On the Structure and the Development of Starch Grains II. The structure and the development in general, the classification and the heredity in the starch grains¹

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(With 6 Diagrams)

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Introduction

In the previous paper the writer reported the results of the structure as well as the developmental studies in several kinds of starch grains. The results lead the writer to conclude that the starch grains not only in the plant cells but also those taken out from the cells can not be separated from the plastid-plasma as long as they have keeping the structure even after they have somewhat damaged. In other words the starch grain is the state of the amyloplast, which is one of the constituent of the protoplast containing its own product in it, but not mere product of the carbohydrate nature. Under this consideration the writer has analyzed the starch grains from the biological standpoint, classified, and gave some consideration on the hereditary phenomena of starch grains in plants. And in addition especially the crystallization of amyllum molecules in the amyloplast and the zonation (the crystal layer formation) by the rearrangement of the amyllum molecules in the primarily formed amyllum crystals in the aggregate of the amyllum vacuoles were discussed, thus a certain modification in the trichite theory is proposed.

Developmental course and composition of starch grains

1. *Amyllum vacuole*. In the very early stage of development of the starch grains there appear vacuoles in the amyloplast as previous investigators already observed (Salter 1898, etc.), though they did not recognized them as vacuoles. I should call them 'amyllum vacuole', because it appears in the amyloplast and the amyllum formation takes place in it (Diag. 2-6, A). Though the process of the appearance of such vacuoles in the amyloplast has not been traced yet, it seems natural to consider that the primordia were present in the plastid as very minute vacuoles containing certain substances, playing the role on the polymerization of glucoses, such as diastase. The observation in *Zea*, *Pisum* etc. suggests that my amyllum vacuole may be the same with the grana in the amyloplast.

The primordia developed as the amyllum vacuoles accumulate glucose

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and other polysaccharoses in them. As the result the vacuoles enlarge absorbing water, due to the increase of osmotic pressure, and became recognizable under the microscope as the amyllum vacuoles. Artificial enlargement mentioned in the previous paper (p. 00) may explain such process in those primordia.

The growth of starch grains therefore must be preceded by the amyllum vacuole formation and also by the enlargement of the vacuoles, then the crystallization of amyllum takes place in the amyllum vacuoles.

2. *Starch granule.* Starch granule is composed mainly of the sphecrocrystal or the crystal of amyllum produced in the amyloplast. There are two kinds of starch granule in plants. One is the primary starch granule and the other is the secondary or aggregated starch granule.

a. *Primary starch granule.* This granule is to be called as the simple starch granule on the standpoint about the composition of the granule, but to avoid confusion with the name 'simple grain' after Nägeli I called it 'primary starch granule'. The primary starch granule is that which was produced in an amyllum vacuole in the amyloplast, such as the starch granules in the amyloplast in the endosperm cells of rice. In those amyloplast generally numerous amyllum vacuoles are produced in an amyloplast simultaneously. The amyllum vacuoles develop directly into the component starch granules, and the accumulation or production of amyllum take place simultaneously in all amyllum vacuoles. Due to a rather small product in the amyllum vacuoles the vacuole-membrane or the plasma-membrane retains certain thickness, recognizable under microscope as the framework of the architecture of the starch grain at the last stage of development (Diag. 3).

The starch grain having such starch granules is to be called the primary or true compound grain. The starch grain in this type has no plasma-center, the so-called hilum, because there has no aggregation of the amyllum vacuoles taken place.

b. *The aggregated or secondary starch granule.* The name 'secondary' correspond with the primary starch granule in the last item, but the 'aggregated' explains the process of development of this granule. The aggregated starch granule is the one which was produced by the aggregation of amyllum vacuoles in the amyloplast (Diagram 6, C).

