MICRURGICAL STUDIES IN THE PHYSIOLOGY OF CELL DIVISION

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INTRODUCTION

Division and growth are the basic phenomena of life. Yet, the mechanics of these processes can never be adequately understood if they be regarded as singularly "vital" activities. For, fundamentally, the cell including all its morphological counterparts, is constituted of the same unit as is the inanimate world, namely the molecule. And, because of this common factor in structure, the reactions involved in an activity as cell-division may be interpreted in terms of those properties of molecular behaviour already investigated and known. Thus, solute molecules in a supersaturated solution which orient and attach themselves to a growing crystal, furnish one approach to the study of gene duplication. Similarly, the folding and unfolding of polypeptide chains, involving the separation as well as the formation of various secondary bonds, may be a basic feature of the structural changes occurring in the chromosome during the mitotic cycle. And, though interpretation in terms of molecules does not afford an ultimate explanation of these phenomena, the process of resolution should be step-wise and not neglect molecular behaviour in preference to that of the more fundamental constituents as atoms and electrons.

The organization of the protoplasmic body is so

complex, that present knowledge of molecular chemistry is insufficient to account for its behaviour. Consider for example, the main unit of structure - the protein molecule. Study of the latter "in vitro" has given us only a partial and somewhat incoherent picture of its nature. The concept of it as a single reactive unit is clearly inadequate. Its many radicles, side-linkages, and internal linkages, coupled with a variety of prosthetic groups and active centres, makes it capable of several simultaneous reactions, which in turn, influence the behaviour of dissociation constants, and consequently affect the behaviour of the protein in solution. Yet, even these various internal relationships do not exhaust the problems of protein behaviour. The "macromolecule", as the protein molecule is often called, exhibits many colloidal properties and the issue of whether it exists in solution as molecule or micelle has created a controversy which has not yet been settled, though it is not improbable that its giant size enables it to react in both fashions. The phenomenon of protein denaturation, which is so prominent in protoplasmic behaviour, has also brought forth several hypotheses. Neurath (1940) suggests that there is a split along the main axis of the molecule, while Lloyd and Schmidt propose internal rearrangements in the molecule including shifts in side-linkages. Other theories of enolization of C:O and N-H- bonds as well as changes in the Hydrogen

bonds have also been proposed.

Further, the living cell is not merely a conglomeration of molecules. It is, on the contrary, a very definite organization of them. Thus, the cell cannot be treated as a homogeneous physico-chemical system, whose reactions permit stochiometrical formulation. And if the protein molecule cannot be resolved at present, the difficulty in interpreting the organization of such cellular bodies as chromosomes or genes becomes obvious. The gene string, for example, is a coherent and relatively stable structure. From chemical considerations, we know that the entire substratum of protein molecules is not likely to constitute the active phase of the gene's behaviour, while cyto-genetic studies have shown various portions of the chromosome to be inert. Hence, we may conceive of a framework consisting of a matrix of relatively inert protein molecules, attached to which are groups of active ones. But, this involves, first, a knowledge of individual molecular constituents such as has been arrived at in the case of the nucleic acids; secondly, it involves a knowledge of intermolecular organization by virtue of which the active molecules are bound to the molecules of the substrate; thirdly, of the manner in which the molecules of the substrate are bound to each other so as to give rise to a more or less permanent structure, which does not suffer any linear derangements in the course of a cell's ordinary development. Goldschmidt's

assertion that the gene acts like an enzyme bears a certain degree of truth in relation to genoneme structure. For, in the enzyme, we have both active and inert groups which crystallize out together, and such a structural relationship probably exists in the gene though the similarity between the two may not be as complete as Goldschmidt would like to have it. But, the difficulties do not exhaust themselves at this point, for we must also explain in what fashion the gene duplicates itself. Molecular orientation and deposition about an existing crystalline structure will cause a growth of the crystal around its axis, but not necessarily the type of growth which will enable subsequent separation into two exactly similar crystals. Such a separation would demand a lamellar structure, probably involving an uncoiling of the protein molecules.

Having created an approach to the molecular aspects of cell-division, one must still resolve the complex problems of microscopic structure. For, inasmuch as we cannot consider isolated protein molecules "per se", when treating vital problems, we must not overlook the behaviour of the larger microscopic organizations. The chromosome, which at the prophase of meiosis appears as a long string, and which at metaphase assumes the configuration of a sausage containing internal coils and manifesting chiasmata at various points along its length, is one of the central units in cell-division. For, after envisaging duplication of the gene string, one must consider the mechanism whereby the genoneme, contained as it is in the

larger chromosome body, proceeds in regular fashion, both spatially and temporally, to distribute its replications at the two opposite poles of the cell. The answer to this involves another unit of physico-chemical behaviour, the colloid particle. For both the chromosome and the cytoplasm have a colloidal meshwork structure, which exists in various states of formation as sol, coacervate or gel.

The type of aggregation existing in a particular region of the cell may be very important in determining the behaviour of the cell's inclusions. Thus, the presence of a sol state, within which movement of consistent bodies may take place relatively easily, constitutes an important factor in the separation of the chromosomes (though the possibility of some form of protoplasmic streaming need not be excluded). The viscosity changes occurring during mitosis, some of which are localized and others general, are probably also effective agents in the mechanics of division. The birefringent spindle, whose absence is usually associated with non-separation of the chromosomes, may prove to be a liquid crystal formation, the orientation being caused in the process of flow. On the other hand, properties of elasticity and extensibility are due to the more consistent state of gel aggregation. The chromosomes, which possess elements of rigidity and elasticity, as well as fluidity, probably consist of more than one

aggregational state. In fact, the role of coacervate in the pattern of chromosome organization is yet to be fully ascertained.

Description of the mitotic process in terms of viscosity and sol-gel- coacervate change, is inadequate. For, even from a microscopic viewpoint, the existence of any of these states is merely the sum total effect of a group of factors. And because of the latter fact, no firm generalizations can be evolved simply in terms of these structural aggregates. Slight changes in ionic adsorption causing changes in surface charge may readily serve to transform a sol to a gel. But, there is no reason to suppose that such a change in aggregation is not a by-product of the divisional process, rather than a causal factor in it. The outer cortical zone of the cell may quite likely change its viscosity several times during the division, but one need not assume that such changes are effective in determining the course of the process. Moreover, even when we establish the effectiveness of a viscosity change in the mechanics of division, we have only served to indicate a starting point of investigation. For our unit is the colloidal micelle and the behaviour of the latter is dependent upon water of hydration, adsorption, surface charge and amounts of freeenergy available at its surface, as well as types of molecular configuration affecting the nature of the surfaces. The latter factors

must always be sought out in any change, for they will not only reveal the particular problem at hand but also serve to interpret other phenomena occurring concomitantly in the cell.

Any form of organization must have a state of dynamic equilibrium. And whenever a change occurs in the form of organization there is also a change in the constant of equilibrium. Thus the transmutation of the thread-like prophase chromosome to the sausage-like one of metaphase, represents a shift in the various equilibria factors of the cell. The source of these equilibrium shifts may well be the initial changes in the molecular arrangement of the genoneme, yet the source might be external to the nucleus. The production of nucleic acid and its subsequent disappearance illustrates the process of equilibrium change. Increase in lactic acid prior to division, as well as evidences of protein denaturation, indicate further that the former point of stability has been somehow shifted. Indeed, it is this delicate balance of forces which permits us to introduce artificial factors so as to enable determination of the possible changes occurring during and prior to division.

Finally, the classical concept of chemical equilibrium has given way to a more modern consideration--that of entropy and free energy change. Both Einstein and Planck have pointed out that under a given set of conditions only a certain amount of energy is available for use. This

limitation in utilizable energy imposed upon a situation by the very nature of things, must be a focal point in the process of cellular division. For, the duplication of the cell is initiated through transfer of energy to its molecular constituents so that they may orient themselves as well as multiply their number. This acquisition of energy is effected through chemical processes which manifest themselves in various physico-chemical changes. To obtain, then, a comprehensive picture of mitosis, one must be cognizant of two aspects of natural behaviour--structural change and energy change. The latter, which includes all chemical reactions will supply the fuel for the process, while the former will be the mechanism by which the various forms of organization are duplicated.

