PHYSIOLOGICAL AND PHYSICAL CHANGES OF PROTOPLASM DURING MEIOSIS AND MITOSIS IN POLLEN MOTHER-CELLS OF TRILLIUM.

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INTRODUCTION (GENERAL)

Ever since the recognition of mitosis[1] as the mechanism of nuclear division, its importance in biological development, whether normal growth and reproduction or pathological production of tumours and cancers, has led to a great deal of inquiry into its nature. The manner of treatment at any period was determined, to a large extent, by the available techniques of investigation and, to a lesser but still important degree, by the facility with which known and utilizable material lent itself to a given type of approach. Accordingly, early research workers concentrated their attack upon the morphology of mitosis, a study which now provides a very detailed and fairly complete description of the microscopically visible structural characteristics of cell and nucleus during their cycle of division. Recent research has, in a sense, leap-frogged to the more ultimate constituents of the cell, namely the protein molecules and their allied substances, and has by means of techniques adapted from those of physics and chemistry investigated molecular structure and orientation in protoplasmic constituents, and analyzed the chemical components of nucleus and cytoplasm. The gap between these mutually remote techniques is one that has to be filled by a study of the cell "in vivo", that is, by means of physiological data. Not that such knowledge is entirely lacking, but as a bridge between the other two approaches it is still highly inadequate.

It is clear, even from casual observation, that the scope of the study of mitosis is very broad. A complete resolution of its mechanism depends not only upon a full knowledge of protoplasmic structure, which by

1. The term "mitosis" is used in its broadest sense, namely, as the all-inclusive expression for the division of nucleus and cell. "Meiosis" is therefore regarded as a special case of mitosis whereby the daughter cells possess half the chromosome number of the parent one.

itself may be regarded as an ultimate in biological research, but also upon a knowledge of metabolic gradients as related to the chemical components and structural characteristics of the nucleus and cell. The latter concept, i.e., the necessity of integrating function with structure, was pointed out by Scarth (1927) who, in demonstrating the presence of structure in the optically homogeneous nucleus, stated that "physically speaking, function cannot be separated from structure. Some material configuration must be assumed or else an agent immaterial". The resolution of the mitotic mechanism further requires what is unique in biological relationships, namely, the conformity of all such data with those of genetics on the transmission of hereditary factors. The deductions made from post-mortem studies of cell structure and cell components are of inestimable value in the resolution of cell-division, but it is clear that such deductions will be less tenuous when interpolation of data from the behaviour of the cell "in vivo" is possible. In fact, such crucial phenomena as the dissolution and formation of membranes, both nuclear and chromosomal, and the movement of chromosomes, cannot be adequately understood without a knowledge of the physiological properties of the cell.

The discrepancy between the mass of morphological data and the accumulating body of physical and chemical data on the one hand, and the paucity of physiological data on the other, is obviously large. Past research has stressed the structural kinetics of cell-division, an emphasis which stems no doubt from the relative ease with which data on the morphological patterns of the cell at any stage of mitosis can be obtained. The fairly simple techniques of post-mortem staining have given rise to an exceedingly large body of information and have consequently made chromosome morphology the focal point of cytogenetic research even to the present day. The empnasis

of present studies has shifted somewhat to the physics and chemistry of the nucleoproteins where the advanced techniques of biochemistry have made rapid progress possible. Physiological research, however, has been to a large extent neglected in the efforts to resolve the mitotic mechanism. It is true, of course, that the subtler conditions of investigation involved in physiological studies do constitute a barrier to treatments as elaborate as those afforded by the other approaches. Yet, it is clear that a knowledge of the physiological behaviour of the mitotic cell is indispensable to a complete understanding of cell-division.

There is, in a sense, a reciprocity of limitation which one branch of research imposes upon the other. The advances in chromosome morphology are halted by the restrictions of sub-microscopic analysis and the latter, in turn, could not have achieved their present degree of progress without having obtained data from microscopic studies. Both these approaches are limited by, and in turn limit, the progress made possible through chemical studies of the nucleus which again is associated with and dependent upon a physiological understanding of cell behaviour. It follows therefore that no single technique of investigation can alone provide the data necessary for a complete resolution of mitosis. It is clear, too, that the studies reported here were not intended as the sole materials for a theory of mitosis, but rather as an aid in interpreting the mitotic mechanism.

REVIEW OF THE LITERATURE

The literature available on the study of mitosis is large indeed, and it would be far beyond the scope of this thesis to attempt any comprehensive review. The morphological investigations which are by far the most complete, in so far as any single phase of the problem can be complete, will not be dealt with in this section, partly because of the many reviews published on the subject (the best critical review on chromosome morphology is to the writer's knowledge that written by Huskins (1942); the most comprehensive one, that of Schrader (1944),) and partly because a detailed account of such work would have little direct bearing on the research reported. What will be undertaken are a brief summary of some of the data on the chemical aspects of division, and as complete an account as possible of the physiology of mitosis.

Chemistry

Several reviews have been published on the chemical constitution of the nucleus (Shulman 1938, Gulick 1941). Essentially, all the analytical techniques (ultra-violet absorption spectra, (Caspersson 1938); enzyme digestion, (Mazia and Jaeger 1939); salt extractions, (Mirsky and Pollister 1943)) agree on the presence of thymonucleic acid, or some close isomer, along with a basic protein, chiefly histone, or in the case of some fish sperm, protamine, in the chromatic portion of the nucleus. Apart from the data of Bensley and his co-workers (see Lazarow 1943) who claimed to have isolated from a 10% NaCl extract a cytoplasmic constituent called "plasmosin" containing a desoxyribose nucleate, there is a further agreement among other workers that thymo-nucleates are found exclusively in chromatic portions of the nucleus. The nature of the protein substrate, though accepted generally

as a histone or protamine, has been challenged by Stedman and Stedman (1943) who isolated what they considered the chief protein component of the chromosomes, "chromosomin". Unlike the histones, this is an acid protein, iso-electric point between three and five, the acid property being due largely to the presence of glutamic acid. It differs furthermore in that it has a large tryptophane content in contrast with the nucleo-proteins described by Mirsky and Pollister (1943), which have none. Stedman and Stedman (1943), though agreeing on the presence of histone in the nuclei, consider it to constitute a less significant fraction of the total protein.

The conception of various locales of metabolic activity within the cell has been put forward by several authors. Moyer (1942) pointed to the different pH regions of the cytoplasm as evidence for different local situations. Claude (1943) fractionating cell extracts by centri-fugation, isolated granules ("secretory granules") which he demonstrated to be capable of d-amino oxidase activity. He also isolated a smaller particle which had about twice the lipoidal content of the first though he had not found it to be associated with any specific metabolic activity. In all particles he detected the presence of ribose nucleic acid. Ballentine (1940) found that by centrifuging Arbacia eggs into two halves he could locate dehydrogenase activity in the granular portion of the cell. The distribution of such respiratory activities in the cytoplasm is paralleled by a pattern of localized metabolic activities within the nucleus. The distribution of the two nucleic acids, namely, desoxyribose and pentose nucleic has been studied by several workers. Schultz (1941) pointed out that the heterochromatic regions of the chromosome, which he considered nearly void of thymonucleate, divided more slowly than the euchromatic ones which he believed contained a relatively large amount of the substance.

Accordingly he speculated that the desoxyribose nucleic acids were responsible for synthesis of fibrous protein within the chromosomes. Dufrenoy (1943) reviewing the data of several investigators upheld the evidence for a higher content of desoxyribose nucleic acid in the euchromatic portions of the chromosomes. The type of enzymatic activity ascribed to the nucleic acids by Schultz (1941) has, to the writer's knowledge, not yet been confirmed by other workers.

There is also an emphasis on certain chemical substances as activators or promoters of cell-division. The substances concerned fall mainly into two categories; the SH compounds and the nucleic acids. Not that the two groups are necessarily unrelated, even though contemporary hypotheses have dealt exclusively with either one or the other. Mayer and Voegtlin (1935), for example, showed that SH compounds such as glutathione and cysteine inhibited action of tumour nuclease, the nuclease being, of course, a hydrolyzer of nucleic acids. Similarly, Waldschmidt-Leitz et al (1933) showed that though sulfhydryl compounds activated enzymes such as cathepsin and papain, they inhibited the hydrolysis of thymo-nucleic acid by phosphatases. The observation, however, that SH groups affect the growth process was made prior to any studies on the behaviour of the nucleic acids. Hammett (1930) suggested that the simple law of Mass Action was involved in growth, the chemical reaction being from the reduced SH groups to the fully oxidized forms. In an extensive series of experiments (e.g., see Hammett, 1938) he demonstrated a quantitative correlation between concentration of sulfhydryl groups and cellular proliferation. Voegtlin and Chalkley (1935) extending Hammett's studies to include the respiratory activities of dividing cells, examined the inhibitory effects of H_2S and HCN and the influence of CO on cell-division, and concluded that the metabolic functions of mitosis were independent of respiration. It was Rapkine

(1931, 1937, 1938) who integrated these findings along with his own into a chemical theory of mitosis. Essentially the theory (see Needham 1933) maintained that the original source of reaction was the SH groups which on being exposed by a denaturation of the proteins lowered the oxidation-reduction potential of the cell. This resulted in a partial anaerobiosis, that is, a glycolysis which accumulated lactic acid, the lactic acid being reduced in the course of division. The hypothesis of SH action is not accepted by all investigators. Morgulis and Green (1932) criticized Hammet's work on regeneration as lacking in accuracy; so did Ellis (1935) maintain that SH groups are not involved in cell-division. Heilbrunn's (1937) rejection of Rapkine's theory seems unjustified since he does so on the basis of evidence that mitosis is independent of respiration, an argument which in no way contradicts the theory since it is precisely what Rapkine demonstrated and emphasized.

The association of desoxyribose nucleic acid with cell-division was originally demonstrated by Caspersson (1938) in his ultra-violet absorption studies, and though he found an increase in nucleic acid content before cell-division and a decrease following it, there has been little evidence to support a more precise definition of relationship between nucleic acid and chromosome reproduction (see criticism of Huskins, 1942). The possibility of increasing proliferation by means of nucleic acids has been shown by Stern (1943) who promoted growth of chicken embryo tissue "in vitro" by addition of these compounds. Histone, which is considered as, in some ways, a factor in the synthesis of nucleates (Dufrenoy, 1943) has been suggested by Stedman and Stedman (1943) as a regulator of mitosis, the protein acting by combining with nucleic acid to prevent the synthesis of "chromosomin".

A complete discription of the chemistry of mitosis is at present

impossible. There is both a lack of data on the constituents of cytoplasm and nucleus as well as a lack of understanding of the mechanisms involved in enzymatic behaviour. From the genetic viewpoint, Waddington (1939), in considering the physico-chemical nature of the gene, maintained that genic interaction was more than an interaction between protein side-chains and extended for much longer distances. That is really a more abstract way of saying what Treffers (1944) demonstrated in his review of the immunological behaviour of the proteins, namely, that the specifity of such biological reactions depends not only upon the particular combination of active or prosthetic group with protein substrate, but also upon the configuration of the active group itself. Thus even a single change in steroid property of the non-protein component, say reversing the order of glycine and leucine, is sufficient to inactivate the entire complex. Considering such intricacies of chemical behaviour, it is easy to see that a complete understanding of the chemistry of gene, chromosome, and cytoplasmic reproduction is beyond the reach of contemporary knowledge.

Physiology

There have been several reviews of the physiology of cell-division. Dalcq (1928) dealt chiefly with membrane formation, sol-gel transformations, and to some extent, permeability; Wasserman (1929) reviewed cell growth and proliferation in much greater detail; Gray (1931) emphasized the physical aspects of mitosis and cleavage; Heilbrunn (1937), though briefer, was more critical and comprehensive in considering the various approaches to cell-division; and Bujard (1941) discussed, both critically and comprehensively, the recent as well as earlier physiological studies of mitosis.

(a) Hydration

Degree of hydration and changes in hydrational property have been

considered by many workers as an important factor in promoting nuclear division. Whether the emphasis is due to the ease with which morphological changes are effected by osmotic differences and various adsorbates or to the obviousness of hydrational changes is not clear since both are inter-related. There is nevertheless a vast amount of literature dealing with the topic of morphology as related to hydration; some of these are mentioned here. Lawson (1911) considered osmotic forces responsible for diffusion of karyolymph from nucleus to cytoplasm causing a contraction of the nucleus and an eventual breakdown of the nuclear membrane. Sinke (1939) produced many refractive changes in nuclei and chromosomes by treating them with salts and acids in hyper- and hypotonic solutions. He interpreted all such effects as due to hydration or dehydration. Kuwada and Nakamura (1934), observing mitosis in living staminate hair cells of Tradescantia reflexa, concluded that vital detection of spiralization and coiling of the chromonemata was dependent upon the degree of hydration. The disappearance of coiling was therefore due to chromonemata swelling during mitosis, the swelled condition persisting until telophase. Pfeiffer (1934), studied Tradescantia cells "in vivo" by observing intra-cellular changes during division under constant extra-cellular conditions. He distinguished various degrees of protoplasmic refractivity during mitosis, a slight lowering of the refractive index before prophase, a graded increase during prophase, and a return to the refractive index of the resting stage before or with entry into telophase. From these observations he concluded that a cycle of hydrational change was associated with nuclear division. Pfeiffer (1936), also made some accurate measurements of refractive indices of mitotic and resting nuclei. From the results obtained he reasoned, rather vaguely, that such changes were due to some transformations in the physical structure of the cell.

The evidence for association of sub-microscopic structural changes with hydrational factors during nuclear division is supported by more direct work on mitosis. Wada (1935), for example, using microsurgical techniques found the spindle to be of a fluid nature. He also observed that the spindle became increasingly visible upon dehydration of the cell. It is not clear, however, that the increased refractivity of the spindle during division is due to an over-all dehydration of the cell. Giles (1939), studying microsporogenesis in Tradescantia, claimed that dehydration suppressed spindle formation. The divergence, however, is more apparent than real. The microspores were dehydrated by sealing off entire shoots and a number of abnormalities resulted suggesting, therefore, that the absence of a spindle might have been due to any number of causes. Moreover, in determining the effects of dehydration, the degree of denydration must be taken into account otherwise there is no standard of comparison. Hence, while a limited removal of water may be involved in spindle formation or at least, in its visual identification, drastic removal of water may equally suppress it. Sigenaga (1937), in fact, had made similar observations on the effects of dehydration. He noted that with a sufficient degree of dehydration there was not only a suppression of spindle and wall formation (as corroborated by Giles) but also a failure of the chromosomes to uncoil, thereby completely inhibiting nitosis. Wada (1936), in contrast, obtained an unravelling of the chromonemata by dehydrating the staminal hair cells of Tradescantia reflexa in sealed chambers through which dried air was blown. He produced the same effect by plasmolyzing the cells, a shrinkage of the chromosomes preceding the unravelling of the chromonemata. There have been, in fact, various experimental techniques used to cause a despiralization of the chromosomes by, presumably, the mechanism of hydration and dehydration (Kuwada (1937), Kuwada, Sinke, and Nakazawa (1939)).

The large body of data on the hydrational properties of the nucleus has led to the formulation of theories associating the division of the nucleus with the state of protoplasmic hydration. Belar (1929) and Mollendorff (1937) both suggested that dehydration of the interior of the nucleus was a precondition of chromosome condensation. Kuwada (1937) claimed that the telopnase unravelling of chromosomes was due to the hydration of the nucleus at that stage. Gustaffson (1939) proposed an even more generalized theory for the interrelation of mitosis and meiosis. He considered the mitotic nucleus to be hydrated in the resting stage and the meiotic nucleus to be hydrated after prophase. He further speculated that chromosome reproduction would not take place until a certain degree of nuclear hydration had occurred.

The data accumulated by the many experiments on nuclear hydration does point, as one would expect, to the importance of water, molecular and micellar, in controlling many mitotic processes. The value, however, of such data alone in explaining mitotic phenomena is questionable. There is first of all a doubt as to the validity of some of the results. Becker (1938), in reviewing the more recent work on cell-division, questioned the conclusions of Japanese workers on the swelling of chromosomes based on dark field illumination. He pointed out that cellular organs might appear equally dark after contracting or swelling when viewed in a dark field. Also, Strohmeyer (1935) claimed to have demonstrated the swelling of chromosomes during cell plasmolysis, a condition which Wada believed to promote dehydration and hence, contraction. There is, moreover, a fundamental objection to resolving mitosis in terms of degree of hydration. It is true that in subjecting cells to varying intensities of dehydration anomalous effects are obtained. It would be very surprising indeed if that were not the case since water is so important and universal a constituent of protoplasm.

When, however, hydration is suggested as a mechanism of mitosis, it follows that a causal relationship exists between the degree of hydration and the morphological pattern of the cell at any given period in its mitotic history. Such a condition is acceptable if considered simply as an expression of the equilibrium existing between water, free, chemically bound, or adsorbed, and the substrate. It is doubtful as a dynamic mechanism since it is much more likely that the molecular changes involved in mitosis (e.g., denaturation, proportions of H-ion and OH-ion, concentrations of soluble metabolites) are to a large extent responsible for changes in hydrational property. Hydrational differences are likelier to be associative than causal, and the data available is of greater use as an indication of ultra-structural change than as the exclusive subject matter of theories of division. The induction of various morphological patterns (e.g., despiralized chromosomes, spindle abnormalities) by altering the degree of cellular hydration does point to the importance of hydrational factors in the equilibrium of cell activity, but the problem which arises out of the accumulated evidence is the nature of the factors themselves, that is, inasmuch as they reveal any regularity of behaviour in the cycle of mitosis.

(b) Physical Properties

Evidence for a cycle of change in the behaviour of colloidally active substances is indicated by the results of viscosity studies on dividing cells. Heilbrunn (1957) showed an over-all decrease in cytoplasmic viscosity prior to prophase and an increase to telophase. Similar results were obtained by Fry and Parks (1954). Kostoff (1950) examining floral buds of tobacco plants found the lowest viscosity values at the resting stage and at metaphase. The criticism applicable to any such over-all measurement of viscosity as determined by the centrifuge has been discussed by Scarth (1927). Moreover, it would appear that mitosis involves, more than

other types of cellular activity, a high degree of localization in consistency. Certainly chromosome, spindle, cytoplasmic matrix, and cortex, are not all represented by a single curve of viscosity change. In fact, a number of investigators have demonstrated and stressed the zonation of protoplasmic structure (Scarth 1942; Chambers 1924, 1938) so that there is no satisfactory basis upon which to evaluate the above results. Their relevance too, in resolving mitotic mechanisms is not very apparent.

Localized changes in the cytoplasm, particularly those involving sol-gel transformations have been studied by many investigators. Chambers (1938) observed a sol-gel change at the equator of the echinoderm egg which he attributed to an inward flow of sub-cortical cytoplasm from the poles to the equator of the cell. Using micrurgical techniques he demonstrated the inhibiting effects of upsetting this sol-gel change on cleavage of the egg. Schechtman (1937) likewise believed that cleavage was due to a process similar to pseudopod formation in which solated cytoplasm moved into a gel region producing a constriction on gelation. Marsland (1939), extending these studies, decreased the efficiency of division by simply applying hydrostatic pressure to dividing egg cells. He was able to show that such treatment resulted in a liquifaction of the cortical gel region. That differences in consistency exist not only within the cytoplasm but between the cellular organs as well has been shown by the techniques of micromanipulation. Chambers and Sands (1925) in studying the pollen mother-cells of Tradescantia virginica found the cytoplasm to be a jelly-like mass though the chromosomes were of greater viscosity. The spindle, however, was found to be more liquid than the cytoplasm. Chambers (1924) also studied the structural properties of the nuclei of Dissosteria spermagonial cells. Among other things he showed that localized injury to the nucleus caused a condensation of the chromosomes as well as a contraction of the

nucleus. Wada (1933) in microdissecting the chromosomes of Tradescantia reflexa found that they would undergo a reversible coagulation, their structure being visible only in the gel state. Bucke and Boche (1938) suggested the presence of a fluid substance within the chromosomes. Their results were obtained by asphyxiating larvae and observing the exudation of fluid from the chromosomes into the cytoplasm, an apparently reversible process, the fluid being reabsorbed when the chromosomes regained their original volume. Scarth (1927) showed that even optically homogeneous nuclei possessed a coherent physical structure.

(c) Electrolytes

The intimate association of such diverse physical aggregates suggests, apart from the many other factors, known and unknown, the effectiveness of ions in maintaining the characteristic heterogeneity of the cell. The importance of salts and H-ion concentration in the solution or precipitation of proteins and the changes in viscosity and hydration of colloids produced by treatment with ionic substances are well known. It is no wonder then that the mitotic cell should show characteristic ionic properties as well as a marked reactivity to the introduction of electrolytes. It is probably the latter reason, more than any other, that has stirred so much physiological study of ionic effects. Some of these studies are listed here; to report all would be far too elaborate a task, so that as in the previous sections there is presented as typical a cross-section as possible, selective only to the degree that it is relevant to the present treatment.

Sakamura (1926) showed that the refractivity of chromosomes could be increased or decreased by appropriate adjustments of the pH of the medium. Yamaha (1935) using Brom-Cresol Green as indicator determined the different pH values of the cytoplasm and nucleus during the meiosis of pollen mother-cells. Yamaha (1937) also reported different electrophoretic properties for nucleus and cytoplasm; thus, while the introduction of an electric

current caused the cytoplasm to swell it caused the nucleus to contract slightly and move towards the anode. Churney and Klein (1937) using a cataphoretic cell found the nucleus electro-positive and the chromosomes electro-negative, the induction of a current causing, as in the observations of Yamaha (1937), an increased refractivity. Strohmeyer (1935) decreased the viscosity of the karyoplasm by treatment with $Ca(NO_3)_2$. He further maintained that the gel-sol transformation caused by the salt was indicative of death. The inference that the sol state of aggregation is necessarily associated with moribundity is, however, questionable since several investigators (Chambers 1925, Scarth 1927) have found many animal and plant nuclei to be highly fluid. The importance of the calcium ion has been stressed by Heilbrunn (1937) who claimed that it is always released upon cellular injury. Mazia (1937) emphasized the release of calcium during fertilization of Arbacia eggs, showing that bound calcium decreased by 15% though the total calcium remained constant. He speculated that the change in bound calcium might be due to a change in the protein and incidentally suggested that the release of calcium ions was responsible for an increase in cytoplasmic viscosity. Sorokin and Somner (1940) investigated the effects of calcium deficiency on roots of Pisum sativum. They found that mitoses were generally disturbed, cells forming with polyploid or constricted nuclei. Increases in the amount of calcium fed to the plants decreased the degree of injury. Churney (1940) studied osmotic and salt effects on mitotic elongation. He showed that magnesium and potassium increased elongation while calcium inhibited it. On the other hand, he found that calcium was essential for furrow formation in egg cleavage. Bancher (1938), in treating nuclei with salts, concluded that the physical structures of the nucleus was affected by the electrolytic properties of the salt rather than the osmotic ones. He also found that the treatment caused a gelation

of the nucleoplasm which became fluid only after continued micro-manipulation. He reasoned as did Strohmeyer (1935) that the nucleus was normally a gel and not a sol. Sigenaga (1940) used salts such as NaCl and KNO_3 to produce an unravelling of chromonema spirals. Kuwada, Shinke, and Oura (1938) obtained the same result with alkaline solutions.