In the aggregated starch granule firstly there appear more or less amyllum vacuoles in an amyloplast, as in the case of the primary starch granule while here the aggregation of amyllum vacuoles take place, resulting an aggregate of amyllum vacuoles and an intervacuolar space in the center of the aggregate, the plasma-center; secondly the amyllum vacuoles produced later in the amyloplast are excluded around the first formed aggregate forming a layer of amyllum vacuoles and then those amyllum

Diagram. 1-2, an amylopectin molecule and the developmental process of the zonation shown. 1, an amylopectin molecule; *a*, part of amylose; *b*, part of amylopectin. 2, developmental stages of amylopectin crystal. *A*, an amylopectin vacuole (circle) in the plastid-plasma. *B*, the same, the later stage the primary amylopectin crystal is formed in the vacuole; the amylopectin molecules arrange radial: amylopectin molecules in the center and the amylose molecules in the periphery of the amylopectin vacuole. *C*, still later stage the shape of the amylopectin vacuole is changed due to the pressure of neighbouring amylopectin vacuoles which grew larger and also due to the increase of the number by the intussusception of later formed vacuoles, and the rearrangement of the molecules took place from the radial to the side by side, resulting zonation. *m*, plastid-plasma between amylopectin vacuoles. In the following diagrams the same letters are used for the corresponding figures *m* is abbreviated. 3-6. Different types of development of the starch grains. 3, Primary or true compound starch grain. Left half (*A*), circles show the primary amylopectin vacuoles in amyloplast. Right half (*B*), primary amylopectin crystal is formed in each amylopectin vacuole which has changed the shape. 4, potato-type derivative grain; the plasma-center is shown centric. Left half shows the younger stage of the development than the corresponding ones in the right half. *p*, plasma-center, the so-called hilum. *B*₁ and *C*, the central aggregate, in *C* the zonation took place already, while in *B* amylopectin vacuoles are in the early stage. *B*₂ and *A*, a later formed amylopectin vacuole layer surrounding the first aggregate. 5, *Pisum*-type derivative starch grain. *B*₂, *B*₃ and *C*₂ show the first aggregate. *C*₁, the first formed amylopectin vacuole layer surrounding the first aggregate, the zonation took place already. *C*₂ amylopectin vacuoles at the periphery of the first aggregate, the zonation took place later than in *C*₁. *B*₃ in which the rearrangement of the crystal form is not taken place yet. *B*₁ and *A*, later formed amylopectin vacuole layer. 6, secondary compound starch grain. *A*, young amylopectin vacuoles in the amyloplast shown; *B*, in which amylopectin vacuoles just before the aggregation takes place are seen. *C*, aggregation as well as the zonation took place already. The later formed amylopectin vacuole layer surrounding the aggregate like in the potato and *Pisum* types is abbreviated in Fig. 6.

vacuoles aggregate; and thirdly the amylum vacuole layer formation takes place in the same manner periodically around the previously formed aggregate, resulting a large aggregate of amylum vacuoles having an plasma-center (Diag. 4 and 5, P). The 'periodical' means the somewhat rithmical intervals in which the density of the amylum vacuole accumulation reaches as that there the recrystallization of the primary amylum crystal takes place.

Such aggregation of amylum vacuoles in the amyloplast may due to the rich and rapid accumulation of amylum molecules in each vacuoles and also the periodical rapid increase of amylum vacuoles in the amyloplast.

The starch granule in the so-called half-compound grain belong to this category, but it has more than one plasma-center surrounded by the common amylum vacuole layers. It is due to the fact that more than one aggregates of amylum vacuoles have been formed first in an amyloplast and later they have been enclosed in common amylum vacuole layers.

3. *The crystallization of amylum and the rearrangement of amylum molecules in the crystal, the zonation.* A. Meyer (1895) proposed the 'trichite' theory to elucidate the formation of layers in starch grains, the trichite of amylose and amylopectin being arrange side by side in the layer in the starch granule. Meyer's amylose is now considered as the amylum molecule in which the configuration of the glucose units is in a single chain. Such molecules arrange themselves radially and chiefly in the periphery of the amylum layer in the starch granule. The amylopectin trichite is the polymerized amylose molecules whose configuration is branch like; the amylose molecules join with the others as the side chain, and also the polymerizes molecules run together with each other resulting in a reticulated configuration. The latter are distributed principally in the inner portion of the layer. Thus amylum molecules are arranged as the anisotropic layer (Frey-Wisseling 1938, K. H. Meyer et alii 1940)*. Diag. 1 shows an amylum trichite.