Review of the Literature

Though the gene is the fundamental unit of heredity, theories of its structure and constitution have been highly speculative due to the lack of a direct experimental approach. Several investigators (Gulick-1938 Waddington-1940) have reviewed the gene from a physico-chemical standpoint, and while such reviews do not contain any conclusive evidence for a molecular pattern of gene structure, they do provide a valuable concept of gene activity. Essentially, the gene is

compared to an enzyme, and is suggested as consisting of a substrate composed of protein molecules, to which are attached prosthetic groups or active centres. The latter are considered responsible for the characteristic reactions of each gene, while the substrate is thought to be relatively inert. Such an arrangement, of course, implies that the gene acts catalytically though it may be argued that an enzyme-like structure need not necessarily behave as an enzyme. However, genetic evidence points to the possibility of the gene effecting qualitative as well as quantitative reactions. Whether the mechanism of the qualitative changes is also quantitative cannot yet be ascertained. But, this conception of the gene does enable one to conceive of mutation phenomena as resulting from some translocation or alteration in the active centres of the gene, rather than involving a complete change in gene structure.

The dynamic behaviour of the gene also has its basis in the molecular structure of the chromosome. Wrinch (1936) described the chromosome as a continuous molecular system in which are contained the "supermolecules" comprising the genes. Schulman (1938) reviewed some of the more recent advances made in the chemistry of the chromosomes, a study which was instituted by Caspersson's investigation of the nucleic acids. Essentially, these advances consist of an analysis of the various components found in the nucleic

acids of the cell as adenine, cytosine, uracil, and thymine. Mazia and Jaeger (1939) showed by partial digestion, using pepsin and HCl, that protamines and histones were present in the chromosomes, and that the framework of the latter was independent of the nucleic acid molecules. Gulick (1941) in summarizing the data of other investigators, indicated that 8% of the dry weight of the nucleus were lipids. He believes, however, that they serve as structural material, rather than metabolic or nutritional.

The application of various reagents to the living cell has provided a very important channel of information for understanding cell behaviour. Both protein molecule and the colloidal micelle are sensitive to their osmotic and electrolytic environment, and therefore may be easily approached by observing their reactions in various media. Though the work performed has not sufficed to present an integrated picture of chromosome or gene structure, it does help to create a framework for some concept. Calvin, Kodani, and Goldschmidt (1940) have studied the effects of certain chemical reagents on the morphology of salivary gland chromosomes. Upon treatment of the latter with alkali, they obtained a lampbrush structure following fixation. Speculating upon the chemical phase of this reaction they suggested that the secondary bonds of the protein molecules were released, probably neutralizing the guanidine ion in arginine residue. Staining with Sudan-IV showed that the

alkali had removed the lipid components. Bancher (1938a, 1938b) approached the problem from the nuclear viewpoint. He treated the nuclei of plant cells with different salt and sugar solutions and concluded that the former affected the nucleus not through any osmotic force but rather through an electrolytic one. He further contended that the nucleus was a type of two-phase system in equilibrium--a sol phase and a gel phase. Salts either caused a hydration of the sol phase (Karyolymph) through ionic adsorption and thus swelled the nucleus, or flocculated the gel phase and separated it from the sol one. These interchanges in phases he designated as the basis for refractive changes in the nucleus. Shimakura (1937) obtained differences in the refractivity of the chromosomes with differences in pH, the former increasing with decrease in the latter. Shinke (1939) performed a large number of experiments on the nucleus, including effects of hypo- and hypertonic solutions, effects of electrolytes as salts, acids and alkalis, and effects of high and low temperatures. The essence of his conclusions is that the changes effected in the nucleus are hydrational ones. Kuwada (1937) experimented upon another effect of hydration showing that spirals would unravel in hypotonic medium, while they would tend to spiralize in a hypertonic one. Sigenaga (1940) obtained the same results by use of neutral salts in solution, claiming that the uncoiling was due to swelling and contraction. Churney and Klein (1937)

and Yamaha (1937) investigated the cataphoretic properties of the cell. Yamaha showed the nucleus to be positive and indicated a difference in electric charge of nucleus and cytoplasm by causing a swelling in the former but not in the latter through the use of a current. Churney and Klein obtained similar results except that they found the chromosomes to be negatively charged and the nuclear volume as well as chromosome refractivity to increase on application of a current.

Chambers and Sands (1923) dissected the pollen mother cells of Tradescantia virginica. They showed the cytoplasm to be a jelly-like mass which liquefied upon continued manipulation with the micro-needle. They also produced evidence to support the idea that the chromosomes are more viscous than the cytoplasm. On the other hand, they found the spindle to be more liquid than the latter. Chambers (1917) found the spindle to be a highly elastic gel. Thus it is difficult to ascribe any fixed state of aggregation to these structures, one should rather consider them as non-permanent states in equilibrium with the environing conditions of the cell. Buck and Boche (1938) succeeded through micromanipulation and asphyxiation, in showing that chromosomes contained some fluid capable of being exuded and reabsorbed.

Approaches have also been made toward an understanding of the dynamics of cell-division. In this sphere,

however, work has been of a heterogeneous nature and no central theme has been followed by the investigators. Thus many of the studies made have not probed the fundamentals of cell-division but rather described some of the activities associated with it. Schechtman (1937) indicated that the immediate cause of cell-division was localized cortical growth. He believed the sol phase of the cytoplasm moved into the gel region and gelated thereby forming a constriction and subsequently causing cleavage . Chambers (1938) pointed out another aspect of the process in echinoderm eggs where daughter nuclei in a more eccentric position caused a movement of the cytoplasm towards the equator where the cortical area increased through a sol-gel transformation. Marsland (1939) corroborated this by demonstrating that hydrostatic pressure on eggs caused a liquefaction of the equatorial gel cortex and decreased the efficiency of division. Dan and Dan (1940) studying the eggs of Strongylocentratus pulcherrimus showed that the division process involved an inward migration of granules followed by the formation of an extra-granular zone. Barber (1939) studied the rate of chromosome movement which he found to be least in the first meiotic division. He attributed this behaviour to chromatid drag. On the other hand, he claimed that viscosity had no effect on movement since large chromosomes moved at the same rate as smaller ones. Changes in viscosity, however, were shown to take place by a host of investigators as Heilbrunn

(1928), Kostoff (1930), and Fry and Parks (1934). Yet results obtained by the various researchers are not in complete accord. Gustaffson (1939) contended that the factor of hydration was not only of great importance in division but that it determined the nature of the division, whether it be meiotic or mitotic. In case of the latter, he believed that the nucleus was hydrated in the resting stage, while in case of the former, it was not hydrated till after prophase. The hydration, he claimed, was essential for chromosome reproduction. Sinnot and Bloch (1940) showed that from early prophase, cytoplasmic strands in vacuolated cells aggregated themselves into a plate (phragmosome) along which the future wall was to be laid. Churney (1940) studying mitotic elongation showed the latter to be affected by O.P. changes as well as various ions. Thus Mg and K aided elongation whereas Ca inhibited it. He believed the latter to be necessary for successful furrow formation.

