this in the case of mitosis in the binucleate cell.

segregation into short rodlike masses and finally into a spireme) can take place in a nucleus which is undergoing constriction. Thus it appears that mitosis can proceed as usual in nuclei partially divided or wholly divided by the amitotic process.

Discussion.

The direct division of the nucleus is associated with certain changes of the cell as a whole. Elongation of the nucleus seems to be a prerequisite, and this apparently is secondary to a lengthening and narrowing of the cell body occasioned by the pull of its processes. It has been pointed out that the centrosphere is situated characteristically in a concavity at one side of the nucleus, and, when the nucleus lengthens, this body sinks deeper into its side; at the same time, judging from fixed preparations, and also from the appearance of the living and dividing nucleus, mitochondria come to lie across this narrow nuclear isthmus. These bodies, the centrosphere and associated mitochondria, seem to play a part in the fission of the nucleus. The exact manner of their action is not clear, but it may be that streaming of the nucleoplasm away from the equator of the nucleus follows upon the mechanical irritation of the nuclear membrane by their movements, or possibly upon local alteration of surface tension from their chemical change. Certainly the position which these bodies take with reference to the constricted nucleus points to their participation in the process of fission.

The centrosphere does not divide, nor does it encircle the nucleus as in the form of division described by Meves ('91). The nuclear membrane remains intact and nothing resembling an amphiaster is formed.

Some theories of amitosis have attempted to place the responsibility for the initiation of the divisional stimulus upon the nucleolus, and it has been found by some investigators that in certain nuclei the nucleolus is the first body to become divided. Such a function of the nucleolus does not obtain, however, in the nuclei studied, for, although in some cases there was a division of the nucleolus into two parts, one of which became alloted to each separate nuclear part, yet, sometimes the nucleolus did not divide, and one of the nuclear fragments was without visible

nucleolar material (Fig. 24). Occasionally two fragments would be found in one nuclear part while the other had none; again it was very frequently found—indeed it was the rule—that a nucleus would have two separate nucleolar fragments without any nuclear division. In short not only was the division of the nucleolus in most cases not followed by nuclear fission, but the latter took place in many cases without division of the nucleolus.

Neither before nor after nuclear fission was there discovered an instance of differential staining of the two halves of a nucleus about to be divided, or already divided, as shown by Child ('07) so that there was no evidence of this kind to support the belief of Child that there may be a physiological independence of the nuclear areas even before they become amitotically divided, manifested by a variation in the staining of the two nuclear halves. The study of living nuclei, too, divulged nothing in favor of this hypothesis.

The result of this process of nuclear splitting was the formation of a cell containing one or more separate nuclei of about equal size, and each of about the same size as the nuclei of mononucleate cells. After nuclear fission the separated nuclear elements manifested the power of growth, and seemed to have metabolic independence. The cell protoplasm also of these cells shows an ability to increase in bulk. This is especially evident in giant cells which can thus become quite large.

It is believed, furthermore, that binucleate cells and giant cells in tissue cultures (except foreign body giant cells which arise by fusion of previously separate cells) do not arise in any other manner than that above outlined, for there is no other adequate explanation of their origin. A careful examination of living and fixed material does not reveal any evidence of fusion of cytoplasm without fusion of the nuclei, so that there are no grounds for admitting this as a possible theory of formation. Although many of the binucleate cells undoubtedly do migrate as such from the original piece, where they are doubtless formed in the same manner as they are in the new growth, yet an increase of over 100 per cent. in their number in the growth of the second day as compared with that of the first (as ascertained by making careful counts of the 20 heart specimens aforementioned) can

hardly be explained by the assumption of an increased emigration of these forms during the second day; it is more probable that some of these bipartite nuclei have originated in the new growth, and the observation of this process here confirms this conclusion.

Mitosis, too, cannot account for the formation of these bi- and multipartite nuclei, for in all the cases of mitosis followed through in the living condition the end result has always been two daughter cells, quite separate except for a narrow connecting process, and each containing a single centrosphere. The only theories remaining for consideration are those of nuclear origin *de novo*, or from chromidial extrusions, and these suppositions are too improbable to discuss here. No other process than that of nuclear amitosis, therefore, can account for the production of bi- and multinucleate cells in the cultures examined.

