SPOROGENESIS IN LILIUM TIGRINUM AND PODOPHYLLUM .

Minor Thesis in Cytology, submitted to Dr.E.A. Smyth, for the Degree of Master of Science in Agriculture

by

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History of Cytology.

Ever since the cell theory has been ennunciated by Schleiden and Schwann in 1838-39 it has become more and more apparent that the final key to all biological problems must be looked for in the cell, the unit of organic structure. By the cell theory the animal and vegetable structures were brought under one point of view, showing their common plan of organization. Through the cell theory Kolliker, Nageli, Hofmeister and others opened the way to an understanding of the nature of embryological development and the law of genetic continuity upon which inheritance is based. Virchow, Max Schultze, and others showed further that all the various functions of the body, in disease and health, are but the outward expression of cell activities. Through the cell theory Van Beneden, Strasburger and others solved the great problem of fertilization of the egg and the mechanism of hereditary transmission. No other biological theory except the theory of evolution has accomplished more for the unification of knowledge. The cell theory must therefore be considered as one of the foundation stones of modern biology.

The theory of evolution grew out of the study of natural history and was definitely shaped long before the ultimate structure of living organisms was well understood. The investigators of that period gave little attention to details of structure but were concerned mainly with the obvious characters of plants and animals such

as form, color, etc., and their classification was based on these characters. The study of microscopical anatomy, on which the cell theory was based, lay in a different field. Only within recent years has the path been cleared for the close alliance between the study of organic evolution and the study of the cell, which has been of such great importance in the latter day biology and in research work. The fact that these two great lines of research, having their beginnings so far apart, have at last met, is a striking evidence of the progress of modern biology. While Schleiden and Schwann are universally recognized as founders of the cell theory, a great number of earlier investigators had contributed to this discovery. The significance of Schleiden's, and especially of Schwann's work lies in the thorough way in which the problem was studied and the conclusions developed, and in their influence upon further research. The theory of organic descent has been shown by an overwhelming mass of evidence, to be the only true conception of the origin of different forms of life. The general outline of the cell theory is as follows:

In all higher forms of life, both animals and plants, the body is composed of a vast mass of minute structural units, known as cells, out of which all parts are built. The tissues are not homogeneous, as they appear to the naked eye, but are composed of innumerable minute bodies. The cell is mainly a minute mass of protoplasm which Huxley called the "physical basis of life". There is no characteristic type of organization common to all these protoplasmic masses, and they may in a way be regarded as elementary organic units out of which the body is formed. The lowest forms of life, like bacteria and protozoa, consist of single cells. Multicellular bodies can therefore structurally be considered as colonies of one-celled forms. In the one-celled organisms all functions are performed by

one cell while in the multicellular bodies the cells differentiate and different groups of cells have different functions.

Researches on the early history of the germ cells and the fertilization of the ovum by Auerbach, Butschli, Van Beneden, and others, raised entirely new questions regarding the mechanism of development and the part of the cell in hereditary transmission. It became apparent that the general problems of embryology, heredity, and evolution were closely connected with those of cell structure and had to be solved by cytological research. The identification of the cell-nucleus as the carrier of inheritance was a very important step in this direction and was made independently in 1884-85 by Oscar Hertwig, Strasburger, Kolliker, and Weismann. To the modern student the germ is simply a "detached living portion of the substance of a preexisting living body, carrying with it a definite structural organization characteristic of the species." By the extreme evolutionists the egg was believed to contain an embryo fully formed in miniature, as the bud contains the flower. Development was for them merely the unfolding of something already present. Schwann and his followers recognized the fact that the egg is a cell with the same essential structure as other cells of the body, and that a single cell may contain the sum total of the inheritance of the species. Kolliker demonstrated that the spermatozoa arise directly from cells of the body and are not parasites. They were found to contain nuclei and cytoplasm like the egg cells and proved to be true cells even if of minute structure. Oscar Hertwig finally established the important fact that fertilization of the egg is accomplished by its union with a single spermatozoon. In sexual reproduction, therefore, each sex contributes a single cell of its own body for the formation of the offspring. Thus sexes play equal though

not identical parts in hereditary transmission and the cell is shown to be the final basis of inheritance.

Morphology of the Cell.

The cell is a rounded mass of protoplasm. In isolated cells, such as unicellular plants and animals or the egg cells, the form is approximately spherical but in by far the greater number of cells this form is modified by the pressure of surrounding structures, or by movements of the cell-substance. The active basis of the cell is the protoplasm, a viscid, translucent substance, frequently finely granular, sometimes apparently homogeneous. As a rule, it has the appearance of a network, the "reticulum". Various lifeless bodies are also mostly suspended in the network, food-granules, drops of oil or water, pigment bodies, etc. These bodies are of minor importance. They are often difficult to distinguish from the protoplasmic granules. The protoplasm includes the entire active cell substance, the nuclear material or karyoplasm, as well as that of the cell body, the cytoplasm. Neither one, however, forms a homogeneous substance, for both consist of several distinct elements.

The nucleus usually is bounded by a definite membrane. There is reason to believe, though, that its structural basis is similar to that of the cell body, and during cell division both come into very close contact. The terms "nudeus" and "cell-body" therefore only have topographical significance. There is, however, a certain chemical contrast between Karyoplasm and cytoplasm.

Structural Basis of Protoplasm.

Under microscopes of average powers the protoplasm appears as a granular substance without definite structure. Careful examination under high power after suitable fixing and staining shows a very complex structure of both nucleus and cytoplasm. The basis of

structure in many forms of protoplasm is a meshwork consisting of at least two substances, the meshwork proper and the ground substance occupying the intervening spaces. Granules or microsomes are formed along the branches of the network. Besides these three elements supposed to constitute the active substance, the protoplasm contains also various passive or metaplasmic bodies of a nutritive nature. These bodies are often difficult to distinguish from the microsomes; it is even uncertain whether there is any reason for distinction.

There have been brought forth several theories about the structure of the protoplasm. The earlier observers regarded the meshwork as a fibrillar structure either forming a continuous network or reticulum or consisting of disconnected threads. The granules have been considered as nodes of the network or microsomes suspended in the net work. Butschli developed the "alveolar theory". He considered the protoplasm as having a foam-like structure and as consisting of separate, closely crowded, minute drops of a liquid "alveolar substance suspended in a continuous interalveolar substance, likewise liquid, but of different physical nature." The appearance of a network is illusory, being due to optical section of the interalveolar walls. A third general view is the "granular theory", most fully developed by Altmann. According to his view, protoplasm is composed of innumerable minute granules which alone form its essential active basis, while fibrillar or alveolar structure may occur, but are only of secondary importance. Many controversies arose about these theories by their respective adherents, but no definite conclusion has been reached regarding the true nature of these protoplasmic structures. It now seems probable that the substance of the meshwork is most active in cell-division, but the ground-substance is certainly also the seat of chemical changes.

The Nucleus.

The nucleus is generally regarded as the controlling centre of cell-activity and a primary factor in growth, development and the transmission of specific properties from cell to cell and thus from generation to generation. A cell without nucleus may live for some time but it is devoid of the powers of assimilation and growth and dies sooner or later. Destructive metabolism may go on for a while without nucleus but the constructive metabolism stops with the removal of the nucleus.

In performing its functions the nucleus passes through two quite different phases, one of which is called the "resting stage", where the nucleus appears as a rounded sac-like body surrounded by a distinct membrane, the nuclear membrane. There is visible also an irregular network inside of the nucleus. The form does not vary much though it may be in some cases elongated or lobed. The nuclear membrane is a well-defined delicate wall, which separates the nucleus sharply from the surrounding cytoplasm. Its staining power varies much but is quite marked in the lily. The most important part of the nucleus is an irregular branching network, the "nuclear reticulum" which consists of two widely different substances, the linin forming the general protoplasmic basis of the nucleus, and the chromatin, a deeply staining substance. The linin is invisible until after treatment with reagents and shows a finely granular structure and stains like the cytoplasm, a chemically related substance. The chromatin, as its name indicates, is a deeply staining substance and the nuclear substance par excellence. It seems to be the only element, in some cases, that is passed directly from cell to cell, and it seems to be capable of producing all the other elements. It often appears in the form of scattered granules

embedded in the linin substance. Commonly it forms a more or less regular network, often hardly distinguishable from the surrounding linin until the approach of mitosis, where it becomes plainly visible.