Finally, some review on the biochemical aspect is in place. Brown (1892) demonstrated that a suspension of yeast cells in maltwort could continue fermentation without any proliferation, thereby indicating a definite independence of cell-division from respiration. Warburg (1908) used phenylurethane to retard cleavage but permit normal respiration. Quastel and Whethar (1924) succeeded in obtaining "Resting Bacteria". Rapkine (1931) employed KCN to stop respiration in dividing eggs but was able to show that despite this, oxidation of the SH group to S-S proceeded

as revealed by the nitroprussate reaction. Hammet (1932) employed $PbNO_3$ to inhibit division in onion root tips and showed that the SH group was involved. Rapkine (1936) was able to inhibit the SH activity by use of monoiodoacetic acid. This reaction had further implications since $I.CH_2COOH$ also affects the production of lactic acid. But Rapkine (1931) had already shown that an accumulation of lactic acid takes place prior to division and thus it could now be inferred that glycolysis was in some fashion connected with the release of SH groups and their subsequent oxidation in the course of cellular division. Needham (1933) has summed up the essentials of the theory of cell-division as proposed by Rapkine. The energy is produced by glycolysis which is brought about by partial anaerobiosis prior to division. This is followed by a lowering of the red-ox potential of the cell. It is believed too, that a denaturation of the protein occurs which exposes the SH groups and reduces the soluble S-S groups thereby causing anaerobiosis.

Scope of the Present Investigation

The numerous aspects of activity associated with cell-division makes a simultaneous experimental treatment of these activities impossible. In view of this, it has been decided to restrict the research to the physiological factors involved in meiosis and to temporarily abandon the morphological and the biochemical. Yet, the physiology of

meiosis involves so wide a range of factors that even this limited field of approach necessitates a restrictive choice. Thus, the physico-chemical changes occurring in the protoplasm during the course of meiosis was chosen as the topic for investigation.

There are many physico-chemical states which probably undergo a definite cycle of change during meiosis. Such properties as water of hydration, osmotic pressure, permeability, surface charge, and state of aggregation, may all be effective in contributing to the mechanism of cell-division. And, the evidence accumulated from cytological data should be complemented by a physico-chemical interpretation of the phenomena involved. These phenomena, which include volume changes of the chromosomes, coiling and uncoiling, and movement through the cytoplasm, most certainly involve alterations in some of the above-mentioned properties.

In the course of reviewing the literature, attention has been drawn to the investigators who have utilized the physiological approach for the study of mitosis. Unfortunately the paucity of research done in this field has made the definition of a problem involving specific physiological processes difficult. The primary task then, of the following studies has been an exploratory investigation along several lines leading to the formulation of more specific problems for later work.

Experimental Work

The present research deals with three properties of the pollen mother-cell; its physical structure, its physico-chemical organization and its permeability behaviour. The first is investigated directly by the use of the microdissection apparatus, and indirectly by observation of Brownian movement in the cytoplasm; the second is studied by suspension of pmc in various solutions, particularly electrolytes and sucrose, and observation of their effects upon such properties as refractivity and cytoplasmic consistency; the third is determined by the effectiveness of external solutes in causing intracellular changes and total volume changes. These techniques, however, cannot be applied independently, for the properties being investigated are highly interrelated. Permeability, for example, may limit or extend the observed effectiveness of an ion in solution, and the physical aggregations determined by the micro-needle may depend largely upon the constitution of the external medium. Thus, as a preliminary measure, it has been necessary to study the interaction of these factors rather than the intrinsic nature of the factors themselves. The results of such a study are compensating in that they define more clearly certain avenues of approach to the more general problem of meiosis. And, though some properties of the meiotic

cell have been revealed in the course of this research, the task of their elucidation has been left to subsequent investigation.

Materials and Methods

Pollen-mother-cells of Trillium erectum and Tradescantia reflexa served as materials for investigation. The first species was used preferentially because its extended meiotic period (four to five months) permits a more intensive study of each particular stage, while, the comparatively large pmc are most suitable for microdissection. However, since the supply of Trillium was limited, Tradescantia was used when the former was not available.

To avoid cell injury, extremely fine needles must be used in microdissection. Those whose penetrating shaft is greater than $1\frac{1}{2}$ microns in diameter are considered coarse, for they almost invariably produce irreversible effects upon the physical structure of the cell. Needles which taper abruptly into a short but extremely fine shaft are the best, since they possess rigidity which permits of facile manipulation.

Pmc suspensions may be prepared for microdissection by squeezing the contents of an anther fragment into a drop of paraffin oil mounted on a cover-slip. The pmc are thus surrounded by a layer of their own anthral sap, the oil preventing any evaporation from taking place. This procedure is not always satisfactory since the anthral sap, at

certain stages, is like a jelly and does not permit any appreciable dispersion of the cells. Moreover, in the absence of a liquid environment, the oil often comes into contact with the cells themselves causing moribund effects. To counteract these factors, a modification of the first procedure was introduced whereby the anther is cut into as many portions as desired, and the fragments placed in a solution of sucrose. When the anther is now squeezed into the oil-drop, the pmc are dispersed in droplets of sucrose solution contained in an oil medium. This technique has wider application since the effects of various ions or other solutes on cytoplasmic structure may be readily studied by previously immersing anther fragments in the desired solution. The use of aqueous solutions without oil as mounting media is not as satisfactory a method. Since the medium is suspended as a hanging drop, specific gravity relationships make the utilization of the high-powered objectives difficult, and evaporation necessitates the use of the moist chamber which is a relatively awkward procedure.

The most suitable physiological medium for micrurgical work is saccharose solution. Isotonic concentrations of salts, either as pure solutions or as balanced ones, are not satisfactory because they cause intracellular changes which are mainly of an irreversible nature. Effects of salts, indicator dyes, and stains, were investigated by adding the

desired reagent to a sugar solution, the final concentration of the latter always being kept constant. To facilitate this procedure a stock solution of 1.0M sucrose is made up in distilled water, and the particular medium required is prepared by diluting the stock solution with a solution of the reagent and with distilled water. The mounting media are usually made up to a volume of ten ccs. and placed in small preparation dishes in which the anther fragments are then suspended. When the pmc are transfered to a slide, care is taken to avoid mechanical injury by cutting across the anther with a sharp blade, and when necessary , teasing the contents apart gently with two sharply-pointed mounting needles.

Results

Permeability

Pmc of T. reflexa show a homogeneous interior when immersed in 0.2M sucrose, no plasmolysis being evident. After a brief period of time, some of the cells increase in refractivity and begin to show chromosomal configurations, their protoplasts "plasmolyzing". Alteration of the sucrose concentration or adjustment of the pH to 7 or 8 does not prevent the plasmolysis of the refractive cells. The reaction is even more striking when diads are observed, for often one cell may be seen expanding at the expense of another whose volume and tonicity decrease while its refractivity increases. Dilution of the media with water produces no deplasmolysis of

the plasmolyzed cells, even though the concentration of the sucrose is reduced to insignificant proportions.

Occasionally, pmc of T. reflexa dispersed in pure sucrose solution possess refractive chromosomes but do not plasmolyze. However, continued observation of these cells shows that their refractivity does not persist, the interior of these cells becoming homogeneous and similar in appearance to the others. Only those cells whose chromosomes have been made refractive by the addition of minute amounts of salts persist in their refractivity and yet do not plasmolyze. (Effects of salts upon the refractivity of the chromosomes will be discussed later.)

"Plasmolysis" of pmc in sucrose solution is not due to the semi-permeability of the cytoplasm. The absence of plasmolysis in normal cells, even when immersed in sucrose concentrations of 0.5M, suggests a high permeability of the cells to this substance. Permeability to polar solutes may be further tested by the use of the sulfonphthalein dyes. It is generally known that these dyes are impermeable to living cells (see Chambers and Pollack; 1927) whether in the ionized or in the un-ionized state. Yet, if a drop of Bromcresol Green is added to a suspension of pmc in 0.2M sucrose, the dye penetrates and stains the cells. Moreover, if the pH of the medium is now increased by addition not only of NH_4OH but also of a strong base NaOH, the absorbed dye changes colour, indicating an alkaline reaction. A similar behaviour was

observed when other dyes of that group were used, such as, Bromcresol Purple, Cresol Red, and Methyl Red. We must conclude therefore that the permeability properties of the pmc differ greatly from those of ordinary vegetative cells in that they permit rapid penetration of polar solutes such as, sucrose, salts, strong acids and bases, and sulfonphthalein dyes. The intracellular changes caused by salts even in very dilute concentration (see later) and the lack of correlation between cell volume and external osmotic pressure point to the same conclusion. And, it is probable that the contractions of the cytoplasm observed in certain media are due to coagulative reactions since they cannot be referred to differences in osmotic pressure.