Though the amitotically-divided parts of the nucleus seem to possess metabolic independence, as noted, they do not appear to have reproductive independence, for the reason that they are never dissociated one from the other to become the nuclei of separate cells. Furthermore they show only one type of cell division, viz., mitosis, in which the process begins coincidently in the two nuclear parts, manifested by the simultaneous appearance in each part of a similar spireme. Although in amitosis there is a mass division of the nuclear material there is no meristic division, and it appears that before a cell containing an amitotically divided nucleus can divide it is necessary that the separated chromatin moieties should recombine. This was done in the specimen examined during life, for the combined product of the two nuclear sacs formed a single equatorial plate of chromosomes. Such a type of amitosis, therefore, is not incompatible with the chromosome hypothesis.

Spiremes in bipartite nuclei, and in dumb-bell-shaped nuclei evidently undergoing amitosis, are not confined to the cells of tissue cultures, for Maximow ('08) has described them in the normally-developing mesenchyme cells of the embryo rabbit, and this author refers to similar cell configurations which Karpow ('04) describes in the leucocytes of urodele amphibia.

Fragmentation.

A note may here be made regarding a pathological change which nuclei subjected to unfavorable conditions undergo, viz., fragmentation. This change consists in a breaking up and degeneration of the nuclei. In cells which had grown for six days in unchanged media, in which food and oxygen had become depleted and metabolic products had accumulated (Fig. 26) and also in cells growing in a medium to which a small amount of ethyl alcohol had been added (Fig. 27), this form of degeneration was seen. Multilobulation of the nucleus appeared to antecede the actual breaking away of the parts. These latter were of different shapes and sizes, often did not contain a nucleolus, and showed no power of growth. The cytoplasm enveloping them did not divide and apparently was incapable of increasing.

In preparations containing forms of this kind no mitoses were seen, and the phenomenon seemed to be quite different from nuclear amitosis which occurred only in healthy cells. It is believed, moreover, that the process of fragmentation has been confused with that of amitosis, and it is possible that it is this confusion which has accounted for certain well-known views which regard amitosis as an evidence of degeneration.

Vital Stains.

Finally, a word as to certain so-called "vital stains." It was hoped that gentian violet would prove to be of value in rendering visible the *minutiæ* of the living cell during its vital changes, since the results following the use of this dye recorded by Churchman and Russell ('14) and Russell ('14) with cultures of frog tissue were so encouraging. Unfortunately the dye proved toxic to the tissues used in a dilution of 1 in 200,000, and, though the nucleoli, nuclear membranes, certain granules and the cell borders were brought into sharp relief this staining was always accompanied by cessation of vital phenomena, and the cells speedily went into degeneration.

Janus green, in a dilution of 1 in 80,000, was also toxic, and, while it stained mitochondria specifically, yet these bodies soon became granular and lost their characteristic appearance. Hence neither of these dyes could be spoken of as acting "*intravitam*,"

and they were of value only in obtaining rapidly information as to the obscure structure of the cell; the cell, however, was thus sacrificed.

Summary.

The observations above described and the interpretations thereof may be briefly summarized as follows:

Amitosis was found to involve only the nucleus and was not a method of cell proliferation. It occurred in normal cells and was characterized by a separation of the nucleus into one or more parts which possessed no reproductive independence.

The process of nuclear amitosis consisted in a unilateral or bilateral constriction, manifested by a narrowing of the nucleus in the region of its equator, and a streaming of its contents toward the poles, with final separation of the two nuclear portions. This phenomenon seemed to be associated with the action of the mitochondria and centrosphere upon an elongated nucleus. There was no amphiaster or spireme formation and no centrosome fission. Division of the nucleolus was not an essential. Repetition of this process leads to the formation of a giant cell.

Not all nuclei which show elongation and constriction divide by direct fission, but many return to their usual rounded or oval form. When, however, the constriction has passed a critical point the division goes on to completion, and this final stage is rapid.

Cells containing nuclei in process of, or the result of, amitosis divide by mitosis. Mitosis in binucleate cells, which are the product of nuclear amitosis, is characterized by the simultaneous appearance in the nuclear parts of a spireme, from which a single equatorial plate of chromosomes is formed. Furthermore, binucleate cells divide as frequently by mitosis as do mononucleate cells, and this was the only form of division found to occur in them.

Since the parts of an amitotically divided nucleus do not become separated as reproductive units but divide only by mitosis, in which the chromatin in the parts is recombined, there is nothing in nuclear amitosis opposed to the chromosome hypothesis.

The type of amitosis in which the nucleus is split by the growth

through it from one side to the other of a membrane was not found. Nuclear figures simulating this proved to be caused by the close apposition of separate nuclear sacs, or by nucleoli, mitochondria or folds of the nuclear membrane.

The dyes, janus green and gentian violet, were toxic and their presence in the cell was incompatible with its continued life. They were, however, of service in quickly studying structural details which were not discernible in the living state.