Now I have some data which I can not elucidate with above theory, the first point is that there is no such sharp border line between zones, one is supposed to be amylose zone and another the amylopectin-zone, but one zone tranfers into the other so easily as we can not draw a clear border line as Frey-Wisseling has illustrated. The second point is that the discrepancy between the consideration about the arrangment of the amylum molecules in the unit amylocrystal and that in the amylum vacuole layer, in the former the amylum molecules arrange themselves radially, so that the amylose molecules enclose the amylopectin molecules. Therefore in the amylum layer, in other words in the amylum vacuole layers we

* For literature, see p. 74 in the Part I of this report.

have to find out at least two molecule layer, the inner and the outer on both sides of the amylopection layer, but they consider two layers only, the inside amylopectin and the outside amylose layer. And the third point is that we can not explain the zonation in the aggregate of amylum vacuoles, on which I have described under the item of *Pisum sativum*, in the previous paper i.e. in the first aggregate in the amyloplast the zonation in the primary amylum crystals takes place centripetally. According to Meyer's theory the zonation must be formed centrifugally.

My consideration on these points is as follows: The primary amylum crystal is formed in the amylum vacuoles before the aggregation takes place. The structure of those primary amylum crystal is spherical in which the amylum molecules arrange radially and the part of amylopectin occupy the central- and the amylose molecules the peripheral- portion (Diag. 2-5, Bs). In other words in the primary amylum crystal the polymerization of amylose molecules takes place in the center of the crystal forming amylopection and at the radiated distal ends of the trichites the amylose, i.e. the amylose molecules occupy the periphery of the spherical crystal.

In the true or primary compound starch grain a primary amylum crystal appears in each amylum vacuole and grows independently (Diag. 3). The growth of the primary amylum crystal may be due to the intussusception of amylum molecules.

In the derivative starch grains such as those in the potato tuber cells, 1) primary amylum crystal appears in the same manner in the case of the primary starch grains in the amylum vacuoles (Diagram 4, B), 2) the aggregation of those amylum vacuoles (the first aggregation) takes place, 3) the rearrangement of the orientation of the crystal form in the spherical primary crystal; the trichite rearrange from a radial to a side by side arrangement, resulting the zonation surrounding the plasma-center (Diag. 4, B₁, C and p); in the outer half of the spherical primary crystal the distal end of the molecule, the amylose, arrange outside and the proximal part of the molecule, the amylopectin, inside, while in the inner half of the sphere the molecules arrange inversely, so that there are formed three zones in which the outer and the inner zones are composed of amylose and the middle zone is composed of amylopectin (Diag. 2 and 4, C). Thus an amylum vacuole layer which is composed of three zones results. In the amylum vacuole which was produced later, surrounding the first formed aggregate mentioned above, the primary amylum crystal formation in each amylum vacuole, the aggregation of amylum vacuoles, and the rearrangement of the crystal form or the zonation take place in the same manner as in the first aggregate (Diag. 4, A and B₁). Consequently the inner amylose zone in the later formed

amylum layer becomes in contact with the outer amylose zone of the first aggregate, forming no sharp border line between these two zones (cf. Diag. 5, C₁ and C₂). In the same manner the successively formed amyllum layers increase contrifugally.