Another class of bodies found in the nucleus is the nucleoli, larger rounded or irregular bodies, suspended in the network. There are one or more of them present; in some nuclei they are entirely absent. The number varies quite much even in the same cell with varying physiological conditions. There are at least two different kinds of nucleoli, the true nucleoli or plasmosomes, and karyosomes. The plasmosomes are true nucleoli and spherical, and reach to stains very much like linin and the general cytoplasm. The karyosomes are the net knots, chromatin-nucleoli which stain like chromatin and are doubtless to be regarded as a part of the chromatin network, since during cell-division they have the same history as the rest of chromatin substance. The nature of the true nucleoli is only imperfectly understood. Some investigators have regarded them as storehouses of material contributing to the formation of chromosomes during division. Other believe them to contain a store of "kinoplasm", furnishing the achromatic part of the mitotic figure.

The ground substance or nuclear sap, also called Karyolymph, is a clear substance that occupies the interspaces of the network and does not take most of the stains that color the chromatin, linin or plasmosomes. It is considered by most investigators as a liquid, filling the spaces between the nucleolar network. The The chromatin network varies very much in different cases. It is sometimes very loose and sometimes very coarse or irregular. Different periods in the same cell may produce great changes in the network both in physical configuration and in staining capacity. Embryonic cells as a rule are characterized by the large size of the

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nucleus. In plants they have also been shown to contain a larger percentage of chromatin than in later stages.

The question has been raised frequently whether the nuclear structures may not be regarded as aggregates of more elementary morphological bodies. Especially the chromatic network shows evidence in this direction. Previous to division this network forms a number of rod-shaped bodies, the "chromosomes", which split lengthwise as the cell divides. These chromosomes are formed by aggregations of minute rounded bodies or microsomes, called "chromomeres" or "ids". As a rule they are quite distinctly visible and very regularly arranged during the "spireme" stage in cell division, when the chromatin is arranged in a thread, or in separate chromosomes. In many cases they are also distinctly visible in the reticulum of the "resting" nucleus. It is still doubtful but nevertheless possible that the chromatin granules have a persistent identity and can be regarded as morphological units of which the chromatin is built up. There are a number of other classes of granules that have been formed by different investigators in the nucleus and which are considered as morphological units. But to this time their true nature has not been fully understood.

Schwarz in 1887 has proposed the following classification of nuclear substances, which has been widely accepted and is open to criticism:

1. Chromatin = the chromatic substance of the network and of those nucleoli known as net knots or karyosomes.

2. Linin = the achromatic network and the spindle fibres arising from it.

3. Paralinin = the ground substance.

4. Pyrenin or Parachromatin = the inner mass of true nucleoli.

5. Amphipyrenin = the substance of the nuclear membrane.

Cytoplasm.

The cytoplasm of unicellular forms is often differentiated into two distinct zones, an inner medullary substance or "endoplasm" containing the nucleus, and an outer substance or "exoplasm" from which the more differentiated organs are derived. In the tissue cells of higher plants indications of such differentiation are often shown. It has to be seen yet whether this distinction is of fundamental importance. It seems that the cytoplasm next to the nucleus shows less differentiation while the peripheral region of the cell contains more differentiated products of cell activity. This fact supports the view that the nucleus is immediately concerned with synthetic metabolism. Great care is necessary in the study of the cytoplasm not to confound coagulation products with the natural structures of the cell. In many cases very deceiving bodies have been formed in cells as results of coagulation which resembled closely bodies found in cells. Careful study has shown though, that the form and arrangement of most elements is almost identical with those in life. Where living and dead cells can be compared the true structures can soon be determined, but in some cases it is doubtful whether the structures have not changed during the killing process. In the cell we find often differentiation of the cell substance into more or less definite structures either constant or transitory. The plastids or protoplasts are the most interesting ones of these. They are capable of growth and division and are handed down from cell to cell. Most important are the chromatophores or chromoplastids, characteristic of plants. They give rise to the starch builder, amyloplastids, chlorophyll-bodies, chloroplastids and other pigment bodies, chromoplastids.

The Centrosome.

The centrosome is usually an extremely minute body, or more commonly a pair of bodies. It stains intensely with haematoxylin and some other stains and it is surrounded by a radiating aster of cytoplasm or by the "attraction-sphere" a rounded mass. As a rule it lies inside the cytoplasm but sometimes it lies inside the nucleus. The relation of the centrosome to the surrounding structures lies still much in the dark. Physiologically it was regarded as the especial organ of cell division or the "dynamic centre of the cell". While centrosomes occur in some of the lower plants it has been impossible for the most competent worker in cytology to discover centrosomes in higher plants.

The Cell-Membrane.

Cell walls play a very important role in plants as compared with animals where they are relatively unimportant. The wall belongs to the passive or metaplasmic products of protoplasm rather than to the living cell. The cell wall in plants becomes very thick and often has a complex structure. It is doubtful whether any cell is devoid entirely of an enclosing envelope. The opinions are divided about the mode of origin of the cell wall. It seems probable that in general it is a secretion-product, though in some cases a direct transformation of protoplasm into the membrane seems to occur. In the formation of plant cells the daughter cells, as a rule, are cut off by a cell plate arising in the protoplasm of the mother cell as a transverse thickening of the spindle fibres in the equatorial region. All available facts indicate that the formation of membranes by secretion is the most common and typical process.

Polarity of the Cell.

In a large number of cases the parts of a cell are arranged symmetrically with reference to an ideal "organic axis". The general conception of cell polarity has been developed in two different directions, one based on morphological considerations, the other one on physiological relations. Van Beneden conceived cell polarity as a morphological attribute of the cell, the organic axis being a line drawn through the centre of the nucleus and the centrosome. Heidenhain maintained that all the structures of the cell have a definite relation to the primary axis. Other authors meant by cell-polarity a polar differentiation of the cell substance caused by adaptation of the cell to its environment in the tissues.

The Cell in Relation to the Multicellular Body.

Outside of the one-celled organisms and the egg cells the cell can physiologically only in a limited sense be considered as an independent unit, because it is very closely connected with the general life of the organism of which it forms a part. While it is capable of independent action within certain limits it is nevertheless a part and a localized area of activity and not a whole. The most important biological question of today is the means by which the individual cells interact and the organic unity of the body is preserved. Around the question revolves not only the problem of inheritance of acquired characters and of evolution, but also our idea of life itself. The cells have to be regarded as local centres of a formative power pervading the whole growing organism. Towards the later stages of embryological development some cells acquire a high degree of physiological independence, but this is only a secondary result of development through which the cells become more or less emancipated from the general control. The life of multicellular bodies has to be considered as a whole.

Cell Division.

Every cell originates from a preexisting cell, just like an animal can arise only from another animal or a plant from another plant. Thus the principle of the law of genetic cellular continuity in the whole series of living organisms is assured. The advance of research is continuously adding new proofs to the conclusion that cells originate only by division of a preexisting cell. There are two different types of division, the direct division or amitosis and the indirect division or mitosis. Amitosis is simple division of the cell starting with the nucleus and is completed by division of the cell body and the cell membrane. It is extremely rare and exceptional. The general method of cell division is the mitosis or karyokinesis and this is an elaborate process.

Indirect Division or Mitosis.

The process of mitosis consists of four successive stages: 1, the prophases or preparatory changes; 2, the metaphase or monaster stage, the most important step in the division of the nucleus; 3, the anaphases, in which the nuclear material is distributed; 4, the tolophases, in which the entire cell divides and the daughter cells are formed. These stages grade into each other and are separated by no well defined limits. Yet the differentiation is sufficient to give us a convenient system for describing the different phases.

