

# Reconstruction of Daughter Nuclei and the individuality of Chromosomal Vesicles during Interkinesis.

By

**J. McA. Kater,**

Botanical Laboratory, State University of Iowa.

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With Plates 4 and 5.

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## INTRODUCTION.

THE problem of reformation of daughter nuclei has proved to be a very elusive one. Even the more superficial aspects of the telophasic processes are, to some extent, matters of doubt and disagreement. It seems likely that elucidation of the reconstructive changes might be achieved by studying them without the presupposition that the components of the metaphase chromosome are equally basophilic. As the mass of daughter

chromosomes becomes transformed into an interkinetic nucleus one notes the appearance of strands or sheets of achromatic material. Many accounts have been given of this period which strongly indicate that such achromatic sheets are derived from the surfaces of the formed chromosomes. Further, that they serve as bags in which the chromatin of the metaphase chromosome is enclosed.

A brief consideration of the facts which lend support to such a view seems well worth while. In the early blastomeres of animal eggs the anaphase or telophase chromosomes ordinarily increase greatly in size and become much less chromatic than the metaphase chromosome. The imbibition of achromatic material from the cytoplasm is apparently responsible for this. The achromatin first appears as small, scattered globules which increase in number until the interior of the chromosome, now a chromosomal vesicle, becomes a veritable network of chromatin enmeshing spherules of achromatin. This achromatization or alveolization brings into evidence a thin sheath of material bounding the vesicle. The sheaths are, in most cases, no longer demonstrable after the vesicles become contiguous, except in so far as they give origin to the nuclear membrane when in contact with cytoplasm. In one case they have been followed through the resting nucleus (Richards, 1917). Chromosomal vesicles have been noted in animal eggs by a number of investigators, but their history has probably been followed more closely by Richards (1917) than any other. For this reason we will give some of his interpretations. He considers that the metaphase chromosome consists of an achromatic sheath (linin) or sac filled with chromatin, the alveolization of which gives rise to a vesicle whose border is merely the distended sheath of the earlier chromosome. In addition, that the linin of the resting nucleus as well as the nuclear membrane is traceable to the chromosomal sheaths. The resting nucleus, accordingly, would consist of contiguous vesicles, and there would be no interchromosomal channels present.

The unusual size of the vesicles and the resting nucleus in early blastomeres is probably responsible for the fact that

reconstruction of nuclei by chromosome vesiculation was first discovered here. Indeed, it was thought for some time that such a method did not exist elsewhere. However, Sutton (1900) found identically the same process in Orthopteran spermatogenesis. His work has since been extended to many grasshoppers by Wenrich (1916) and others. Wenrich also noted the formation of vesicles in somatic mitosis. The vesiculation of telophase chromosomes in somatic mitosis in animals has been observed in *Rana pipiens* and *Mus norvegicus albinus* by myself (Kater, 1927 *a*, 1928). It is to be remembered that the above view does not preclude the possibility of amoeboid processes being thrown out by the chromosomes during the period of reconstruction, but it does exclude the possibility of vesicles in the interkinetic nucleus being held apart, as it were, by such pseudopodium-like projections, so that achromatic channels would intervene.

Animal cytologists, for the most part, have come to regard the alveolization of chromosomes as a method of nuclear reconstitution of very frequent occurrence, to say the least; also its corollary that the resting nucleus is composed of contiguous vesicles. On the other hand many plant cytologists, probably the majority, look upon the metaphase chromosome as a more or less homogeneous body, at least not as a mass of chromatin enclosed in a sheath of different constitution. They hold that the reconstruction of daughter nuclei is a process involving not only alveolization of the chromosomes, but also branching and anastomosing of these units. According to their conception the chromosomes first draw together into a compact mass and later loosen up so that cytoplasmic channels intervene between the chromosomes. These channels are bridged by branching and anastomosing of the chromatin. This view has been especially championed by the Gregoirian school of cytologists (Gregoire, 1906; Gregoire and Wygaerts, 1904; Martins-Mano, 1905; Sharp, 1913, 1920, 1926; Robyns, 1924).

Although chromosomal vesicles are not commonly thought to occur in the somatic cells of plants they have been distinctly figured. Wager (1904) in his illustrations of nuclear division in

the root-tip of *Phaseolus* shows vesicles as distinctly as they have ever been seen in early blastomeres of animal eggs (see fig. 29 in his account). The present writer found his illustrations to be wholly justified. And further, traced the linin sheath of the vesicles through interkinesis in this same plant (Kater, 1926). Although Wager was not interested in chromosomal individuality and in his interpretations overlooked it, he merits credit for so accurately figuring his observations that really have a direct bearing on this important subject. The condition in *Phaseolus* is especially interesting when one recalls that its resting nucleus has been previously described as possessing interchromosomal channels which arose in the telophase (Martins-Mano, 1905).

It seems desirable to the author to investigate the reconstruction of daughter nuclei in some of the plants that have been used by the Gregoirian school with the object of determining whether or not linin can be differentiated in the reconstruction period, and to what extent the condition in certain other plants is comparable to *Phaseolus* and many animals, to express it conservatively. This will also afford an opportunity to evaluate the theories of telophasic duality.

The very great importance of this problem can readily be recognized. Firstly, its direct bearing on the subject of chromosomal individuality makes it of paramount interest, owing to the fact that our modern theories of genetics rest entirely upon the assumption of chromosomal individuality. In addition, any theories which we might have concerning cell physiology must be based upon a correct idea of the structures involved, if they are to have any semblance of verity.