In the amyloplast of the cotyledonary cells of *Pisum*, 1) in the first formed amyllum vacuoles several primary amyllum crystals appear before they aggregate (Diag. 5, B₂), 2) amyllum vacuoles excluded later from the amyloplast plasma form an amyllum vacuole layer (the first vacuole layer) around the first aggregate, 3) primary amyllum crystals in the amyllum vacuoles in the first vacuole layer are formed and then the aggregation of the amyllum vacuoles takes place, 4) the rearrangement of the orientation of the trichites from radial to the side by side takes place in the primary amyllum crytals, forming the zones in the first vacuole layer (Diag. 5, C₁), 5) on the outside of the first formed amyllum layer the periodical increase of the amyllum vacuole layers centrifugally and the zonations take place, 6) simultaneously at the peripheral portion in the first aggregate the rearrangement of the orientation of the crystal form from the radial to the side by side takes place, resulting the zonation in the same manner as those mentioned above (Diag. 5, C₂) so that the outer amylose zone in the first aggregate comes in contact with the inner amylose zone of the first vacuole layer. The zonation in the same manner takes place centripetally in the first aggregate (Diag. 5, B₃), finally it reaches the plasma center (Diag. 5, p.)

4. *Intravacuolar substance of amyllum vacuoles.* In the amyllum vacuoles there must be present certain substances filling the interspace of the amyllum crystals. Of these substances there is no view proposed yet. Generally speaking the investigators in the field of chemistry are considering that this interlocating substance is water.

From the biological standpoint, however, this substance should be the mother liquid formed in the amyllum vacuoles or grana in which the amylose and the amylopectin or their primordial substances were produced. Thus this interlocating substance ought not to be the mere water, and such a mother liquid can not be of the same quality in the different kinds of amyloplast in various plants; and also it may be stated that the cellular conditions surrounding those amyloplasts should not be the same in varous plants.

Such different conditions of the amyllum vacuoles may play the role in the crystallization of amyllum molecules, just as in the case of the crystallization of calcium oxalate in plant cells. The pH differences in the cell sap determine the crystal system of calcium oxalate as is well known. Katz (1930, 1934 etc.) found that there are three different crystal system in the natural reserve starch (β starch). They give A-, B-, and C-type

of X-ray diagram respectively. All jellified starch grains in hot water become amorphous, which is called α starch. When α starch is dehydrated with absolute alcohol and crystallized it does not reverse its own crystal system, but give V-type X-ray diagram regardless of what was its original crystal system (Katz and Itallie 1930). After several retrograde experiments on certain jellified starches Katz considered that such differences in the crystal system among natural starch grains may be due to the different conditions in which they have crystallized (Katz and Derksen 1933). In their experiments the very high and low temperature, high percentages of alcohol etc. played the role on the modification of the crystal system, but we can not suppose such an extreme condition is present in plant cells. So we have to find out certain factors which can play in the normal temperature.

5. *The architecture of the starch grains.*

a. *Plasma-center.* The so-called hilum has been hitherto considered as the crystal-center which specially appear in the amyloplast in the early stage of development of starch grains. However, as far as my material is concerned the so-called hilum does not appear by itself in the amyloplast, but is produced as a secondary product as the result of aggregation of amyllum vacuoles. It is the intervacuolar space enclosing a piece of the amyloplast-plasma or the plastid-plasma, as the result of the compact aggregation of the amyllum vacuoles (Diag. 2-6, *ps*). Therefore the hilum is not the specific organ formed in the amyloplast. We will consider such intervacuolar space including the piece of the plastid-plasma as the plasma-center of starch grain or amyloplast. In some starch grains the plasma-center looks like as if it is playing the role on the amyllum crystallization.

b. *The framework.* In the true compound grains the plastid-plasma which separates the amyllum vacuoles retain until the last stage of development of amyloplast, and it constitutes the framework of starch grains with the outer-plasma-layer of the amyloplast (Diag. 3).

However, in the aggregated starch granules it appears as if the plasma-membrane between the vacuoles is ruptured when the development of the granule proceeded and the vacuoles swelled. But we may assume that the plasma-membrane is retained in a very thin form in the later stage and remain so long until the starch granules are digested. Because such plasma-membrane is very soft and so flexible as it cannot be ruptured by a pressure due to the swelling of the vacuoles, and also it can spread so thin as a monomolecular membrane (Diag. I, 2, *M*). So that in the case of the derivative grains the framework of the architecture of starch granule may retain as in the case of the primary or true compound grains.