1. The Prophase. At the time when the cell prepares for division a very conspicuous change of a physical as well as chemical nature takes place in the nuclear substance. The staining power of the chromatin substance increases rapidly and a definite number of separate bodies are formed, known as "chromosomes". These are usually rod-shaped and have a high staining power. The chromatin net resolves itself gradually into a more or less twisted thread,

the spireme or skein. It is at first fine and closely convoluted, forming the open spireme. Sometimes there is one continuous thread, in other cases there are a number of separate pieces or segments, known as "segmented spireme". This is the case in the nucleus of the megaspore of the lily. In either case the spireme breaks finally transversely forming the chromosomes, which are mostly rod-shaped, either straight or curved, and sometimes ovoid. The nuclear membrane gradually disappears in the meantime and the chromosomes lie naked in the cell, while the ground substance of the nucleus combines with the cytoplasm. It has been established with high probability that every species of plants as well as animals has a certain number of chromosomes, characteristic of the species, which constantly reappears in the division of all of its cells. In all forms produced by sexual reproduction the number is even. Thus in the lily the number is 24, in onion 16. The even number is due to the derivation of one half of the number from each parent. The chromatin nucleoli contribute to the formation of the chromosomes while the true nucleoli sooner or later disappear. They are supposed not to take part in the chromosome-formation. While these changes in the chromatin are going on, a complicated structure known as "amphiaster" appears in the position formerly occupied by the nucleus. It consists of a fibrous spindle shaped body the "spindle" and an "aster" of rays, radiating into the cytoplasm is formed at both ends of it. The centre of the aster is occupied by the "centrosome", a minute body. The chromosomes group themselves in a plane between the two poles and form the equatorial plane. The structure produced by these changes is called the "mitotic figure" and the chromosomes form the chromatic figure, called thus on account of its high staining power as compared with the achromatic figure, consiting of the spindle and

asters, which does not stain very deeply.

2. Metaphase. The prophase so far is only preparatory for the metaphse which forms the initial step of actual division. Each chromosome splits lengthwise into two exactly similar halves which are transported in opposite directions and here the daughter-chromosomes give rise to two daughter nuclei. The splitting does not always take place at this stage, but often the splitting takes place in the earlier spireme stage, or sometimes even earlier. But the main fact is that the chromatic network changes into a thread - mitos - either continuous or discontinuous, which splits into two exactly equivalent halves. The word mitosis was derived from the Greek word "mitos", meaning thread. This splitting is the essential part of the cell division because by it the chromatic network is exactly halved and both daughter nuclei receive a precisely equal amount of chromatin from the mother nucleus. It does not matter whether the cell body divides equally or not.

3. Anaphase. As mentioned above, the daughter chromosomes form two groups and separate in opposite directions to the opposite poles of the spindle, where they crowd closely to the centre of the aster. While they separate the two groups of daughter chromosomes are connected by strands of achromatic fibre, known as the "connecting fibres". Plant cells show almost invariably a series of deeply staining thickenings in the equatorial plane of the mitotic figure, called the cell-plate, which are quite conspicuous.

4. Telophase. This is the final stage of mitosis and the entire cell divides into two in a plane passing through the equator of the spindle. Each of the daughter-cells receives one of the two groups of chromosomes and one half of the spindle, and a daughter-nu-

cleus is formed in each cell from the chromosomes it received. At first the daughter-nuclei are of equal size, but if the division of the cell has been unequal the nuclei become finally also unequal, corresponding to the size of the cells. In plant cells the cell plate finally stretches all across the cell and forms two layers between which the new cell membrane, separating the two daughter cells, is formed, probably by secretion.

The essential features of mitosis are: (1) The formation of chromatin and achromatic figures. (2) The longitudinal splitting of the chromosomes or spireme thread. (3) The transport of the chromatin halves to the corresponding daughter cells. There is a great variety in these processes but essentially they are the same. The effect of mitosis is to cause an equal distribution of the chromatin of the mother-cell to the daughter-cells and this process is characteristic of all vegetative and all germ cells.

Development of the Floral Organs of Angiosperms.

Before taking up the development of the germ cells we will consider first the development of the organs which produce these reproductive cells. The angiospermous plants outnumber all other plants combined. They are especially adapted to the present conditions upon the earth and we find them in mostly all places where vegetable growth is possible. A great help for the success of the angiosperms in the struggle for existence has been the fact that they are utilizing animals, especially insects, for distributing their pollen and seed. They are well prepared to attract insects by their colors, odor, honey, or peculiar shape. The most characteristic structures of the angiosperms are the flowers. They differ from those of gymnosperms in having the ovule always protected within the ovary. The essential parts of the flowers are the stamens and

carpels, known as "sporophylls", which in the lower floral forms are borne in separate flowers or even on different plants. These flowers are called dioecious. In higher floral types such as the lily and the podophyllum, stamens and carpels are usually borne in the same flower. These flowers are called perfect or hermaphrodites. Characteristic of angiospermous flowers is the presence of a series of sterile leaves, the floral envelope or perianth. The perianth serves for protection but at the same time it may be conspicuously colored, and thus make the flowers attractive for insects. The floral parts are arranged either spirally or in whorls about the apex of the shoot or floral axis. The outermost leaves or sepals are usually green and constitute the calyx. The members of the second series, consisting of the petals, are usually larger and more conspicuously colored and form the corolla. Inside of the corolla are the stamens which bear the pollen sacs, and the innermost parts are the carpels. The development of the typical flowers follows closely that of a vegetative shoot. The apex of the shoot is the floral axis. Around this the different series of floral part are grouped either spirally or in whorls.

It is not always easy to delimit a flower strictly from a vegetative shoot; there are numerous gradations between foliage and floral leaves. The naked flower, with one or more free sporophylls, may be regarded as the most primitive form; it is an angiospermous flower without the floral leaves. From this stage a series could be constructed showing the gradual development of the floral envelopes. Among the more primitive flowers the floral axis has a tendency to elongate and the floral leaves are arranged along a low spiral. In more highly developed flowers the growth of the axis is checked early and the spiral becomes lower and lower until it passes into cycles,

the number of which gradually decreses. Sometimes not all menbers of a single flower attain the cyclic arrangement and thus sometimes stamens and carpels are spiral while the floral leaves are cyclic. There is a tendency for members of a set to form a congenital union as is the case in the sympetalous corolla. It is merely the development from the meristematic zone at all points instead of at certain points only. It is being shown first of all by the carpels, resulting in syncarpy. Among the more primitive flowers each cycle arises separately from the growing point and it is inserted definitely below the next inner cycle, and a hypogynous flower is the result. The tendency to zonal development is carried farther when two or more cycles of floral members are produced by a region, as in the case of stamens inserted upon the tube of the corolla, due to the tendency of petaliferous and stameniferous cycles to have a common origin. Somtimes the region of the growing part of the carpels cease to develop while the rest continues to develop and forms a cup-like growth, from the edge of which the three outer sets develop, froming the perigynous flower. Far more common is epigyny where the check of apical growth and the continued growth of the outer part of the growing point produce a cavity gradually roofed over by the carpels. From the top of the ovary thus formed the four sets of floral members develop.

Description of Sporophylls.

As mentioned above the sporphylls of spermatophytes consist of two kinds, stamens and carpels. The stamens bear the pollen sacs or microsporangia, which contain the microspores or male germ cells, while the carpels contain the ovules or macrospores. The stamen is mostly a true foliar organ but exceptionally it is developed directly from the axis. It is usually differentiated into two distinct regions,

the anther and the filament. The anther is the region bearing the sporangia and is usually composed of four pollen-sacs or microsporangia. It represents a morphological complex of sporangia and sporophyll tissue. There are six stamens present in the tiger lily, and 12 to 18 in the podophyllum, which are true foliar organs and represent spore bearing leaves and the pollen grains formed in the anthers are the microspores of the plants. The microsporangium corresponds in its development with that of the higher Pteriodphytes and Gymnosperms. The walls of the sporangium, when nearly ripe, consist of three layers of cells, the inner becomes very much disorganized at maturity, while the middle one develops thickened bands, fibrils, which are hygroscopic and aid in the dehiscence of the pollen-sac. The filament is a stalk like structure which supports the anther.