The investigation described in these pages was begun at St. Louis University and completed in this department. I wish to thank Professor R. B. Wylie for the many courtesies that have been extended me here.

#### MATERIAL AND METHODS.

Somatic tissue from three plants has been used in this study. They are *Solanum tuberosum*, *Lycopersicon escu-*

lentum, and *Allium cepa*. Attention has been confined to the root-tips as they probably afford a greater variety of nuclear phases for the bulk of tissue used than any other part of the plant. These three plants were selected because of the gradation of chromosome size which they exhibit: *Solanum* having very small chromosomes, *Lycopersicon* having medium-sized ones, and *Allium* having very large ones. With these varieties of chromosomes it seemed that it would be possible to determine to what extent homologies can be drawn between these types and also to what degree the conditions in such representative plants are comparable to that of *Phaseolus* and most animals.

The root-tips were fixed either in Bouin's picro-formol-acetic or Flemming's strong mixture. Impregnation was done with low melting-point paraffin and embedding in hard paraffin. Sections were cut at 4 micra to 6 micra. The stains employed for most of the sections was iron-alum haematoxylin counterstained with light green. Other dyes were found to be of less value, though in *toto* staining with saturated eosin in 95 per cent. alcohol followed by haematoxylin after sectioning produced very nice results.

#### SOLANUM TUBEROSUM.

Reconstruction of Daughter Nuclei.—The chromosomes of *Solanum* are extremely small, as compared to those of most higher plants. According to Lutman (1925) the diploid number is thirty-six. No careful attempt has been made to determine their exact number, but tentative counts would indicate that Lutman's determination is, to say the least, close to correct. In the early anaphase these numerous small chromosomes are rather irregularly arranged in a band as they move towards the poles of the spindle. It is to be recalled that *Phaseolus* also possesses quite small chromosomes, though not nearly as small as those of *Solanum*, and in their migration to the poles of the spindle a very orderly arrangement is maintained, their long axes being consistently parallel. The same relationship is still found after they become contiguous.

This is mentioned because it may help to explain the apparent differences in the process of reconstitution in the two plants. Since they do not form a perfectly even band in the anaphase nor are their long axes parallel in *Solanum* (fig. 1, Pl. 4), they become enmassed at the poles ('*tassement polaire*') in a very unordered fashion. Some of them even seem not to be in contact with the cytoplasm. Rarely the chromosomes form a regular band at the poles (fig. 2, Pl. 4). After arriving at the poles the chromosomes draw together into such a compact mass that individual ones cannot be identified (fig. 3, Pl. 4). The same type of '*tassement polaire*' is formed in *Phaseolus*, but the chromosomes can be traced by the indentations on the surface of the basophilic mass (Kater, 1926). This is not true for *Solanum*. It is true that there are numerous indentations on the surface of the chromosomal mass, but the chromosomes are so small and their number so great that any such conclusion would be purely on the basis of homology with the bean.

This fact makes it evident at the outset that it will be impossible to follow the alveolization of individual chromosomes in this plant. However, that does not preclude the possibility of its study throwing much light on the subject of nuclear structure. It would seem that such a '*tassement polaire*' should afford excellent material for determining whether chromosomes draw apart to permit the formation of achromatic channels between them. This is undoubtedly true. In fig. 4, Pl. 4, one notes that the chromosomal masses exhibit a very even outline, and the proximal side is beginning to give evidence of alveolization. Spherical areas are becoming less basophilic, apparently the result of the appearance of many small achromatic spherules throughout their extent. The distal portion of the mass shows no evidence whatever that the chromosomes are becoming 'less closely packed' (Sharp, 1926), as it is still evenly and deeply basophilic. In addition, the achromatic areas in the proximal sides are not of such a shape that they could well be considered to be slits between adjacent chromosomes.

In some cases the outline of the '*tassement polaire*' is not so even. Projections are occasionally present which appear

very much as if they were derived from a part of a chromosome. Such a projection towards the equatorial side is illustrated in one of the daughter nuclei in fig. 5, Pl. 4. This whole body is becoming achromatized, as well as many other areas in both nuclei. The protuberance exhibits about the same nature and degree of chromaticity as the other alveolized areas, and it seems logical to assume that the cause of decreased basophilia is the same in both cases.

It is appropriate to inquire at this time what cause underlies the processes of reconstruction as far as they have been outlined above. There are two features of this process to be explained, namely, the increase of the size of the reconstituting nuclei and the decrease of basophilia in certain areas. Such an increase in size must be at the expense of the cytoplasm, that is, material must be absorbed from the latter. This assumption would explain, at the same time, the decrease of basophilia. The very small spherules that can be seen suspended within the chromatin are entirely achromatic. This would fit in with the above assumption. It seems probable that temporary permeability of the limiting portion of the nucleus to some cytoplasmic constituents is responsible for this absorption.

The last sentence introduces the topic of the limiting boundary of the nucleus. We might emphasize at this point the importance of studying the nuclear changes at the earliest point possible. It is probable that many of the erroneous conceptions of nuclear structure take origin in investigations which disregarded the very early steps in the transformation of chromosomes into an interkinetic nucleus. Such has been shown to be the case with regard to the origin of the nuclear membrane in the previous work on reconstruction in the bean, frog, and rat (Kater, 1926, 1927 *a*, 1928). It was distinctly shown that the nuclear membrane arises from the sheath or limiting membrane of the chromosomes. Let us return to fig. 5. The membrane which is visible over the achromatized areas and especially the projection from one of the daughter nuclei distinctly appears to be continuous with the surface layer of the non-alveolized portions. This is very indicative of a chromosomal-sheath origin for the nuclear

membrane, but is not nearly so clear as in the bean, frog, or rat. The evident condition in *Solanum*, however, is sufficient to entirely justify homology with *Phaseolus* with the consequent conclusion that the nuclear membrane in the former also arises from that part of the sheath of the chromosomes that happens to be in contact with the cytoplasm.