The biological effects of ions is apparent not only morphologically, but also in the chemical behaviour of the cell. McDonald and Kuntz (1941) for example, showed how effective calcium and other ions are in the formation of an enzyme such as trypsin. Similarly, the results of Burk (1942) demonstrate the influence of various ions in promoting or inhibiting protein denaturation. That such dual effects of electrolytes can be demonstrated emphasizes one of the previous criticisms, namely that the property of hydration, or, for that matter, viscosity, cannot be satisfactorily accepted as an adequate cause of mitosis.

(d) Physiological Cycles

The description of mitosis in terms of a morphological cycle naturally leads to a search for its physiological counterpart. It is from this viewpoint that our knowledge of its nature, as has been suggested earlier, is most inadequate. The data reported in the preceding paragraphs chiefly concern morphological changes produced either by application of various substances directly to the living cell or by subjecting the plant to different temperature and hygroscopic conditions. As contributions to a knowledge of protoplasmic structure they are, of course, directly relevant and important. The bearing of such data in formulating a physiological cycle of mitosis is, however, less apparent. Of the three paramount physiological properties of the cell, structural consistency, osmotic pressure, and membrane permeability, only the first has been resolved to any degree. The

remainder of the data would, if integration were attempted, form a network so tenuous as to be of doubtful value. And it is precisely that integration of differences in physiological property into a systematic and consistent cycle of change that is necessary. The cyclical pattern followed in the cell's morphology must have its counterpart in the sphere of physiology, the alternative is, to repeat, "an agent immaterial". This alternative is unnecessary. To the extent that any attempts are made or have been made in revealing the physiological dynamics of the cell, they constitute a very important basis for further studies. For while Strassburger provided an adequate description of the chromosomes as early as 1875, and thus laid a pattern which subsequent morphological research had merely to broaden by increased and more refined observation, the physiology of cellular division lacks such a pattern even to-day. There is fortunately some information suggestive of such a cyclical pattern. Because of the direct bearing of the data on the experiments performed, they will be treated in conjunction with the experimental observations, rather than with the more general review.

The manner of reporting the literature has in a way anticipated the results of the present investigation. As such, there are both intentional and unintentional omissions, though in the writer's opinion, their insertion would in no way alter the general picture of the problem. As comprehensive a treatment of mitosis as that of Schrader (1944) concludes "Not any one of the many hypotheses broached has in it the definite promise of a final solution . . . it is almost impossible to affect a given structure or process of the mitotic mechanism, be it by operation or physico-chemical means, without simultaneously affecting several others". There have been, for example, omissions in reporting some micrurgical studies of the nucleus (Sands 1925, Duryee 1938, Cohen 1937, Harris 1939) and other physiological experiments (Lillie, 1931, 1934, Becker 1933, Politzer 1934, Schrader 1934,

Sinnot and Bloch 1940, Brumfield 1942). Yet, in absence of any definite frame of reference it is difficult to discriminate between the relevant and irrelevant, for what appears at present to be of only distant relationship to the physiology of mitosis, may be of extreme importance for its analysis in the future. References to the literature have, therefore, not been grouped according to any graded scale of importance apart from those results suggestive of physiological cycle. The merits, of course, in stressing physiological cycle will be determined by future research.

It follows that the research to be reported has been limited to searching for the framework of a physiological cycle, that is, inasmuch as so diffuse an objective can be said to impose a limitation. But it is precisely the very nature of the problem, its lack of definition, that has made the work exploratory and in a sense, both crude and elementary. Still, that is no drawback in light of the main objective. If the outlines of some methodical behaviour can be revealed, then what follows is simply the task of correcting, refining, and increasing the amount of data on the subject until a clearer resolution is possible. That is the best formulation of the scope and nature of this investigation. For the sake of achieving some outline, albeit dim, of physiological changes, much has been overlooked or consciously sacrificed in the way of refinement of technique. It is hoped that future work will serve both to correct and amplify.

MATERIALS AND METHODS

The experiments described in this paper are based chiefly on the pollen mother-cells of Trillium erectum (L). The long meiotic period of these cells and their large size make them very suitable for micrurgical investigation. Physiological changes, which, occurring in rapid succession, are difficult to detect in the mitosis of most vegetative cells or in the meiosis of many spermagonial cells because of their short duration, are more readily observed in the relatively long meiotic phases of the pollen mother-cells of Trillium. Though this advantage is limited by the heterogeneity of stages which is present in a single anther during the later phases of division, it has been possible to discover a cycle of physiological changes associated with meiosis.

Plants of Trillium erectum were collected in the early fall of each year in the vicinity of the Island of Montreal. They were planted in flats containing an ordinary soil mixture and stored in a refrigerator at 2° C. Single corms were transferred periodically to small crocks so that they could be removed for examination. No plant was exposed to room temperature for more than eight hours at a time; in most cases the period was considerably less. To check the possibility of temperature effects, however, several experiments were repeated at the end of the day.

Four properties of the pollen mother-cells were studied: the physical structure of the cells, their plasmolytic behaviour in different concentrations of sucrose, the stability of their cytoplasm and nucleus, and the changes occurring in their plasma-membrane. Physical structure was determined chiefly by microdissection. The other properties were observed by immersing the cells in solutions of sucrose, which is the most suitable physiological medium. 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 ions, where such were investigated, were determined by adding the desired concentration to a sucrose solution.

Buds were opened by a single longitudinal slit and a glass vial was inverted over each bud to prevent desiccation. One half anther was removed at a time and cut into three or four pieces each of which was floated in a small preparation dish containing the sucrose solution. When cells were required for microdissection the contents of the anther fragment were squeezed into a drop of paraffin oil mounted on a cover-slip. The mixture of solution and anthral sap is dispersed in the oil as little droplets, the oil preventing both evaporation of the solution and abrupt movements of the pmc such as occur when microdissection of pmc's is attempted in pure sucrose solutions. Plasmolytic tests were made in moist chamber slides. The chamber consisted of a ring, 25 mm. in diameter and 6 mm. deep, fastened to a microscopic slide by DeKhotinsky cement. A cover-slip suspending a drop of solution was sealed to the ring with petroleum jelly and a little of the solution was placed at the bottom of the chamber to reduce evaporation from the hanging droplet. To test for exosmosis of solutes some preparations were perfused before being sealed into a moist chamber. This was done by placing the cover-slip on a microscopic slide and drawing a steady stream of solution past the cells. The technique, however, was only applicable to early prophase when the pmc's are embedded in a gel matrix and are not drawn off with the current of solution; in later stages the anther fragment was immersed in a large volume of solution for a given period of time so as to permit an outward diffusion of solute and then sealed into the moist-chamber slide.

Several precautions were observed in the techniques employed. To avoid injury, extremely fine needles were used in microdissection.

Needle shafts of diameters greater than 1 1/2 microns were discarded. Those needles which tapered abruptly into short but very fine shafts were most satisfactory as they possessed a rigidity necessary for easy manipulation. To remove traces of salts and other impurities cover-slips and slides were rinsed in boiling distilled water and dried between layers of clean filter paper. All stock solutions were sterilized in the autoclave and their containers were attached to sterile burettes. Cover-slips and moist-chambers were made as aseptic as possible to avoid fungal or bacterial growth in prolonged experiments. It was found impracticable, however, to work under completely aseptic conditions, so that in most cases bacterial growth appeared in the moist chambers after 48 hours.

Meiotic stages were determined by fixing the preparations at the end of each experiment and then staining with aceto-carmine. In many instances, however, degeneration was so great that this procedure was of little value. Fixation of an untreated anther fragment was satisfactory in prophase stages, but of little value in later stages because of the variability in different portions of a single anther. In order to simplify the expression for degree of mitotic development a method of calculation has been employed based on the assignment of numerical values to the different morphological stages. The magnitude of the values is a function of the time interval of a particular stage since it is assumed that with all other conditions constant meiosis is a function of time. The computation of stages is, however, of secondary importance to the results reported, and a detailed description of the method has been deferred to Appendix I.

EXPERIMENTAL RESULTS

I. Structure: Appearance and Consistency

In view of the relation between the appearance of the pmc and the nature of its external medium, it has been necessary, in order to facilitate the investigation of other physiological properties, to determine: (1) the normal appearance of pmc's; (2) the type of medium promoting such normality; and (3) the physical pattern corresponding to a given optical pattern of the cell. While much work has been done on the structural properties of dividing cells (Wilson (1925), Chambers (1924), Wada (1933)), it will be seen that the maintenance of normal physiological environment is of extreme importance in micrurgical studies of Trillium pmc's, a condition not emphasized by other investigators.

The resolution of physical structure has an additional advantage by way of physiological studies. The determination of normal physical texture and its corresponding optical pattern makes it possible to use the appearance of the cells as an index of internal physico-chemical change. Changes in appearance may serve as an assay for the activity of foreign substances or, given a constant environment, as a sensitive indicator of intra-cellular change. In anticipation of subsequent investigations, much of the study reported here deals with the relation between medium and physical texture, and between physical texture and appearance.

Appearance

PMC's suspended in a sucrose solution may be optically homogeneous or heterogeneous, completely granular, or irreversibly coagulated. The particular morphological pattern depends upon the concentration of sucrose, the stage of meiosis, and, if other factors are included, the pH of the medium as well as the presence or absence of dilute concentra-

tions of ions. Representative forms of the cells are shown in Plate I, Figs. 1, 2, 3, 4, 5, and for simplicity of reference they are classified as A, B, C, and D.

Type A, which is optically homogeneous except for a narrow granular peripheral zone, and type B, in which both nucleus and cytoplasm are refractive, are the uninjured or completely viable forms. Their existence as such in the anther is strongly indicated by suspending a portion of its contents in an oil drop within which the pmc's remain surrounded by their own anthral sap. Under such conditions only types A and B are seen.

Type C is a highly granular cell. It has been observed only in late prophase to metaphase and is probably related to certain peculiar properties of the cytoplasm at one or all of these stages. Though the granules appear like swollen chromonemata when viewed through the microscope, they are not actually so, as can be shown by staining the cells with aceto-carmine. Finally, type D is an irreversibly coagulated type, which often shrinks on coagulation so that it appears like a plasmolyzed cell. Such cells do not deplasmolyze however, and are clearly moribund forms. The gradations, in fact, between types B and D are numerous, but their relation to viability will be discussed in a later section.

Appearance vs. External Medium:

At a particular stage of meiosis the appearance of the pmc depends upon the concentration of sucrose in the medium. In the earlier phases of division the relation between solute concentration and optical pattern is fairly well defined. In pre-leptotene cells, for example, type A cells are rarely found in solutions below 0.5M, thus suggesting a critical concentration for the production of a type A pattern. The value

drops during the prophase stages and type A cells are then found even in 0.2M solutions, though often not for any extended period of time. (Immersion in such concentrations may be accompanied by degeneration) At diakenesis and metaphase the relation between appearance and medium is complicated by major physiological changes which will be discussed later. An increased refractivity is apparent at relatively high sucrose concentrations, and in some, though not all metaphase cells, media of 0.2M or less produce type A patterns. (The dual behaviour of metaphase cells will be treated more thoroughly in succeeding sections.)

The nature of the relation between solute concentration and appearance is of particular interest here. Contrary to the usual behaviour of plant cells, (see Shinke 1939) there is no increase in cell refractivity with increased concentration of medium. Though normally, hypertonic solutions in producing a plasmolysis cause an increase in refractive index by partial dehydration of the protoplasm, pmc's immersed in such solutions show neither plasmolysis nor high index of refraction. Pre-leptotene cells retain their type A pattern even in 2.0M sucrose concentration. Dilution of the medium is paralleled by a decrease rather than an increase in refractivity. Thus, types A and B may be reversibly interchanged at this stage by lowering the sucrose concentration to obtain B and raising it to obtain A. The gradual addition of distilled water to a suspension of pmc's causes an increase in cell refractivity and ultimately, upon sufficient dilution, a coagulation of the protoplasts.

The critical balance of factors associated with the optical refractivity of cells is illustrated by a few slide experiments with pmc's of Tradescantia reflexa. When $CaCl_2$ is present in a 0.2M sucrose suspension of pmc's 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 concentration, two limits are set between which the minimum probably lies. Below is listed the threshold values for various cations.

Ion	Molarity	Ion	Molarity
Ba	.005 - .0025	La	.000062 - .00003
Sr	.00125 - .0062	Al	.00005 - .000025
Ca	.00025 - .000125		

The reactions are reversible though the conditions governing the reversibility are not clearly defined. Thus often, the $CaCl_2$ effect is reversed with time particularly if the salt is present in low concentrations, and cells which have been refractive become homogeneous once more. KCNS also causes reversals, the reaction proceeding best in the presence of an alkali buffer. Extruded prophase nuclei demonstrate the phenomenon clearly. The nuclei are refractive in the presence of .0002M $CaCl_2$. Addition of KCNS and alkali cause a reversal to the homogeneous state which is similar morphologically to the nuclei described by Chambers and Black (1941) as "phantom nuclei". The precondition of plasmolysis claimed by these authors does not apply to the pmc. Since they were dealing with vegetative cells it is probable, as they themselves suggest, that the deplasmolysis increased the permeability of the cells. The property of low permeability is not present in pmc's, and so the membrane offers no barrier to the penetration of ions. These results are again in agreement with the evidence of Scarth (1927), that the refractivity of the nucleus could be changed reversibly by altering such factors as the pH of the medium.

Appearance vs. Stage of Meiosis:

It is possible to trace the changes in optical property during meiosis by comparing the appearance of pmc's at different stages in similar sucrose media. The type A pattern found in pre-leptotene cells suggests a comparatively low refractive index. The drop in the critical sucrose concentration necessary to produce type A during early prophase indicates, though not too clearly, a lowering of the refractive index during this phase of division. The change from optical homogeneity to heterogeneity at diakenesis and metaphase points to an appreciable increase in index of refraction. The relatively high value does not persist through the remainder of division since the tetrad stage is characterized by low refractivity at higher sucrose concentrations. The changes in optical property during meiosis may also be stated in terms of cell type. The prophase is generally characterized by type A. In the course of division this homogeneous pattern is replaced by type B which is most pronounced at metaphase. Subsequently, there is a reversion to the A pattern. It is probable, however, that the above descriptions represent only the average trend, for experimental evidence indicated the possibility of an oscillating rather than of a steady change in values. It is interesting to note that the inferred cycle of changes in refractive index corresponds closely to the results of Pfeiffer (1936), who made quantitative determinations of the phenomenon in mitotic cells.

Consistency (micrurgical expts.)

It is clear from the above that the type of physical structure which may be revealed by microdissection will depend partly on the medium in which the pmc's are suspended. Actually, the existence of sol or gel, and the localization of the different physical aggregates

within the cell are determined to a large degree by the concentration of sucrose in which the cells are manipulated. Conclusions reached on the physical structure of the pmc without regard to the dissection medium are faulty. Why so sensitive a relationship should exist between the cell interior and the environment will be better understood from the later consideration of other physiological properties. The results obtained at different stages of meiosis are as follows:

The Pre-Leptotene Cell: The pre-leptotene pmc is structurally differentiated into three zones corresponding to its optical differentiation. In the A type cell, which is present in suspensions of 0.6M and 1.0M sucrose, micromanipulation is easy, the only barrier to penetration of the needle being the elastic jelly-like cell-wall. When a needle is pressed against it, the wall dents at the point of contact but recovers its original shape immediately upon penetration of the needle, the cytoplasm offering no resistance to the movement of the shaft. The liquid character of the homogeneous interior is clearly demonstrated by the ease with which a needle is moved about without causing any visible disturbance in the adjacent parts of the cell. The fluidity is by no means a degenerative effect of continued manipulation as found by Chambers and Sands (1925) in their microdissection of Tradescantia pmc's, the sol-like aggregation is evident at the moment of penetration and is the same in any number of cells. Gel texture is apparent only in degenerate preparations, where coagulation of cytoplasm has occurred.

The internal zone of protoplasm is, of course, the nuclear zone. Its coherence despite its fluidity is demonstrated by rupturing the cellular membrane and wall. While the outer regions of the cell are dispersed in the medium the internal zone remains a coherent mass which flows out through the rupture and rounds up, though not to complete

sphericity, in the surrounding solution. When stretched by two micro-needles it shows no rigid elastic properties but resumes its original shape slowly behaving as though it were a fluid enclosed in some viscous membrane. Of all the zones it is least affected in its physical consistency by changes in sucrose concentration, and in all type A cells studied its behaviour was more nearly the same even though sucrose concentrations differed.

The whole cell, however, is not entirely of a fluid consistency. There is an outer gel layer of variable thickness which borders on the periphery of the narrow granular region. Its presence can be demonstrated by various methods. If a needle which has penetrated the cell is pushed across it, the tip contacts the inner margin of the gel layer and strands may be drawn from it into the interior of the cell (see Fig. 1). The strands are very thin, because of the diameter of the needle shaft, and the presence of granules can be detected only at their very base. The gel-like strands of cytoplasm are distinctly elastic and snap back completely on detaching themselves from the micro-needle. The presence of an outer gel zone may also be demonstrated by pinching a cell with two blunter needles so that the opposite walls are almost in contact. In presence of an entirely fluid cytoplasm one would expect a complete flow of protoplasm out of the pinched region. On examination of the "isthmus" however, a thin layer of granular cytoplasm is seen between the walls, thus suggesting a gel-layer. Physically, this region is very responsive to changes in sucrose concentration so that upon immersing pre-leptotene cells in 0.2M sucrose, the diameter of the zone increases as judged by the distance from which it is possible to draw out strands into the cell interior. The increasing gelification of the

outer region of the pre-leptotene pmc is, in fact, paralleled by the increasing lethality of diluter solutions.

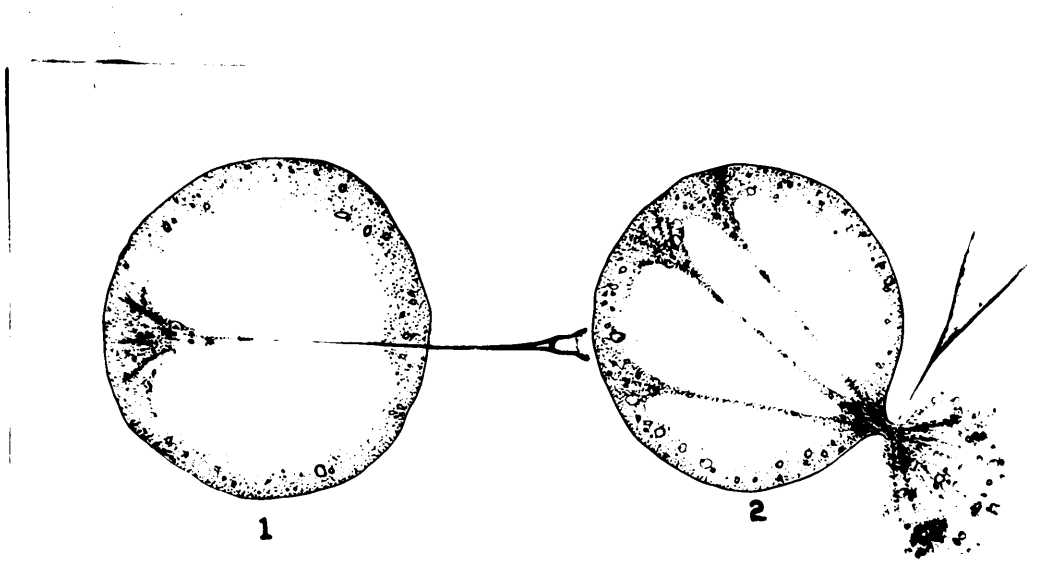


Fig. 1. Microdissection of pmc of Trillium in sucrose solution.
1: demonstration of peripheral gel zone in pre-leptotene pmc, the fluid interior may also be inferred;
2: the fluidity of the outer granular zone shown by rupture of the membrane.

The granular region is not entirely gel. Though its true condition has been difficult to determine, it is clear that it often behaves as a fluid. Thus, upon tearing of the cellular membrane, the granular cytoplasm invariably flows out and disperses in the medium, leaving no semblance of any structure. Often, upon injuring the cell at a single point, currents of granular cytoplasm flow from various directions across the cell through the narrow rupture dispersing in the medium as soon as they reach it. It is significant to point out that in all observed cases of cellular injury where any disintegration did take place (see Plate I, Fig. 6), the nuclei remained coherent while the cytoplasm dispersed in the solution.

The Late Prophase of Meiosis (Leptotene to Diakenesis): At this stage even if the cells are immersed in 0.2M sucrose solution, there is a markedly lower viscosity of the cytoplasm than in the previous stage. Apart from microdissection, the lower consistency is indicated by the rapidity of Brownian movement in the cortical region. There is often no cell-wall and the cell behaves as a naked protoplast. It is much more susceptible to mechanical injury than in the pre-leptotene stage, the slightest rupture in the cell membrane causing a complete dispersal of the contents of the outer cytoplasmic zones. In many instances only the nucleus remains. This fluid property of the cytoplasm is significant in the anomalous behaviour of some pmc preparations at this stage, particularly in cellular fusions, a phenomenon which will be discussed later in relation to membrane properties of the meiotic cell.