The carpels may be separate but more often they are united and form a compound pistil. The typical pistil consists of three portions, the ovary at the base, which bears the ovules or macrosporangia, the style, a more or less elongated and stalk-like region arising from the top of the ovary, and the terminal stigma, upon which the pollen is deposited. It consists of specialized tissue and produces a viscid secretion which serves to hold the pollen and to induce its germination. This tissue is part of the tisue lining the ovarian cavity, known as placenta, and of the tissue extending through the style, known as conductive tissue. In the tiger lily there are three single pistils which are fused together into one compound pistil with a three-celled ovary at its base. Podophyllum has one pistil and a one-celled ovary with one parietal placenta.

The Germ Cells.

Every living thing arises from a single cell, the germ which is derived by the division of a parent-cell of the preceding

generation. In unicellular plants this happens by simple fission of the entire parent body with the formation of two new individuals like the parent. In the multicellular plants the cells of the body sooner or later become differentiated into two different classes of cells. These are the "somatic" cells, which are differentiated into various kinds of tissues to perform the functions of individual life and which constitute the body, and the germ cells, which are intended to give rise to new individuals by being detached from the parent body. The distinction of these groups is however only relative. Both have a common origin in the parent germ cell and arise also by mitosis, and both have practically the same structure and often both have the power of development. They differ in their physiological functions and though the differentiation is the most important one of the multicellular body they are to be regarded as differing only in degree from the distinctions between various kinds of somatic cells. In higher types of plants the germ cells are more or less definitely arranged in groups and are nourished and supported by special somatic cells forming distinct sexual organs, the ovaries and spermaries. Here the germ cells develop and prepare for their future functions. At first the progenitors of the germ cells do not differ from the somatic cells surrounding them. Later on they differentiate from the somatic cells and finally the two sexes undergo remarkable changes of structure to fit them for their special functions. This differentiation is only the result of physiological division of labor. The female germ cell has to be large because it supplies most of the material for the embryo while the male germ cell supplies only a small amount of substance to the embryo. Therefore, we have at time of maturity a great difference between the ovum and sperm cell.

In the lower forms of plant life both sexual and asexual reproductive cells may be produced on the same individual, but in the angiosperms we find that the plant exhibits two phases in its life cycle, one of which is the sexual stage, and the other the asexual stage. The sporophyte is the asexual phase while the gametophytes representes the sexual phase. The regular alternate occurrence of gametophyte and sporophyte is called the antithetic alternation of generations and is a constant feature of higher plants. There is a great difference in the relation of these two phases to each other in the different classes of plants. In the lower types such as the thallophytes, the gametophyte is by far the most predominant part while the sporophyte is only a small structure and lives as a parasite upon the gametophyte to which it remains nearly always attached. In the mosses or Bryophytes both forms are nearly equal in size and development but the sporophyte is still always parasitic upon the gametophyte. When we come to the Pteridophytes or ferns we find a reversal of form, and the sporophyte has become the predominant form while the gametophyte has been reduced to a very small size. In the spermatophytes finally this phenomenon has become still more pronounced. The sporophyte has now become very large while the gametophyte is only a very minute structure, recognizable only through the microscope.

Sporogenesis.

Sporogenesis is the process of development and maturation of the germ cells or spores by the parent plant or sporophyte and falls under two distinct heads, namely, oogenesis, which includes the formation and maturation of the female germ cell, the ovule or macrospore, and spermatogenesis, comprising the corresponding phenomena in the development of the male germ cell, the pollen grain or

microspore. The type of maturation is practically the same in both sexes. In both sexes the number of chromosomes is finally reduced in the course of the last two cell divisions, known as maturation divisions, which result in the definite germ cells. Each one of the four cells formed has only one half of the original number of chromosomes. While in the male all four cells become functionally germ cells, in the female only one cell forms the germ cell while the other cells are incapable of development. The tiger lily has been used as an object for studying oogenesis while podophyllum was used for the study of spermotogenesis.

Oogenesis.

Both pollen and egg cells take their origin from cells known as primordial germ cells which become distinctly differentiated from the somatic cells at an early period of their development and at first show no distinctions between the two sexes. There seems to be strong reason to believe that in most cases the primordial germ cells are sexually indifferent, and their change into male or female germ cells seems to be due to external stimuli. Food seems to be a very important factor. We are not yet able to tell whether there is one common cause to all cases of sex determination.

The megasporangium is of hypodermal origin, being derived from the outermost layer of the periblem. While it is an embedded organ it becomes very distinct on the surface by the growth of the cells around and beneath it, and the whole structure constitutes the ovule. The ovule may arise from any free surface within the cavity of the ovary and may be foliar or cauline. In the different groups of angiosperms they are borne in very definite ways. In the tiger lily the ovules are of cauline origin and are attached to

the axial placenta of the ovary. At first the epidermis where the ovule is going to appear is even and in the hypodermal layer the archosporium may be evident. There is usually a single hypodermal initial cell which soon can be recognized among the other hypodermal cells by its larger size and a difference in appearance of the cell contents. This large initial cell divides usually by a periclinal wall into two cells and the inner one of these is the primary sporogenous cell. This cell does not form any more sporogenous cells but constitutes the megaspore mother cell. It divides, forming a tetrad by two successive division which are the reduction divisions. The tetrads form a linear row and the three outer cells are lost while the innermost of the row becomes the embryo-sac. In the lily this process is somewhat shortened as the initial cell does not divide and is thus the primary sporogenous cell or mother cell. There are no tetrads formed but the megaspores are represented by four nuclei, three of which are lost while the fourth one becomes the nucleus of the embryo-sac. In the meantime, a slight protuberance is developed by cell-divisions and it becomes more and more prominent and forms the nucellus of the nascent ovule. After the nucellus has become prominent a ring like outgrowth begins at its base and soon develops into the inner integument which as a rule covers the nucellus. Soon after the inner integument is well started the annular growth of the outer integument becomes visible. Finally the integuments overlap the nucellus and where they close over it there is left a narrow more or less elongated passage way, the "micropyle". There are three different positions of the ovules on the stalk or funiculus on which they are borne. The first one, the orthotropous form, is stright, outward with straight axis and the micropyle di-

rected away from the origin of the ovule. In the ampylotropous form the micropyle is directed towards the surface of origin and the whole body of the ovule is curved. The third form which we have in the tiger lily is the anatropous form. The ovule is at first straight but soon develops a curvature near the base of the integument, the curvature increases with the growth of the integuments and finally the nucellus is inverted against the funiculus. For this reason the outer integument is not developed in this case on the side toward the funiculus. The micropyle is now directed towards the surface of the origin of the ovule and the funiculus appears as a ridge along one side of the ovule.

The history of the female gametophyte is considered to begin with the division of the mother cell or in case of the lily with the division of the nucleus of the mother cell. As mentioned above, three of the nuclei formed in this division are lost while the fourth one forms the nucleus of the female gametophyte. During this division the number of chromosomes is reduced to one half. Thus the gametophyte contains only one half the number of spores as the sporophyte does. By the fusion of the male and female gametes the original number is established. The exact significance of this phenomenon is as yet not fully understood though a number of theories have been advanced. The physiological advantages are evident for by the constancy of numbers the parent is enabled to transmit an equal number of chromosomes to the offspring and by the reduction a constant increase in the number of chromosomes is prevented. As the reduction takes place in the mother cells of the spores these have to be regarded as the beginning of the new generation. The megaspore during its growth and development absorbs the other nuclei and some of the surrounding sterile tissue

and becomes very much enlarged. The history of the development of the gametophyte from the megaspore to the mature egg stage is very uniform. The nucleus divides by mitosis into two daughter nuclei which move to the opposite ends of the embryo sac, and in turn by a second mitosis produce two more cells and these finally each two more so that at the end we have a gametophyte with eight nuclei, four at each end. Those near the micropyle are called the micropylar nuclei while those on the other end are called the antipodal nuclei. The antipodal polar nucleus and the micropylar polar nucleus move toward each other and fuse near the centre of the embryo-sac forming the primary endosperm nucleus. The remaining three micropylar nuclei form the egg apparatus, one of the nuclei forming the female gamete which by the union with the male gamete gives rise to the embryo. The remaining two nuclei are called the synergids, meaning helpers because they assist in the process of fertilization. The three antipodal nuclei form the antipodal bodies. They are either epidermal or form a very active tissue. There seems to be no question to the view that the antipodal bodies are vegetative cells of the gametophyte. Their polarity, as compared with that of the egg apparatus, confirm this view. Their function seems to be to supply the embryo-sac with nutritive material absorbed from the outside at a time when the endosperm has not been developed.