As the cell progresses farther in division, as evidenced by the formation of the cell plate, the above-mentioned processes continue. The alveolization of some areas is so great that entirely achromatic spots appear. As a consequence of this dechromatization inward extensions from the nuclear membrane become visible (fig. 6, Pl. 4). The illustration just referred to is very similar to fig. 4 of my paper on *Phaseolus* (1926) except that the outline of the nucleus is perfectly even and the direct indication that such strands are the borders of chromosomal vesicles is not quite so strong. However, the similarity is so striking that we will assume, for the present, that these rather achromatic strands are the vertical walls of two adjacent chromosomal vesicles. The nuclei at this stage show a decided tendency to have the non-alveolized portions of chromatin collected into one or a few clumps.

Further changes resulting in a return to the resting condition consist in increasing the extent and degree of alveolization and a rearrangement of the densely chromatic particles to form the nucleolus. In fig. 7, Pl. 4, the daughter nuclei are divided into a number of compartments by the sheets of linin and the larger non-alveolized masses of chromatin show a tendency to come together. Fig. 8, Pl. 4, shows the larger masses of chromatin lining up in the nuclei, preparatory to joining. Shortly after this stage the cell plate is completed and the remains of the spindle disappear (fig. 9, Pl. 4). At this time the nucleolus is formed, arising by the juncture of a number of non-alveolized chromatin masses, and the vesicles have become so rearranged that their sheaths radiate about the nucleolus. The vesicles contain numerous small granules of chromatin.

Before leaving the topic of reconstitution of daughter nuclei it is well to summarize the steps in the transformation of a



chromosome into a chromosomal vesicle. As mentioned above, when the chromosome first begins to take up achromatic material the chromatin appears to be the continuous phase while the achromatin is suspended in the form of minute spherules. The somewhat dechromatized areas in figs. 4 and 5 are of this nature. The continuation of alveolization reverses this condition. For example, in figs. 7, 8, and 9 the achromatin is continuous and the chromatin takes the form of granules suspended in the former. Whether or not this is a true reversal of phases cannot be stated. It seems possible that the preparation of tissue for study involving fixation, &c., might break delicate sheets of chromatin connecting the larger granules. Such an assumption would relegate this apparent reversal of phases to the realm of artifact. On the other hand, we know that a true reversal of phases is possible in some emulsions. Such may be the case here, but, at the present time, we must leave this feature unsolved.

Even as early as the stage represented in fig. 9 the nucleolus is slightly less chromatic than the metaphase chromosome. The nucleolus here is comparable to the alveolized areas in fig. 4. That this body is typically alveolized in the same manner as the reticulate portion of the nucleus, though to less degree, becomes more apparent in the resting phases. The figures accompanying this account show quite clearly that the nucleolus of *Solanum* is derived from slightly alveolized portions of chromosomes, and does not arise suddenly and from an unknown source in the late telophase, as Martins-Mano (1905) concludes. Lutman (1925) also seems to assume that the nucleolus does not have a chromosomal origin. This erroneous conception can very well be understood upon examination of their illustrations. They apparently skip too many early stages and consequently overlook the repeated coalescence of large chromatin bodies which results in the appearance of the nucleolus. Although the chromosomal origin of the nucleolus is evident in *Solanum* it is not nearly so clear as in *Phaseolus*. In the latter the distal portion of the chromosomes do not become alveolized but round off to form the nucleolus (Kater, 1926, figs. 4-8). The

development of this body in the potato is more similar to the condition in the frog and rat than to that of the bean.

The Interkinetic Nucleus.—The distinction between interphase and resting nuclei is a rather arbitrary one. It is also making differentiation and perhaps senescence a part of the definition of 'rest'. However, the terms are valuable providing their exact connotation is understood. Interphase, then, refers to the interkinetic period in rapidly dividing cells, such as the promeristem of a root-tip. Resting nuclei, on the other hand, are from cells that are dividing slowly and which consequently are somewhat differentiated or even senescent. The term for intermediate forms would be a matter of choice.

The interphase nucleus of *Solanum* contains one or more large nucleoli, the size being inversely related to the number. Relatively large achromatic globules are frequently found suspended in the chromatin substratum of the nucleolus (fig. 10, Pl. 4). The vertical walls of the chromosomal vesicles are again visible where they traverse the wide, hyaline perinucleolar zone. Sometimes they can be traced through the reticulum to the nuclear membrane, but in the majority of cases the reticulum, which is more darkly stained than they, obscures them. The sheaths of the vesicles give the impression of a mechanism for suspending the nucleolus in the midst of the perinucleolar zone. This hyaline zone is sharply delimited from the reticulum which occupies the rest of the space out to the nuclear membrane.

The dispersed chromatin (reticulum) presents an aspect quite different from the condition described above for the late telophase. The 'granules' are much smaller and are in continuity with each other, the chromatin again being the continuous phase. Achromatic spherules are suspended in its meshes. Within the continuous chromatic element one can discern more densely basophilic granules of variable size. Some of these are relatively large (fig. 10, Pl. 4).