The micromanipulation of the nucleus, now large and refractive, is even more instructive structurally. The operation is difficult because it is hard to manipulate a needle within such a nucleus without causing either a contraction and subsequent gelation of the nucleus or a disappearance of the chromatid strands so that the structural differentiation is no longer visible. If, however, a very fine needle (it must have a very fine shaft of fairly constant diameter or mechanical injuries cause changes instantaneously) is manipulated into the nucleus, the fluid character of the nucleus is apparent. Movements of the needle cause displacements only of the adjacent chromonemata, the local disturbance demonstrating clearly the low consistency of the organ. Scarth (1927), showed that optical homogeneity and fluid consistency was no indication of a lack of structural identity in the resting nuclei of plant cells. The identification of a fluid consistency combined with

an optically differentiated structure is a reciprocal demonstration of that same phenomenon. The sol-like character is also demonstrable in non-refractive nuclei whose outlines are discernible unlike the completely non-refractive ones in the type A cell. The operation is aided by the presence of a refractive nucleolus. Manipulation of a needle within the nucleus produces no corresponding displacement of the nucleolus unless the needle is brought up close to it. Such conditions of movement are possible, or course, only in the presence of both a structural frame-work and a low consistency. It is suggested here that the structure visible in the type B cell persists in the non-refractive types as well. Moreover, it confirms a point of view previously expressed that the changes in visible structural pattern of the cell are not necessarily related to osmosis and gelation. That the refractivity does increase with more drastic physical change such as gelation has already been indicated. More evidence is provided by refractive though contracted nuclei which have a gel-like consistency; movement of an inserted needle here results in a movement of the entire nucleus about the cell.

Metaphase and anaphase: The fluidity of the cytoplasm is similar to that of the previous stages though the existence of a more coherent internal structure is evident. Abrupt movements of the micro-needle cause corresponding movements of the chromosomes, though it is possible with careful manipulation to make small movements in the cytoplasm without affecting their position. Micro-dissection of the chromosomes was not attempted because of the difficulties involved in the operation. Refractive chromosomes tend to gelate completely so that careful analysis of their structure becomes impossible, while often, where such coagulation does not take place, manipulation promotes a fusion.

Discussion

The structural diversity of the living cell points, more clearly than any other single factor or cell behaviour, to the importance of ion and solute molecule in maintaining the characteristic heterogeneity of cell protoplasm. Furthermore, the fact that intra-cellular reactions must produce an unequal distribution of metabolic products and thereby cause a selective and localized concentration of chemically and colloidally active substances within the protoplasm suggests, with equal emphasis, the interdependence of the various cell organs in preserving their structure. In view of the importance of such extra- and intra-cellular conditions in affecting the structural pattern of the living cell, it follows that the determination of physical texture by means of micromanipulation requires not only a control of the immersion medium, but equally, an investigation of individual organs within the intact cell rather than as freely suspended aggregates in an artificial environment.

The degree to which the nature of the external medium affects the internal character of the cell depends, of course, both on its permeability and on its intrinsic degree of stability. Spirogyra, for example, may be exposed to tap water for several hours without causing any detectable deterioration in its structure. Pollen mother-cells of Trillium, on the other hand, when subjected to similar conditions, degenerate and coagulate instantaneously. Even dilute concentrations of sucrose, producing morphological types characterized as intermediate between "B" and "D", cause an increased gelation of the cytoplasm. In fact, all forms described in the preceding section vary to a greater or lesser degree in their physical texture according to the sucrose

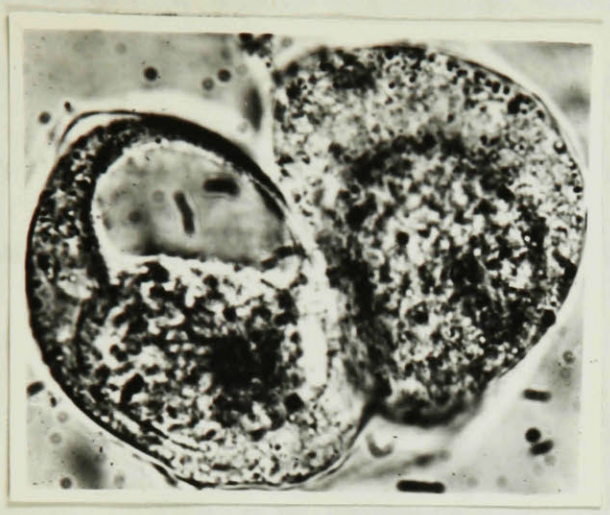
concentration of the medium. Whether this condition alone accounts for Chambers and Sands (1925) finding the cytoplasm of Tradescantia pmc's to be a jelly-like mass is not clear, since only Trillium pmc's were examined and possibly, the viscosity of Tradescantia pmc's is normally greater than that of Trillium. The results Buck (1942) obtained on the elasticity of the salivary gland chromosomes of Chironomus are, however, open to much criticism since he treated them with 2% Osmic acid for 18 - 24 hours at 5° C (he himself suggests that the chromosomes are not normal). Similarly, Wada's (1953) stretching of meiotic chromosomes in a solution of sucrose and KCl is far from being a reliable indicator of chromosome texture. Metaphase chromosomes of Trillium pmc's, made optically refractive by the addition of some salt to a sucrose solution, fuse easily when manipulated within the intact cell. In fact, by staining with Brilliant Cresyl Blue the partial fusions are readily seen. Chromosomes, however, which are withdrawn from the cell gelate with time and retain their morphological identity exhibiting properties similar to those described by Wada (1953). The inference is obvious. Any deductions made concerning the physical properties of protoplasm as revealed by microdissection in mechanically injured cells, or even in partially injurious media, require serious scrutiny since such conditions generally promote changes in property markedly different from that of the normal cell.

The effect of upsetting the intra-cellular equilibrium is illustrated by puncturing the prophase nucleus with a micro-needle. Unless performed delicately, as described in the experimental section, there is an immediate contraction of the organ accompanied by a rapid gelation. Whether this phenomenon is due to a sudden collapse of structure resulting from mechanical agitation, a behaviour observed in

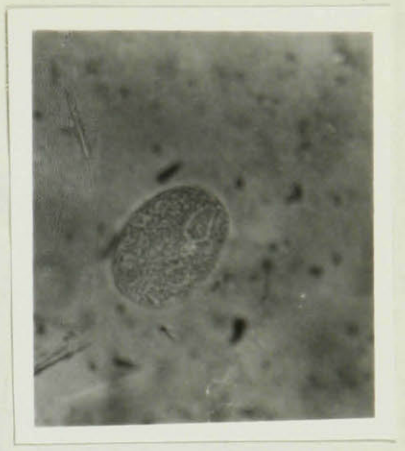
some uni-cellular organisms (Seifriz 1942), or to the rupture of a semi-permeable membrane is not as evident, though the difference in colloidal and solubility properties between the two zones is readily inferred. The production of a clear hyaline area upon contraction of the nucleus indicates, at least, the presence of a mobile fluid component which is released on injuring the organ. It is not necessary, however, to injure the membrane mechanically in order to produce this phenomenon; injurious solutions of salts and sugars behave in similar fashion. (Fig. 2b). It would appear that the unequal accumulation of cellular substances, as revealed by the changing volume of the nucleus, depend either partially or completely on the presence of a selectively permeable nuclear membrane and/or a differential swelling power of the nuclear proteins. The rapid shrinking of the nucleus on slight mechanical rupture of the membrane suggests the former as the more likely factor. So too does the rapid outflow of cytoplasm on injury of the plasma membrane, indicating as it were, some internal pressure within the cell. Such pressure may well be due to an osmotic gradient between nucleus and cytoplasm resulting from the differential permeability of the nuclear membrane and the unequal distribution of soluble products.

The difference in physical property between nucleus and cytoplasm may also be related to the kinetics of division. While it is clear that contraction of the chromosomes would hardly be possible in a gelated nucleus such as is produced on injurying the nuclear membrane, the structural effects of chromosome condensation are equally relevant. The chromosome strands disentangling themselves from the matrical framework in the course of contraction, would, by decreasing the intricacy of micellar association, decrease its consistency. The matrix, now being statistically of a different chemical constitution,

may well show a physical homogeneity with the cytoplasm especially upon dissolution of the nuclear membrane.



(a)



(b)

Fig. 2. (a) Pmc of Trillium in .5M sucrose. One of the nuclei has contracted and the clear hyaline area is readily seen.
(b) Nuclear fragment in sucrose solution. One nucleus may break up into many such fragments.

The difference in physico-chemical property on either side of the nuclear membrane is apparent on examining reactions to the immersion medium. Though both regions are of fluid consistency, the nucleus and cytoplasm show different degrees of solubility and dispersability. Cytoplasm disperses completely when in contact with a sucrose solution. The nucleus, on the other hand, shows no such tendency even when it disintegrates into a number of fragments, for even these fragments retain their identity (Fig. 2b).

Not only the solubility, but also the sensitivity of the respective components to the colloidal activity of the ions is significant. In Tradescantia pmc's, very dilute concentration of ions is sufficient to effect a refractive change in the nucleus without visibly altering any other character of the cell. Why such selective adsorbabilities

should exist is of course to be explained by the chemical or colloidal properties of the cell. Where a difference in ionization of protein exists between cytoplasm and nucleus, it is feasible that an unequal distribution of mobile ions should result. If pH is any indication of such differences then the measurement of Chambers and Pollack (1927) showing the nucleus to be more alkaline than the cytoplasm is further evidence of the situation. So too are the different solubilities demonstrated in the fractionation of cellular proteins (Mirsky and Pollister 1943; Lazarow, 1943).

If, the structural character of the living cell is to a large degree dependent upon the maintenance of a gradient of solute concentration, it follows that the micrurgical investigation of organs removed from the cell is not indicative of a normal condition. This consideration applies particularly to the manipulation of chromosomes removed from the cell. Moreover, the condensation of metaphase chromosomes involves more than a contraction of elongated polypeptide chains since the presence of such bodies indicates, at least, a newly formed interface between chromosome and substrate. The fusion of chromosomes in micromanipulation is evidence of the activity of the surface layer and it is probable that the intervening cytoplasmic matrix is responsible for maintaining the identity of these bodies. Probably the synapses observed in meiosis are products of such surface fusions.

The physical differentiation of the pollen mother-cell resembles closely the pattern of protoplasmic differentiation described by Scarth (1942), apart from the absence of a vacuole. The point of departure, however, in assessing the physical properties of the non-dividing and the mitotic cell lies in the relative stability of each. Whereas the structural pattern of the non-dividing cell remains relatively

constant, that of the pmc is in continual flux. Changes in consistency, sol-gel transformations, condensation and redispersion of chromosomes and membranes, all point to the relative instability, or more correctly, sensitivity, of the various protoplasmic aggregates to physical change.

The different morphological types described are, in fact, indicators of fundamental physical changes in the protoplasmic proteins. In the absence of more refined physico-chemical techniques for the determination of colloidal or chemical properties of the pmc protoplasm, it is possible, by the use of structural criteria, to identify some of the more radical intra-cellular transformations occurring during meiosis. The mechanism of refractive changes of proteins is not entirely clear. Craig and Schmidt (1932) have made some proposals in that direction. The reactions recorded here can be explained in their terms of hydration, and, particularly, ionization. But, apart from the actual mechanisms involved, the over-all morphological changes occurring in the cells are used as indicators of cytoplasmic stability. The stability of the cytoplasm, which is in itself a result of an aggregate of factors, will be considered in the next section.

II. Stability

One of the characteristic properties of Trillium pmc's is their extraordinary sensitivity to various solutions. While most plant cells can be immersed in many different media containing such solutes as $CaCl_2$ or other salts, balanced and unbalanced, or merely in tap-water, attempts to do so with the meiotic cells of Trillium were consistently abortive. Their rapid coagulation in such media, which itself is indicative of singular protoplasmic instability, is related not only to the composition of the medium, but also to cycles of other physiological changes associated with meiotic division. Thus, these peculiarities of behaviour, though at first regarded mainly as technical problems, are of much greater interest as indicators of intra-protoplasmic transformations occurring throughout the entire cycle of nuclear division. From this standpoint, the work reported is merely preliminary to a more fundamental and thorough study of the mechanism of stability change.

Criteria of Viability:

Cells which have been distorted by coagulation or destroyed by degeneration are easily distinguished optically and are readily classified in terms of viability. It is more difficult, however, to classify the many grades of viability which lie along the gradient between healthy normality and death. The only consistent indicator of viability, which nevertheless involves some subjective interpretation, is the degree of refractivity of the cell and its contents. It is an almost general rule that as the moribundity of a cell increases so does its refractivity. Preparations containing highly refractive pmc's survive for a much shorter period of time than those containing non-

refractive or B type cells. Yet, though this is used as a general criterion or classification, there are exceptions to the rule. Occasionally cells are killed completely without any evidence of high refractivity. This occurs often in alkaline media; it is generally the case when injury results from an outflow of protoplasm, as in the formation of hyaline vesicles; it may occur in sucrose solutions of very high concentrations. Taking such exceptions into account, however, the use of degree of refractivity and of protoplasmic distortion as criteria of viability is the most satisfactory method.

Since there is a continuous gradient of refractivity, it follows, assuming an interrelationship of the two properties, that there is a parallel gradient of degree of viability. It is, however, impossible to measure the refractive indices of the cells in a single suspension, so that in evaluating a preparation a rough but simpler method of categorizing the cells is used. Healthy normality and irreversible coagulation are assigned values of 100% and 0% respectively. Highly refractive (HR) cells which may lie anywhere along the gradient between the two extremes are considered 50% viable. Thus, in determining the viability of preparations, cells are classified as "Non" (non-injured), "HR" (highly refractive), and "D"(dead). The classification obviously involves a considerable margin of error. Since degree of refractivity is not expressed quantitatively, preparations which are assigned similar percentages of HR cells often differ in their average refractivity. This is particularly true when various concentrations of sucrose are used for similar preparations of pmc's, the dilute concentrations generally possessing a higher degree of refractivity, though the percentage of HR cells in the two preparations is nearly the same.

Suspension Media:

Solutions of sucrose are the most suitable physiological media for pmc's of Trillium. Though better media are probably obtainable by addition of various salt mixtures and by adjustments of H-ion concentration, pmc's suspended in pure sucrose solutions remained alive for as long as thirty days. In fact, cells in the early prophase of meiosis are quite stable in solutions of pure sucrose. The concentration may be varied from 2.0M to 0.5M without appreciably affecting the viability of the cells. It is when the cell enters into the more rapidly changing morphological phases of division that differences in viability are apparent and solutions of pure sucrose are inadequate. Prior to the latter stages, the only differences in behaviour are those related to the range of optimum sugar concentrations. Generally, preparations made from anthers of the same plant several weeks apart show that the later preparations are more viable than the earlier ones in relatively low concentrations of sucrose. Such advanced prophase cells may be kept in 0.5M and 0.2M sucrose for many days and even weeks, providing no bacterial infection occurs. In most preparations which are kept for such extended periods of time the cell wall dissolves and the cells gradually break away from the aggregate mass to which they adhered (see Plate II, Fig. 1.).

In view of the complete viability of pre-leptotene pmc's in solutions of pure sucrose, the use of such media to trace the presence of changes in cell viability has been of great advantage. By maintaining a constant environment it has been possible to limit the variables to the cell itself. Thus, while later stages of meiosis are very unstable in sucrose suspensions, their behaviour is nevertheless indicative of intra-cellular changes related to meiosis. What follows

is therefore a study of the variations in protoplasmic stability associated with nuclear division as observed in pmc's suspended in sucrose media.

Cycle of Viability Changes (Stability):

The stability of Trillium pmc's varies markedly according to the stage of meiosis. The differences in percentage of viable cells found in a series of preparations are hardly random but follow a definite pattern of change pointing strongly to a close relationship between meiotic stage and degree of stability. The results supporting this conclusion are based on cell counts of preparations made from one to three hours after immersion. (It will beshown later that differences between one and three hours immersion do not affect the trend.) All the individual values are plotted as dots in the graphs of Figure 5. The average results for the different stages are tabulated in Table I.

(a). Limitations. 1. The lack of uniformity in meiotic stage of individual preparations has been unavoidable. The variation in degree of meiotic development and the impossibility of determining stage until after having made cell counts are both contributing factors. The points are therefore scattered unevenly along the curves so that in some places interpolation has been necessary.

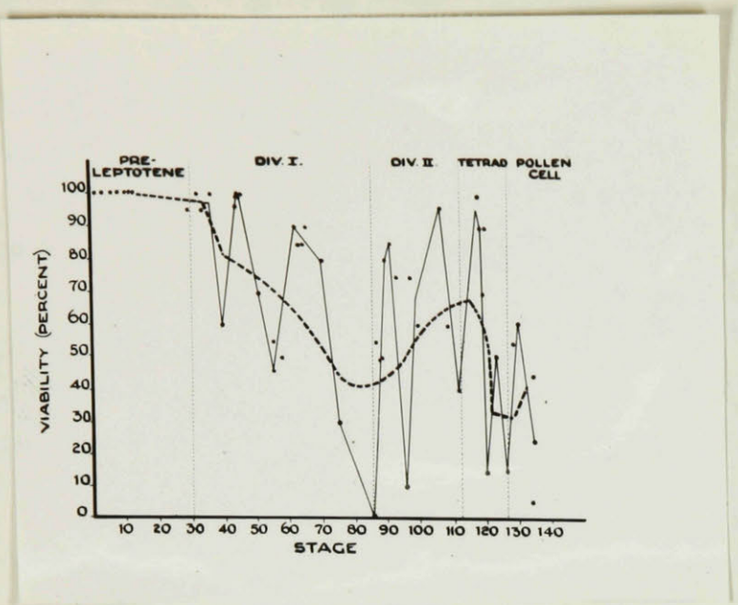
2. In the early prophase of meiosis the pmc's are embedded in a stiff gel matrix. The characteristic condition of the anthral sap at this stage interferes with the penetration of the surrounding medium so that changes resulting from the activity of a solution appear first only at the periphery of the pmc mass, and later, occasionally much later, in the interior. This is easily demonstrated by adding a stain or a salt to a suspension of pmc's; the resulting change in colour or refractivity spreads gradually

TABLE I

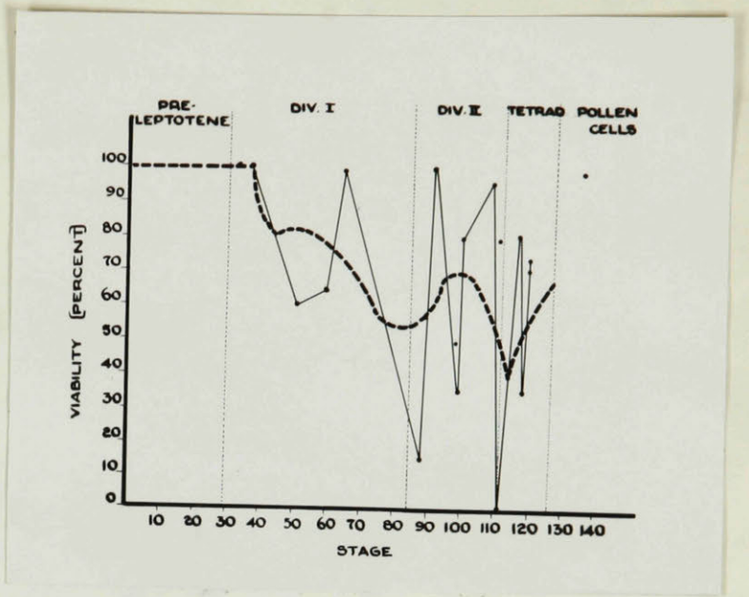
VIABILITY OF TRILLIUM RED IN SUCROSE SOLUTION (1-3 hrs.)

VIABILITY

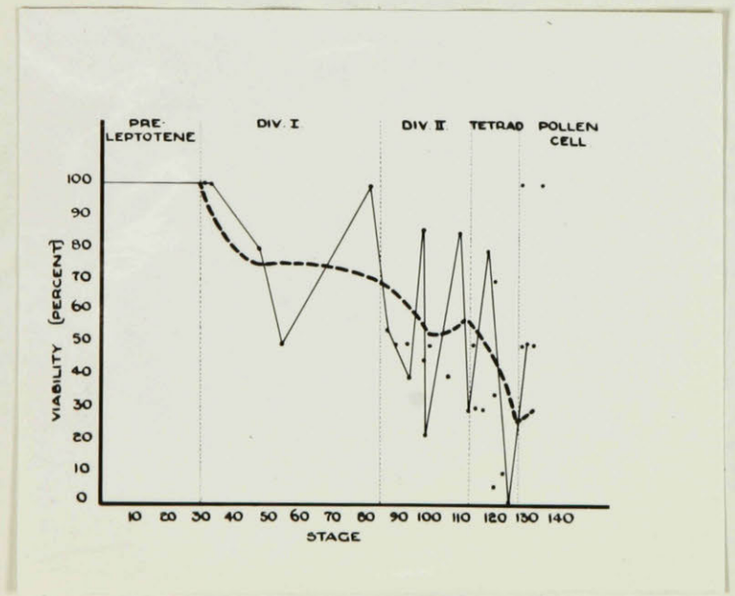
	Sucr. Conc. stage	1.2M-1.0M	0.8M	0.5M	0.2M-0.1M	0.08M-0.05M
FIRST DIVISION	0-30	100	100	100	100	65
	30-35	100	---	100	100	80
	36	100	---	100	---	10
	40	60	---	---	---	--
	43-48	95	---	---	80	--
	50-60	55	60	65	50	--
	62-72	85	---	100	---	--
	75	30	---	---	---	--
SECOND	79-82	---	95	---	100	100
	86-89	40	40	15	55	--
	90-93	80	85	100	50	--
	94-96	50	90	---	40	--
	97-105	70	75	55	50	45
	107-110	80	---	90	85	--
TETRAD	111-116	40	60	0	35	40
	117	95	90	80	80	--
	118-120	80	55	60	30	30
	122-131	40	---	---	45	45
	133	25	90	100	100	80



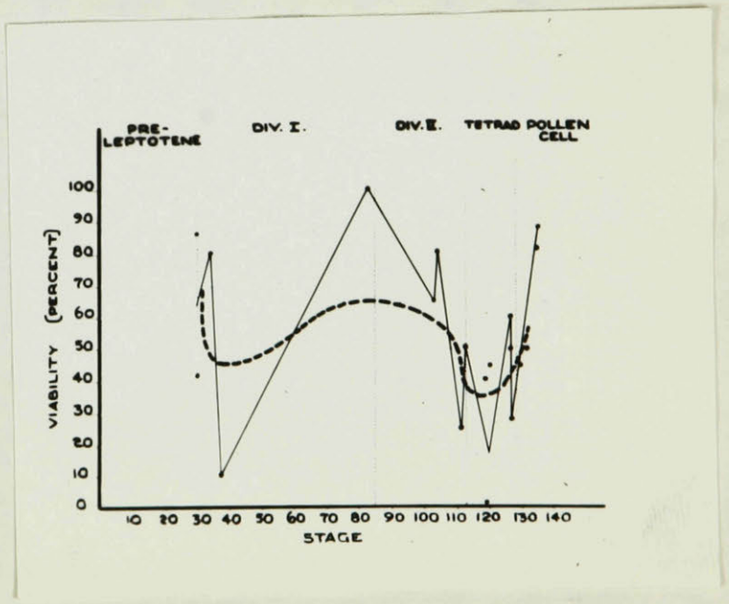
a. 1.2M - 1.0M



b. 0.5M



c. 0.2M - 0.1M



d. 0.08M - 0.05M

Figure 3. The viability of Trillium pmc's in sucrose solution. Readings taken after 1-3 hours immersion.

through the mass, the order of change being from the outer edge to the interior.