Spermatogenesis.

Like the megasporangium also the unicrosporangium is of hypodermal origin and is also derived from the outermost layer of the periblem. In general the microsporangia are produced upon lateral members or sporophylls and they may therefore be called

foliar. Some sporangia however are derived from the periblem of the axis and are of cauline origin. The organs producing the sporangia are the stamens. The layer just underneath the epidermis, the hypodermal layer, is potentially sporogenous. As a rule it becomes actually sporogenous however only in four regions where the conditions are favorable for this development, and these regions form the microsporangia. Here we find a varying number of initial cells which really form four longitudinal hypodermal bands or plates. These plates of hypodermal cells become differentiated in each lobe, and differ from the adjacent cells by their larger size, their elongation, their larger nuclei, and their different reaction to stains. In transverse section the plate consists of a single row of cells of varying number. In longitudinal section the band extends about the length of the anther. Each one of these bands of initial cells divides periclinically almost simultaneously forming an outer layer, primary parietal layer, and an inner layer, primary sporogenous layer. The primary parietal layer divides further, forming several wall layers. The inner layer produces the sporogenous tissue. The outermost parietal layer is usually much modified and forms the so-called "endothecium" which assists in the dehiscence of the sporangium. The innermost wall layer, as a rule, becomes a part of the "tapetum", a jacket of nourishing cells around the sporogenous tissue. The tapetum has no definite origin but is derived from sterile cells of different origin, next to the sporogenous tissue, and may include a variety of morphological elements. The cells of the primary sporogenous layer divide as a rule, two or three times in every direction, and form the spore mother cells. The mother cells and their nuclei become very much enlarged and dif-

fer from the adjoining cells also in their staining power. The cells become more or less rounded and separate slightly from each other. The mother cells stay close together in the sporangium. The enlargement of the cavities of the sporangium results in a reducing of the sterile tissue which separates the two sporangial cavities on each side of the anther to a thin layer, which finally may disappear, causing the two cavities to fuse into one. The mother cell stage is regarded as the end of the sporophyte stage, because now follows after a shorter or longer rest the reduction division of the mother cell. The mother cell produces in two rapidly succeeding divisions tetrads with a simultaneous reduction of the number of chromosomes to one half, and the reduced number of chromosomes is a characteristic of the gametophytic tissue. This division therefore marks the beginning of the gametophyte. The reduced number of chromosomes appears during the first mitosis in the pollen mother cell, which has therefore to be considered as the first gametophytic cell. The result of the two divisions is four microspores. It is a very interesting fact that cytologically these two mitoses agree very closely with those of the megaspore mother cell. The pollen mother cell can be identified by the synapsis stage before any rounding off takes place. During the spireme stage the chromatin thread splits longitudinally in its whole length and the resulting double thread is divided transversely into the number of chromosomes characteristic of the gametophyte of the respective species. After the two divisions the four microspores are surrounded by a delicate wall independent of the wall of the mother cell. This new wall soon develops two layers, the inner one, the "intine" consisting of cellulose and forming later on the pollen tube. The outer layer or exine is cutinized and often covered with ridges and warts. In

most cases there are thin spots of varying number in different species for the exit of the pollen tube. As a rule the mature pollen grains become entirely free from each other and form a powdery mass. In some cases as for instance in the Asclepiadaceae the whole contents of a sporangium form one single mass, the pollinium.

The germination of the pollen grain starts with the division of the nucleus. This always takes place before dehiscence. At first the daughter nuclei are, as a rule, of the same size and form, however, in most cases the tube nucleus soon increases much in size and the two nuclei become differentiated. The tube nucleus has a large nucleolus and a rather small amount of chromatin network, while the generative nucleus is smaller and has denser chromatin. A generative cell is formed by the cytoplasm being arranged more or less distinctly around the generative nucleus and is often cut off from the other parts of the spore by a distinct wall. The generative cell is the primary sporogenous cell, and it divides only once, producing two equal male cells. At this time the pollen is ready to pollinate the stigma.

Pollination.

Under pollination we understand the transfer of the pollen from the stamen to the stigma and it always must precede fertilization of the ovule. There are many ways by which pollination can be brought about, natural and artificial ones. When the pollen grain has reached the stigma it begins to germinate under the action of the secretion of the stigma and it develops a pollen tube which grows from the stigma through the conductive tissue of the style into the ovarian cavity where it enters the embryo-sac through the micropyle. After the pollen tube has entered the embrye-

sac it discharges the two male cells. One of them passes to the egg and fertilizes it while the other one passes on to the primary endosperm nucleus and fuses with it. The fertilized egg cell then gives rise to the embryo.

Material used for the Investigation.

The material used for this work was largely taken from the supply prepared by Dr. Smythe for laboratory use. Some material was collected by the writer and prepared for microscopic examination. The ovaries were taken from tiger lilies growing in Dr. Smythe's garden on the V. P. I. campus. For the study of spermatogenesis podophyllum buds were used which are growing wild in the woods around Blacksburg. A description of the plants used shall follow at this place.

The Tiger Lily. LILIUM TIGRINUM.

The tiger lily is an angiospermous plant belonging to the monocotyledons. It is a native of China and Japan and has been cultivated for a long time in the United States and has escaped from cultivation in some localities. It belongs to the Liliaceae and has a number of nodding flowers borne in panicles, consisting of a showy and regular perianth of three sepals and three petals of equal size and all of an orange-red color with purple spots. The parts of the perianth are lanceolate, papillose, recurved, and about three to four inches long. There are two sets of three stamens each, one slightly shorter than the other hypogynous, each one of the six stamens slightly attached to the base of a perianth segment. The filaments are filiform, the anthers two-celled, linear and versatile, and the pollen sacs longitudinally dehiscent. The ovary is three celled with numerous ovules arranged in two vertical rows in each cell. The pistil is compound, with a three lobed stigma,

and a long style. The seed capsule is oblong and has numerous flat, horizontal seeds, packed in two rows in each cavity. The stem is stout, purple or nearly black, while pubescent above, about 2 to 5 feet tall, and leafy nearly to the base. The leaves are lanceolate, alternate and glabrous. Some of the upper leaves bear bulblets in their axils. The plants bloom about in mid-summer.

Podophyllum peltatum. May Apple.

Podophyllum is an erect herb about 1 to 1½ feet high, with a horizontal poisonous rootstock, large peltate, palmately-lobed leaves and a single white, perfect nodding flower with six sepals, that droop when flower opens, and 9 to 6 obovate spreading petals. There are many stamens with anthers linear and longitudinally dishiscent. There is one pistil many ovuled ovary which forms a large, fleshy berry. Seeds are numerous, obovate and enclosed in fleshy axils. The flowering stems appear from different root stocks, and have one to three leaves. The flower is about two inches broad and starts from the base of the upper leaf. The fruit is ovoid and yellowish, about two inches long and edible. It grows in low woods from Quebec and Ontario to Florida.

Collecting the Material.

In the collection of plant material for the study of sporogenesis buds have to be cut at different stages of maturity, beginning with the earliest stages until all stages have been secured. The early stages of development were found in very young flower buds, while the last stages were found in flowers in full bloom. For this work is needed a sharp knife and a bottle with killing fluid, which has to be fresh in order to get best results. Immediately after cutting the bud or ovary from the plant, drop it into the killing fluid as otherwise changes of the cell structures

may take place before the cell activities are stopped. If the material is not too large it may be dropped into the killing solution right away, but if the pieces are rather large they have to be cut into smaller pieces to allow a more rapid penetration of the killing fluid into the tissues. The way of cutting the pieces depends upon the object aimed at.

Killing and Fixing the Material.