In a resting nucleus, such as those found in the zone of maturation of a root, the nucleolus is much smaller than in interphase and has to a great extent lost its affinity for basic dyes. Occasionally it is visibly, though finely, vacuolated, which is

probably connected with the loss of basophilia. The perinucleolar zone is less extensive than in interphase, and the reticulum suspends much larger globules of achromatin and is itself much more homogeneous. The linin sheaths of the vesicles are visible in the hyaline perinucleolar zone and occasionally in the midst of the reticulum (fig. 11, Pl. 4).

The most important feature of the late telophase and interkinetic period as described above is the persistence of the chromosomal sheath in an apparently unaltered condition from the time that it first becomes microscopically visible in conjunction with alveolization of the chromosomes until the nucleus has reached not only a resting condition but a somewhat senescent one (fig. 11, Pl. 4). In addition it has been shown that the vesicles are contiguous, and are not separated by achromatic channels.

The difference in the reticulum of interphase and resting nuclei may be due to a greater degree of physical dispersion in the latter, but it seems probable that the early onset of senescence has actually decreased the amount of chromatin present. Lutman (1925) has shown that in distinctly senescent cells of the potato there is a great loss of chromatin, as compared to meristematic ones. The same phenomenon is well shown by fatigue in the cells of animals (Dolley, 1913, 1925).

It may be well to anticipate the suggestion that if the 'strands' were sheaths bounding chromosomal vesicles they would not appear so strand-like. This is not true for the same reason that the nuclear membrane is hardly discernible in surface view while in optical cross-section it is very apparent. Likewise the curved surfaces of the vesicle sheath that are nearest to vertical are the most evident. Refraction, of course, plays a part in this.

**The Prophase.**—The very early prophase varies to some extent depending upon the condition of the nucleus during the preceding interkinesis. When an interphase nucleus enters the prophase the disperse chromatin becomes collected into relatively few granules of appreciable size. This invariably exposes the linin sheaths of the vesicles so that they can be seen through-

out their extent from the nucleolus to the nuclear membrane (fig. 12, Pl. 4). At this early period the nucleolus is still quite chromatic. In resting nuclei that are entering division the same processes occur, but, naturally, the picture presented differs in the same manner as the resting antecedent, the chromatin granules being extended over almost the entire nucleus and the linin not being quite so evident. The nucleolus soon loses its basophilia, its chromatin content apparently being drawn off to give rise to more densely basophilic chromatin granules. These granules increase in number and size until the nucleus is fairly filled with them (fig. 13, Pl. 4). Naturally this procedure more or less obscures the linin.

An additional word might be said concerning reversal of phases in the nucleus. It will be recalled that it was not decided above whether the seeming reversal in the telophase is actually correct or is an artifact. In the early prophase when the reticulum disappears and the chromatin takes the form of granules, discrete or connected to form a beaded chain, we are justified in making a little more definite interpretation. Considering the fact that a little later all of the chromatin will be aggregated in a few chromosomes it would seem that this is most surely a true reversal of phases, the chromatin becoming the discontinuous or disperse phase.

The granular effect is soon lost and the chromatin becomes collected in more or less ribbon-like bands (fig. 15, Pl. 4). The bands seem to be located along one side of the vesicles, hiding the linin strands. Like the chromosomes, however, the ribbons are short, frequently not reaching the nucleolus, and the linin can be seen extending inward from their ends. It is especially noteworthy that these bands are single when they first arise. This would discredit the theories of anaphasic and telophasic duality. The nucleolus still remains as a very slightly chromatic body. Shortly after the stage represented in fig. 15, Pl. 4, the splitting of the chromosomes becomes evident.

Figs. 16 and 17, Pl. 4, are two sections of a single cell. These are very illuminating figures. It is to be noted in each that the chromosomes are double and that the sheaths of their vesicles

are still intact and can be seen stretching about through the interior of the nucleus. A strand never runs to the dividing point between two daughter chromosomes. If there were such a thing as a true duplication of the chromosomes in the telophase they would have to be separated by a linin partition, in the light of the present work, and we would expect to find linin sheaths separating them in figures such as those under discussion. The linin sheaths in these figures also show that the vesicles maintain their integrity even after the chromosomes are formed. In fact they are still visible in some areas after the nuclear membrane has started to disappear (fig. 18, Pl. 4). The nuclear membrane and the internal linin eventually dissolve and the contents of the nucleus and cytoplasm become mixed.

How does the new linin sheath of the chromosomes arise? Can continuity be applied to it also? These questions cannot be answered. The present condition is very similar to that found in the rat (Kater, 1928) and in that case it was suggested that the new sheath might be formed from one side of the old one. The fact that the prophase chromosomes always appear along one side of their vesicle might be interpreted to support such a suggestion. The nature of this problem, however, will make it very hard to solve. It is perhaps impossible to do so on the basis of morphological study alone. It may be, of course, that the new linin is differentiated anew from the surface of the chromatin as a precipitation membrane, but one is ordinarily loathe to suggest *de novo* origin unless there is something to support it.

In *Solanum* there is always a vestige of the nucleolus remaining after the chromosomes are formed. During the process of the development of the latter one finds a progressive decrease of the basophilic character of the nucleolus. This gradual transition is illustrated in figs. 12-18, Pl. 4, beginning with a chromatin nucleolus and ending with an acidophilic plasmosome. After the linin has dissolved and the chromosomes are lined up on the equator the nucleolus takes a dumb-bell shape and also extends across the equator (fig. 19, Pl. 4). It very quickly disappears.