Structural Properties of the Pollen Mother-Cell

The pmc consits of several cytoplasmic zones, including an outer granular zone, which may be a sol or a gel, an inner hyaline one, which possesses characteristics of both aggregational types, and a nuclear one whose structural pattern suggests not only sol and gel, but coacervate as well. Since their relative volumes and consistencies vary with the nature of their external medium, the behaviour of each zone is treated independently, except for the inner hyaline zone and the nuclear one which are combined because of the difficulty in distinguishing the two.

The Outer Granular Zone: (Citrate-phosphate and soda-borax buffers were used wherever pH control is indicated.

However, the pH's mentioned are not necessarily the true pH's of the suspensions under consideration, since no account was taken of the effects of the anthral sap and the pmc upon the concentration of the H-ion. Occasional references to the pH of the slide preparation showed it to be lower than that of the original solution.)

Tetrads of Tradescantia reflexa suspended in 0.2M sucrose solution at a pH of 5.0 possess a wide granular periphery and a high concentration of granules or droplets (referred to later as granules) within the cytoplasm. If the tetrads are distributed among a graded series of H-ion concentrations ranging from pH 4.0 to pH 10.0, a decrease is observed in the volume of the granular periphery and in the concentration of the granules. At a pH of 9.0, the interior of the cell is quite homogeneous and the granular periphery is very narrow. Addition of acid at the edge of the cover-slip reverses the process and a wide granular periphery results. It is not clear whether the changes in degree of granularity are due to dispersion and flocculation of the granules or simply to changes in the refractive index of either matrix or granule. But, it is certain that the H-ion concentration affects the zonation of the cytoplasm, the acid side increasing visible structural differentiation. Such behaviour is not confined to tetrads, similar observations having been made at other stages as well. However, variation in cell structure during the development of

meiosis makes quantitative generalizations impossible, and, unfortunately, lack of time did not permit adequate studies of stages other than that of tetrads.

Changes in H-ion concentration affect the viscosity of the cytoplasm as well as its structure. The granules contained in the outer zone of the pmc show an active Brownian movement and may be regarded as indicators of the region's viscosity. Since the rate and degree of Brownian movement is not constant in a given suspension of cells, conclusions must be drawn from a statistically significant number, though even that procedure is limited by the lack of quantitative data on the actual rate of movement of the particles. Brownian movement of the granules reaches a maximum at a pH of 8.0, the rate falling off on both sides of that point. Thus the viscosity of the cytoplasm being a reciprocal of the rate of Brownian movement, it may be said that the viscosity of the outer zone reaches a minimum when the pH of the external medium is 8.0.

When a pmc of Trillium erectum is pierced by a micro-needle, the outer granular region shows no resistance to its movement. Manipulation of the needle within this zone causes no corresponding movement of the granules. If the cell wall and membrane are torn by inserting both needles within the cell and then pulling them apart, the contents of the granular zone flow out and mix completely with the surrounding medium. There is no indication of a

coherent structure except for the periphery adjacent to the cell wall. But, the micro-manipulation performed did not reveal the nature of that portion of the region. The only evidence obtained for the presence of a more coherent structure was the change from sol to gel with increasing acidity of the medium and in the microdissection of pmc tetrads in a 0.2M sucrose medium, where no outflow of the granular contents was obtained when the cell membrane was torn. When the granular region is sol, however, the adhesion of the protoplast to the wall makes it most difficult to distinguish between cytoplasmic periphery and inanimate wall. And since the latter is of a jelly-like constitution, properties of high viscosity and consistency displayed by the outermost region may be due to it and not the cytoplasm. It is nevertheless likely that a peripheral gel cortex is present in the zone and is in equilibrium with the sol portion. Transformation from sol to gel may be brought about by alteration of the pH of the medium (this has already been indicated in Brownian movement observations) as well as by the natural changes which occur during the cycle of meiosis.

The Inner Hyaline Zone: Pmc of both experimental plants possess a homogeneous inner zone when suspended in appropriate concentrations of sucrose. The cells of Tradescantia do not vary much in their reaction to the sugar concentration with the stage of meiosis, though differences do occur in the percentage of viable cells. Trillium pmc vary

considerably in refractivity when different stages of meiosis are observed in a fixed concentration of sucrose. Thus, 0.2M sucrose almost invariably gives rise to optically homogeneous cells in Tradescantia while the same concentration of sucrose, when applied to Trillium produces such cells only at leptotene, the other stages needing either increased sugar concentration or addition of alkali for the maintenance of optical homogeneity.

A micro-needle which has penetrated into this region shows no evidence of a consistent structural framework. Though no movement of particles can be seen when the zone is in the homogeneous state, tearing of the cell-wall allows the whole region to be manipulated out of the cell. If this cytoplasmic mass is now stretched slightly, there is a contraction upon release of the needles indicating elastic properties. The recoil, however, is in the nature of a flow and not that of a stiff elastic contraction. Thus an element of fluidity is present in the inner zone. This is demonstrated more clearly when metaphase I pmc of T. erectum are dissected. Abrupt movements of the needles cause corresponding displacements of the chromosomes, though more delicate manipulation of the needles, so that the chromosomes are not contacted, produces no displacements. Such behaviour indicates the existence of a coherent structure (presumably the spindle mechanism as well as the chromosomes) within a fluid matrix. The limited elasticity of the con-

sistent bodies within this region is demonstrated by stretching the cytoplasmic mass across the microscopic field. Under such treatment the zone shows incomplete recovery, the mass remaining partially stretched and swelling while suspended in the medium.

Thixotropic behaviour may also be seen in this region. Individual chromosomes when stretched between the micro-needles show elastic properties. The apparent resilience of these chromosomes when tapped by the needles is suggestive of the presence of a gel in their structure. If however, the needles are manipulated sufficiently within this region, then the chromosomes fuse. Vital staining of the chromosomes with Brilliant Cresyl Blue shows that they do not fuse completely, the proportion of their fused areas increasing with continued manipulation. And, if protruding ends of the chromosomes are stretched with the needles, they show a flow contraction similar to that of the mass as a whole.

The matrix of this inner zone does not possess a high degree of structural consistency though its cohesion in one mass when pulled out of the cell suggests the existence of some form of coherent structure at the interface of the inner and outer zones. But, as in the case of the outer zone, the consistency of its structure varies with the nature of the medium. Gelation of the entire region may be brought about by reducing the sucrose concentration

below its optimum value or by lowering the pH as well as by allowing the pulled-out mass to remain suspended in the surrounding medium even though this medium does not favour gelation of the region when it is within the cell.

The Action of Electrolytic Agents

If a drop of Neutral Red is added to a suspension of pmc of T.erectum in 0.2M sucrose, the dye is only slightly absorbed in the minute vacuoles of the cell giving the cytoplasm a faint pinkish tinge. Injured cells show a high absorption of Neutral Red by the cytoplasm, which becomes dark red in colour. Occasionally, however, viable cells with refractive chromosomes absorb Neutral Red strongly. The stain is highly preferential for the chromosomes since the remainder of the cytoplasm is lightly coloured, thus permitting a fairly clear resolution of chromosomal structure, the coils of the chromonemata being quite distinct. The staining intensity does not progress to a constant but shows an initial increase followed by a gradual decrease until the entire dye disappears and the interior of the cell becomes optically homogeneous. Addition of $CaCl_2$ at the moment when the dye is strongly absorbed will catalyze the process and the stain will disappear almost instantaneously.