5. For reasons of technique, there is an absence of completely aseptic conditions in moist chamber preparations. The growth of bacteria in preparations after 48 hours, and occasionally after 24 hours, makes the values of viability where such growth occurred unreliable indicators of the effects of the medium.

(b) General Observations. Examination of either curves or tabulated results reveals a pattern of fluctuating viability values ranging from 0 to 100%. The amplitude of these fluctuations leaves little doubt as to the reality of the observed differences, and points, without the aid of further analysis, to the existence of a cycle of variation in protoplasmic stability simultaneous with the morphological division of the cell. In view of the presence of a constant external environment, whatever the nature of stability, it is clear that the factors promoting variation are intra-cellular, the extra-cellular factors being equal at all stages of meiosis. The curves drawn in heavy broken lines join the midpoints between the extremes of viability, thus representing the over-all trend in division. Their slopes show clearly the tendency for stability to decrease as division proceeds, the principal deviation from this downward trend being a rise in values during Meiosis II.

An interesting aspect of pmc behaviour in individual preparations is the appearance of 1st and 2nd division cells depending on their proportions in the suspension. Generally, though not always, where there is a relatively large number of 1st division cells and few 2nd, almost all the diads are healthy. When the proportions are reversed, however, so is the situation, such that the diads are healthy and the 1st division cells moribund. This phenomenon, though actually implicit in the graphs, is

described because of its vivid demonstration of the changing properties of the pmc. The extremes in behaviour of cells removed from a single anther and contained in the same suspension strongly suggest that the reactions observed are by no means the products of some generalized pathological effect, but are clearly the resultants of a given physiological condition.

(c) The First Meiotic Division. The pre-leptotene of meiosis is stable in sucrose solution. Apart from dilutions below 0.08M, the cells are completely viable from one to three hours immersion. At this stage it is difficult to assess, however, the viability of the suspensions, since the presence of the gel matrix previously described interferes with the diffusion of solutes, although as will be shown later, there is no significant degeneration even after twenty-four hours.

At stage "40" a viability of 60% has been recorded. Unfortunately, there has been only one preparation at this stage so that it is difficult to make any generalizations. Fixation of the cells showed 47% at leptotene-pachytene, 41% at diakenesis, and 12% at metaphase. From unrecorded observations such a pre-metaphase drop is considered probable, although more evidence is required to establish it. There is a high viability from 45-48 followed by a drop which has been observed at several concentrations and in at least eight preparations. The low values ranging from 50-65% are found between stages 50 and 60. That this interval represents chiefly the metaphase or its adjacent stages is evident from examination of fixed preparations (where such were possible). Association of the metaphase stage alone with poor stability is incorrect however, since observation of single preparations reveals rather a duality of behaviour. Some metaphase cells remain stable for very long periods of time, others degenerate almost immediately. Thus, the apparent inconsistency is

indicative of a fundamental relation between the morphologically observable and the physiologically detectible changes, namely, that what appears as a single stage morphologically may imply the co-existence of more than one phase of physiological property. The relationship will be seen to hold equally true for permeability behaviour. Whatever the time of change, it is clear that there are marked variations in stability associated with the metaphase and its adjacent stages.

There is a rise in stability as the cells approach completion of the first division cycle. Near the border-line the stability is well defined. Again, just prior to completion, at stage 75, one preparation shows a viability of 30%. If it were valid to draw an exact parallel between 1st and 2nd division, the presence of the drop would be substantiated. Unfortunately, the conditions under which the 1st and 2nd phases terminate differ markedly in their morphological aspects, so that it is inadvisable to presume an identity of physiological behaviour.

(d) The Second Meiotic Division. The trend of stability values in the second meiotic division is essentially, though not completely, similar to that in the first. Chiefly, meiosis II is more rapid and involves fewer stages. The interval between the first and second division of meiosis is characterized morphologically by very little interruption of activity. The common resting nucleus is not found at the time of diad formation, in fact, no nuclear membrane is formed. Thus it is not surprising to find a drop in stability at the very beginning of 2nd division (86-89). It is interesting to note, too, that the degeneration is intensified, viability now ranging from 15-55%, a set of values much lower than that found in 1st division. The interval (94-105) following the next period of high stability (90-93) is less sharply defined. The average indicates a drop in stability, and except

for one preparation showing a value of 90%, all the others are below 75%.

Apart from a more precise evaluation, the trend to increased stability in the latter part of meiosis II is indicated by the high values of stages 107-110. The latter period is just before tetrad formation and leaves no doubt as to the drop in value associated with the beginning of tetrad development (111-116) where values range from 55-60%.

Though there is an interval (117) of high stability in tetrad cells, this stage of meiosis is generally characterized by a very definite and large degree of instability. Extreme distortion of the cytoplasm is common, pointing strongly to the poor viability of the cells. Not only does the internal zone of cytoplasm show a high sensitivity to change, but also the membrane. Formation of hyaline vesicles occurs frequently at the surface resulting in injury and subsequent death of the cell. This phenomenon (to be treated in detail in a later section) is most frequent at the time of tetrad separation (122-131) into individual pollen cells (135). The latter are generally stable and will not be considered in any detail.

The tetrad stage is clearly an endpoint in meiotic division. The nuclei of tetrads are optically identical with those of other normal non-dividing cells. Thus, the differences in stability between the end of 1st division and that of 2nd division are understandable in light of the dissimilarity in morphological patterns. The high degree of instability as well as the peculiar surface activity of the tetrad stage point to intra-cellular changes which are, at least, intensified at the completion of division. But though such intensification of changes exists, the similarity of cycles in 1st and 2nd division is by no means obscured; the extended period of high stability at pre-leptotene is paralleled by a short period after diad formation, and the

brief interval of low viability at the end of 1st division is paralleled by a longer interval following tetrad formation.

Stability vs. Time:

The differences in percentage of viable cells present after one hour and after three hours immersion in solution is not large enough to warrant any special consideration. The results of counts made at intervals of one or two hours revealed no significant changes after the first hour of immersion. The change, however, is of appreciable magnitude when preparations are examined about twenty hours after the original count. Generally, there is a decrease in percentage viable. The less stable preparations, however, show a greater increase in moribundity than the more stable ones, and consequently, the differences both between stages of meiosis and between concentrations of solute are accentuated. Unfortunately, many preparations were infected by bacterial or fungal growth after such time and their results are not included, leaving, therefore, a smaller number of samples for analysis.

The dots in Fig. 4 represent individual values, the averages for the various stages being tabulated in Table II. It is significant that the trend of stability after twenty-four hours immersion is similar to that found after three. Both mid-first and 2nd division drops in viability are clearly indicated. Stages 51-55, for example, possess values ranging from 0-45% and the transition from 1st to 2nd division (85-87; 88-90) is characterized by 0 viability except for one preparation (Stage 90) in 1.2M sucrose concentration. The persistence of such differences emphasizes the significance of stability properties in relation to meiosis. By prolonged immersion the effects due to differences in permeability are reduced to a minimum, suggesting thereby, that the observed fluctuations in viability are more nearly, apart from all other factors involved, a function of the intra-protoplasmic changes associated with the

TABLE II

VIABILITY OF TRILLIUM FMC. IN SUCROSE SOLUTION (24 Hrs.)

VIABILITY

	Conc. Stage	1.2M-1.0M	0.8M	0.5M	0.2M-0.1M	0.08M-0.05M
FIRST	0-30	95	85	75	100	--
	30-35	85	--	80	65	25
	45-45	85	--	--	---	--
	51-55	45	0	25	0	--
	65-66	85	0	95	---	--
	70-80	80	85	--	---	--
	85-87	0	--	--	0	--
	88-90	100	0	0	0	--
SECOND	92-94	90	0	95	---	--
	98-100	--	--	0	15	--
	106-111	60	0	55	15	0
TEST	112	0	74	0	0	0
	117-120	80	0	0	0	0

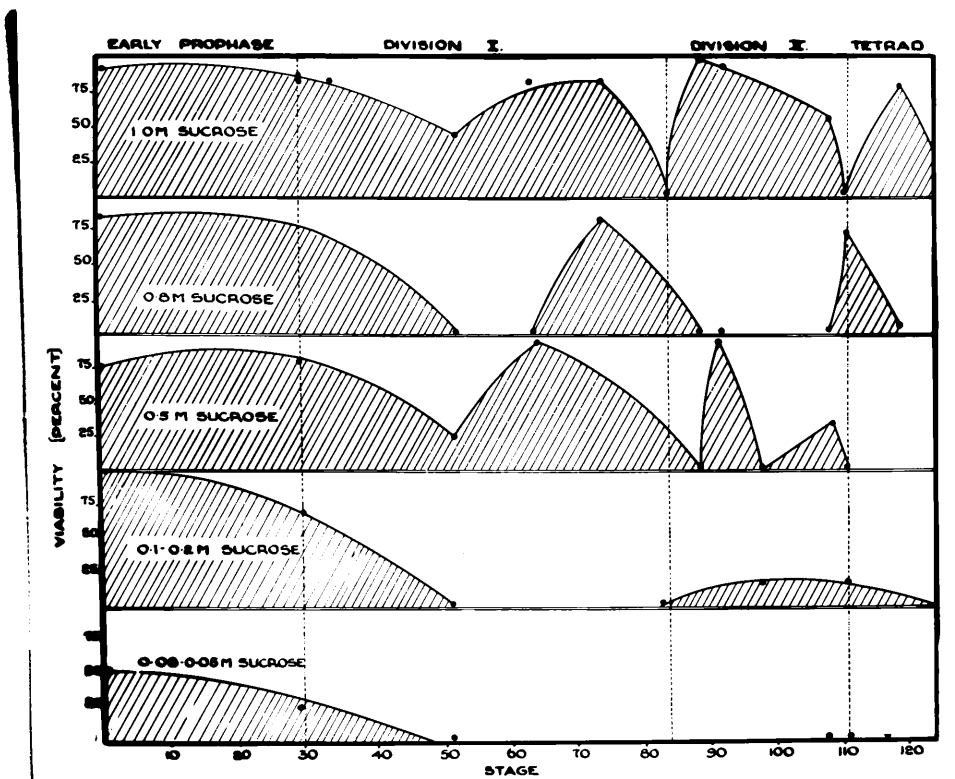


Figure 4. Viability of pmc's of Trillium in solutions of sucrose after twenty-four hours immersion. Shaded areas represent viable cells.

meiotic process.

Factors in PMC Stability:

(a) Sucrose. One feature of pmc behaviour in sucrose media is the relation of viability to the concentration of solute employed. It is apparent on examination of Fig. 4 or Table II that the differences associated with stage of division are superimposed on differences associated with sucrose concentration. The higher concentrations of sucrose are clearly more effective in maintaining cellular viability. In fact, at low concentrations there is relatively little viability at any stage. The effectiveness of high molarities of sucrose is evident not only in the aggregate counts but also in the lesser degree of

cellular granulation, there being an inverse relationship between refractivity and sucrose concentration, at least within the range of molarities investigated. That this relation is not a simple function of osmotic factors is clear from the absence of plasmolysis in prophase cells immersed in hypertonic solutions, and from the higher viability of plasmolyzed tetrads over non-plasmolyzed ones.

(b) Exosmosis of Solutes. Because of the aggregation of pre-leptotene pmc's into one gelatinous mass, it is possible to perfuse such cells without their being washed away. This condition was taken advantage of in investigating the factors governing stability. By perfusing a mass of cells for a sufficient length of time (see "Materials and Methods" for account of technique, p. 20) the exosmosed cellular products are removed and the effects of their removal can be observed. Pre-leptotene and probably leptotene pmc's are completely viable when immersed in sucrose solutions of molarities ranging from 1.0 to 0.5. If, however, they are first perfused with these solutions and then suspended in the corresponding medium, distinct morphological changes occur. The rapidity of such changes is governed chiefly by the concentration of sucrose employed, molarities of less than 0.1 having an almost instantaneous effect. Concentrations between 0.5M and 1.0M do not produce any appreciable changes until two hours after perfusion, but for longer periods of time the cells invariably show progressive changes from the A type through B to D. Complete mortality is reached within twenty hours.

The removal of intra-cellular solutes which have migrated into the medium is suggested by the behaviour of the gelatinous mass. In all cases the cells at the periphery of the mass react first. These change from type A to B and finally to D. Moreover, cells nearest the source of the perfusion current react before those located distantly from it.

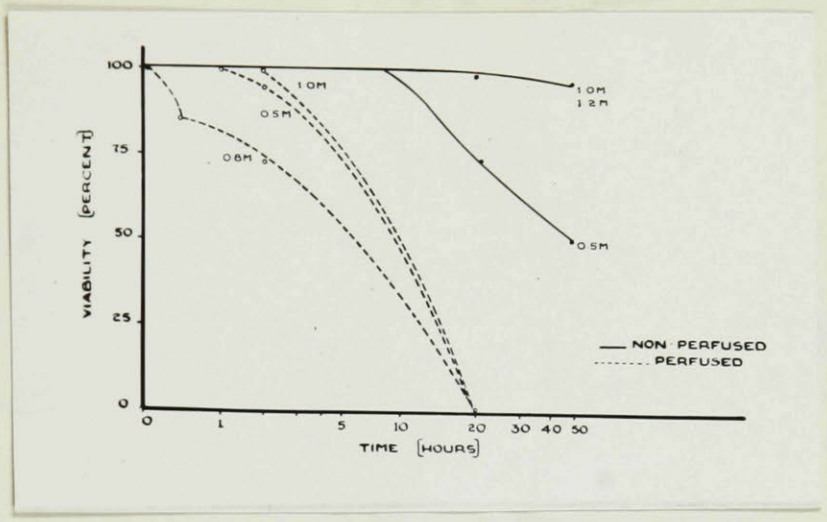


Figure 5. Viability of pmc's of Trillium in perfused and non-perfused sucrose media. All cells are in pre-leptotene stage.

TABLE III

Behaviour of Perfused and Non-Perfused PMC in Sucrose Solution. (Pre-leptotene; 30/9 - - 19/10).

Conc.	Sucrose Treatment	Prep. No.	Viability				
		Hours	0	0.25	0.50	2	20
0.5M	Perf.	13	100	100	100	95	0
"	Non-Perf.	4	100	---	---	100	75
0.8M	Perf.	14	100	100	89	75	0
"	Non-Perf.	6	100	---	---	100	60
1.0M	Perf.	8	100	100	100	--	0
"	Non-Perf.	10	100	---	--	--	90

The effects of the treatment proceed inward with time until, eventually, all pmc's are similarly affected. Simultaneous reaction of the entire mass occurs only where effects of the medium are drastic, such as those of very dilute sucrose solutions.

The results (Fig. 5 Table III) indicate, furthermore, the importance of solutes other than sucrose in maintaining cellular stability. Since the experiments performed are based mainly on the behaviour of pmc's in media of pure sucrose, it is clear that this procedure can be used to study only a limited number of aspects of physiological behaviour. The effects of various ions and pH's are undoubtedly of importance, but only a brief survey of their behaviour has been possible.

(c) Salts. Solutions of pure salts are definitely injurious to the pmc's. (See Table IV) In presence of sucrose, addition of salts in concentrations of .1M or less, to the medium produces no visible degeneration. Whether there is an increased viability, has not been determined. Certainly balanced solutions of ions should have a stabilizing effect.

(d) pH. The effects of pH were studied in late diads and tetrads. Since both these stages are unstable in pure sucrose solutions they were very suitable for purposes of this investigation. Quantitative estimations of viability in acid media were not made inasmuch as preliminary experiments showed all low pH's to exert a deteriorative influence on the viability of pmc's. This was first made apparent in some exploratory work on the metaphase I cells of Trillium grandiflorum. Survival in sucrose solution was only possible by addition of alkali; addition of acid to neutral solution increased the degree of coagulation. A similar behaviour was found to occur in some experiments (unreported) on the culture of anthers "in vitro". Examination of anthers retained

TABLE IV

EFFECTS OF $CaCl_2$ AND $NaCl/CaCl_2$ ON VIABILITY OF PMC'S.

(Pre-leptotene)

Sucrose conc.	$CaCl_2$ Conc.	Viability				
	Hours	0	2	10	20	45
---	0.4M	0				
0.4M	0.2M	---	0			
0.6M	0.1M	100	--	0		
0.8M	0.01M	100	100	100	100	100
	$NaCl/CaCl_2$ (9:1)					
0.8M	0.005M	100	--	--	75	--
"	0.01M	100	--	--	76	71
"	0.1M	100	--	--	90	85
0.7M	0.1M	100	95	--	91	84

for four weeks in artificial media showed that the more alkaline cultures permitted the most rapid division as well as the least injury.

Similar to its effect on other plant cells (See Scarth and Lloyd, 1930), the presence of alkali decreases the refractivity of the Trillium pmc. Since there is no break in the tendency towards optical homogeneity with increasing alkali concentration, it is occasionally difficult to differentiate injured from non-injured cells at higher normalities. The use of Neutral Red was therefore helpful, as the cytoplasm of dead cells stains deeply whereas that of living ones does not. The dye, however, was not used universally, since being an ionizable solute, it too affects

protoplasmic stability. It was therefore used to check counts, and fortunately, the results both with and without Neutral Red corresponded.

The results tabulated in Table V and illustrated in Fig. 6 show clearly the influence of alkali on cellular vaibility. The effects of other factors already discussed, such as concentration of sucrose and stage of development, are also demonstrated in the data obtained. In similar solutions of alkali and sucrose the viability of diads is consistently greater than that of tetrads. A parallel situation exists where the alkali concentration is kept constant, for here 0.5M sucrose is clearly more stabilizing than 0.1M. The latter is vividly demonstrated by comparing individual cells in different preparations. When several suspensions are made of one anther, the plasmolyzed tetrads found in the higher concentrations of sucrose are more stable than the non-plasmolyzed ones at lower concentrations, showing, at least, the absence of direct relation between internal vs external osmotic pressure and cellular stability.

The pH of the suspensions were not those of identical NaOH concentrations in distilled water. They were, in fact, considerably lower as revealed by occasional measurements with indicators. It is clear, however, that alkaline pH's are responsible for increase in cellular viability. The curves in Fig. 6 suggest, at least, a rapid rise in viability above a certain pH. Were the experiments more extensive it would have been possible to trace a more accurate relationship between the two factors. It is significant, though, for the present, to point to the effectiveness of alkaline media in promoting stability.

TABLE V

BEHAVIOUR OF TMC IN ALKALINE MEDIA

Plant No.	Conc. Sucrose	Normality NaOH	- log. Conc.	No of Cells Counted	% Viable
		TETRADS			
G	0.1M			248	5
G	.1M	.00005	431	178	1.5
B	.1M	.00005	431	570	5
H	.1M	.0001	400	758	29
G	.1M	.0005	331	296	14
C	..1M	.005	231	105	43
C	.1M	.01	200	518	85
G	.1M	.01	200	90	65
A	0.3M	.00005	431	94	50
A	"	.0001	400	151	33
A	"	.0005	331	126	71.5
B	"	.0005	331	109	41
A	"	.005	231	189	18
B	"	.01	200	300	80
		DIADS			
F	0.1M			294	4.7
G	"	0.000005	531	314	10.0
G	"	0.00001	500	205	25
G	"	0.00001	500	267	1
G	"	0.00005	431	310	6
G	"	0.0001	400	158	59
F	"	0.0001	400	167	57
G	"	0.0005	331	76	96
F	"	0.001	300	294	87
G	"	0.001	300	224	80
E	0.3M			263	17
E	"	0.00001	500	146	16.5
F	"	0.00001	500	476	24
F	"	0.00005	431	626	44
E	"	0.0001	400	203	37
F	"	0.0001	400	367	65
F	"	0.0005	331	344	85
F	"	0.001	300	376	84
F	"	0.005	231	90	92

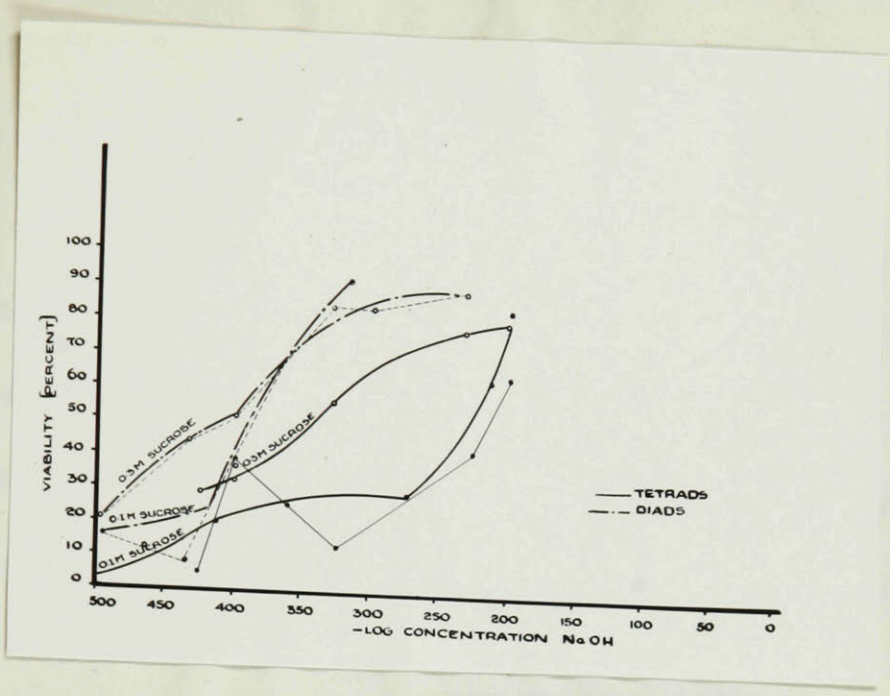


Figure 6. Viability of pmc's of Trillium in alkaline media after one hour immersion.