The immunological tests show that the different kinds of starch grains give different kinds of serum. The immunological experiment on several

different kinds of starch grains by Nodzu (1926) shows us that they give different kinds of antibody. Though his consideration about that data, the different kinds of plant give different kinds of amylose which act as the antigen-, was corrected by Fujimura (1935), the data still show us that the different kinds of starch grains, have their own specific substance which play the role on the production of specific antibody except amyloses. Fujimura confirmed in Merck's soluble starch that the producer of the antibody is the other substance than amylose and he has considered the former as the impurity which is left after the amylose was taken out from the starch grain by digestion. These data with the results of the observation in this work let the writer to consider that the substance which act as the antigen is nothing but the plastid-plasma in the plasma-center together with the plasma-membrane of amylum vacuoles.

Under these considerations a starch granule is not mere crystal of amylum, but a system composed of the framework made of mother substance of amyloplast, the substance of amylum vacuoles and the amylum crystal in the compartment of the vacuole.

Here I wish one thing to be emphasized. It is that the amyloplast is not a mere constant mechanical organ in which the amylum is produced, but it is a living plasmic organ and it develops during the developmental process of the amylum or rather to say the process is the part of the developmental or vital phase in the plastid.

Classification of starch grains

Now I will classify starch grains into three types; 1) the primary or the true compound type, 2) the derivative type, and 3) the secondary compound type.

1) *True compound starch grain.* This type of starch grain has most primitive architecture; the framework of the architecture, i.e. the plasma-membrane of amylum vacuoles and the peripheral plasma of the amyloplast, being retained until the last stage of development of the grains and is visible under the microscope, each compartment of the amylum vacuoles being occupied by a primary amylum-crystal. Diag. 3 shows the diagram of the developmental process.

2) *Derivative starch grain.* The typical starch grain in this type has one starch granule in an amyloplast. The starch granule is the aggregated granule consisting of the plasma-center containing a piece of the plastid-plasma, the framework of the architecture consisting of the plastid-plasma which becomes indiscernible under the microscope at the last stage of development, and the amylum vacuole layers. In each amylum vacuole the amylum molecules, trichites, arrange themselves side by side, forming three zones, the inner amylose, the outer amylose and the

middle amylopectin zones. Thus the starch granule forms a crystal system in an amyloplast (Diag. 4 and 5).

In certain kinds of derivative starch grains, the plasma-center is excentric, but in others centric, while in still others elongated in the central part of the grain.

3) *Secondary compound starch grain*. The amyloplast has more than one, rather a few of aggregated starch granules. Thus the architecture of the component starch granules is the same with that in the derivative starch grain.

Nägeli (1858) has classified the reserve starch grains into two groups generally, the simple (my derivative starch grain) and the compound grain (my true or primary compound grain together with my secondary compound starch grain). Besides these two, he has recognized the half-compound grain (the grain having my semi-aggregated starch granule). And he has considered that the simple grain is the primitive form of the starch grain and the compound grain is the derivative of the simple grain. As far as my observations concern, however, his simple grain has a few larger or numerous small amyllum vacuoles in the first stage of development just like true compound grains, and it is to be considered that his simple grain is a derivative one.

Genetics in starch grains

Nägeli has also classified the plants by the characteristics of their reserve starch grain into two groups; one having simple grains and the other having compound grains. His classification rests on a genetical concept. This concept is now more extended; in the pea the wrinkled seed (sugar-seed) has compound grains, while the smooth seed (starch-seed) has simple grains, and the latter character is dominant over the former in the F_1 generation (Bateson 1905).

The present study shows that the simple grain in pea is the derivative starch grain and the compound grain is the secondary compound grain. Therefore their difference is that of the degree of the aggregation, probably due to the quantity of the amyllum formation, but not the difference in the real character of the starch granules in the amyloplast. So that the genes concern with the allele, the smoothness and the wrinkledness of the seed in *Pisum*, are to be the agents which determine the amount of amyllum production in amyllum vacuoles, but not that which directly determine the number of the starch granules in the amyloplast.