A variety of salts produce marked refractivity changes in the cells of T. reflexa . When $CaCl_2$ is present in a 0.2M sucrose suspension of pmc, the homogeneous interior becomes refractive and the chromatin strands are

visible. Only a very low concentration is necessary to produce this change, and though it is difficult to determine the precise minimum quantity, two limits are set between which the minimum lies. Below, is a table showing the threshold values for various cations:

Ion	Molarity	Ion	Molarity
Ba	.005-.0025	La	.000062-.000031
Sr	.00125-.00062	Al	.00005-.000025
Ca	.00025-.000125		

These reactions are reversible though the precise conditions governing the reversibility are not clearly defined. Thus, often the $CaCl_2$ effect is reversed with time as in staining with Neutral Red, particularly if the salt is present in low concentrations, and cells which have been refractive become homogeneous once more. KCNS also serves to reverse the system, though the action is carried on efficiently only in presence of an alkali buffer. If 0.1M KCNS is added to a pure sucrose solution, there is a slight shrinkage of the cells after which the nuclei become refractive. $CaCl_2$ reverses this effect if applied in the presence of an alkali. Extruded prophase nuclei demonstrate the process very clearly. (Such nuclei are obtained readily by slight pressure on the cover-slip.) These nuclei are refractive in the presence of .0002$CaCl_2$. Addition of KCNS and alkali cause a return to the homogeneous state which is similar to that of the nuclei described by Chambers and

Black (1941) as "phantom nuclei".

It is doubtful however, that the reversals produced are due to the interaction of KCNS and $CaCl_2$. The indispensability of the alkali with either salt suggests a more active role of the hydroxyl ion. This belief has been confirmed by observation of the effects of alkali upon cell structure. It has already been stated that alkali buffers decrease granularity of the Tradescantia pollen mother-cells. The viability of Trillium pmc at metaphase I and meiosis II is greatly increased in presence of alkali. It has also been observed that alkali decreases the optical heterogeneity of these cells, though in this case the latter condition was not brought about by application of salts. The active role of the alkali in decreasing granularity and in increasing optical homogeneity indicates the possibility of causing reversals of salt effects by addition of alkali without the presence of any salts whatsoever. Such experiments have not yet been performed.

Cultivation of Exised Anthers

Some preliminary experiments were designed to study the possibility of culturing anthers in solution as well as of observing the effects of pH on meiotic division. The former has already been attempted by Gregory (1940) while pH has been recognized as an important factor in many growth experiments in vitro. The solution used was similar to that employed by White (1933) except that the two percent sugar

solution was replaced by a six. In the first series of experiments the cultures were placed in Erlenmeyer flasks (125 ccs.). A modification was introduced in the second series. In order to have a random distribution the anthers of each rhyzome of T. erectum were divided among the various flasks so that a single flask had no more than one anther of the same plant. For the adjustment of pH, NaOH and HCl were used, since they are often preferable to buffers. (White, 1933)

The apparatus used in the second series is indicated in Fig. 3.

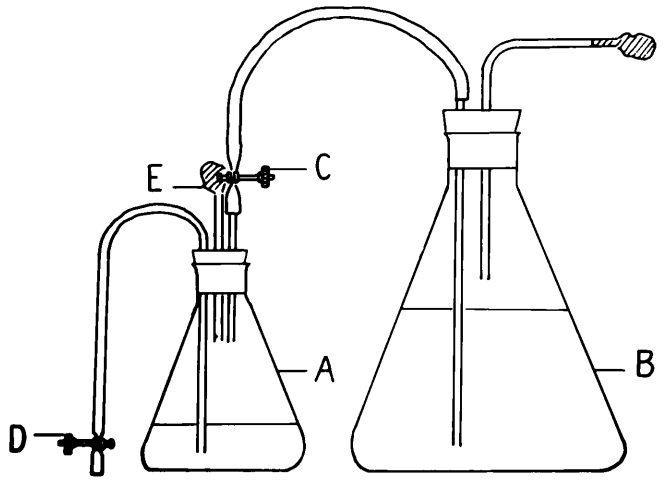

Fig. 3. Apparatus for cultivation of excised anthers.

A-Culture Bottle; B-Stock Solution; C,D,- Siphon Clamps; E-Opening for inserting anthers.

This arrangement helped to retain aseptic conditions by permitting a change of solution without exposing the culture to the atmosphere and to the possibility of contamination. Periodic tests of the pH were made by withdrawing a small portion of the solution. The procedure was partially successful in that it helped maintain a more constant pH than that in the previous set; however, there was the

accidental disadvantage that the Trillium rhyzomes used were much more advanced than the first set and therefore did not provide a wide range of stages.

The anthers were both removed and grown under aseptic conditions and were at a constant temperature of about 5°C. The following were the results observed:-

Series 1.

This series remained in cultivation for a period of 23 days, most of the rhyzomes being at the prophase of meiosis at the time of anther excision.

Culture No.	Orig. pH	Final pH	Stages observed at end of period
A	6.4	3.3	Diplotene, Metaphase I, Telo. I
B	7.1	3.2	" " Rest. Micr.
C	8.2	3.4	Meiosis II prevalent
D	8.5	3.0	Mainly tetrads, Some Meiosis I
E	9.2	5.0	Microspores, mainly Resting Stage
F	9.7	4.2	All post-tetrad stage

Series II. (Examination made after 14 days in solution)

Solution	Orig. pH	Final pH	Stages observed
A	5.8	5.6	Prophase, Metaphase, Anaphase, Some tetrads present.
B	8.3	7.4	Tetrads, Metaphase II, Microspore Resting Stages.
C	9.4	7.8	Post tetrad and Micr. Resting Stg.

Discussion

The advantage of a large variety of techniques gained in the research on so general a topic as the physiology of cell-division is duly compensated for in the disadvantage of being unable to assess properly results obtained or even to obtain any very significant results. In this aspect the discussional phase of the thesis must suffer from having few new results, none of which may even have fundamental significance. To offset this, it will attempt to view the whole problem in perspective and undertake the task of considering in general terms the possible meaning of the phenomena here investigated in relation to the process of cell-division.

Physical Structure

The protoplasm of the mitotic cell has frequently been described in terms of its physical properties as consistency, viscosity, and elasticity. Such knowledge, of course, is indispensable for understanding the mitotic phenomena of chromosome expansion and contraction, of chromonema coiling and uncoiling, and of chromosome movement through the cytoplasm. There are, however, limitations inherent in the use of these terms, since there is a tendency to regard them as absolute in the cycle of division rather than relative and possibly specific to the species of cells being investigated. In contrast to the vegetative cell

whose protoplasmic pattern is comparatively stable, the organization of the mitotic cell is in a state of flux. The factors, then, which contribute to the physical aggregations of the latter, are more likely to vary with differences in species of plant or animal, as the particular conditions under which the cells divide are not generally the same.

The truth of this situation is evident in a survey of the literature (see Review of Literature). The spindle, for example, has been found to be either sol or gel, depending upon the species of the cell examined. Chambers and Sands (1923) claimed that the cytoplasmic matrix of Tradescantia pmc was a gel, whereas the author has found that of Trillium to be a sol. Such apparent inconsistencies are not irreconcilable since it is not certain that the absolute viscosity of the cytoplasm, structural or Newtonian, affects the course of division. (Barber's findings that the rate of chromosome movement is independent of the viscosity of the cytoplasm may be recalled here.) Relative consistencies of the cellular organs or localized changes in the cytoplasm may be far more effective in determining the mechanics of the division.