The killing agent is used to bring the life processes to a sudden termination and the fixing agent serves to preserve cells and other structures in a condition as near as possible to the condition during life. By the fixing process the material is hardened in order that the various elements may retain their natural conditions during the succeeding manipulations and procedures. Usually the same reagent serves for both killing and fixing while in some cases different reagents are used for these two processes. Some of the usual fixing and killing agents are: alcohol, chloroform, Osmic acid, acetic acid, chromic acid, corrosive sublimate, and others. In this work the following solution was used:

Chromo-Acetic Solution.

Chromic acid,	1 gram.
Glacial acetic acid,	3 cc.
Water,	100 cc.

Chromic acid alone will cause plasmolysis of the cells, while acetic acid has a tendency to cause swelling rather than shrinking, thus counteracting the action of chromic acid. The collected material was left in this chromo-acetic solution for about 24 hours.

Washing the Material.

After the fixing has been finished the chromo-acetic solution has to be washed out thoroughly because some reagents leave precipitates and for this purpose the material was removed from the solution and washed in running water for about 24 hours. The process can be shortened by using luke-warm water.

Dehydrating the Material.

The object of dehydration is to get rid of all the water in the material in order to prepare it for embedding in paraffin or celloidin. Also objects to be mounted in balsam have to be dehydrated. A slight trace of water can become very fatal for the preparation. Alcohol is the most used reagent for dehydrating. It completes the hardening process and dehydrates at the same time. The process has to be very gradual in order to prevent injury to the delicate parts. Material taken from water or aqueous solutions has to be passed through a series of alcohols beginning with not more than 15% alcohol. In this the material was allowed to stay for six hours then the alcohol was poured off and 35% was poured on and left for about the same length of time. Then it was replaced by the 50% alcohol which was left for about 12 hours, when 70% alcohol was poured. In 70%, 85%, 95% and 100% alcohol the material was left for 24 hours each. The time depends on the material to be dehydrated. If for any reason the process has to be interrupted, the material can be left in the 70% alcohol for considerable time without injury. The lower grades of alcohol may be used several times, but the absolute alcohol should not be used again for the same grade but may be saved for rinsing slides. Be sure that the dish is absolutely dry before pouring the absolute alcohol into it, and keep it tightly corked or covered. The following formula is

quite useful in making up different grades of alcohol from 95% alcohol:

95	95	95	95	95
15	35	50	70	85
80	60	45	25	10

Subtract 15 from 95 and 80 remains the number of cubic centimeters of water to be added to 15 c.c. of 95% alcohol to get 15% alcohol, and so on.

Clearing of Material.

Clearing agents are used to render objects transparent. Preceding infiltration in paraffin a clearing agent is used to replace the dehydrating agent with a solvent of paraffin. Before the clearing agent is added the material ought to be perfectly dehydrated with absolute alcohol. The clearing agent used in this work was xylol. The material should be brought into xylol only very gradually. For this reason four different steps were used. At first the material was transferred to a mixture of alcohol and xylol containing three parts of alcohol and one part of xylol and left in this solution for 24 hours, and then transferred to stronger solutions of xylol in the following manner:

Absolute alcohol 3 parts. Xylol, 1 parts. Leave for 24 hrs.
" " 2 " " 2 " " " 24 "
" " 1 " " 3 " " " 24 "
Pure xylol, " " 24 "

The pure xylol should not be used again for the same purpose, but may be saved for dissolving paraffin ribbons.

Infiltration.

In the process of infiltration the object is to permeate the tissue of the material with paraffin. This should also be a very gradual process. A convenient way is to drop a piece of paraffin into the clearing agent with the material and allow the paraffin to dissolve gradually and the infiltration starts very gradually. The bottle ought to be kept lukewarm and tightly corked. Add no more paraffin than will go into solution and the temperature may be increased in order to get more paraffin in solution. If the paraffin is allowed to crystallize out it is liable to damage the tissues of the object. A convenient way is to keep the bottle with the material on the top of the oven for 6 to 12 hours.

The Paraffin Bath.

In order to complete infiltration the xylol has to be entirely replaced by paraffin. This is accomplished by placing the material in a small pan with soft paraffin of low melting point, which is kept melted on the oven. The oven is so constructed that the heat can be kept nearly constant for a long time by a gas flame which can be regulated very closely. This is of importance as all unnecessary heat should be avoided, because it is liable to impair the preparations. All we need is to keep the temperature slightly above the melting point of the paraffin. The soft paraffin has a melting of about 45°C. while the hard paraffin melts at about 54°C. The material is left in the melted soft paraffin until the last trace of xylol has disappeared. In this work the material was left about 24 hours or longer. It is safer to use prolonged periods in order to be sure of complete permeation of the tissue with paraffin.

Imbedding.

For imbedding, the material was transferred into little trays of paper, about half inch deep and of a width to hold comfortably the material to be imbedded without crowding it too much. Hard paraffin was melted and poured into the tray. Then the material was transferred into it as quickly as possible and arranged conveniently for cutting. The tray was now cooled quickly in cold water, in order to prevent crystallization of the paraffin which is liable to occur in slow cooling. The material should be well covered with paraffin.

Cutting.

As soon as the paraffin is hardened thoroughly the material is ready to be cut. Blocks were cut out of the paraffin, with the object in suitable position for cutting, as nearly rectangular or square as possible. A rotary microtome of the minot type was used for cutting. The blocks intended for cutting were fastened to the disk by warming the disk and the bottom of the paraffin block and pressing them together. Then the block was fastened still more by placing small pieces of paraffin at its base and touching them with a hot needle and rinsing the block with cold water. The disk was now fastened to the microtome. Great care has to be taken to have the surface of the block parallel to the direction of the knife in order not to lose any sections of the material. The knife ought to be kept perfectly sharp and a dull knife is liable to tear the sections. The blocks ought to be trimmed to perfect rectangles in order to secure good ribbons. If the upper and lower sides of the block are not parallel the ribbon will be crooked and is liable to cause trouble in mounting it on the slide. The knife ought to strike the whole edge of the

block at once and not obliquely. Great trouble may be caused by the paraffin not being in the proper condition for ribboning. If it is too hard, warm the knife slightly and if possible go to a warmer room. If it is too soft, cool for a while in cold water. Avoid any draft during cutting as the ribbon may be blown against other objects or may be torn and partly lost. In removing the end of the ribbon from the knife it is best to use a small camel's hair brush which is not very apt to injure either ribbon or the knife, while the other end may be held with a pair of forceps. The lily ovaries were cut 14 microns thick, the podophyllum buds 6 microns thick, Marchantia 8 to 10 microns thick, and Chara 6 microns thick.

Fixing Sections to the Slide.

It is of great importance to have the sections firmly attached to the slide as otherwise they are liable to be washed off during the different processes of staining, washing, etc. The fixative used was Mayer's albumen fixative, made according to the following formula:

White of egg, (active principle),	50 c.c.
Glycerine, (to keep it from drying up),	50 c.c.
Salicylate of soda, (antiseptic, to keep out bacteria)	1 gram.

A small drop of this fixative is put on a clean slide and smeared evenly with the finger over the surface. Wipe off all excess until only a scarcely perceptible film remains. Now put a drop of distilled water on the slide enough to float the sections of the ribbon put on the slide. Heat gently until the paraffin spreads out and forms a smooth surface. Be careful not to melt the paraffin as the albumen will coagulate and the sections come lose.

Land recommends the use of a 1% solution of gum arabic and about 1% or less solution of potassium bichromate which form in light an insoluble compound and do not coagulate by heating. After the sections have become smooth let the water run off carefully without disturbing the sections and dry the slides for several hours before further procedure.

Removal of the Paraffin.

To remove the paraffin from the slides and out of the sections the slides were put into a vessel with xylol and allowed to stay there for 10 to 30 minutes, according to the time required by the xylol to dissolve the paraffin. Then the slides were put into 100° alcohol for about 5 minutes to remove the xylol from the slide and further transferred to 95% alcohol for the same length of time. The slides were now ready for staining.

Staining.