In the case of *Zea mays*, the sugar grain as well as the starch grain have equally the secondary compound grains. The phenotypical difference of grains is due to the number of starch grains in cells, so that the genes concerning the allele, the smoothness and the wrinkledness, are to be the

agents which determine the number of starch grains in the cell but not that which determine the number of starch granules in an amyloplast.

6. *Regeneration of starch grains and starch granules.* Many investigators described about the suspension of the development of starch grains, the digestion of amyllum crystal and further about the resumption of the growth in such starch grains (Schimper 1881, A. Meyer 1881, Ebert 1885, Salter 1896 etc.). The same facts were confirmed by the writer in the reserve tissue of the potato tuber, and of sweet potato. And even in the developing tissue such antagonistic processes occur in different starch grains independently in one and the same cell. In those storage tissue of those plants the reversion between crystallization and the degeneration of amyllum crystal occurs in both directions throughout the storage season resulting the modification of the structure of the starch grains and also some extreme growth of starch grains in the sacrifice of other grains in one and the same cell. While preserving sweet potato and potato a large starch grain is seen to occupy almost whole space in the cell.

Conclusion

1. The starch grain in general is composed of starch granule and the plastid-plasma or amyloplast-plasma. In the earliest stage of development of starch grains the amyloplast has a few or numerous amyllum vacuoles in it (Diag. 3, A). This structure of the amyloplast is to be called the primary architecture of amyloplast, all starch grain formation in various plants as far as I have studied pass through this stage. Later amyllum formation takes place in each amyllum vacuole; and still later the amyllum crystallizes in the vacuole (Diag. 2, B). The first formed amyllum crystall is called the primary amyllum crystal.

2. The aggregation of amyllum vacuoles in the amyloplast takes place except the primary or true compound grains. The later formed amyllum vacuoles are excluded from the plastid-plasma on the surface of the previously formed aggregate, and results a layer of amyllum vacuoles (Diag. 4, B₁ and A₂). The primary amyllum crystal in those aggregated amyllum vacuoles changes the molecular arrangement from radial to the side by side resulting three zones (Diag. 2, C), the outer, the inner and the middle zones. It is considered that the former two zones consist of amylose and the middle zone of amylopectin. In the successively centrifugally formed vacuole layers the crystallization, the aggregation and the zonation take place in the same manner generally, while in the first aggregate in certain amyloplast, i.e. in *Pisum*, the zonation takes place centripetally.

3. The starch grains in the reserve tissue may be divided into three groups, according to the differences of the structure and the develop-

mental processes:

a. *The most primitive type of the starch grain, the true compound grain.* The starch grain retains their primary architecture until the later stage of their development. The accumulation or the production of amyllum takes place simultaneously in all amyllum vacuoles. Due to the small product in the amyllum vacuoles, the vacuole membrane (the plastid-plasma-membrane between the amyllum vacuoles) forming the framework of the architecture of the starch grain retains certain thickness recognizable under the microscope even in the last stage of development. Starch grains of rice, oat etc. belong to this type.

b. *The most differentiated starch grain, the derivative grain.* The amyloplast has a single aggregated starch granule having complicated architecture. A derivative starch grain having a small plasma-center, the so-called hilum, surrounding the plasma-center the layer consisting of amyllum vacuoles is produced. Another kind of derivative starch grain has a large plasma-center surrounded by a small number of large amyllum vacuoles. Further structure is described under the items of *Solanum tuberosum*, *Pisum sativum* in the previous paper (p. 02 and, 06) and Discussion 2.

c. *Secondary compound starch grain.* The so-called compound starch grains found in the tuberous root of sweet potato etc. having rather small number of large starch granules in an amyloplast belong to this category. The composition of starch granules and the course of development are the same with that of the derivative starch grain. However, in this type of starch grain the more than one intervacuolar spaces retain their existence in an amyloplast, resulting in more than one plasma-center in it. Surrounding those plasma-center the successive layers of amyllum vacuoles develop periodically, consequently more than one starch granules, separated with visible plastid-plasma, develop in an amyloplast.