Nuclear fragmentation, which differs in many ways from nuclear amitosis, is a pathological condition, and occurs in degenerating cultures.

It is believed that the facts brought to light through the tissue culture method may be applied to the interpretation of the phenomena of normally developing cells.

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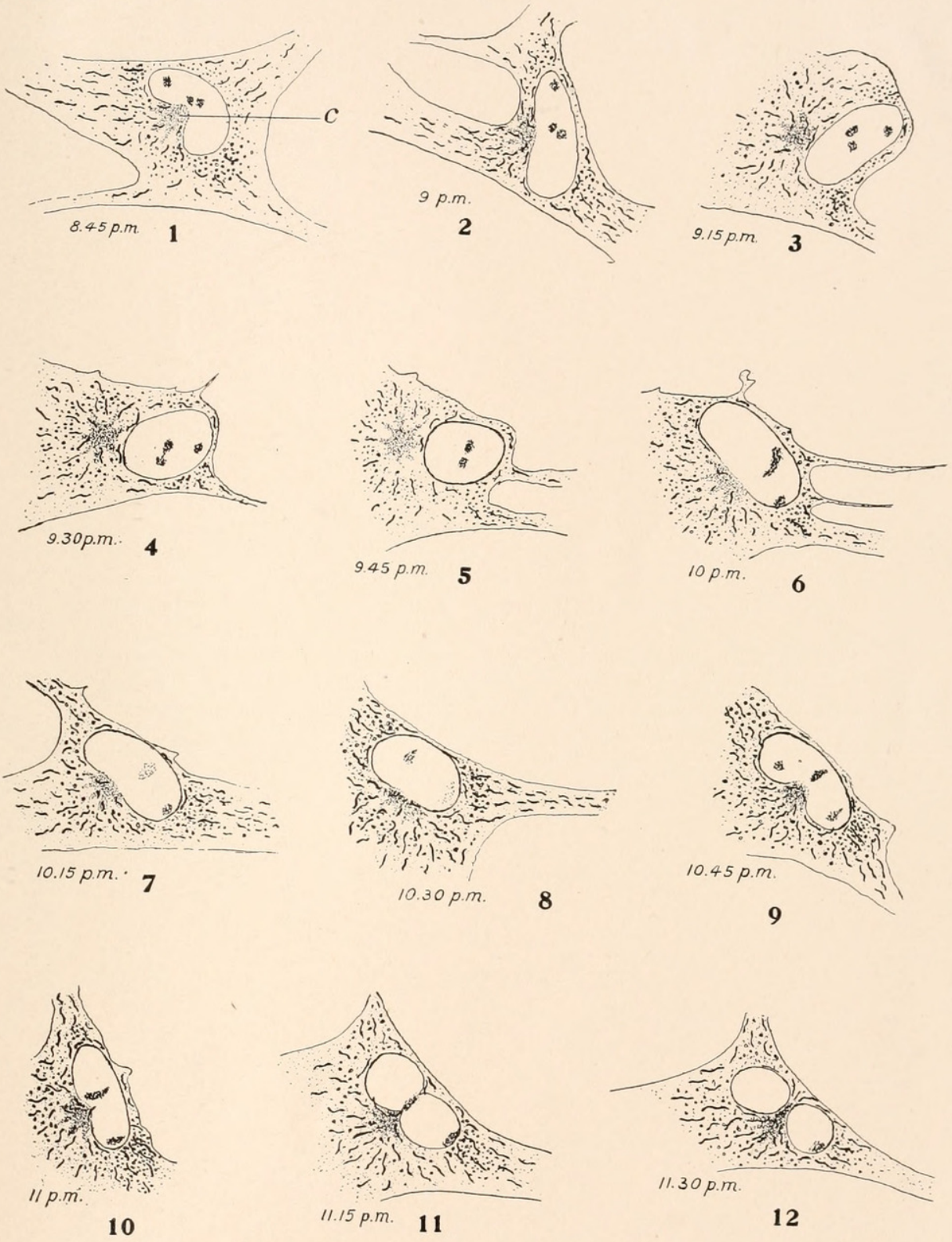
EXPLANATION OF PLATES.

PLATE I.

FIGS. 1 TO 12. Successive stages covering a period of 2¾ hours in the life history of a connective tissue cell from a 57-hour culture of 5-day chick heart, growing in Locke solution (0.5 per cent. dextrose with extract of chick embryo). The nucleus finally divides by direct fission. Small circles represent fat globules and short threads mitochondria. *c*, Fig. 1, points to the centrosphere. Free-hand drawings × about 900. (Description in text.)


c
8.45 p.m.
1
9 p.m.
2
9.15 p.m.
3
9.30 p.m.
4
9.45 p.m.
5
10 p.m.
6
10.15 p.m.
7
10.30 p.m.
8
10.45 p.m.
9
11 p.m.
10
11.15 p.m.
11
11.30 p.m.
12

C. C. MACKLIN.

PLATE II.