The existence of cytoplasmic "zones" possessing different structural consistencies has been shown in the microdissection of Trillium pmc. In prophase, at least, the outer granular layer is a sol, while the inner hyaline one, is also a sol but is more viscous and contains a

structurally consistent framework. It is therefore questionable whether the over-all viscosities, as measured by the centrifuge technique, are of any real significance in the mechanics of mitosis. Moreover, if there is a co-existence of different structural aggregates in the cell, it is highly probable that localized changes may also occur, particularly in view of the localized accumulation of metabolic products, as the nucleic acids. The changes, then, which are local in character would be more important than the measurement of aggregate change, since the latter does not define any single process. And, unfortunately, the centrifuge technique only measures over-all change.

There is a further observation to be considered in the investigation of physical structure, namely, the relationship between the medium and the structural pattern of the cell. Differences in viscosity of the outer granular layer were found when the pmc were suspended in media of different pH. A similar effect was noted when the cells were microdissected in various concentrations of sucrose. Thus alteration of the environing solution, as to pH or sucrose concentration, often changed the consistency of the outer granular zone. In lower concentrations of sucrose the zone was a gel, whereas at higher ones it was always sol. And, since higher sucrose concentrations (.2M to .5M) and alkaline pH's (about pH 8) were associated with a greater stability and viability of the pmc, microdissectional results obtained

in these media were regarded as representative of their natural state.

The properties of flow and elastic rigidity manifested by the chromosome are evidence for the existence of a two-phase system within it. Its highly elastic behaviour when stretched slightly is probably due to the prominence of the gel zone in the rapid contraction, while the flow which sets in when it is pulled across the microscopic field may be considered as resulting from an incomplete reversibility of the gel phase and a prominence of the sol one. Yet the gel zone is not very stable, for it will be recalled that even gentle mechanical agitation is sufficient to cause thixotropy, and hence a fusion of several chromosomes. Whether the localities of these fusions are random or determined by the nature of the region has not been ascertained, though such a knowledge would be helpful in understanding the physical mechanism of the exchange of chromatids in crossing-over.

The possibility of the chromosome existing as coacervate need not be excluded. There is ample evidence for the presence of a consistent structure in the hyaline region at the metaphase stage but there has been little indication of it at the prophase of meiosis, though at no time was a structurless behaviour observed similar to that of the outer granular zone. Thus, the prophase chromosome may be in a liquid state of aggregation and exist as a

coacervate droplet within a liquid matrix. The detection of such a property by the micro-needle is rather difficult, since manipulation inevitably produces physical changes within the protoplasm. These changes are not necessarily moribund; they may even be quite subtle, as a denaturation of the protein. But the instability of these structures may accentuate the effects and the slight transformations may become qualitative in character.

A grosser example of such an effect is provided in the swelling of the hyaline region when withdrawn from the cell. The phenomenon is not osmotic since it does not occur unless the mass is previously stretched. The alternative, then, is an increased micellar hydrophily. Since protein is known to increase its viscosity and consequently its hydrophily upon denaturation, it is probable that stretching of the cytoplasmic mass by micro-needles produces a similar reaction.

The evidence accumulated here for the existence of localized physical aggregates as well as for changes in these aggregates with changes in the composition of the environing medium. points to a condition of unstable dynamic equilibrium in the structural pattern of the cell. This instability, associated with a high sensitivity to various solutes as well as to concentration of H-ions, probably becomes more exaggerated by the numerous metabolic by-products and the many molecular transformations occurring

during the process of cell-division. And, in view of the rôle which the actual molecular structure plays in the determination of such properties as viscosity, consistency, and elasticity, the probable changes in the configuration of the protein molecules, particularly those of the chromosomes, assume an importance equivalent to those produced by electrolytes and solutes. A phenomenon such as denaturation then, (and chemical studies of mitosis show that such a protein reaction does occur) not only affects structure through its release of sulfhydryl, amino, and carboxyl groups, but also through its unfolding of the globular polypeptide chains. This and other similar intracellular physico-chemical reactions, indicate the inadequacy of simplified structural versions of the mitotic process, as well as the need for a more accurate appraisal of the effects of the suspension medium upon the observed physical structure of the cell.

Permeability

Pollen mother-cells seem to possess to a degree unparalleled by any other type of cell (as far as records known to the writer reveal) a high degree of permeability to polar solutes, even those of relatively large molecular size. This character strictly limits the media in which they retain normality and is probably responsible for conflicting reports as to the physical state of their protoplasm, but it also affords exceptional opportunity

for studying the effect of various substances on the cells.

The lack of plasmolysis of normal pmc in hypertonic sugar solutions, the penetration of sulfonphthalein dyes, and the internal effects of low concentrations of salts, constitute the evidence for a high passive permeability. The latter thesis, however, contradicts the assertions of both Shinke (1939) and Shimakura (1938) who believed the lack of plasmolysis to be due to a mechanical injury of the membrane followed by a penetration of solute and a swelling of the cell which consequently produced optical homogeneity. Such an hypothesis implies first, a complete reversibility of the pmc from the plasmolyzed to the deplasmolyzed state, secondly, a high sensitivity of the membrane to mechanical injury, and thirdly, an irreversibility of the homogeneous state, which would be indicative of an injurious effect. Treatment of "plasmolyzed" cells in the course of these experiments has shown them to be incapable of deplasmolysis. The change from refractive state to optical homogeneity, when cells are immersed in Neutral Red and sucrose, occurs over a period of time when no mechanical interference is possible. Finally, the homogeneous condition is reversible to the refractive one by use of dilute concentrations of salts, or by varying the concentration of H-ions.

The permeability tests made were confined mainly to the prophase of meiosis. It is more than likely that so high a passive permeability is not permanent but is subject to

some cycle of change. Whether the rise occurs at the inception of active division and the fall at its completion, has not been determined. The period of this physiological state may be much shorter. Its duration is, however, probably correlated with the duration time of the cell's meiosis. Thus, Trillium would be expected to have a much longer period of high permeability than Tradescantia. If physiological conditions of mitotic and meiotic division be similar, then this property might be extended to the mitotic cells. Perhaps the lack of success of investigators in determining permeability changes of the mitotic cell, (see Heilbrunn-1937) is due to its very short period of duration.

High permeability implies an intimate relationship between the cell and its physiological environment. In this sphere, the factor of organismic influence assumes an importance in the interpretation of meiosis. By means of such a mechanism, enzymes and hormones necessary for the activation of cellular division may be produced not necessarily intra-cellularly but in different localities of the plant or animal body, from where they could be transported to the meiotic cells. The presence of these substances in the environment of the cells would be immediately followed by their inward diffusion because of the tendency towards physico-chemical equilibrium. The importance, then, of a high permeability mechanism cannot

be denied, though its precise significance in the scheme of cell reproduction is by no means clear.

The Action of Salts

Inasmuch as the results indicate that pmc are freely permeable, both to the entry and exit of sugars and also of salts, it may be presumed that they are probably permeable also to outward diffusion of salts. Furthermore, it would seem unlikely that a physiological mechanism of active accumulation of ions through the plasma membrane can function side by side with free outward diffusion of the same. The thesis of high passive permeability to salts or ions of pmc implies therefore that the concentration of all inorganic ions inside and outside the cells tends toward a state of true Donnan equilibrium in which only the presence of large indiffusable organic ions (namely protein) in the cells causes departure from equal distribution. It follows that a normal colloidal condition of the cell interior, let alone the cell surface, can only be preserved for any length of time with a proper balance of ions in the external medium.

Salts may act in two ways upon a protein; they may either react with the Zwitterion of the protein molecule and form new chemical complexes, or they may be adsorbed at the surface of the protein micelle and cause changes in such surface properties as charge, hydration, and surface tension. Insofar as the solution aspect of the salt's behaviour is

concerned, the Coulomb forces play an important role. The latter depend upon such properties as valency, effective diameters of the ions, ionic radii, and dialectric constants of the solutions. It is these forces which affect the ions or Zwitterions of less soluble compounds and draw them into solution. From the colloidal viewpoint, the hydrophilic and adsorption properties of the ions are the most prominent, their surface action being responsible for the aggregation of the molecules into various colloidal states. It is this duality of action which must be remembered when considering the interaction of salts upon any organ of the protoplasm. Thus, when a salt reacts with the chromosome proteins, it may do so molecularly as well as colloidally. In fact, the molecular reaction is very important since salts often cause molecular changes in the protein which appear as some microscopic morphological change.