Summary of Observations on *Solanum*.—(1) The numerous small chromosomes come together at the poles to form a densely basophilic mass in which the individual chromosomes cannot be distinguished. (2) The early alveolization of this mass shows that the nuclear membrane is derived from its border and that there are no interchromosomal channels formed. (3) Extension of alveolization discloses the linin sheaths of the vesicles which extend inward from the nuclear membrane. (4) The nucleolus arises from non-alveolized portions of chromosomes. This body gradually becomes achromatized and a vestige of it remains until the next prophase. (5) The chromosomal vesicles maintain their integrity during interkinesis and well through the next prophase. (6) The present interpretations would discredit the theory of telophasic duality.

#### LYCOPERSICON ESCULENTUM.

Mitotic Processes compared with those of *Solanum*.—The chromosomes of *Lycopersicon* are of medium length and not so numerous as in *Solanum tuberosum*. In their migration to the poles they typically assume a U-shape (fig. 20, Pl. 4). When they become contiguous at the poles of the spindle these U's are frequently compressed, having the two arms of the U pressed in upon one another. Consequently some of the chromosomes form two bands across the 'tassement polaire'. Their arrangement at the poles is far from regular, one arm of a U occasionally extending out into the cytoplasm in a manner similar to the 'finger-like' processes which Boveri has described in the reconstituting nuclei of *Ascaris megalocephala* (fig. 21, Pl. 4). Such processes in the daughter nuclei of the tomato are admirably suited to a study of the reconstitucional changes. We find that their alveolization results in the formation of a vesicle as described above for the potato (fig. 22, Pl. 4). Very few illustrations have been taken from the tomato. For the sake of economy they have been kept at a minimum, only enough being introduced to show that the conditions are fundamentally the same as in the potato. Fig. 22, Pl. 4, represents about the same stage of division as

fig. 5, Pl. 4. It is to be noted that in the former there is more inclination to restrict the early alveolization to the proximal side of the chromosome mass. In this respect *Lycopersicon* resembles *Phaseolus* (Kater, 1926, fig. 4). In fig. 22, Pl. 4, the bands separating the alveolized areas are quite chromatic. However, continued imbibition of achromatin converts the chromatin on the bands into the developing reticulum and leaves achromatic linin sheaths in exactly the same manner as in the potato, resulting in figures comparable to 6.

The nucleolus arises from non-alveolized portions of chromosomes and in the late telophase and interphase is very basophilic (fig. 23, Pl. 4). The apparent reversal of phases is again found. The disperse chromatin of the late telophase appearing as discrete granules scattered about in the vesicles. The latter radiate from the nucleolus as before.

The interphase nucleus of *Lycopersicon esculentum* is identical with that of *Solanum tuberosum* except that the perinucleolar zone is not nearly so large (fig. 24, Pl. 5). With a picture in mind of the hyaline perinucleolar zone as it appears in the interphase of both the potato and tomato we might consider whether or not it is an artifact. The condition in the onion is quite different and will be dealt with below. Many are inclined to consider this an artifact (Sharp, 1913; de Smet, 1914). However, in the two plants under discussion it seems to me that such is probably not the case. In the first place the perinucleolar zone is always present in interphase nuclei, but not invariably so in resting nuclei. In the latter state the reticulum is probably normally attached to the nucleolus. In addition to being an invariable accompaniment of interphase the area outside this hyaline zone presents a very normal alveolar structure. If the so-called reticulum had contracted from the nucleolus we would expect the achromatic globules of the disperse phase to be tangentially flattened. Such is not the case. We might emphasize here the fact that the interkinetic nuclei of both the tomato and potato are not reticulate as in some plants and animals, but foam-like.

The prophase presents no differences from the account given

for *Solanum tuberosum* except that the nucleolus is a little more ephemeral, usually vanishing before the nuclear membrane is entirely gone (figs. 25, 26, and 27, Pl. 5). In fig. 27, Pl. 5, two chromosomes are embedded in the remains of the nucleolus, as if they had been formed there.

#### ALLIUM CEPA.

Mitotic processes have probably been followed by the human eye more frequently in the root-tip of *Allium* than in any somatic tissue of other organisms. Therefore it might seem presumptuous for one to undertake the study of such material with the hope of elucidating problems of cell structure. However, the general lack of agreement on fundamental questions relating to nuclear reconstruction makes any organism a legitimate field for investigation. Perhaps there is more opportunity for correlating the previous and current ideas from material which has played a part in the controversies.

Reconstruction of Daughter Nuclei.—It is a matter of common knowledge that the chromosomes of *Allium* are relatively very long and thick, and that in their migration to the poles they typically assume a U- or V-shape with the bottoms of the V's towards the pole to which they are travelling (fig. 28, Pl. 5). As a consequence of this, when they become enmassed at the poles ('tassement polaire') each one is folded back upon itself, pushing the arms of the V's together. Thus each chromosome gives rise to two bands across the 'tassement polaire', parallel with each other and with the long axis of the spindle. During this procedure of bending the chromosomes back upon themselves they are more or less contiguous, some, however, being occasionally separated from the rest (fig. 29, Pl. 5).