FIGS. 13 TO 23. Graphic record of the process of mitosis in a living binucleate cell, from a 19-hour culture of 7-day chick heart, growing in Locke solution (1 per cent. dextrose with extract of chick embryo). *c*, Fig. 13, points to the centrosphere. Camera lucida drawings. × 1,333. (Description in text.)

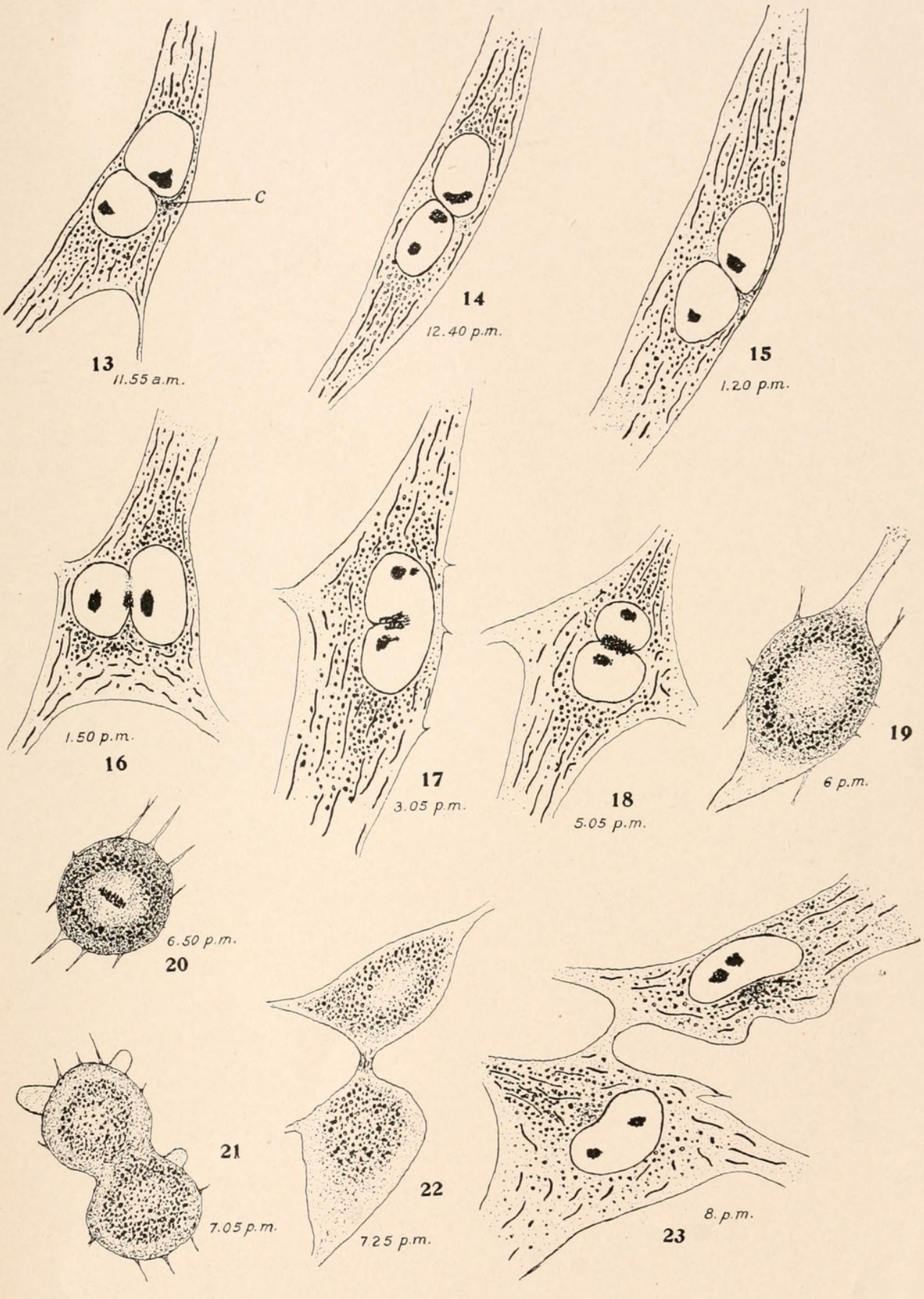

C
13
11.55 a.m.
14
12.40 p.m.
15
1.20 p.m.
1.50 p.m.
16
17
3.05 p.m.
18
5.05 p.m.
19
6 p.m.
6.50 p.m.
20
21
7.05 p.m.
22
7.25 p.m.
8. p.m.
23