The stain used for the lily ovaries and podophyllum buds was Flemming's triple stain. This is a combination stain consisting of safranin, gentian violet, and orang G. Safranin has long been a famous stain for mitosis. These stains belong to the group anilins, and are some of the most brilliant and beautiful stains in use. They are especially valuable for cytological work because they are specific stains. In staining intricate structures as those involved in mitosis and development of germ cells specific stains are imperative to differentiate the different types of structures and tissues. The procedure in the triple stain is as follows: Stain for 5 to 24 hours in a 50% alcoholic solution of safranin. In most cases the stain is too deep and has to be reduced in 50% alcohol to

the desired strength. Then wash in water and put the slide into a 1% aqueous solution of gentian-violet for 10 minutes, wash again and stain for 1 minute in 1% aqueous solution of orange G. Dip the slide a few times into 95% alcohol until the gentian violet stops streaming off the sections. Rinse shortly with absolute alcohol to dehydrate and add a drop of oil of cloves to clear the sections. In order to facilitate clearing heat gently over a flame. The oil of cloves is drained back into the bottle and any remnants of it have to be rinsed off with xylol as otherwise the preparations are likely to fade. Be careful that the sections do not get dry during the procedure and before the xylol has evaporated put a drop of balsam on the slide and the cover glass on, pressing it down gently with a needle in order to expel any air bubbles under the cover glass. Put a clamp on the slide and cover glass until the balsam has dried to prevent slipping of the mount. A short discussion of the stains used may follow here.

Safranin.

There are two kinds of safranin on the market, one soluble in water and the other soluble in alcohol. The alcoholic solution is made by dissolving 1 gram of the alcohol soluble safranin in 100 c.c. of 95% or absolute alcohol. The aqueous solution is made by dissolving 1 gram of water soluble safranin in 100 c.c. of water. For the present work the following formula was used: Equal volumes of a 1% solution of alcoholic safranin and of aqueous safranin were mixed and thus a 1% solution of approximately 50% alcohol was obtained. Safranin is the most useful red stain for general work and, fortunately, keeps well. It stains liquified, suberized and cutinized membranes a brilliant red, also chromosomes and nucleoli.

Gentian-Violet.

Gentian-violet is a very important stain used in histology. It is particularly good for achromatic structures, but stains also chromatin, starch and chromatophores in varying degrees. It shows the chromatin granules in the ovaries and the mitotic figures by giving them a brilliant violet color. It is of great importance in giving clear views of the minute structures. The solution used was a 1% aqueous solution, which keeps indefinitely.

Orange G.

A 1% solution of this stain was used. It is a useful staining for staining plasma and is often used in combination with safranin and gentian-violet.

Laboratory Apparatus Required.

The laboratory apparatus used in this work consisted of a rotary microtome, a compound microscope with high and low power objectives, a hand-microtome, a paraffin bath with gas burner, a scalpel, a camel's hair brush, a pair of needles, a pair of scissors, a pair of forceps, a section lifter, a number of stender dishes, a series of bottles, watch glasses, microscopic slides 1 x 3 inches, several sizes of square and long cover glasses, labels, slide boxes. A piece of glass was found to be very handy to protect the stage of the microscope when examining slides that were still wet from different reagents used. Sheets of heavy paper are useful to spread the ribbons on before mounting them on the slides. The reagents should always be pure and fresh to obtain best results. It is very important to pay a good deal of attention to all details of the work if we want to get satisfactory returns for our work.

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ILLUSTRATIONS.

Fig. 1. Transverse section of ovary of Lilium tigrinum, showing two daughter nuclei on the embryo sac.

Fig. 2. Transverse section of ovary of Lilium tigrinum, showing four daughter nuclei at the end of the second maturation division.

Fig. 3. Transverse section of ovary of Lilium Tigrinum, showing the eight daughter nuclei at the end of the last division.

Fig. 4. Transverse section of ovary of Lilium tigrinum, showing the four nuclei stage. Microphotograph.

Fig. 5. Transverse section of ovary of Lilium tigrinum, showing the eight nuclei stage. Microphotograph.

Fig. 6. Longitudinal section of the anther of Podophyllum peltatum, showing the spore mother cells in the spireme stage at the beginning of the mitosis.

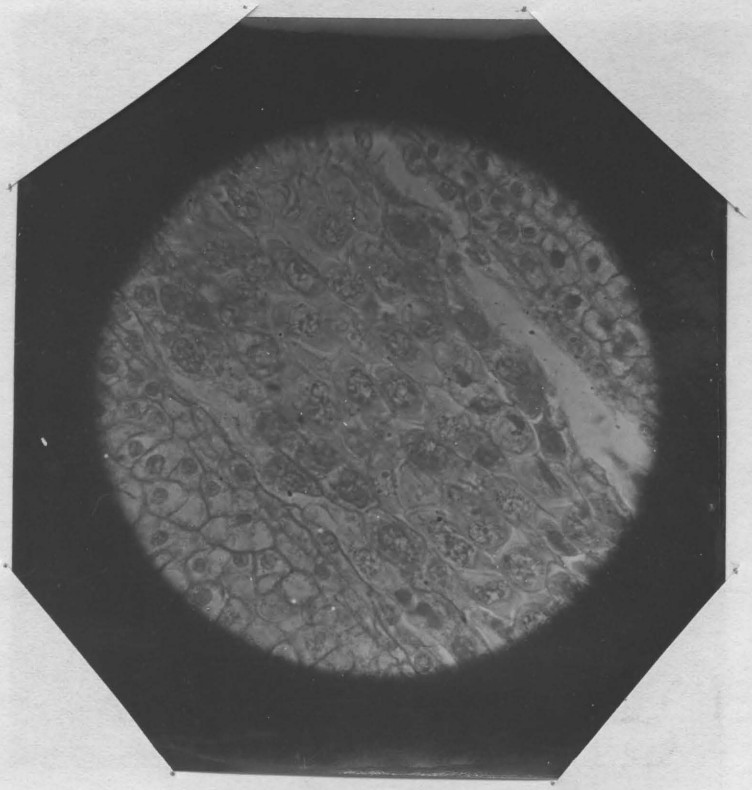


Fig. 6.

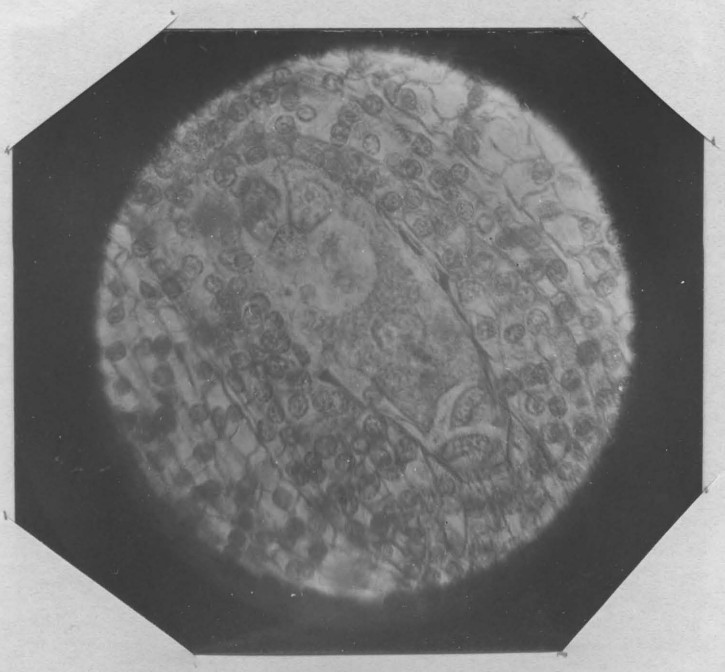


Fig. 5.