In large chromosomes such as those of *Allium* one can discern appreciable alveolization in the early anaphase. The chromosomes lose the homogeneous appearance which characterizes them at metaphase and exhibit small achromatic spherules suspended in their chromatin substratum (fig. 28, Pl. 5). The imbibition of these achromatic globules continues



as the migration proceeds, so that when the 'tassement polaire' has been formed the globules are quite large and numerous (fig. 29, Pl. 5). The condensation of the chromosomes draws them so closely together that individuals cannot be distinguished except where their ends project freely from the general mass. This packing of the chromosomes so transposes them upon one another that the achromatic spherules pass out of view. By far the greater bulk of the chromosomes is yet basophilic, and such an obstruction of the view of small achromatic globules is to be expected. We have, however, every reason to suppose that they are still present throughout the mass. The individual ends of chromosomes that can be seen show even more alveolization than in preceding stages (fig. 30, Pl. 5). This condition quickly changes. The solid mass of chromatin becomes broken up into alternating light and dark bands (fig. 31, Pl. 5). The figure referred to is almost identical with a photomicrograph of a reconstituting nucleus in *Trillium* as published by Gregoire and Wygaerts (1904). It is on just this type of picture that the Gregoirian school bases their theory of the 'loosening up' of the chromosomes. The appearance of alternate chromatic and achromatic bands in the reconstituting nucleus must be explained, and they do so by assuming that the ends of the chromosomes, forming nuclear membrane, remain in contact while their inner portions draw away from each other permitting cytoplasmic material, probably spindle substance, to rush in between the chromosomes. The formation of the reticulum is explained by supposed branching and anastomosing of chromosomes by way of bridging these cytoplasmic intranuclear channels. According to this conception the achromatin of the resting nucleus would be both interchromosomal and intrachromosomal. This theory served its purpose at the time when no better explanation was suggested, but since there is absolutely nothing to support it, it is high time for it to give way to a conception based on observation of more favourable material. There is nothing in the discernible behaviour of the reconstituting nuclei of *Allium* that will absolutely disprove the above assumption, but there is a great deal that argues

against it. In the first place, the ends of the chromatic bands are frequently farther separated than the inner part (fig. 31, Pl. 5). If the nuclear membrane is derived from the chromosomes, as is widely believed, including Gregoire's school (Robyns, 1924), it would be hard to understand this condition. Secondly, the channels are never entirely achromatic. The strongest argument against this 'loosening up' idea will be shown in the description of the prophase.

*Allium* does not afford the best of material for investigation of nuclear reconstruction, but, as outlined above, the few discernible steps in the process indicate that the conditions are the same as in more favourable forms, such as *Phaseolus* and *Mus norvegicus*. In both of these organisms the vesiculation of contiguous chromosomes is the means of reconstruction (Kater, 1926, 1928). In the former alternating bands are not formed in the vesicles, one end remaining solid chromatin, while in the latter the chromatin and achromatin banding appears exactly as in *Allium*. In addition the linin sheaths of the vesicles can be seen in this stage of division in the rat. The individual vesicles can be followed on the surface of the nucleus by indentations and extending inward from these points of contiguity of adjacent chromosomes one can see the linin sheaths of the vesicles, in case they traverse one of the achromatic channels (Kater, 1928, fig. 1 b). The similarity of the processes in the onion to those found in mitosis in the cell of the rat is sufficient to justify concluding that they are fundamentally the same, that is, the achromatic channels are merely greatly alveolized sections of chromosomal vesicles. However, I am willing to admit that on a basis of my observations on *Allium* alone I would be at a complete loss for any explanation of the conditions depicted by the accompanying illustrations. Plants with large chromosomes, it seems, afford less opportunity for solving the intricate problems of nuclear structure than those with smaller ones, such as *Phaseolus*.

The parallel chromatin bands soon have their relationship modified, their distal ends drawing together at one point,

forming a relatively large chromatin mass from which the bands radiate. At the same time they become more slender (fig. 32, Pl. 5). The chromatin mass formed from the ends of these bands is the nucleolus. This becomes evident as the bands become entirely alveolized while the central body remains. Linin sheaths occasionally can be seen radiating from the nucleolus also (fig. 33, Pl. 5).

Rarely the chromatin bands seem to be made up of a double row of granules. The appearance which has led to the theories of telophasic duality. Such a seeming duality is unquestionably exhibited by some reconstituting nuclei, but how far we are justified in going with interpretations is another matter. In the middle of the upper nucleus in fig. 33, Pl. 5, a linin sheath extends from the nucleolus out between a row of double granules. This suggests to me that the duplicity is produced by rows of granules happening to be located along the contiguous sides of two vesicles. This would make the two halves of a double row products of different vesicles. The condition illustrated in fig. 33, Pl. 5, is very rare, which would deter one from basing much on it. In addition, the origin of chromosomes in the prophase is antagonistic to the idea of telophasic duality.

As alveolization of the remaining chromatin proceeds the reticulum becomes more complete. By the time the cell plate has extended entirely across the cell the nuclei are practically in the interkinetic condition (fig. 34, Pl. 5).

The formation of the nucleolus is not always as regular as fig. 32 would indicate. In such a case only one is formed, while the more frequent number in the late telophase is two (fig. 34, Pl. 5).

**The Interkinetic Nucleus.**—The interkinetic nucleus is typical for plants with large chromosomes. Many animal nuclei are also of the same type. In both interphase and rest the chromatin is the continuous phase and presents a reticulate aspect. The reticulum is made up of moderate-sized granules of deeply basophilic chromatin which are connected by thin sheets of oxychromatin. The granules with their connexions form triangular or rectangular figures, quite at variance with

the foam-like state of stained nuclei in the potato and tomato. The resting nuclei of young somatic cells of animals are of the same type as the onion nucleus. The heavy reticulum entirely obscures the linin sheaths of the chromosomal vesicles in well-fixed material (figs. 35 and 36, Pl. 5).