Discussion

Although under the conditions of the experiments the property studied in this section is, in fact, the apparent viability (or reciprocally, moribundity) of the cells, the idea of stability is preferred to viability for several reasons. Cells left in their normal environment are perfectly viable and the association of meiosis with a cycle of changing viabilities is strictly speaking, incorrect, since under natural conditions no such change occurs. Furthermore, some of the experimentally induced abnormalities used in classifying the cells might have been reversible if the cells could have been returned to their normal environment. Hence, while such abnormalties were considered as indicators of a decreased cellular stability, the potential viability of the cells was in no way altered. The term viability, though used interchangeably with stability in reporting the experiments, is actually too inadequate in its meaning to represent the phenomenon observed.

The argument for the conception of general stability is that with the progress of the meiotic cycle there is a parallel variation in resistance to a diversity of abnormal conditions, both chemical and physical. Such resistance stems, no doubt, from the peculiar structural associations, molecular and micellar, within the living cell. Yet, inasmuch as present techniques exclude the possibility of limiting intracellular variables to a single factor, the characterization of the resistance described must be limited to some general descriptive term, namely, stability. This does not, of course, exclude speculation as to the nature of the intra-cellular change based on analogies with "in vitro" data. The results, in fact, point to known mechanisms and will therefore be discussed in the latter part of this section. Meanwhile, stability, as used in this discussion. means the resistance of the cell to change under any set of abnormal conditions.

That the stability of dividing cells is lower than that of non-dividing ones is suggested by the results of various workers. Kuhn (1937) found that pollen cells burst in solution irrespective of the osmotic pressure of the medium, pointing therefore, to conditions of stability different from that found in normal non-dividing cells. Shimakura (1937) observed that the pmc's of Trillium Kamtschaticum were abnormal when immersed in sucrose media. Eker (1937), Sax and Swanson (1941), as well as many others, found that dividing cells were more susceptible to the action of x-rays than resting ones. Steinitz (1944), in studying the effect of low oxygen tension on pmc meiosis, concluded that meiotic cells were more sensitive to oxygen deficiency than mitotic ones, and that the latter were more sensitive than resting cells.

Whether it is the cytoplasm or nucleus which is the less stable during meiosis cannot be deduced from the data obtained. Certainly, the

effect seems general. Although the disintegration of the cytoplasm proceeds more rapidly than that of the nucleus when either is exposed directly to a sucrose solution, the immediate gelation of the nucleus upon slight mechanical disturbance, or its sensitivity to dilute concentrations of salt (See Section I), suggests an equivalent instability of both zones. It is more likely than not, however, that changes in one region of the cell, particularly degenerative ones, affect the viability of the other, so that isolation of unstable zones is not always possible.

The nature of conditions under which instability is identified will of course partly determine the type of cycle observed. In view of the diversity of external factors employed - x-rays, heat and cold, oxygen tensions, non-physiological media - deviations from the cycle reported here are to be expected. Also, the criteria used to characterize instability are of importance. The biological effects of x-rays, for example, deal chiefly with the problem of mutation. Thus, Sax and Swanson (1941) classified induced changes as either "primary" or "secondary", the secondary grouping including all chromosomal aberrations, and the primary, which they did not study, all the other aspects of protoplasmic change. The use of particular cellular organs or regions as means of identifying change introduces, therefore, an additional source of variation.

Although variations in type and degree of instability are probable in view of the differences in external factors, it is instructive to compare the results obtained here with data from other workers. The conception of cyclical change was, to the author's knowledge, first forwarded by Lyons (1902) who claimed the presence of a cycle of susceptibility to heat and cold in the fertilized eggs of the sea-urchin. Herlant (1920) discovered a similar cycle in relation to the action of

salts. Less direct evidence is obtainable from the work of Shimakura (1934). In attempting to promote division of Tradescantia pmc's in artificial media, he found that only those cells which had already reached the metaphase stage and plasmolyzed in solution would divide. Cells in earlier stages were found injured. Such results suggest, of course, a period of low stability extending until, or partially into, the metaphase stage. The work of Gregory (1940) points to similar conclusions, in that only those Lilium anthers excised at or beyond the diplotene would permit a normal division of the pmc's in artificial culture media. (Unlike Shimakura, Gregory placed the entire anther in the culture solution.) Steinitz (1944), using chiefly chromosome abnormalities as indicators of pmc sensitivity to lack of oxygen, suggests the prophase to be the most sensitive of all mitotic or meiotic stages.

Sax and Swanson (1941) find that the sensitivity of cells to chromosome breaks reaches a maximum at mid-prophase. Marshak (1939) found the maximum number of chromosome abnormalities at pachytene. Whiting (1939) suggests that condensed chromosomes are more sensitive to x-rays than diffuse ones. Eker (1937), in a more comprehensive study of the male germ cells of the grasshopper Tachycines asynamorus, points to various degrees of sensitivity. He considers the anaphase stage to be highly resistant to radiation, while late prophase and metaphase to be most sensitive. His results are in accord with other investigations that the resting stage is the least sensitive. Both Eker (1937) and Stone (1939) show that x-rays exercise a "blocking effect" on the germ cells in the resting stage. Stone suggests that a "physiological reaction" is set up which is inhibitory. Eker, after more careful investigation, shows that such a period is followed by one of abnormally rapid division.

In view of the sources of variation, the similarity of results

obtained in this investigation with those of other workers strongly support the thesis of a stability cycle. The suggestion of a mid-prophase drop in stability is maintained by much of the x-ray data, as well as the metabolic data of Steinitz (1944). The instability of the stage at or about metaphase is evident from the results of Shimakura (1954) and those of Eker (1957). The peculiarities of tetrad behaviour are supported by the data of Lewis (1942). Clearly, the changing stability of the pmc is a real physiological characteristic of meiosis. The nature of the change will be considered briefly below.

The data accumulated on the behaviour of pmc's in suspensions of sucrose or other solutes point to the presence of denatured protein as the principal cause of cellular instability. It is, of course, difficult to assign a specific cause to so broad a phenomenon as cell stability, even more so since denaturation itself is a very comprehensive term including any one or more of several known characteristic changes. The 'in vitro' evidence for decreased solubility and increased susceptibility to electrolytic action along with other physico-chemical properties of the denatured protein suggest, in view of the similar changes found within the cell, that a denaturation of the protein substrate is associated with the observed drop in cell stability.

(1) It has been shown by means of perfusion that the drop in cellular viability can be partly accounted for by the exosmosis of cell solutes. Such a mechanism, however, cannot account for the differences in viability under the conditions of observation. The changes, for example, in percentage viability after the cells had been immersed for twenty-four hours in sucrose solution are hardly due to differences in permeability, since the effects of such differences would be nearly

equalized after so long a period of time. Moreover, the coincidence of high permeability with high viability in the pre-leptotene of meiosis rules out any positive correlation between low stability and solute exosmosis. It is apparent, therefore, that the changes in pmc stability are due, not to the entry or exit of solute by virtue of which coagulation of the protein might occur, but to the changes within the protein molecule itself which has rendered it less soluble and therefore less stable. This conclusion is emphasized by the rapid coagulation of the pmc in tap or distilled water and in pure or mixed solutions of salts.

(2) The parallel behaviour of pmc and protein extracts in sucrose solutions suggests, as in the previous case, protein solubility to be a factor determining the viability of the cells. Though the literature on sugar-protein relationship is by no means extensive, there is sufficient data to establish the more obvious effects of the sucrose molecule. Lidforss (1907), for example, showed that precipitation of egg albumin when frozen in salt solution could be prevented by addition of 10% sugar. Similarly, Newton and Brown (1931) found that sucrose acted as a protective agent against precipitation of proteins from press-juice of leaves. To the writer's knowledge there is no adequate explanation of the mechanism. Northern (1940), in discussing the effects of sucrose of Spirogyra, suggested that it acts on the secondary linkages of the proteins, but such a view is purely speculative. It is apparent, however, that the presence of sucrose does inhibit protein precipitation. This, in fact, is the most likely factor determining the increased stability of the pmc when immersed in high sucrose concentrations. Osmotic pressure or internal solute concentration, as has been mentioned earlier, is hardly possible as an explanation, since hypertonic solutions of sucrose, where such were possible to determine, showed greater stability

than iso- or hypotonic ones.

(5) The effects of alkali on the viability of pmc's is equally, if not more instructive. Not only are the higher pH's associated with a larger percentage of viable cells but the stage of meiosis is an important limiting factor. While the prophase cells are stable in pure sucrose solution, tetrads are generally unstable so that the presence of alkali at the end of division is much more effective. A comparison of the viability of diads and tetrads in Fig. 5 indicates the same trend. Since alkali acts to prevent a coagulation of the protoplasm which is rendered less stable in the course of meiosis, the significance of alkali activity may be sought in studies of protein extracts. Mirsky and Anson (1936) claimed denaturation to be reversed by means of alkali, though Neurath et al (1944) questioned the completeness of the process. However, the increase of iso-electric point in denatured protein is accepted univer-sally, so that where the pH of a solute is on the alkaline side of its IEP, addition of a base would prevent its precipitation. This, in fact, is suggested as the principal effect of alkali when added to a pmc suspension.

It is difficult, to obtain any unqualified proof for the presence of denatured protein. Chiefly, because denaturation itself provides so many alternative changes that a fixed characterization is impossible. The essential feature of change in molecular shape is of no direct value since such changes vary in magnitude (so does denaturation) and detection of the spatial pattern is only possible by means of x-ray analysis, a technique hardly applicable to living cells. The importance, however, of resolving the intra-molecular changes in biological systems cannot be over-estimated. Extraction techniques alone, or even 'in situ' chemical tests, will nor furnish the complete answer. For where living

cells are no longer in a physiological environment it can be assumed that deteriorative changes, however small, have already taken place. And while it is difficult to resolve the nature of such changes under physiological conditions, it is at least possible to detect them with greater accuracy. This has been the approach in the reported experiments. The suggestion of denaturation as the cause of the observed behaviour came only from analogies with the more controlled "in vitro" systems.

The role of denaturation may also be extended to the process of cell-division. The existence of a protein substrate in the chromonema to which are attached various reactant or prosthetic groups, particularly nucleic acids, is accepted universally. From a molecular viewpoint, the somewhat clumsy structure of the long polypeptide chains and the interaction of atomic forces produce configurations as yet incompletely resolved. It has, however, been suggested (Wrinch 1936; Gulick 1939) that polypeptide chains, in order to duplicate themselves, must assume longitudinally extended configurations even if partial. Such stretching of the molecule is associated with denaturation (Bull 1938, Mirsky 1941) a phenomenon which has both chemical and physical implications. Chemically, the exposure of the SH groups has been considered by Rapkine (1931) to be an important metabolic factor in cell-division. Physically, the changes in viscosity and hydrational property (Schmidt, 1938) may be responsible for the microscopically visible alterations in chromosome morphology. The increase, for example, in chromonema length and the suggested spiralization within a pellicle (Huskins 1942) points, among other things, to hydration as a likely factor. Thus, it is not inconceivable that denaturation is the hub of widely divergent phases of mitosis.

Both the physiological significance and the mechanism of pmc instability leave several alternative possibilities. The low viability of the cells immersed in artificial media suggest the more detailed problem of associating a particular region with a particular degree of susceptibility. Or, and this is the more likely situation, of characterizing a certain stage of meiosis by sensitivity of a certain organ and correlating these localized sensitivities with the entire mitotic process. The general deterioration observed is hardly convincing evidence for universal instability. Mechanical injury at any point in the surface is sufficient to destroy the whole cell. So are the formation of hyaline vesicles. (Surface changes will be treated later). The high passive permeability to polar solutes is also effective in decreasing cellular viability. There are, indeed, many sources of change which can affect the stability of the pmc. And, there is little doubt that all such mechanisms are active at one time or another in the course of meiosis. The organization of the cell suggests limiting but not single factors of operation. The pattern of metabolic activity is hardly a function of a single phase system. The long polypeptide chains folding into characteristic configurations, and exposing now some metabolically active groups, now others; altering steriod patterns to conform with changing requirements of primary and secondary linkage resonances, while simultaneously attracting or repelling water molecules and ionic complexes; releasing polar groups and associating with apolar ones, thus acquiring new surface properties and creating new interfactial conditions; strengthening or weakening the tertiary linkages binding one protein complex with another, and thereby withdrawing from or associating with the different colloidal phases; all these point, indeed, to the simultaneous operation of many factors. Changes in stability represent only one characterization,

and at that, a composite one. The significance of cellular equilibrium in relation to mitosis will become clearer only as the many component changes are more fully resolved.

III. PERMEABILITY

The importance of permeability in biological phenomena is suggested by the increased permeability of cells in resisting frost, drought, and infection by rusts (Scarth 1939, 1944). Recent metabolic studies have indicated that it is also associated with actively growing tissues (Prevot and Steward 1936, Steward 1937) and with tissues subjected to anaerobic conditions (Brauder, Brauner, and Hanson 1940). In view of the metabolic and structural changes involved in mitosis it is not surprising, therefore, to find that distinct differences in permeability are also associated with division. Lyons (1902) originally suggested an increase in the permeability of fertilized marine eggs. McClendon (1915), investigating the outward diffusion of ions, claimed a rise in the permeability of frog's eggs either after fertilization or after electrical stimulation. Heriant (1920), in a more extensive study, pointed to an increased permeability in the eggs of the sea-urchin to salts and other substances. He also suggested a cycle of change consisting of an interval of high permeability at prophase, followed by one of low permeability extending for the remainder of division and interrupted only by a brief interval of high permeability at telophase. Lillie (1916, 1917, 1918) suggested an increase in water permeability after normal or artificial activation of sea-urchin eggs. He estimated, in fact, the water permeability of fertilized eggs to be four times as great as that of unfertilized ones. Faure- Fremiet (1925) discussed the above results more fully, though in his own investigations of Sabellaria eggs he was unable to demonstrate permeability changes as distinct as those found by Heriant. Spek (1918, 1920) demonstrated an increased permeability to both water and salts in Nereis eggs following fertilization. Stewart

and Jacobs (1952) found fertilized eggs of Arbacia to be more permeable to ethylene glycol than unfertilized ones, though they could detect no corresponding difference in Asterias eggs. Shapiro (1941) examined the Chaetopterus egg and found an increased water permeability after fertilization.

Despite the above data, the extent to which permeability has been considered as an important aspect of division may be judged from more recent criticisms. Heilbrunn (1951) dismisses permeability as an activating factor, a judgement which is probably valid, but in reviewing the entire subject of division he does not refer to permeability in any other capacity. Schrader (1944), though considering physiological factors such as hydration and viscosity in his very thorough review of mitosis, does not include permeability in the discussion of mitotic mechanisms. To the writer's knowledge, the only recent treatment of permeability in relation to division has been by Bujard (1941). Why so important a physiological property should be neglected may be due to the lack of sufficient evidence. The rapidity of the mitotic process in most cells does limit the possibilities of physiological study. The choice of Trillium pmc's is therefore very fortunate, since the period of division is exceptionally long and the physiological changes are much easier to observe.

A. General

More than any of the other physiological properties of mitosis, the plasmolytic changes in pmc's of Trillium are the most outstanding and, probably, the most unique in the behaviour of plant cells, at least as far as records known to the writer reveal. The absence of any plasmolysis in pre-leptotene cells immersed in sucrose solutions of concentrations as high as 1.5M by volume, the equivalent of 66 atmospheres of pressure, is

in itself indicative of the very unique plasmolytic properties at meiosis. That this phenomenon is due largely to the high passive permeability of the cells rather than to osmotic factors, emphasizes even more the distinctive physiological characteristics of nuclear division.

The evidence for the possession of so high a degree of permeability is broad, and can be found in the peculiar behaviour of a variety of solutes. Traces of salts ($AlCl_3$, $CaCl_2$ - - See Section I) which penetrate normal non-dividing cells slowly, if at all, diffuse speedily through the pmc membrane. Their rapid penetration is easily verified in Tradescantia pmc's where the salts produce distinct refractive changes. Even the large organic molecules of the sulfonthalein dyes, ordinarily impermeable (Collander and Holmstron 1951), and usually applied to the cell interior by micro-injection (Chambers and Pollack 1927), penetrate readily into the pmc's of either Tradescantia or Trillium. The high passive permeability of the meiotic cells is demonstrated by adding a drop of the indicator (Brom Cresol Green, Brom Cresol Purple, Cresol Red, Methyl Red) to a sucrose suspension. If, in fact, the drop is applied at the cover-slip edge, the advancing wave of dye is seen to be followed by a rapid staining of the protoplasts. The presence of the indicator in the cell interior may be further verified by altering the pH of the medium, the cell colour changing as the electrolyte diffuses through the solution. The passage of solute through the membrane is not restricted, however, to an inward migration, for there is an outward one as well. The deterioration in viability of cells suspended in a flowing rather than a static medium (See Section I) indicates clearly the absence of a strong barrier to solute escape.

Parenthetically, it is interesting to record the behaviour of pmc's in presence of Neutral Red, a vital stain which ordinarily accum-

ulates in cell vacuoles. Upon penetrating the pmc it stains the cytoplasm lightly and accumulates, under conditions not fully resolved, on the chromosomes. The phenomenon is complicated, though, by the gradual disappearance of the dye. Within five minutes, the chromosomes of some metaphase cells have been seen to stain gradually with increasing intensity, reach a maximum, and then fade until the chromosomes became invisible. No detailed study of the phenomenon has been attempted, although it is a rather vivid illustration, not only of the free passage of solutes through the membrane, but also of the peculiar surface properties of the meiotic cell.

Permeability vs. Osmotic Pressure.

Since there are two cellular variables determining plasmolytic behaviour, a resolution of these two components, namely permeability and internal osmotic pressure, is necessary. In presence of a high passive permeability, however, the osmotic factor is elusive and cannot be determined by ordinary plasmometric or incipient plasmolysis techniques. So rapid, in fact, is the penetration of the sucrose molecule into pre-leptotene pmc's, that no contraction of the protoplast occurs even in osmotic pressures of 60 atmospheres. Only in the later phases of division are plasmolyses observed, and even here, apart from pollen-cell mitosis, determination of the intra-cellular solute concentration is dependent upon the resolution of so many associative factors that its value is questionable.

The considerations of osmotic changes in meiosis are, therefore, essentially theoretical. Clearly, in view of the high permeability of the cells, large fluctuations in intra-cellular osmotic pressure would require parallel fluctuations in the anthral sap, and such extremes are

most unlikely. Again, were rapid changes in sap concentration a determinant factor, then the variation in plasmolytic behaviour within a single preparation would not occur. Although a gradual increase or decrease in concentration of cell-sap is possible, and even likely, it is hardly probable for large fluctuations to occur. It is suggested, therefore, that the plasmolytic changes in meiotic cells are very largely due to parallel changes in their membrane permeability. Thus, the evidence accumulated on degrees of plasmolysis are actually expressions of permeability property. This, in fact, is further supported by the data on pollen-cell behaviour where deplasmolysis time can be determined.

Meiosis vs. Mitosis:

While hypertonic solutions induce a plasmolysis of pollen-cells at almost all stages of mitosis, meiotic pmc's plasmolyze only at certain stages of division. The difference is hardly due to external factors since in both cases sucrose is used as suspension medium and molarities are the same, though a wider range of concentrations has been used in meiosis. Moreover, while the pollen-cells deplasmolyze within three hours after immersion, no such deplasmolysis has been observed in the plasmolyzed meiotic cells. Obviously, there are distinct differences in degree of permeability between the meiotic and mitotic cells. Their behaviour, however, requires clarification.

The degree of semi-permeability* rather than of permeability may be said to determine the plasmolytic behaviour of the dividing cells. Actually the property studied is not the membrane permeability to the single substance sucrose, but is the difference in rates of penetration, inward

* Semi-permeability is preferred to differential or selective permeability because: (1) It commonly refers to the penetrability of one substance, water, and the selectivity of the pmc membrane to water has been the chief factor in plasmolysis. (2) As the difference in rates of penetration increase, the cell approaches a condition of truer semi-permeability. Increase in degree of semi-permeability refers to decrease in solute/water penetration. It parallels therefore a decrease in permeability of the membrane.

and outward, between two substances, namely, sucrose and water. It would appear that this difference is almost negligible in meiotic cells. In mitotic cells, however, the differences in penetration rates of sucrose and water is appreciable, so that an initial plasmolysis does occur followed by a deplasmolysis in the same hypertonic medium. Such behaviour suggests, chiefly, a higher degree of permeability in meiotic than mitotic cells. The conclusion is further supported, though indirectly, by the fact that the high instability characteristic of meiosis is not repeated in pollen-cell division.

Why pollen-cells should deplasmolyze consistently and the fraction of plasmolyzed pmc's should not, is due to the operation of an additional intra-cellular factor. It is presumed that the osmotically active cell solutes are predominantly sugars, and compared with water, are so similar to sucrose that they may be regarded as such from an osmotic standpoint. In presence of a high degree of permeability the diffusion of solutes through the membrane would be nearly equal in either direction, limited only by the concentration gradient between cell interior and exterior. Apparently, this is of no significance in the early stages of meiosis. It is obvious, however, that there is no exosmosis of solute equivalent to the inward movement of sucrose in mitotic pollen-cells, or else no deplasmolysis would occur. The absence of deplasmolysis in pmc's is related to the exchange of solutes between interior and exterior. That such cells can be deplasmolyzed is clearly shown by adding distilled water to a suspension; the reaction is immediate. Even the presence of pure water at the bottom of a moist chamber will occasionally, though not always, produce a deplasmolysis. In absence of a more complete body of data it is suggested that, though the difference in rates of water and sucrose penetration is large enough to cause a plasmolysis, the inward and outward diffusion of osmotically active solutes are nearly equal so that no deplasmolysis of the cell occurs.

With these more general characteristics of the dividing cell in mind, it is appropriate to consider the detailed changes observed in degree of property.

B. Meiosis

Limitations:

(a) Time. As in the estimations of viability (Section II), the time intervals of examination were not constant. Viability and plasmolytic counts were, in fact, made simultaneously. Periods of immersion prior to counting ranged from 10 minutes to 4 hours (the time intervals are all recorded in Table VI.). The differences, however, are not as significant as they might appear to be. In view of the peculiar plasmolytic behaviour of meiotic cells, in most cases there is no appreciable change in the percentage of cells plasmolyzed after several hours immersion. Thus, while a greater accuracy might have been obtained by rigidly maintaining constant conditions, the latter was sacrificed to what was considered to be the more important demands of extensive inquiry. Certainly, the general features of a permeability cycle are clearly discernible.