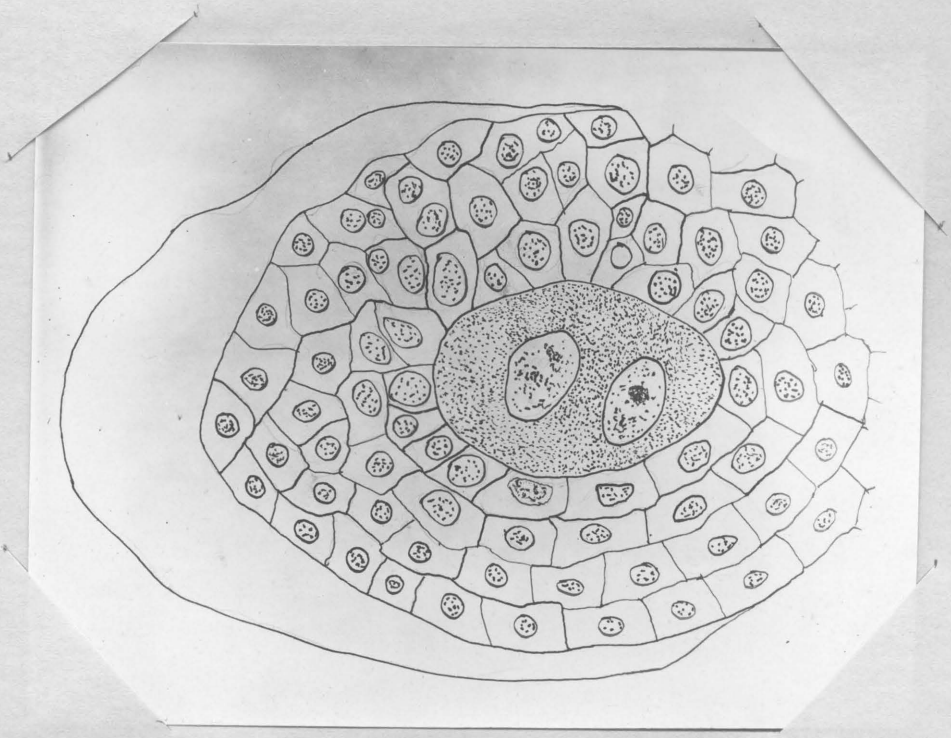
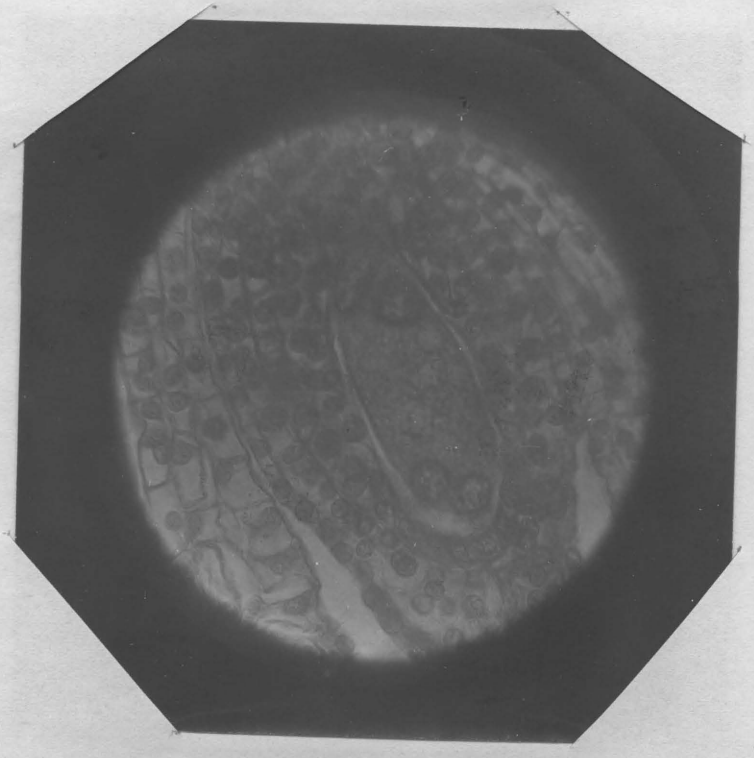


Fig. 1.



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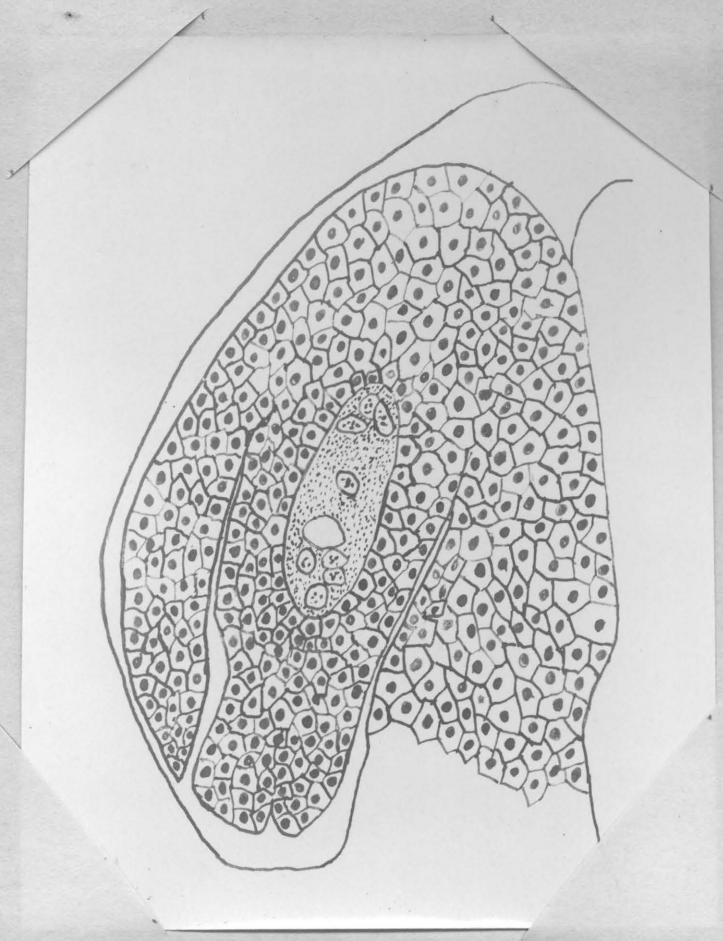

Fig. 3.

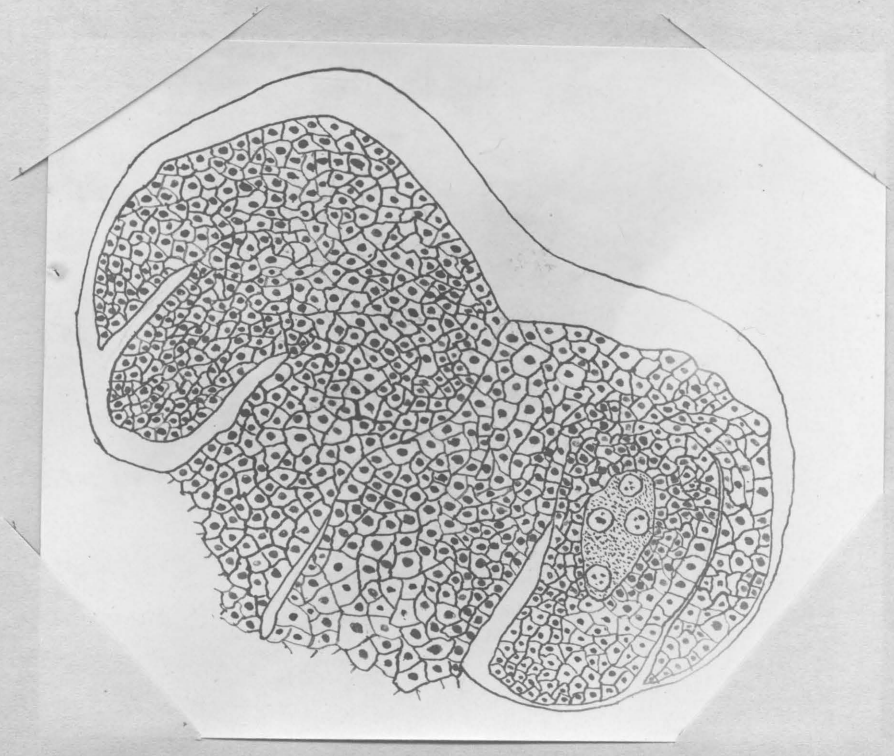

Fig. 2.