The most prominent change brought about by dilute concentrations of salts in a sucrose solution is a refractive one. This phenomenon not only indicates a rapid and free penetration of cations through the protoplasmic membrane, but also a high sensitivity of the nuclear structure to ionic factors. Moreover, the extreme dilution employed excludes the possibility that the reaction is an osmotic one. Changes in the refractive index of protein solutions have been discussed at length by Craig and Schmidt (1932). Since

refraction is due to the interaction of the electrons of the molecules and ions with the transmitted light, it follows that factors causing hydration, ionization, ionic interactions, and molecular associations will alter the refractive index of the solution or dispersion affected. In the case of chromosomes, where the protein constituents are only slightly ionized, association and solvation become predominantly important.

The series Ba-Sr-Ca-La-Al offers two important points for consideration. First, it follows the lyotropic order of ionic activity, and therefore may be viewed as causing some surface change. Secondly, since it is the more hydrated ion which is the most effective, adsorption cannot be the principal mechanism of the reaction. However, Craig and Schmidt (1932) have pointed out the importance of solvation in bringing about refractive change of protein solutions. Moreover, x-ray studies of crystalline cephalin (Palmer et al, 1941) have shown the effectiveness of Ca in bringing about solvation changes. Crystalline cephalin has a bimolecular leaflet structure, the distance between the leaflets increasing greatly when solvated. When Ca is added the water is expelled and the structure collapses. Thus, the penetrating ions may cause a withdrawal of bound water (either molecularly or colloidally bound water) and bring about a change in the refractive index. Since water competition is the central factor, it is understandable why the most hydrated

ion should be the most effective.

There is, however, another hypothesis which might explain adequately the refractive changes occuring in the nucleus. It has been suggested previously that the physical structure of the chromosomes is best represented by a two-phase system in equilibrium consisting of sol and gel. Under such conditions, one of the factors controlling the refractivity of the chromosomes would be the relative volume and hydration of either region. But, Docking and Heyman (1939) have pointed out that hydrated ions increase dispersion on being adsorbed. Thus, the structural change contemplated here could be brought about by the action of the adsorbed ions, the most hydrated ones again being the most effective.

It is pertinent at this point to consider the factor of hydration, particularly as related to chromosome refractivity. The amount of free water contained within the chromosomes is at present a matter of speculation. Certainly, apart from some evidence for the existence of a fluid interior (see Review of Literature) there are no direct data on the subject. On the other hand, the presence of adsorbed or bound water may be considered a certainty, for it would be impossible to conceive of a disperse system of proteins without it. It is not unlikely, therefore, that the adsorbed water should constitute a

high fraction of the total water present. That this bound-water need not be thermodynamically inert has been pointed out by Chandler (1941) in his criticism of the bound-water concept.

Water of hydration is also related to other adsorbates. Ionized solutes will combine with water by virtue of their charge and upon being adsorbed at an interface, will increase the latter's hydration. However, the increase in stability of the disperse phase resulting from the increased hydration is counteracted by a tendency of the ions to compete osmotically for water with micelle or protein molecule. This antagonism, when carried to an extreme, brings about the well known 'salting-out' effect. The relationship is further complicated by the fact that interfaces possess a charge. The addition of a particular ion, then, may not always increase this charge, but may often neutralize it. The neutralization in turn may cause a loss of adsorbed water as well as a destabilization of the disperse phase.

The physical structure and hydrational properties of the chromosome, as well as the comlexity of inter-ionic activity do not point to a simple osmotic relationship as put forth by Shinke (1939). Though he suggests an increased swelling of the chromosomes in hypotonic media until they become optically indistinguishable, it is doubtful whether the chromosomes are surrounded by a semi-permeable membrane, particularly in view of the high permeability

possessed by the outer membrane of the pmc. Moreover, the refractive changes induced by salts make it clear that no osmotic dehydration of the chromosome is necessary. It is most probable that the structural transformation occurring in the chromosome involve a change in water distribution. But, these changes need not be quantitatively large, nor need they involve any dehydration. They may simply be an intra-chromosomal phenomenon, the total water-content remaining constant. This, at any rate, is suggested by the experiments reported here.

Conclusions

The most prominent structural feature of the pollen mother-cell is its zonation. This property is shared not only by the cytoplasm but also by the chromosomes whose sol-gel equilibrium may prove to be of vital significance in their cycle of elongation and contraction. The peripheral gel layer, the outer structurless granular zone, and the more consistent sol-like hyaline region containing a coherent framework, provide a pattern of protoplasmic organization whose significance is yet to be disclosed.

The high passive permeability of the membrane during the meiotic prophase indicates the existence of some physiological cycle related to membrane penetrability. The effectiveness of extremely dilute concentrations of ions in causing refractive changes also suggests peculiar properties of protein stability, which may very likely vary with the stage of division.

The combination of physiological and structural results point to a clearer definition of one underlying problem of meiosis. This problem may be stated as the resolution of the cycle of permeability change and its relation to intracellular solutes, both of which are integrally bound with the structural character of the meiotic cell.

Summary

(1) A study of the physical structure of the pmc of Trillium erectum has been made by means of the microdissection apparatus. Physical injury to the cells is avoided by using needles whose shafts are about 1 micron in diameter.

(2) The pmc of Trillium erectum and Tradescantia reflexa consist of several cytoplasmic zones. Microdissectional studies of Trillium have disclosed the following properties:

(a) An outer granular zone which is a sol and which is miscible with the external medium.

(b) A gel periphery (not fully confirmed) which is adjacent to the cell-wall and which is in equilibrium with the structurless sol region.

(c) An inner hyaline zone which is more consistent than the outer one but which also possesses sol-like properties.

(d) A coherent structural framework within the hyaline region, comprising the chromosomes and spindle (when present) as well as the periphery separating the hyaline zone from the granular one. The properties of fluidity and elasticity manifested by the chromosomes are suggested as due to the presence of a two-phase system consisting of sol and gel.

(3) Variation in volume and viscosity of the zones have been shown to occur with changes in the pH of the medium or in the sucrose concentration.

(4) Observation of the behaviour of Trillium and Tradescantia pmc at the prophase of meiosis in solutions of sucrose, sulfonphthalien dyes, and salts has shown them to be freely permeable to these substances. Plasmolyses observed in hypertonic concentrations of sucrose have been due to some coagulative effect of the

cytoplasm.

(5) Application of very dilute concentrations of salts has caused refractive changes in the nucleus. Effectiveness of the ions bringing about this reaction is increased with increasing hydration.

(6) Culture of excised anthers in nutrient media has suggested a possible relationship between pH of the the medium and rate of meiosis, the latter increasing with rise in pH.

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Plate 1

Fig. 1 A pre-leptotene cell of T. erectum suspended in 0.2M sucrose.

Fig. 2 A pmc of T. erectum whose nucleus has been made refractive by addition of 0.0001M $CaCl_2$.

Fig. 3 A contraction of the nucleus in a pmc of T. erectum. Note the clear hyaline area.

Fig. 4 "Plasmolysis" of a pmc of T. erectum. This cell was originally not plasmolysed, but was allowed to remain in solution for twenty-four hours, after which the protoplast shrank.