(b) Degree of Plasmolysis. Measurement of the diameters of cells and protoplasts would have been useful in establishing the osmotic pressure of the cells. The variations, however, in size and shape of both cell and protoplast were so great that such measurements were impracticable. It was preferable to count the number of plasmolyzed and non-plasmolyzed cells so that examination of a preparation could be accomplished within a reasonable period of time.

(c) Plasmolytic Values. In estimating the "plasmolytic value" of preparations cells were classified not only into categories of plasmolyzed

and non-plasmolyzed, but also into sub-categories of "HR", "Non", and "D" (See Section II). The HR cells were included in almost all calculations. For although non-plasmolyzed HR cells indicate, at least, a lack of plasmolysis, the presence of plasmolyzed HR cells does not always indicate a low permeability. A shrinking of the cytoplasm often occurs in a moribund cell (See Plate I Fig. 4) and this is demonstrable by the failure of deplasmolysis upon dilution of the medium. When a large number of "plasmolyzed" HR cells were present, deplasmolysis tests were made, and if no deplasmolysis occurred, the cells were not included in the calculations. Fortunately there were not many such cases so that their exclusion or inclusion would have had no major influence on the trend of the results. The percentages of plasmolyzed and non-plasmolyzed are relative, as dead cells are excluded from all calculations. Whether the number of dead cells is related to the degree of permeability or to the osmotic pressure of the cell cannot be ascertained. That some relationship does exist has already been suggested, but lack of any mathematical formulation of such a relationship makes their inclusion in the plasmolytic calculations inadvisable.

The Permeability Cycle:

The results of plasmolytic tests are contained in Table VI and illustrated in Figure 7 (a, b, c, d, e, f). Average values for stages grouped according to degree of plasmolysis are listed in Table VII. In view of the variables associated with the experiments, prefixes have been used in Table VI to indicate conditions other than those generally present.

F= Preparation fixed in aceto-carmine. Meiotic value of preparation derived from frequencies of individual stages. Result compared with value calculated from the "rougher categories". (See Appendix I).

TABLE VI

PLASMOLYSIS OF PMC'S IN SUCROSE SOLUTION

CONC. = 1.2M (45.4 ATM)

Slide #	Value	Time Hrs.	Time Mins.	% Non	Rel % Non	Slide #	Value	Time Hrs.	Time Mins.	% Non	Rel % Non
W4.21.1C	F51.1	1	10	67	68	5.4.2C	104.4		12	80	81
5.20.1C	F54.5	1	15	92	95	6.4.2C	106.6	4	25	91	92
4.20.1C	F59.8	2	30	57	85						
5.20.1C	F(45.0)	3	00	71	19	5.25.1C	112.4	2	55	0	0
6.20.1C	F44.0	1	50	60	61	5.4.2C	115.5		12	33	33
5.25.1C	74.4	2	55	15	46	6.4.2C	110.5	4	25	71	12
5.25.1C	90.5	2	55	0	0	W4.25.1C	(119.5)	3	15	0	0
						2.17.2C	(154.5)		15	2	50

CONC. = 1.0M (24.6 ATM)

8.20.1C	F(45.0)		15	66	69	W2.25.1C	F56.2	1	00	44	15
W4.22.1C	45.4	2	05	86	93	W2.27.1C	60.4	1	00	80	93

Slide #	Value	Time Hrs.	Time Mins.	% Non	Rel % Non	Slide #	Value	Time Hrs.	Time Mins.	% Non	Rel % Non
1.20.1C	F43.1		20	68	69	2.20.1C	62.5	2	35	95	100
2.22.1C	44.2		55	85	90	6.9.12B	71.4	3	00	15	100
1.21.1C	F44.6		10	83	84	5.5.2C	78.5		45	70	95
1.20.1C	50.2		50	84	92	W2.22.1C	89.5		35	85	91
5.21.1C	51.3	1	35	41	82	1.20.1C	81.3		20	56	58
1.11.12B	AV54.5	1	30	51	59	1.20.1C	81.5		50	65	74
1.21.1C	F88.1		10	95	93	1.9.2C	AV111.9		30	59	62
1.11.12B	AV88.8	1	30	45	50	5.11.12B	119.2		45	1	2
2.21.1C	90.1	1	00	11	100	4.13.1C	121.9	1	40	2	12
2.20.1C	91.3	2	35	91	100	5.13.1C	122.6	2	15	0	0
5.5.2C	96.1		45	89	85	13.10.2C	125.7	2	30	11	89
8.9.2C	108.3	1	30	83	19						
1.9.2C	AV109.8		30	81	93	4.13.1C	129.4	1	40	9	11
W5.11.12B	111.5		45	0	0	5.13.1C	130.1	2	15	40	58
						13.14.1C	(134.5)		25	65	81
5.5.2C	112.4		45	95	95	14.14.1C	(134.5)		40	15	88
8.9.2C	116.3	1	30	82	83						

CONC. = 0.8M (25.5 ATM)

Slide #	Value	Time Hrs.	Mins.	% Non	Rel % Non	Slide #	Value	Time Hrs.	Mins.	% Non	Rel % Non
5.27.1C	91.8		15	81	84	W 6.27.1C	88.1		25	100	100
W 6.27.1C	92.3		25	86	91	12.19.1C	91.9		30	91	91
12.19.1C	84.1		30	81	95	W 2.5.2C	AV 95.1	1	0	90	99
1.5.2C	AV 79.1		20	66	94						
						1.5.2C	AV 94.0		20	81	95
5.27.1C	88.0		15	45	80	9.10.2C	107.2	1	0	85	89
W 2.10.2C	108.0	1	0	51	91	W 2.5.2C	AV 115.0	1	0	76	100
11.10.2C	111.5	2	0	0	0	9.10.2C	110.9	1	0	58	60
						W 2.10.2C	111.0	1	0	49	11
1.5.2C	AV 112.0		20	82	88	11.10.2C	119.0	2	0	5	21
						12.14.1C	(134.5)		20	98	100

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CONC. = 0.5M (14.5 ATM)

1.27.1C	F55.1		30	95	91	5.13.1C	98.0	1	0	18	59
W 8.27.1C	F50.0		50	92	94	2.13.1C	99.0		55	10	40

Slide #	Value	Time Hrs.	Time Mins.	% Non	Rel % Non	Slide #	Value	Time Hrs.	Time Mins	% Non	Rel % Non
8.19.12B	(30.0)	3	30	89	99	W8.10.2C	99.9	1	10	90	100
6.19.12B	(30.0)	3	30	98	100	9.10.2C	107.9		40	96	98
6.19.12B	(30.0)	4	30	100	100	7.10.2C	110.2	1	0	100	100
3.11.12B	91.0	1	49	81	91						
9.19.1C	(63.8)		10	99	99	3.11.12B	111.8	1	49	19	100
						9.10.2C	110.9		40	93	99
3.11.12B	88.6	1	49	19	100	1.13.1C	117.9		20	0	0
9.19.1C	(91.7)		10	99	99	7.10.2C	118.6	1	0	89	96
W8.10.2C	118.6	1	10	90	100	9.14.1C	(134.9)		35	100	100

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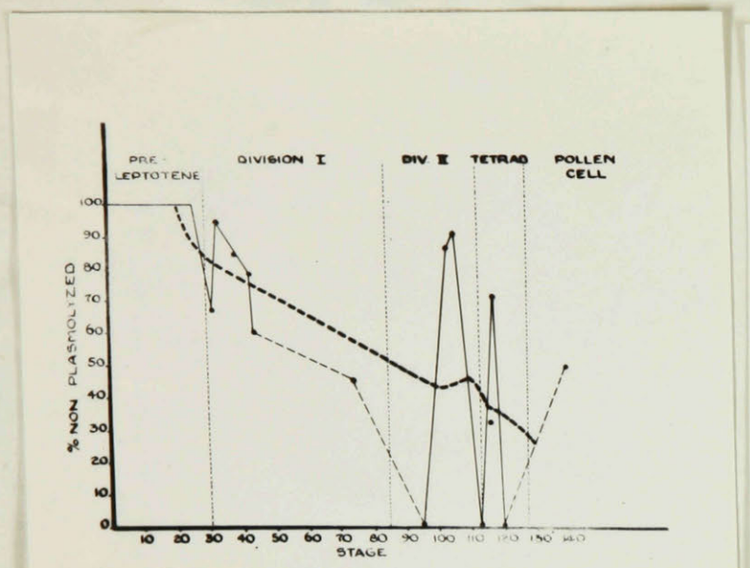
CONC. = 0.2M (5.3 ATM)

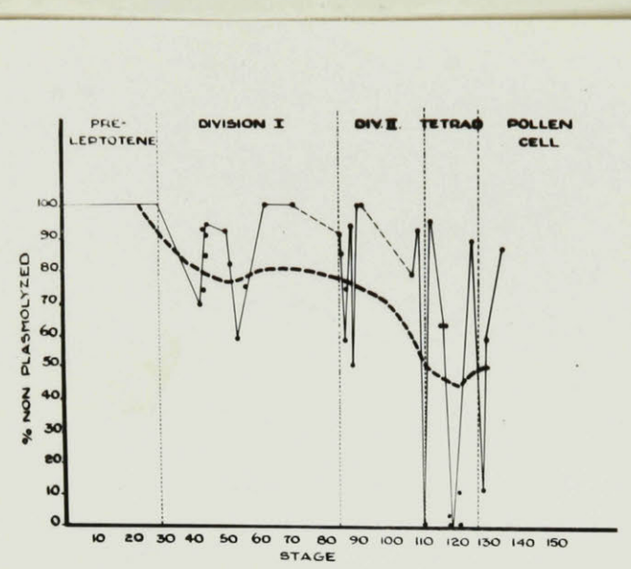
15.27.1C	F(30.0)		30	93	100	11.27.1C	88.9		20	79	100
W12.27.1C	F(30.0)	1	05	92	100	1.11.12B	93.0		45	80	100
W10.27.1C	F(30.9)	1	05	91	100	2.12.1C	98.1		40	94	89
1.28.12B	(33.0)		20	100	100	3.12.1C	98.1	1	0	49	89

Slide #	Value	Time Hrs. Mins.	% Non	Rel % Non	Slide #	Value	Time Hrs. Mins.	% Non	Rel % Non
2,258,128	55.0	50	100	100	4,12,16	98.9	1 50	21	80
4,268,128	(55.0)	50	100	100	1,12,16	109.1	25	44	100
5,288,128	(55.0)	5 15	82	100					
2,11,128	48.2	5 50	90	100	1,11,128	115.0	49	0	0
11,21,16	54.8	20	88	100	1,12,16	115.1	25	11	29
1,11,128	59.0	49	85	100					

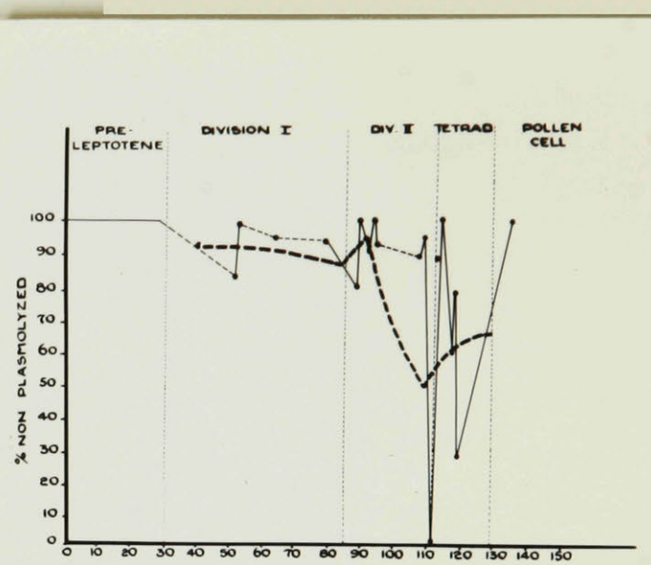
Fig. 7. PERMEABILITY CHANGES IN PMC'S OF TRILLIUM
(PURE SUCROSE MEDIA)



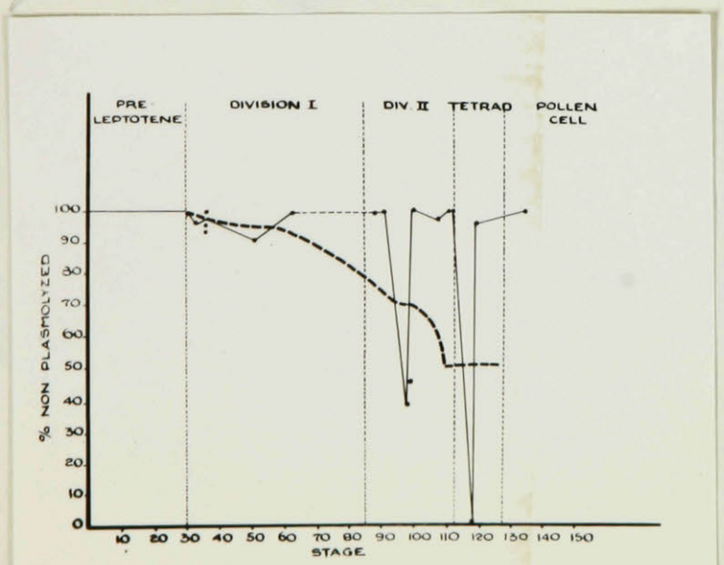
(a) 1.2M



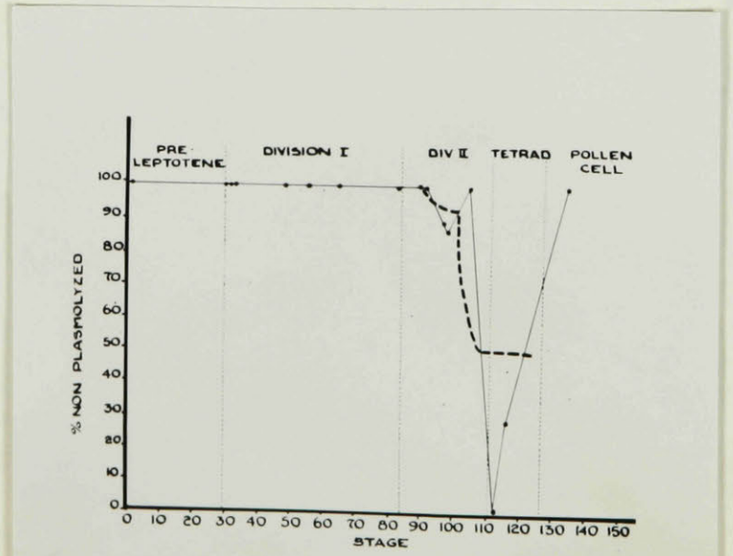
(b) 1.0M



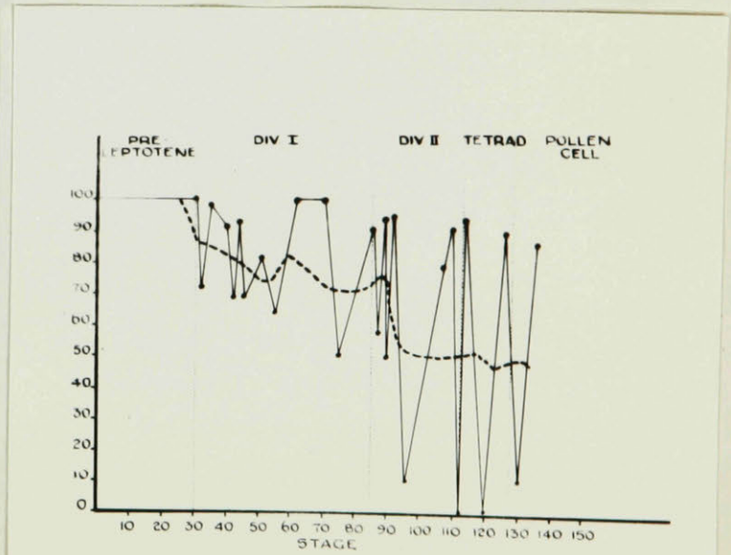
(c) 0.8M



(d) 0.5M



(e) 0.2M



(f) combined results. 1.0M used as basic trend

TABLE VII

PLASMOLYSIS OF PMC'S IN SUCROSE SOLUTION (AVERAGE VALUES)

% NON-PLASMOLYSED (RELATIVE)

	Conc. / Stage	1.2M	1.0M	0.8M	0.6M	0.4M
	0-30	100	100	100	100	100
	30-33	68	-	-	-	100
	34-43	85	86	-	97	-
FIRST	43.1-44	61	69	-	-	-
FIRST	44.1-53	-	87	90	91	-
FIRST	54-55	-	59	-	-	100
	56-57	-	75	-	-	-
	59-64	-	97	95	99	100
	74-75	46	-	-	-	-
	78-79	-	93	94	-	-
	85-86	-	91	-	-	-
SECOND	81-81.4	-	58	-	-	-
SECOND	81.5-90.1	-	83	92	99	100
SECOND	90.5-99	0	-	-	45	88
	100-110	90	86	90	99	100
	111-111.5	-	0	0	-	-
	111.8-112	-	-	88	100	-
TETRAD	112.4-116	16	95	100	-	15
TETRAD	116.5-118	72	62	68	50	-
TETRAD	118-119	-	-	-	98	-
TETRAD	119-154	0	30	27	-	29

AV= Difficulty in distinguishing between 1st division cells and diads (occurred occasionally). Several counts were therefore made and the results averaged.

()= Only 1st division cells present but fixed slide so poor that only a rough estimation of stage was possible.

w= Pure water and not solution present at bottom of moist chamber. The reason for this departure from the usual procedure has already been discussed.

A cursory examination of the data might suggest that an almost random variation in the degrees of permeability is associated with meiosis. The numerous fluctuations are, however, more regular than they first appear to be, and their reality may be judged from two particular conditions: (1) That the differences in degree of plasmolysis are distinct. Though many preparations show intermediate values the more homogeneous ones, such as pre-leptotene or late tetrad cells, show nearly all or none reactions. (2) That the presence of several meiotic stages within a single suspension would be expected to promote different degrees of plasmolysis. Such behaviour, in fact, strongly supports the conclusion of a permeability cycle. What appears to the writer as the most crucial problem in resolving permeability is a more rigid control of meiotic stage which would permit a clearer correlation between morphological and physiological behaviour.

(a) General. The heavy broken lines in Fig. (join the mid-points between the extreme permeability values and so represent the over-all trend in meiosis. The trend is significant to the extent that it indicates the decreasing degree of permeability with the progress of meiosis, a behaviour paralleled in 1st and 2nd division, though it is

obvious that the fluctuations observed are not included in the over-all trend. Such fluctuations are, of course, important in determining what degree of property is associated with a given morphological stage. It can be seen, moreover, that their amplitude increases with the progress of meiosis, a phenomenon significant in indicating the changing physiological properties of the dividing cell. What emphasizes even more the significance of the variations in permeability is the parallel behaviour of pmc's in different concentrations of sucrose, the only difference being that of an average decrease in degree of plasmolysis with increasing dilution of the medium.

(b) Meiosis I. In tracing the cycle of permeability, evidence is available from observation of fixed preparations which is helpful in correlating morphological stage with degree of physiological property. It has been mentioned earlier that the determination of stage by fixation was not always reliable since, often, cytoplasmic distortion was extreme leaving few cells with recognizable stages. There have been, nevertheless, a sufficient number of preparations to indicate the probable relation between meiotic development and permeability value.

The first observed decrease in permeability occurs beyond the pre-leptotene of meiosis (30-33). 32% of the cells are plasmolyzed and the preparation (4.2/.1C) contains 11% diakenesis, 5% metaphase, 84% leptotene-pachytene. Whether there is an early drop in permeability is difficult to judge from the single preparation available. There is, however, a far more definite change between stages 43 and 44. Here several slides have been examined to indicate the trend. Preparation 6.20.1C (1.2M) contains 39% plasmolyzed cells, showing 50% diakenesis and 49% metaphase. A suspension in a slightly earlier phase of meiotic development (5.20.1C - 41% diakenesis, 25% metaphase) contains only

21% plasmolyzed cells. Pmc's immersed in 1.0M sucrose behave similarly. Where 42% of the cells are in diakensis, 30% in metaphase, and 1% diads, 51% of the 1st division cells are plasmolyzed. The decrease beyond this point in the number of cells plasmolyzed (16%) is indicated by slide 1.21.1C. Here, 11% of the preparation is in 1st division, and of this category 17% is diakenesis and 66% metaphase. The distribution of stages in the above preparations points to a pronounced change in degree or permeability somewhere between diakenesis and metaphase. Moreover, it is clear that the metaphase stage is not associated with one particular physiological property but with, at least, two degrees of the same property. This phenomenon, in fact, is emphasized by observation of individual preparations where some metaphase cells are plasmolyzed while others are not. An even more vivid demonstration is possible by using Neutral Red, though in this instance, factors of viability are also involved. Some metaphase cells remain nearly white in a solution to which Neutral Red has been added, the whiteness being accentuated as the concentration of Neutral Red is increased.

The increased degree of permeability persists until stages 54-55 where 41% of the cells are plasmolyzed. No fixation of these preparations was possible so that the only indication of actual stage is obtainable from a suspension of pmc's in a later phase of meiotic development (56.2-2.25.1C, 1.0M) with 25% of the cells plasmolyzed. 62% of the 1st division cells are in anaphase, 24% in metaphase, the remainder in Diakenesis-leptotene. It is probable, therefore, that a change occurs somewhere between metaphase and anaphase. The only other observed drop in permeability prior to completion of the 1st division cycle is at stages 74-75 (5.25.1C - 1.2M), where 54% of the cells are plasmolyzed. There are no data, however, for a more precise correlation.

There are at least three (possibly four) points of change in degree of permeability during the 1st division of meiosis. The degrees of plasmolysis at these points (43-44, 54-55, 74-75) show an increasing intensity from 31-39% plasmolyzed to 41%, and finally to 94%. While one change is most certainly located between diakenesis and metaphase, the others may be less clearly assigned to the metaphase-anaphase stage and the final phase of the cycle.

(c) Meiosis II. It is more difficult to follow the permeability cycle in diads than in 1st division cells. This results partly from the shorter duration of stages, and partly from the increased instability making reading of fixed slides nearly impossible. Though fixation after a few hours of immersion may have yielded better results, all preparations, it will be recalled, were kept for at least twenty-four hours before being discarded. Thus, it was necessary to base all estimations of meiotic development on the frequency of categories present (1st div., diads, tetrads). No attempt is made, therefore, to associate the cycle with morphologically defined stages.