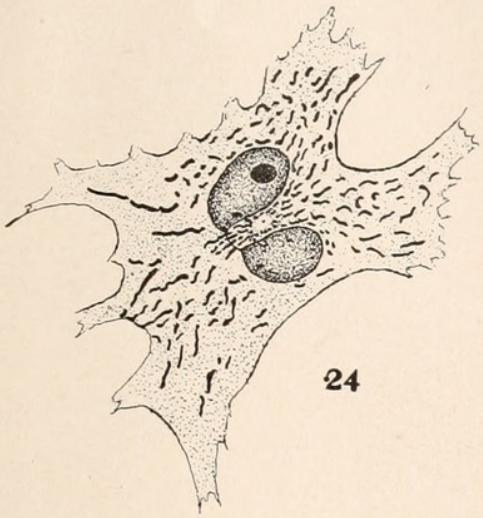
PLATE III.

FIG. 24. Direct nuclear division in a connective-tissue cell, final stage. Centrosphere between the nuclear parts. Across the slender filament joining these is a strand of mitochondria. Nucleolus has not divided. Camera lucida drawing from Preparation No. 2, from 5-day culture of 7-day chick heart in Locke solution (1 per cent. dextrose); osmic acid vapor and iron hematoxylin. × 915.

FIG. 25. Spireme in a bipartite nucleus. Prophase of mitosis. Nuclear membrane and nucleoli are disappearing. Camera lucida drawing from Prep. No. 14, 9–1–15 (Lewis collection). Heart from 6-day chick grown in Locke (0.5 per cent. dextrose) with a little yolk; fixed on third day of growth in Zenker; stained with iron hematoxylin (this culture was originally stained with Mallory's connective tissue stain). On account of the method of fixation the cytoplasmic details are not shown. × 915.

FIG. 26. Nuclei showing fragmentation. Camera lucida drawings from Prep. No. 23, 12–1–15 (Lewis collection). 5-day chick stomach in Locke (0.5 per cent. dextrose). Zenker; Mallory connective tissue stain. Culture grown for 6 days in the same media. × 1,012.

FIG. 27. Nuclei showing fragmentation. Camera lucida drawings from Prep. No. 23, 24–11–14 (Lewis collection). 6-day chick stomach in Locke (1 per cent. dextrose) to which ethyl alcohol had been added to make approximately 1 per cent. 3-day culture. Osmic acid vapor and iron hematoxylin. × 1,500.



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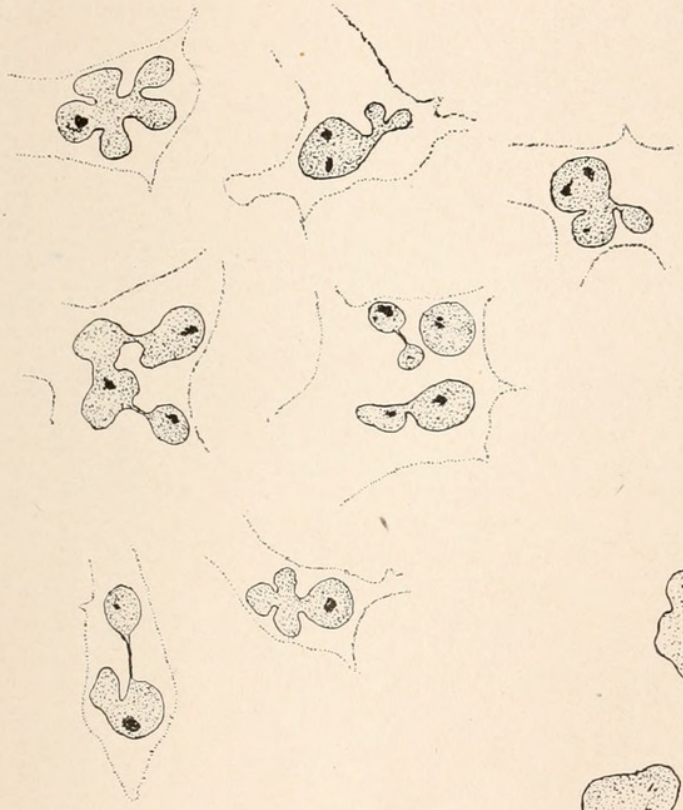

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