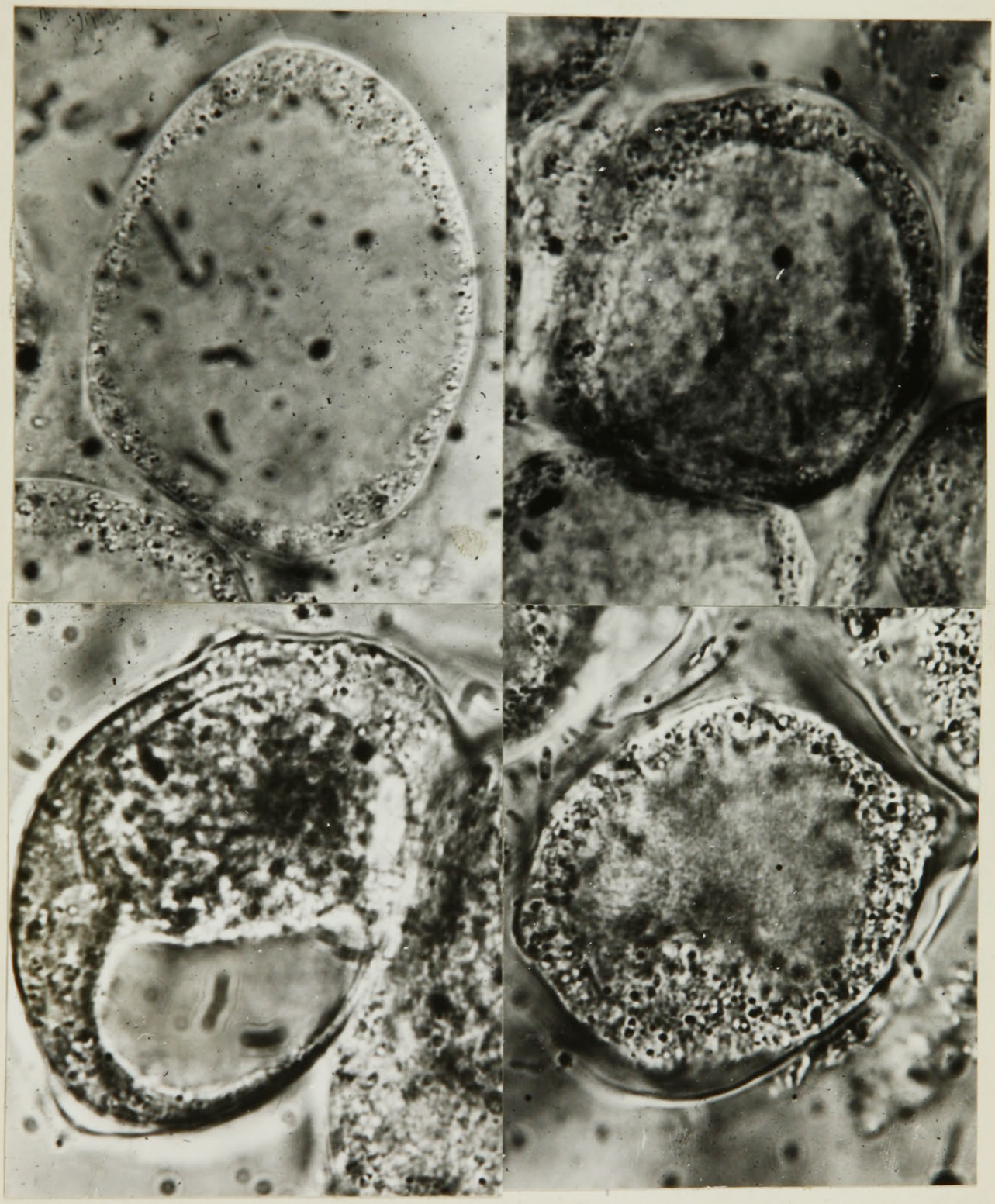


Plate II

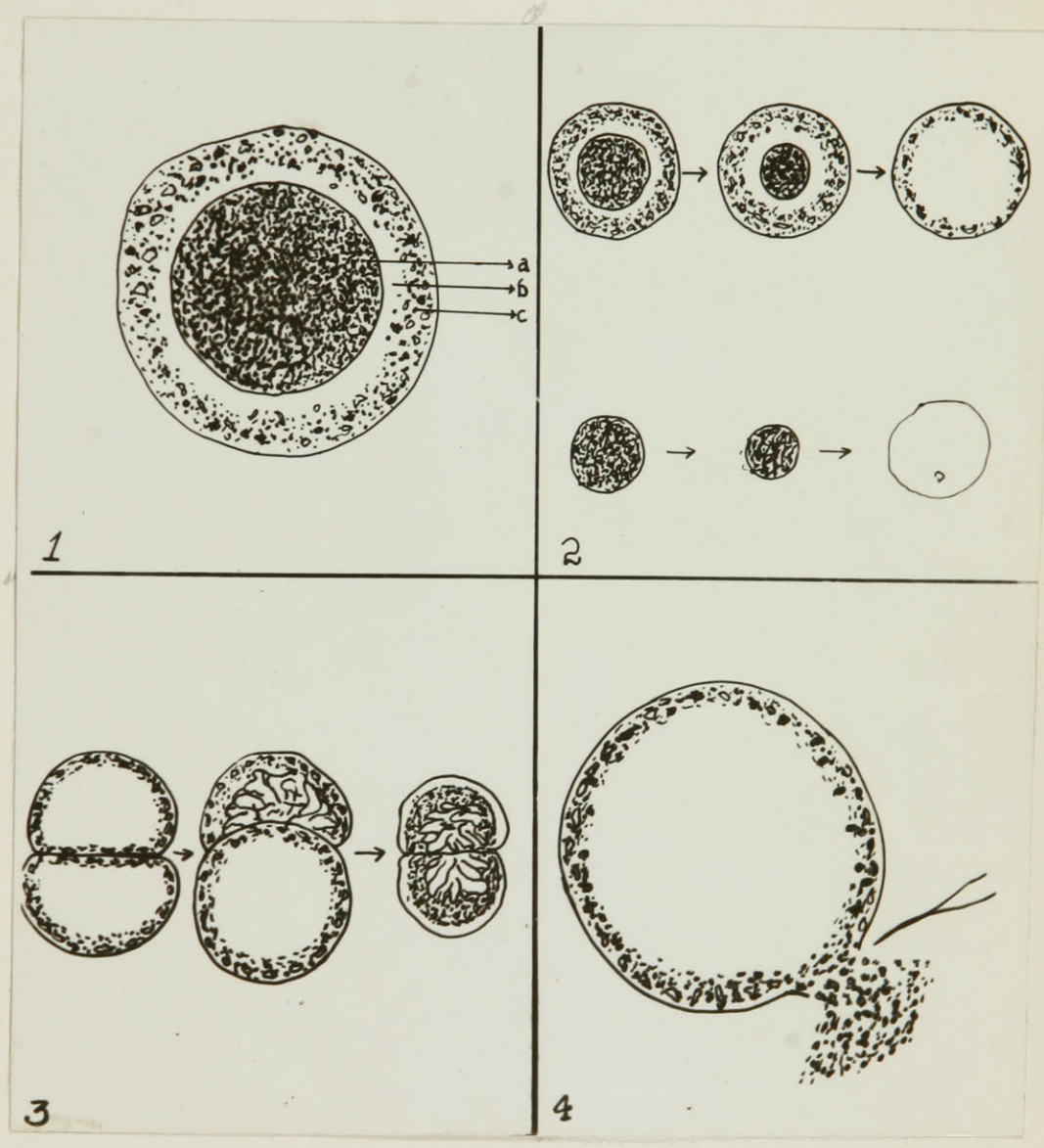
Fig. 1 Diagrammatic representation of a prophase pmc of *T. erectum.*

(a) Nuclear Zone
(b) Hyaline Zone
(c) Outer Granular Zone

Fig. 2 Series of changes occurring upon treatment of refractive cell and isolated nucleus with KCNS and alkali. Note that KCNS first causes a shrinkage of the nucleus.

Fig. 3 Diad suspended in a sugar solution which has been injured and has become refractive, then contracted.

Fig. 4 Tearing of the membrane of *T.erectum*. Note that the contents of the granular zone flow out and mix with the medium.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