It is apparent that the behaviour of diads parallels somewhat that of 1st division cells. A decreased permeability occurs just beyond diad formation (87-87.4) where 42% of the cells are plasmolyzed. This change is comparable to the diakenesis-metaphase one in 1st division, in view of the absence of interkinesis between the two cycles. An even more pronounced drop in permeability is located at stages 96-99, where pmc's in 0.2M sucrose show a 12% plasmolysis. It is probably valid to draw a parallel between this and the metaphase-anaphase of 1st division. As in Meiosis I, the greatest drop in permeability occurs just before completion of the cycle. In concentrations of either 0.8M or 0.9M the plasmolysis of pmc's at this stage (111-111.9) is 100%.

The formation of tetrads cannot be properly compared to any stage in the 1st division cycle since, as has already been noted, there is no completion of division between the 1st and 2nd phases of meiosis. Thus, the low permeability characterizing tetrads may be regarded as a feature of the termination of active division. There are exceptions to this property in some tetrad preparations, but it is difficult to draw conclusions from early phases of tetrad development since only a few tetrads are present. Furthermore, the low viability of tetrads (Section II) complicates such counts even more. Generally, it may be said that the tetrad stage is one of low permeability, a condition in marked contrast to the pre-leptotene of meiosis where osmotic pressures greater than 60 atmospheres produced no plasmolysis at all. The separation of tetrads into individual pollen-cells (154.5+) will not be considered, since the plasmolytic properties of the pollen cell were not studied extensively. Investigation of the interval between meiosis and mitosis is limited to the period preceding active mitotic division when physiological changes are apparent.

C. Mitosis

Pre-mitotic pollen-cells are fairly uniform in behaviour when suspended in sucrose solutions. Generally, they are not unstable and little or no degeneration is visible even after several hours immersion. In fact, the high instability characteristic of meiosis is not found at any stage of mitosis, except for the period of transition from grouped tetrads to single pollen-cells when hyaline vesicle formation is common. The absence of rapid deterioration in a suspension facilitates the study of plasmolytic changes. Of equal value is the sphericity and thick wall of the pollen-cell making plasmometric measurement easy. The only

limitations are, as in meiosis, stage heterogeneity and unequal cell-size during active division, so that upon initiation of prophase the plasmometric technique is no longer applicable.

Osmotic Pressure:

The more extreme changes in osmotic pressure occur during the pre-mitotic period. Though it is difficult to trace the increase in concentration of cell sap in association with mitotic development because of the absence of morphological change, an alternative method is possible. The osmotic pressure of pollen-cells in individual anthers can be measured at different time intervals, and, if comparisons are restricted to anthers removed from the same plant, it is valid to draw certain conclusions. That this method is by no means without possibilities of error is evident from the variation in degree of development between anthers of the same plant. In plant 5 (Table VIII), for example, the pollen-cell diameter is smaller in the anther removed four days later. Although cell size is not rigidly correlated with degree of development, the heterogeneity even in a single flower is obvious.

Calculation of osmotic pressure in interkinetic cells is simple. The cells are fairly uniform, and measurements of a relatively small number of cells is necessary, the number depending on the degree of variation within the preparation. The maximum difference between the two extremes in any one preparation was one unit. Thus, by measuring the internal diameter of the cell (dB) and the diameter of the protoplast (d_A) the osmotic pressure of the cell (OP_c) may be calculated, since the osmotic pressure of the solution (OP_s) is known.

$$OP_c = \frac{d^3_A}{d^3_B} OP_s$$

TABLE VIII

OSMOTIC PRESSURE CHANGES DURING PRE-MITOTIC PERIOD OF POLLEN-CELL DIVISION

Plant No.	Time of Examination	dA	dB	d^3_A / d^3_B	OP_c (atm.)
2	24/3	12.3	13.2	.3302	14.24
2	29/3	13.0	13.2	.0291	21.04
3	20/3	11.0	13.0	.3001	12.43
3	30/3	14.0	13.23	.1813	21.03
1	26/3	11.0	13.3	.3373	12.30
1	2/4	13.0	11.3	.0314	22.33
8	26/3	12.3	11.0	.3413	13.13
8	2/4	13.0	11.0	.0803	23.16

The initiation of active division is accompanied by several factors making the plasmometric technique undesirable. Permeability increases so that the diameter of the protoplast changes as the cell deplasmolyzes. Since measurement of individual cells requires an appreciable period of time, the method is not applicable. Moreover, the increasing diversity in cell-size adds to the difficulty of the technique. In view of these factors, incipient or near-incipient plasmolysis was studied. That is, observation of degree of plasmolysis was made immediately upon immersion of the cells in solution. Though subject to error, this appears to be the most reliable method.

The increase in osmotic pressure preceding active mitosis is clearly shown in Table VIII. The difference is very marked so that even an increased permeability in the latter phases of interkinesis would hardly account for the change. That the internal osmotic pressure at the end of the resting stage is about 23-34 atmospheres is supported by incipient plasmolysis tests carried out on actively dividing cells. The values are listed in Table IX, and it can be seen from an examination of the data that little change occurs in the osmotic pressure of the cells during division. The trend alters only at the conclusion of division where there is a drop from 23.3 to 21.3. The extent to which this trend continues has not been checked by further experiments.

Permeability

The physiological activity of pre-mitotic pollen-cells is demonstrated not only by the changes in internal osmotic concentration but by the increase in permeability as well. In early interkinetic phases deplasmolysis was not observed in hypertonic sugar solutions, although both urea and glycerol diffused through the membrane. Deplasmolysis time in 1M urea solution ranged from 20 minutes to 0, the

TABLE IX

OSMOTIC VARIATIONS DURING THE MITOTIC CYCLE

(Medium - 0.8M Sucrose)

Plant No.	Plasmolytic state	Stage Prophase	Metaphase	Bi-nucleate	Mitotic value	Approx. Isotonic conc.	Approx. O.P. in atm.
19A	P_s	10.1	2.4	10.2	.8554	0.15M suc.	23.44
18	P_s	38.4	6.4	17.0	.2816	0.15M suc.	23.44
18	P_s	31.0	5.5	17.2	.3220	0.15M suc.	23.44
18	P	15.9	4.95	15.5	.8900	0.1M suc.	21.49
18	P	6.2	1.38	91.8	.9662	0.1M suc.	21.49

P_s = slight plasmolysis

P = definite plasmolysis

increasing OP of the cells undoubtedly being a factor. At a later phase, the cells become permeable to glucose so that deplasmolysis time can be clearly measured. At this point, however, it was possible to make a more precise study of permeability in relation to morphological development.

The results obtained on the deplasmolysis time of pollen-cells are listed in Table X and illustrated in Figure 8. In view of the heterogeneity of the cells, it was found advisable to measure 90-100% deplasmolysis time rather than 50%, for at some stages nearly half the cells deplasmolyzed immediately. The complications as to stability of cytoplasm do not occur here as they did in meiosis, since no deplasmolysis exceeded six hours, and that, in fact, was infrequent.

1.25M glucose was used along with its osmotic equivalent 1.0M sucrose. The results obtained from a comparison of the behaviour of the two solutes support the hypothesis previously made, that the size of solute molecule is an important factor determining the different patterns of plasmolytic behaviour between meiosis and mitosis.

As in meiosis, there is a high increase in permeability during prophase. Though the high permeability appears much later in mitosis than in meiosis, it is interesting to observe that the sharpest change in property occurs just before or at the metaphase stage. This, in fact, has been the stage (Diakenesis-Metaphase) at which the pronounced variations were observed in the meiotic cycle.

TABLE X

PERMEABILITY CHANGES DURING THE MITOTIC CYCLE

Plant No.	Plasmolyzing solution	Stages (%) Prophase	Metaphase	Bi-nucleate	Stage value	Time for 100% deplasmolysis
16B	1.0M Sucrose	0	0	0	0	620
16B	" "	0	0	0	0	540
1/A	" "	0	0	0	0	275
1/A	" "	0	0	0	0	260
1/C	" "	0	0	0	0	260
1/B	" "	1	0	0	.6	180
1/B	" "	1	0	0	.6	180
15	" "	15.2	21.6	5.8	.29	155
18	" "	47.2	5.5	2.8	55.5	90
1/B	" "	66.0	1.03		40.4	85
1/B	" "	66.5	7.7		46.1	immediate (1-10 min.)
18	" "	51.4	18.0	18.0	65.2	" "
1/B	" "	46.0	20.4	25.0	67.0	" "
19A	" "	4.0		90.0	92.4	120
19A	" "	2.42	1.22	96.5	98.9	60
1/A	1.5M Sucrose	0	0	0	0	600
1/C	" "	0	0	0	0	550
1/B	" "	1	0	0	.6	255
5	" "	1.1	6.6	4.95	10.9	260
18	" "	51.6	6.25	14.2	50.2	225
1/B	" "	76.2	2.6	5.2	51.0	85
1/B	" "	54.0	15.3	15.5	59.9	immediate
19	" "	3.56	2.14	90.0	93.5	180
18	" "	1.7	8.5	88.2	96.0	120
16B	1.25M Glucose	0	0	0	0	420
16B	" "	0	0	0	0	400
1/A	" "	0	0	0	0	155
17C	" "	0	0	0	0	100
1/B	" "	76.2	2.6	5.2	51.0	20

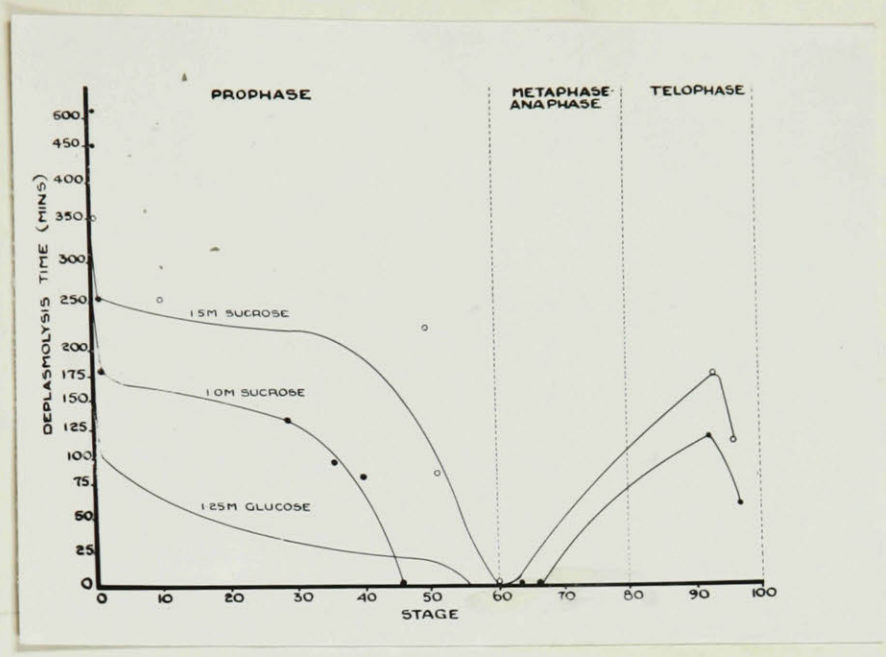


Fig. 8. Changes in permeability of Trillium pollen-cells during Mitosis

Discussion

The unique function of semi-permeability in limiting the passage of substances either into or out of the protoplast poses, of course, the question as to what role the considerable loss of this property plays in division. Obviously, it is difficult to assign some universal function to a property which occurs under a diversity of conditions. The organic environment of the anther in which pmc meiosis proceeds is not strictly comparable to the inorganic environment of fresh- or sea-water where algal filaments or marine eggs divide. To extend the high passive permeability characteristic of Trillium pmc's to the mitotic cells of other biological forms would be, unless qualified, a dubious association. To deny such association, however, would be equally invalid, in view of the evidence obtained both here and in the results of other workers.

In resolving the function of an increased permeability, the

principal question arising is whether the phenomenon acts as a causal factor or whether it is simply a by-product of mitosis. Heilbrunn (1937) believes, at least, that a higher degree of permeability in no way stimulates division. The lack of stimulating effect does not rule out, however, the possibility that increased permeability does contribute to the mitotic process by permitting a more rapid penetration of metabolites, organic and inorganic. Such a function is very probable in view of the evidence previously reported that increased metabolism is associated with a higher permeability, and that the division of a cell involves a higher degree of metabolic activity. Division necessary substances - a very general term including most any constituent of living matter - are clearly not limited to organic materials. The calcium ion, as shown by Sorokin and Sommer (1940), is necessary for normal mitosis. It is also important in the formation of enzymes (McDonald and Kuntz 1941). If then, cell-division does require an increased rate of solute penetration, it is equally applicable to all forms, marine or land. The function, therefore, of permeability in cells developing in an organic environment is paralleled by cells exposed to an environment such as sea-water. What distinguishes the different cells is the duration and degree of permeability, rather than the more general property of an increase in permeability.

A few examples may be drawn from the literature to illustrate the presence of a higher permeability in dividing cells. Shimakura (1934) found that 15% of metaphase I Tradescantia pmc's did not plasmolyze in hypertonic saccharose solution. He also observed that a deplasmolysis occurred in glucose media. Yet, he interpreted the behaviour as pathological, suggesting that the permeability resulted from an injury of the membrane by the sucrose. Apart from what seems to the writer to be

an error of interpretation, the peculiar sensitivity postulated should have merited some special consideration. Unfortunately no such analysis was made. (The reason for division of only plasmolyzed cells has already been suggested - Section II.) The results of Kuhn (1937) also point to the mechanism of permeability. He concluded that the failure of pollen-cell division, either in solution or even on thick layers of gelatin, was due to the loss of "division necessary" substances. Since the latter term might include anything that would affect the viability of the cell, it is safe to presume that the increase in cell-permeability was the chief reason for loss of substance. The very same reasoning is applicable to Shimakura's (1937) observation that pmc's of Trillium Kamtschaticum were somewhat abnormal when immersed in sucrose solution.

Though the relation between permeability and stability is deferred to the more general discussion, it is important to remark on the time-factor in permeability. It is clear that, apart from other factors, the amount of solute diffusing through the membrane is a function of time as well as degree of permeability. Thus, even where degrees of permeability are nearly equal, the inward or outward passage of solute will be very small if the interval of high permeability is of short duration. The differences in stability between Tradescantia and Trillium pmc's are explicable on that basis, since the meiosis of the former is much more rapid. To a lesser degree the lack of extreme injury in mitosis can be interpreted as partly due to the shorter interval of high permeability. While the time-factor in no way necessitates a reinterpretation of the permeability phenomenon, it does explain the lack of uniformity in the behaviour of different mitotic cells, in view of the variation in duration of mitoses.

From the accumulated data it is difficult to make any definite

generalizations concerning the physiological differences between meiosis and mitosis. If a comparison of pmc's and pollen-cells is at all representative, it can be said that meiosis involves a higher degree of permeability than mitosis, and that, probably, the rise in permeability occurs earlier in the meiotic cycle. Insofar as degree of property is concerned, it is probable that it is generally greater in meiosis, though the writer has found little in the literature to corroborate that view. As to the relation between the physiological and morphological cycle, associations are less apparent because of the differences in time and form of the divisions. The mitotic cycle proposed agrees largely with Heriant's (1920) observations on marine eggs, suggesting a decrease in permeability after prophase and a rise with telophase, though the data obtained from studies of Trillium pollen-cells point to a mid-mitotic increase earlier than that shown by Heriant.

IV. SURFACE ACTIVITY

The experiments reported here deal briefly with two types of abnormal behaviour which have been observed in Trillium pmc's suspended in sucrose media. These are the formation of hyaline vesicles and of multi-nucleated pmc's. Though generally, neither of these phenomena occur under natural conditions, apart from the presence of certain genic or extreme environmental factors, their production in artificial media is of interest in the physiology of meiosis. For their occurrence suggests a marked surface activity in association with nuclear division and, in view of their restriction to certain stages of the meiotic cycle, suggests, more specifically, a change in the surface energy of the cell.

Hyaline vesicles are simply protoplasmic extrusions which become completely spherical, detach themselves from the protoplast, and float about in solution. A detailed study of their formation in Paramecia has been made by Bungenberg De Jong and Hartkamp (1938). A parallel phenomenon has been found to occur in cells undergoing mitosis. Lewis (1942) summarizes the evidence for the formation of "blebs" in fibroblasts and sarcoma cells in the anaphase and telophase stages. Though unlike hyaline vesicles in their effects on cell viability,-the formation of vesicles results in a killing of the cell - they are very similar in their development. The chief difference is, of course, the extrusion of the vesicle out of the protoplast; the mechanical separation of protoplast and bleb does not occur.

The formation of polynucleate pmc's has genetic as well as cytological implications. A study of the phenomenon yields, therefore, not only information on the physiology of meiosis, but also on conditions, artificial or natural, responsible for the production of polyploid

progeny. The cytogenetic interest in the problem is suggested by the work of many investigators. De Mohl (1929), for example, made a comprehensive study of meiosis in the tulip, and concluded that the formation of diploid and tetraploid pollen grains was due to the failure of wall formation. Reeves (1928) reported a similar condition in some pmc's of Zea Mays. Beadle (1932) traced the phenomenon in Zea to a recessive gene which caused a failure of cytokinesis when homozygous. Lebedeff (1940) was able to reproduce the effect by heat treatments. A different explanation was advanced by Levan (1941) who believed that the formation of polynucleated pmc's in Phelum pratense was due to cell fusion rather than to a failure of wall formation.

Hyaline Vesicles:

(a) Period of Formation. Vesicle formation occurs most abundantly in the tetrad stage. Within this stage it is restricted chiefly to the period of transition from grouped tetrads to individual pollen-cells. It has also been observed, though rather infrequently, in diads. It would seem, therefore, that the production of hyaline vesicles is associated with the termination of the division cycle, though it may also occur at the end of meiosis I.

(b) Conditions of Formation. No particular treatment is required for the production of vesicles apart from the immersion of cells in sucrose media. Addition of salts or alkali do not inhibit their formation, though, unlike the effects of the solutes on Paramecia (Bungenberg de Jong and Hartkamp 1938), they do not promote vesicle formation at stages other than those listed. The process is rapid and is complete several minutes after immersion of the cell in the medium. Because of its rapidity, it is difficult to trace the events preceding

the formation. In some cases protoplasmic currents have been observed converging at a point where the vesicle forms. These currents are very similar to the "lesion currents" described by De Jong and Hartkamp (1938). Such observations have been too few, however, to justify any general conclusions.

(c) The Nature of the Vesicle. The vesicles are formed outside the cell-wall which is fairly thick at the tetrad stage. They are clearly the products of an exudation of protoplasmic fluid since any "pinching-off" process is hardly possible, and where observation was made, the spherules could be seen to grow in dimension. Detachment of the vesicles may occur without use of any external agent, but they are readily removed by the aid of micro-needles. Their fluidity is easily demonstrated by micro-manipulation, and if some granules are present, by vigorous Brownian movement.

It is not certain that the fluid forming the vesicle is cytoplasmic in origin. The disintegration of the cytoplasm in pre-leptotene and prophase pmc's suggests the alternative of nuclear origin (See Plate I, Fig. 6). Moreover, unstable pmc's, when injured by aceto-carmine, exhibit a nuclear rather than a cytoplasmic flow (Plate I, Figs. 7, 8, and 9). Further, though limited, evidence is obtainable from the deep staining of some vesicles in aceto-carmine. However, the lack of identity between 1st division cells and tetrads, and the very few observations of vesicle staining, prohibit any conclusions as to zone of origin.

Polynucleate PMC'S:

(a) Period of Formation. Although formation of multi-nucleate cells has been observed only from pre-leptotene to diakenesis,

it is difficult to determine the stages to which the process is restricted in view of the increasing instability of the pmc with the development of meiosis. It can be said, however, that the formation of such multi-nucleated cells does occur from pre-leptotene to diakenesis. Whether other stages possess similar tendencies cannot be determined from the data obtained.

(b) Conditions of Formation - External. PMC'S suspended in pure sucrose media often lose their walls after a period of time varying from twelve hours to several days. The removal of the external cell-wall is more pronounced in pre-leptotene cells since there is generally little cell-wall at later stages. If again, the cells are aggregated in a mass, as they are in the pre-leptotene of meiosis, they gradually loosen themselves from it, assume a completely spherical shape, and float about in solution (Plate II Fig. 1). The concentration of sucrose employed seems to have no particular influence, polynucleate cells forming in media ranging from 0.5M to 1.5M. Addition of $CaCl_2$ NaCl, and NaOH, or mixtures of these, in no way inhibits the process, if anything, the salts promote it. The latter conclusion, however, would require a much more detailed study.

(c) Conditions of Formation - Internal. The production of multi-nucleated pmc's is due to fusion of the cells in sucrose solution. Since the process is rather slow it has been observed and photographed in sucrose media (Plate II Figs. 2,3,4, and 5). The number of fusions possible appears to be unlimited though, actually, cells have not been observed with more than about 32 nuclei. The resulting giant cells are completely spherical and their volumes are roughly proportional to the number of nuclei present. For example, in one preparation the diameter of a uni-nucleate cell measured 20.5, and that of a bi-nucleate cell

measured 26. The ratio of their cubes is 8615/17,576, which roughly represents a volumetric ratio of 1:2. The relation is general.

Apart from the absence of an external cell-wall, a fluidity of the cytoplasm appears to be essential in view of the completeness of the fusions and the sphericity of the products. The cytoplasmic fluidity at these stages has already been demonstrated by micro-manipulation (Section I). The primary reaction, however, occurs between the membranes of the fusing cells. This is strongly suggested by the dispersion of the cytoplasm where any rupture occurs in the memorane. Where destruction of the membrane takes place, as in some poorly viable preparations, only the nuclei remain coherent (Plate I Fig. 6), so that the fusion of cells by membrane rupture appears unlikely. What particular property of the membrane is responsible for the reaction is not evident from the results obtained.