According to the current conception of protoplasmic structure the reticulate appearance of the nucleus must be an artifact, while the nucleus of *Solanum* preserves more nearly its normal condition.

The nucleolus at interphase is distinctly a chromatin nucleolus or karyosome (fig. 35, Pl. 5). As one follows a single row of cells up the root it is observed that as the cells pass into rest the nucleolus becomes less chromatic (fig. 36, Pl. 5). The net-knots of the reticulum also decrease in basophilia, but not to as great an extent. The nucleolus is typically connected to the reticulum, apparently being merely a gigantic net-knot. The conclusion of Sharp (1913) and de Smet (1914) that the perinucleolar zone is an artifact is probably correct in the case of *Allium*. Perhaps it is true for all plants having similar-sized chromosomes. Cells which give evidence of distortion from fixation have an appreciable hyaline area surrounding the nucleolus (fig. 37, Pl. 5). Whether or not this is an artifact, its occasional incidence is fortunate because it throws light on the structure of the nucleus. The statement was made above that the heavy reticulum obscures the linin sheaths of the chromosomal vesicles. The pronounced visibility of these sheaths in the perinucleolar zone shows that they are present here as well as in the potato, bean, rat, &c., and justifies speaking of them as present entities.

The Prophase.—Although the telophasic conditions in *Allium* are of themselves not conclusive they are indicative of homology with *Solanum*, *Phaseolus*, *Rana*, *Mus*, &c. The origin of the chromosomes in the prophase makes the similarity even more striking. When the reticulum breaks up in the early prophase and the chromatin becomes aggregated in masses or cords one can frequently see that these aggregates are limited by vesicle walls (fig. 38, Pl. 5). There is an inclination

for the nucleolus to be located at a meeting-point of the vesicles. This is only partly true in the figure just referred to. The aggregations of chromatin become more chromosome-like as the nucleolus becomes less basophilic (fig. 39, Pl. 5). By the time the polar caps have appeared the chromosomes are fully formed and it is to be emphasized that they are not double. The nucleolus has become, at this stage, very acidophilic and the sheaths of the vesicles are still present (fig. 40, Pl. 5).

The splitting of the chromosomes takes place shortly after the polar caps appear and before the nuclear membrane begins to disappear. They are distinctly double in fig. 41, Pl. 5.

Summary of Observations on *Allium*.—(1) The large chromosomes become enmassed at the poles in such a manner that each one forms two loops across the nucleus, parallel with the long axis of the spindle. (2) Vesiculation of the chromosomes begins in the anaphase. (3) The underlying process is not at all clear in the formation of alternate chromatic and achromatic bands in the nucleus, but the observable facts indicate that it is probably homologous with the condition in *Mus norvegicus*. (4) The nucleolus arises from non-alveolized portions of the chromosomes and gradually changes from a karyosomic to a plasmosomic state. (5) There is no perinucleolar zone in well-fixed interkinetic nuclei. (6) The linin sheaths of chromosomal vesicles are visible in the perinucleolar zone, when the latter is present, and also become apparent in the prophase. The splitting of the chromosomes occurs in the prophase.

#### DISCUSSION.

The Chromosomal Cycle.—The anaphasic and telophasic processes in the somatic cells of these three plants apparently correspond identically with those same processes as disclosed in my earlier studies on *Phaseolus*, *Rana*, and *Mus* so far as fundamentals are concerned. The details of the telophasic transformations in *Allium* are very like the condition in *Rana* and *Mus*, while *Solanum* more nearly resembles *Phaseolus*. The underlying physical process is

the same as in the early cleavages of the egg, namely, imbibition of achromatin, which is probably an osmotic phenomenon. The reason for thinking it an osmotic transfer of material is that the amount taken up seems to be proportional to the amount of cytoplasm present. If part of the cytoplasm of an early blastomere is removed the developing nucleus will be much smaller and more chromatic than its sister nucleus, which can well be interpreted to mean that the amount of achromatin imbibed by the nucleus depends upon the equilibrium point of an osmotic interchange. In the budding of yeast the same number of chromosomes enter the bud as remain in the parent cell yet the resulting nucleus is smaller (Kater, 1927 *b*).

This conception of the vesiculation of contiguous chromosomes does not preclude the possibility of the chromosomes sending out amoeboid processes before the formation of the 'tassement polaire', but it does mean that the chromosomes are not separated by achromatic channels which are bridged by anastomosing projections from the chromosomes. The Gregoirian school has mistaken lateral alveolization of the chromosomes for 'becoming less closely packed' (Sharp, 1926). Although, as shown in the present work, there is nothing in the nature of proof with regard to anything obtainable from the study of *Allium*, the conditions so closely resemble those found in *Mus* that homology is justified. Bolles Lee has lately written (1924) on the reconstruction of nuclei in *Paris quadrifolia*. He considers that the chromosomes of the 'polar clump' ('tassement polaire') are separated in their mid-region by the appearance and growth of achromatic globules between them. In the interphase these large alveoles lose their integrity and their substance becomes disseminated through the nucleus. In the light of the present series of studies it can be safely said that the filaments which he interprets as chromosomes are only non-alveolized bands or axes of the chromosomes. The globules are doubtless present, but are probably inside the linin sheaths of vesicles, and instead of pushing the chromosomes apart are merely distending individual

vesicles and consequently the nucleus increases in size and the chromatin bands become spaced farther apart.