4. In the starch grains the course of crystallization is reversible during the development as well as in the storage. Due to such reversibilities the structure of reserve starch grains is reversible in the storage tissue.

5. From the above data we can consider that starch granules in the reserve starch grains are not composed of amyllum crystals only, but they contain more or less the plasma of the mother plastid as such as the plasma-center and the framework of the architecture of the grain. Different results of a chemical analysis by several workers in this field may be due partly to difference of materials. Biological as well as the chemical composition of the plastid must be different in various plants.

6. Differences in the X-ray diagrams, in other words the differences of the crystal types of different kinds of natural starch grains (β starch)

may be due to the different conditions under which the crystallization takes place in different plants.

V-type X-ray diagram is considered as that of the crystal type of the amyllum when crystallized in alcohol.

7. The plastid-plasma in the starch grains is considered as the antigen which plays the role on the formation of the antibody when the starch grain is injected into animals. Different kinds of antibody produced by the injection of the different kinds of starch grains must be due to the constitutional differences of their plastid-plasma.

8. The endosperm cells of corn without exception have secondary compound starch grains, though the plasma-membrane in those starch granules of smooth grains is more indiscernible when compared with that of sugar corn, due to the rich accumulation of the amyllum in the amyllum vacuoles. The smoothness of the grain, however, is rather due to the increasing of the number of the amyloplast in a cell. In contrast in *Pisum* the smoothness of the seed is determined mostly by the amount of amyllum in each amyloplast.

9. The present writer wishes to emphasize here the starch grain in the living plant cells is a state of the plastid (amyloplast) and the starch formation is a living process resulting the amyllum formation, but not the simple mechanism of the polymerization of glucose molecules.

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BIBLIOGRAPHY

(cf. p. 74)

Addendum

The report on the antigenic study on *Paramecium* (Sonneborn, T.M. Beyond the gene. Amer. Scientist 37: No. 1, 1949) shows that there were found 8 different kinds of heritable antigenic types in two different sources. They give 8 different kinds of antibodies in the injected rabbits. Sonneborn has considered that the formation of those different antibodies is due to the presence of the antigenic plasmagenes in the respective type of *Paramecium*, and that on one hand each plasmagene is adaptable to each special living condition, and on the other hand the appearance and the maintenance of certain plasmagene is due to the presence of the special gene or genes in the nucleus.

The comparison of the above results with those in the starch grains shows us the similarities in them as follows:

1. They act as the antigens; 2, as to the different types of *Paramecium* give the different kinds of antibody, so the different kinds of starch

grains from different kind plants give the different kinds of antibody; 3, the bodies of amyloplast transfer from cell to cell independent to the nucleus when the cell divides like the plasmagene does in *Paramecium*; 4, certain physiological activities and development of amyloplast depend upon the gene in the nucleus like the plasmagene especially confirmed in the F-plasmagene in *Paramecium*.

These similarities lead the writer to consider that the antigenic plasmagene in *Paramecium* ought to be certain protoplasmic constituents, e.g. the plastosome, behaving like the amyloplasts.

Here the writer wishes to propose a name "extranuclear gene" for the gene in the cytoplasm though the real locus in it is not certain yet instead of 'plasmagene', and another name "intranuclear gene" for the gene in the nucleus. Because the word plasma means the component of the protoplasm as we call the nuclear substance as karyoplasma but not limited to mean the cytoplasm. To avoid such ambiguity firstly the writer wishes to use those names which could draw a line between the genes in the nucleus and outside the nucleus, and secondly, in each occasion when the real locus or the nature for any of the extranuclear genes is determined we will give it the name which shows the real nature such as the plastid-gene for the gene located in the plastid.