The metaphase nucleus, then, consists of a specific number of chromosomes which are linin sacs filled with chromatin, while the telophase or interkinetic nucleus consists of the same number of chromosomal vesicles, each one of which arose from a chromosome by a process of alveolization. In the alveolization the linin sheath is unaltered except in the matter of distension. The chromatin remains continuous and the achromatin constitutes the disperse phase of the nuclear system. Therefore the reticulum is made up of a single substance, chromatin. This conception of the reticulum has been especially advanced by Gregoire and his school. The only advance in this direction that the present studies has made is the discovery that the reticulum of each vesicle forms a unit and is not directly connected to the reticulum of the adjacent vesicles. This is especially evident in the young oocytes of the rat. Animal cytologists have been especially inclined to consider the reticulum as a framework of linin with attached particles of chromatin. Richards (1917), who very credibly succeeded in tracing the chromosomal vesicles through interkinesis in the early blastomeres of *Fundulus*, thought that the linin sheaths of the vesicles sent branches inward to form the reticulum. The structures which he saw were probably thin sheets of chromatin separating alveoli.

The origin and fate of the nucleolus in the three plants used in this investigation are the same as in the rat. It arises from non-alveolized or only slightly alveolized portions of the chromosomes. As the cell progresses through the resting and prophasic stages the nucleolus gradually becomes achromatized, changing from a karyosome to a plasmosome. This is in harmony with the growing idea that the nucleolus is derived from the chromosomes, whether it be a chromatin nucleolus or a true nucleolus (Wilson, 1925). The work on senescence and fatigue (Dolley, 1913, 1925; Lutnam, 1925), as well as that on metabolic activity (Huie, 1897), indicates that the nucleolus is a storehouse for chromatin which is inactive.

The exact origin of the chromosomal sheath is yet obscure. The prophase chromosomes are the endogenous offspring of vesicles, but whether the new sheath is formed from the old vesicle wall or is formed *de novo* as a precipitation product of the chromatin, or what not, is unknown. This problem is beyond the scope of the present investigation, although it is a very important one and would merit a great deal of consideration should a fruitful mode of attack be devised.

**Chromosomal individuality.**—Genetic continuity has been the central feature of every problem in cytology. The continuity of the cell as a whole was naturally the first great step in the achievement of the early cytologists. This was followed some two decades later by the conclusion of Flemming and Strasburger regarding the continuity of the nucleus. Rabl (1885) suggested a theory of chromosomal continuity which has been supported in one way or another by almost every cytologist. Most of the evidence that has been advanced is of an indirect nature, such as constancy of number and form of chromosomes, maintenance of polyploid and aneuploid conditions, hybridization studies, &c. Although this type of evidence is very strong the subject of chromosomal individuality must remain hypothetical so long as it is based on inferential evidence.

It remained for Richards (1917) to bring forward direct evidence for chromosomal continuity. He succeeded in tracing the chromosomal vesicles, with distinct outlines, through interkinesis and found a prophase chromosome appearing in each and every vesicle. In the present series of papers direct evidence for genetic chromosomal continuity has been obtained from the somatic nuclei of the bean, frog, potato, tomato, and onion, and from somatic and germ nuclei of the rat. In addition, the same resting structure which is evident in these forms has been seen in the nuclei of a number of other organisms. It is not only probable that the universal method of nuclear reconstruction in Metazoa and Metaphyta is by chromosome vesiculation, but also that the vesicles maintain their integrity during interkinesis. Chromosomal individuality no longer rests solely on



an inferential basis, but now has direct evidence to support it. The chromosome can be elevated to the same position as the cell whose continuity was expressed as a fact by the dictum 'omnis cellula e cellula'.

#### GENERAL SUMMARY.

1. The reconstruction of daughter nuclei in *Solanum tuberosum*, *Lycopersicon esculentum*, and *Allium cepa* is by the vesiculation of contiguous chromosomes.

2. The integrity of the chromosomal vesicles is maintained during interkinesis, and a prophase chromosome is formed in each vesicle before its walls disappear.

3. The anaphasic and telophasic alveolization consists in the imbibition of achromatic globules which become the disperse phase of the nuclear system.

4. The reticulum is considered to be a single substance, chromatin. The variation in staining reaction is probably due to physical dispersion.

5. A plasmosome is merely an achromatized chromatin nucleolus. The nucleolus is derived from chromosomes.

6. It is suggested that chromosome vesiculation is probably the universal method of nuclear reconstruction, and also that the vesicles probably maintain their integrity throughout interkinesis.

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## EXPLANATION OF PLATES 4 AND 5.

All figures drawn with aid of Abbe camera lucida. Figs. 1-27 inclusive from sections 4 micra thick; figs. 28-41 inclusive from sections 6 micra thick. Figs. 8, 11, and 28-41 from root-tips fixed in Bouin's picro-formol-acetic; remaining ones from material fixed in Flemming's strong. All sections stained with iron-alum haematoxylin and counterstained with light green, eosin, or orange G. Magnification 1,800 $\times$ .

## PLATE 4.

Figures 1-19 inclusive are of *Solanum tuberosum*.

Fig. 1.—Anaphase. The chromosomes are irregularly arranged on the spindle.

Fig. 2.—The chromosomes are becoming contiguous at the poles of the spindle. Note that the chromosomes are larger than in fig. 1.

Fig. 3.—The chromosomes have apparently fused into a single mass, 'tassement polaire'.

Fig. 4.—Slightly alveolized areas are appearing in the proximal ends of the chromosome masses.

Fig. 5.—Extension of alveolization.

Fig. 6.—Alveolization has disclosed the presence of the linin sheaths of the chromosomal vesicles.