DISCUSSION

In a very general way, the tendency of early meiotic cells toward fusion, that is, to decrease surface area, and the tendency of tetrad cells toward hyaline vesicle formation, that is, to increase surface area, point to an over-all decrease in the surface energy of the cell. The changes in surface energy, often local rather than general, have been discussed by Faure Fremiet (1925). He stresses the very obvious difficulty of measuring actual surface tension of a cell, since among other things, protoplasmic cohesion is a factor. There is an agreement among some workers, however, that the average surface tension of the cell does decrease with the development of the division cycle (See Faure Fremiet 1925). This conclusion is doubted by Faure Fremiet

who believes that the question is of a theoretical rather than of a practical nature in view of the techniques of measurement. The observations recorded here, nevertheless, support the conclusion of decreasing surface energy, although the evidence for it is negative and hence, limited. The fusion of cells would be expected wherever naked protoplasts exist without a peripheral gel layer. The argument based on fusion, therefore, requires no assumption of increased surface energy. So that, the remaining point of reference is the production of hyaline vesicles only at the telophase, a phenomenon probably, though not necessarily, implying a decreased surface tension. The relation of the change to other physiological factors will be discussed later.

The production of polyploid pollen, either by physical or genetic agents, is, in some cases, certainly due to a fusion of the cells. Although Levan (1941) found the multi-nucleated protoplasts to appear like plasmodial masses when observed in fixed preparations, there is no doubt that the loss of sphericity was due to fixation. Cells with ten or more nuclei are extremely large, and are very liable to mechanical injury. Such cells invariably lose their spherical structure on staining with aceto-carmine and appear, as Levan had found, like multi-nucleated plasmodial masses. In solution, however, the polynucleated cells are perfectly spherical and show no form of intra-cellular injury.

It is probable, that failure of cytokinesis is an alternative mechanism to fusion. Physical agents, however, such as temperature (Lebedeff 1940), and oxygen starvation (Steinitz 1944), suggest a destabilization of the membrane and a decrease in consistency which seem to be a precondition of fusion. Clearly, the low stability of pmc's would result in a high sensitivity of the cells to external agents. This in fact, would partly support the conclusions of De Mol (1929):

"What we suppose is this: by certain treatment, chemical or physical, changes may happen which can create a sensitiveness such as to be sure of the origination of diploid and tetraploid pollen grains at a certain temperature during meiosis". The correction to be applied to De Mol's conclusion as suggested by the results obtained would be, that a sensitiveness already exists by virtue of the intra-cellular changes associated with meiosis.

DISCUSSION (GENERAL)

The most striking physiological characteristic of mitotic phenomena is the cyclical pattern of property change. In no other aspect is there so close a parallel obtained as between the corresponding stages in morphological and physiological development of the dividing cell. Though the degree of permeability and the peculiarities of stability point, in contrast with non-dividing cells, to the unique physiological properties of mitosis, the pattern of fluctuations in stability and permeability is by far the most important feature of cell-division. Upon identification of the various cycles of change- morphological, physiological, chemical- and their integration, depends the ultimate resolution of the mitotic process. That physiological cycles can be shown to exist simplifies the problem of division.

If, as has been suggested, the greater stability of the pre-leptotene cell compared to the other stages of division is due to a corresponding lack of denatured protein, then, among other changes, an increased hydration of the protoplasmic proteins is to be expected. Not only are native proteins generally more hydrated (Schmidt 1938), but the increased stability alone suggests a greater hydration of the labile protein fraction, in view of the behaviour of colloids in relation to hydration. Such a condition is, in fact, suggested by the work of Gustafsson (1939) who believed hydration to be necessary for chromosome reproduction. The refractive index studies of Freiler (1934, 1936) point to a similar conclusion, since his proposed cycle of refractive changes includes a slight lowering of the refractive index in early prophase followed by a graded increase until the metaphase. A low refractive index clearly involves an increased sorption

of water. The low refractivity of pre-meiotic or early prophase cells of Trillium immersed in sucrose media points to the same conclusion. Thus, within the limits of division - a comparison with the resting cells not having been made - it can be said that the early phases of meiosis are the more hydrated, a condition consistent with the data obtained here on the stability of the cells. Why there is no continuous rise in stability corresponding to the steady drop in refractive index following metaphase as found by Pfeiffer (1934, 1936), cannot be explained at present. In meiosis, at least, the return to greater stability occurs after tetrad formation.

Assuming that the increased hydration of the protoplasm extends to the protoplasmic surface, a very useful parallel may be drawn with plant cells which have been hardened to injury by frost. Scarth, Levitt, and Siminovitch (1940) point to an increased hydration of the membrane as, at least, partly responsible for the increased permeability to water and polar solutes. The situation is quite similar in the hydrated pre-leptotene pmc's where the permeability of the membrane is probably greater than at any other stage in the life history of the cell. It is possible, therefore, to integrate the more general features of pmc physiology. The higher stability of the prophase can be associated with the greater degree of hydration referred to in the literature and in the experiments reported here. The latter condition can, in turn, be integrated with the property of high permeability. What makes the parallel with frost resistance even more interesting is that the hardy plant cell would be expected to possess a greater stability than the non-hardy one, so that the two main features of the dividing cell, permeability and stability, obtain the same relationship between early and late meiosis as between the hardy and non-hardy states. It is clear,

of course, that the parallel cannot be extended unlimitedly. The resting cell is certainly more stable than the pre-mitotic one, though the former is the less permeable. It is important, however, to consider the special metabolic conditions of division which would clearly justify the special properties found.

The identity in protoplasmic properties between hardy and non-hardy plant cells and certain phases in meiosis is further supported by the unpublished work of Siminovitch (Scarth 1944) who found a much larger proportion of soluble protein in hardy than in unhardy cells. It has already been suggested that the higher stability of the pre-leptotene stage is associated with a greater solubility of the labile protein components. Thus, it is possible to associate a physico-chemical change with changes in physiological properties. To what extent and in what manner these diverse factors are related is hardly obvious, and its resolution is certainly beyond the scope of this work. It would appear, however, that an intimate association does exist between protein solubility on the one hand, and permeability and stability on the other.

The changes that occur in the various physiological properties during the development of meiosis may all be interrelated as above. The decrease in stability points to a drop in the solubility of the protein components. A lower solubility has already been noted to be associated with a lower permeability, both in frost hardened cells and in pmc meiosis. It also involves a decrease in hydration, a condition demonstrated by the increase in refractive index and suggested by the probability of the presence of denatured protein. The conditions responsible for changes in surface energy are less obvious. The formation of hyaline vesicles at the termination of division points to a lowering of surface tension. Apart from that, however, there is no evidence beyond the

probability of a decreased hydration of the membrane.

The evidence accumulated points, therefore, to a characteristic pattern of physiological changes in association with cell-division. It is believed, furthermore, that this pattern of physiological cycles is essentially, at least, applicable to all dividing cells, though detectability of the pattern may be severely limited by specific conditions, such as duration of stage, cell size, and the like. There is little to suppose that similar conditions do not exist in organisms other than Trillium erectum. Where references to the literature have been possible, the data, in fact, uphold the conclusions set forth.

Finally, it is emphasized that no rigid physiological characterization can be made of any artificially defined morphological stage. There is no fixed stage of division except as a reference to development in point of time. In view of the continuity of change underlying the divisional process, the physiological properties of the cell would be expected to vary according to degree of development, but hardly, not to vary within the limits of morphological stage. Thus, parallel to the cycle of structural changes there occurs a cycle of physiological changes, both cycles being characteristic of the divisional process and both being essential to an understanding of it.

SUMMARY

I. Appearance and Consistency.

(a) At any particular stage of meiosis the optical and physical patterns of the pmc are dependent on the nature of the medium employed. In sucrose suspensions it has been shown that the relation between appearance and solute concentration is not a function of osmotic pressure. The critical balance of factors associated with appearance has been demonstrated by the effectiveness of extremely dilute salt solutions applied to the pmc in presence of sucrose.

(b) The appearance in terms of refractive index changes with stage of meiosis. There is an increase from a relatively low refractive index at pre-leptotene to a relatively high one at metaphase, at which point it is probably at its maximum value.

(c) The pmc is structurally differentiated into three zones corresponding to its optical differentiation; an outer gel layer of variable thickness, and inner peripheral layer which is fluid in consistency and granular in appearance, a clear hyaline area of similar consistency and the internal nuclear zone.

(d) The large refractive nucleus examined at stages between leptotene and diakenesis is of fluid texture, even when refractive. Mechanical injury, however slight, causes a rapid contraction and gelation.

(e) There is a decrease in cytoplasmic consistency from leptotene to metaphase.

(f) At the metaphase stage mechanical disturbance of the chromosomes within the cell promotes a fusion. Withdrawal of the chromosomes into the medium causes their gelation.

(g) Tearing of the membrane results in a dispersal of the cytoplasm in the medium. Only the nuclei remain coherent. This is

interpreted in terms of differences in physical property between nucleus and cytoplasm.

II. Physiological Cycles.

Paralleling the cycle of morphological changes is a cycle of changes in the permeability and stability of the dividing cell.

A. Stability

(a) There is an over-all drop in protoplasmic stability from the pre-leptotene to the tetrad stage of meiosis. Actually, the cycle observed in Meiosis I is essentially repeated in Meiosis II.

(b) The pre-leptotene is the most stable stage. There are marked drops in stability at diakenesis-metaphase, metaphase-anaphase, the beginning of 2nd, mid-2nd, and the tetrad stage. The degree of instability at these points increases with the development of the meiotic cycle.

(c) The pattern of fluctuations does not alter if based on cells immersed for 24 hours.

(d) The following factors have been found to be associated with decreasing viability of a preparation:

(1) Exosmosis of cell solutes

(2) A decrease of sucrose concentration in the external medium. Increased sucrose concentration is associated with increased viability.

(3) A low pH. Alkaline pH's increase viability.

(4) High concentrations of salts.

It is suggested that protein denaturation is the factor responsible for the intra-cellular changes observed during division.

B. Permeability

(a) The fluctuations in degree of permeability are similar to those of stability. The pre-leptotene of meiosis is characterized by a very high degree of passive permeability to polar solutes. With initiation of active division there is a drop in value which, though fluctuating, continues that trend until the termination of division.

(b) The points of change in permeability property are presumed to occur at diakenesis-metaphase, metaphase-anaphase, and at the end of the cycle. A similar trend occurs in Meiosis II. Generally the tetrads show a low degree of permeability.

(c) A cycle of permeability change has also been found in pollen-cell mitosis. The fluctuations - if there are such - have not been traced. The permeability increases until the pre-metaphase stage, at which point it is at its highest value, and after which, there is a decrease to telophase.

C. Osmotic Pressure.

(a) Studies of O.P. changes in pollen-cells indicated a rise in O.P. preceding division, a fairly constant O.P. during division, the value dropping off toward telophase.

(b) From indirect evidence it is presumed that a similar cycle occurs in pmc meiosis.

III. Surface Phenomena

(a) Fusion of 1st division cells when immersed in media of pure sucrose or of salt-sugar mixtures in stages ranging from leptotene to diakenesis has been observed. Among the factors responsible for the fusion are the absence of any external cell-wall (generally lost while the pmc's

are suspended in sucrose) and the fluidity of the cytoplasm. In view of the physiological factors associated with pmc development, this is suggested as a mechanism of polyploid pollen formation.

(b) Hyaline vesicle formation is common at the time of tetrad separation into pollen-cells. Among other things, it is considered as probably indicative of a decreased surface energy.

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APPENDIX I

DETERMINATION OF THE DEGREE OF MEIOTIC AND MITOTIC DEVELOPMENT

It may be assumed *a priori* that the morphological changes occurring in the pmc's are associated with changes in its physiology. But the identification of a certain morphological stage in meiosis with a particular degree of a physiological property is somewhat ambiguous since, as will be shown later, any one microscopically distinguishable stage of meiosis may possess within itself different degrees of a physiological property, such as high and low permeability or high and low osmotic pressure. It is ideal, therefore, to characterize the stage of each preparation quantitatively in terms of a graduated scale of meiotic development. This is theoretically possible since with constant external factors, meiotic development may be considered as a function of time. And given the time intervals of each grossly defined morphological stage, the "meiotic value" of a preparation may be determined by appropriate calculation. To the writer's knowledge, however, no accurate determinations have been made of the time intervals of meiotic stages in *T. erectum*. Neither has it been possible, due to the tremendous variations within this species, to make any such determinations in the course of this research. Consequently, a cruder method of evaluation has been used which, though not accurate, does give an approximate scale of change.

It would have been ideal, of course, to study homogeneous preparations. Since there is a marked diversity of development within a single anther, this has been impossible. To express the physiological data as functions of morphological development it has been necessary to integrate the several mitotic stages into one single expression. It is

strongly emphasized, however, that the arithmetic calculations employed here are not intended as a means of defining more clearly a stage of development, but solely as a method of simplifying the expression for the several stages found in a single preparation.

The limitations in identifying the exact stage of a preparation have already been mentioned. It is recognized that such limitations are simply ones of procedure and could be overcome. For the present research, however, at least the following categories have been clearly identified in all preparations: early prophase, 1st division, 2nd division, tetrads, and the mitotic stages of the pollen-cells. Since one or more of these categories occur in a single preparation, a method of determining approximately the degree of development within each category is possible. That is, it can be stated in a rather arbitrary fashion whether each of the categories present is in an early or late phase of its development. The assumption involved, which is partially justified by the data obtained, is that the various stages in a single preparation are interdependent so that a high frequency of cells in an advanced category is indicative of a late phase of development in the preceding one.

This relationship may be expressed mathematically by either assigning arbitrary values to each of the categories, or by, what is considered more instructive, assigning values roughly proportional to the time intervals of these categories in meiosis. In this way the relative rates of the different stages is taken into account. For, no matter how approximate the values may be, there are more instructive as indicators of meiotic and mitotic development than an arbitrary designation of numerical values. A list of values, equal to the mean value of the approximated time interval of each category is listed in Table I.

It is necessary, therefore, to designate the degree to which the presence of one category or stage indicates an increase or decrease in the median value of another category. For example, given 85% 1st and 15% 2nd, the amount by which the 15% of the 2nd division increases the mean value of the 1st and that by which the 85% of the 1st decreases the mean value of the 2nd must be found. This is done by introducing a set of "determinants", and multiplying the percentage frequency of the 2nd division by its determinant and adding the product algebraically to the mean value of the 1st division. The values of the "determinants" are derived on purely mathematical grounds. If meiotic development is represented by a straight line, then the determinant of a category is equal to the distance between its own midpoint and the boundary of the adjacent category. Theoretically therefore, as the frequency of the adjacent category approaches 100%, the value of the first category approaches that of the boundary. Thus, where 50% of the cells are 1st division and 50%, 2nd division, the mean value of the 1st division cells is not 42.5, but 42.5+(.5 x 42.5) which equals 64, a point half-way between the mid-point of 1st division and the beginning of the 2nd. Where, however, the determinant of a category relates to a non-adjacent one, it must be increased or decreased by some factor. In the absence of any knowledge of the mathematical relations between such categories, the determinant was increased by a percentage equal in value to the determinant of the adjacent category as shown in Table I. Thus in slide 5.29.1C 25% is 1st div.; 00% 2nd div.; and 9% tetrad. The 1st division value is 42.5+(42.5 x .00) + (48.5 x .09) = 74.9; the value of the 2nd division is 98.5+15.5 (.09 - .25) = 96.5; the value of the tetrad stage is 115.5-(8.5 x .25) - (7.5 x 00) = 115.5. In the case of pollen cells the procedure is much simpler. Each stage value is multiplied by its percentage frequency in the preparation and the products totalled to give the mitotic value.

TABLE I

MEIOTIC AND MITOTIC VALUES

PMC		1st Div.	2nd Div.	Tetrads	Pollen Cells
Median value		42.5	98.5	119.5	134.5
DETERMINANT	1st		-15.5	- 8.5	
	2nd	+42.5		- 1.5	- 8.5
	Tet	+48.5 (42.5 / +42.5 x 15.5)	+15.5		- 1.5
	P.C.		+14.5	+ 7.5	

Pollen-Cell Mitosis

	Prophase	Metaphase - Anaphase	Bi-Nucleate
Mitotic value	0.6	0.8	1.0

The median or mean value is least in 1st division and highest in pollen-cells. Thus, the more advanced a preparation the greater is its total value. Such a total is arrived at by considering the percentage of each stage present in the preparation. The greater the percentage of the more advanced stages, the greater the total value of the preparation. Another corrective factor is also possible. It is clear that where a large fraction of the cells are, for example, in 2nd division that the remainder of those in 1st division are more likely than not to be more advanced than if there were few diads. In other words, the mean value of a single category, say 1st division, is alterable, the change being proportional to the frequency of other stages present.

APPENDIX II

Slide #	% Frequency of Stages			
	1st Div.	2nd Div.	Tetr.	Pollen-Cells
6-9-12B	32	68	0	0
1-11-12B	63	15	22	0
2-11-12B	81	11	8	0
3-11-12B	87	11	2	0
5.11.12B	0	4	96	0
7.11.12B	57	43	0	0
6.19.12B	100	0	0	0
8.19.12B	100	0	0	0
1.28.12B	100	0	0	0
2.28.12B	100	0	0	0
3.28.12B	100	0	0	0
4.28.12B	100	0	0	0
1.12.1C	0	51	49	0
2.12.1C	6	91	3	0
3.12.1C	3	97	0	0
4.12.1C	0	100	0	0
1.13.1C	0	21	79	0
2.13.1C	0	96	4	0
3.13.1C	0	99	1	0
4.13.1C	0	0	68	32
5.13.1C	0	0	59	41
5.14.1C	0	0	0	100
12.14.1C	0	0	0	100
13.14.1C	0	0	0	100
14.14.1C	0	0	0	100
1.15.1C	0	0	0	100
9.19.1C	50	50	0	0
12.19.1C	49	51	0	0
1.20.1C	82	18	0	0
2.20.1C	53	47	0	0
3.20.1C	97	3	0	0
4.20.1C	96	4	0	0
5.20.1C	100	0	0	0
6.20.1C	99	1	0	0
7.20.1C	83	17	0	0
8.20.1C	100	0	0	0
2.22.1C	96	4	0	0
4.22.1C	98	2	0	0
2.25.1C	98	2	0	0
3.25.1C	25	66	9	0
4.25.1C	0	0	100	0
1.27.1C	96	4	0	0
2.27.1C	58	42	0	0
3.27.1C	100	0	0	0
4.27.1C	100	0	0	0
5.27.1C	78	22	0	0
6.27.1C	77	23	0	0
7.27.1C	98	2	0	0
8.27.1C	100	0	0	0
11.27.1C	71	29	0	0

APPENDIX II (Cont'd)

Slide #	% Frequency of Stages			
	1st Div.	2nd Div.	Tetr.	Pollen-Cells
12.27.1C	99	1	0	0
15.27.1C	99	1	0	0
16.27.1C	98	2	0	0
1.5.2C	13	72	15	0
2.5.2C	1	87	12	0
3.5.2C	16	80	4	0
5.9.2C	0	53	47	0
6.9.2C	0	40	60	0
7.9.2C	0	15	85	0
8.9.2C	0	42	58	0
2.10.2C	0	25	75	0
5.10.2C	0	26	74	0
7.10.2C	0	13	87	0
8.10.2C	0	12	88	0
9.10.2C	0	35	65	0
11.10.2C	0	4	96	0
13.10.2C	0	17	83	0
2.17.2C	0	0	0	100
1.10.3C	0	0	0	100

PLATE I

Fig. 1. PMC of Trillium erectum. "A" type of cell in 0.2M sucrose.

Fig. 2. Trillium PMC in 2nd division. "A" type of cell in 0.2M sucrose.

Fig. 3. Same cell as in Fig. 2, but after some perfusion has become "B" type.

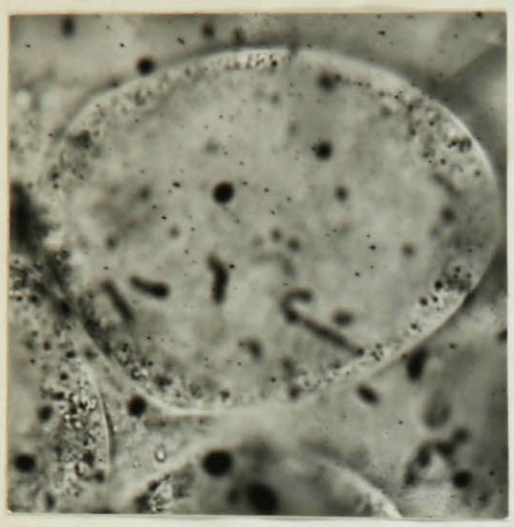
Fig. 4. "HR" cell of Trillium - 24 hours in 0.5M solution. It did not deplasmolyze when treated with hypotonic solutions.

Fig. 5. Type "D" cell in 1.5M sucrose

Fig. 6. Spherical fragments of nuclei and completely disintegrated cytoplasm in 0.5M sucrose.

Figs. 7,8,9. Fixation of unstable cells. No pressure was applied to the cover-slip on fixation. The flowing of the nuclei results from an injury rather than mechanical pressure.

PLATE 1



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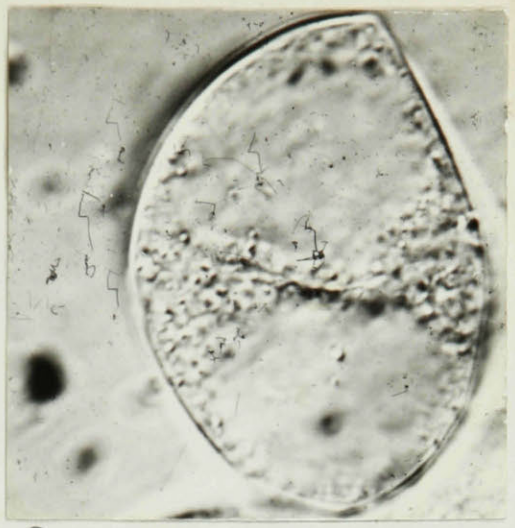

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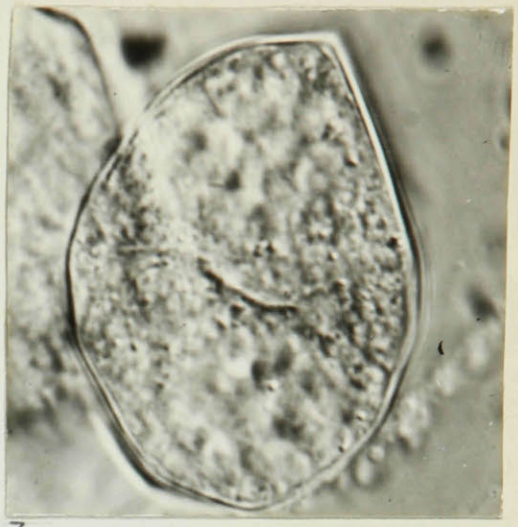

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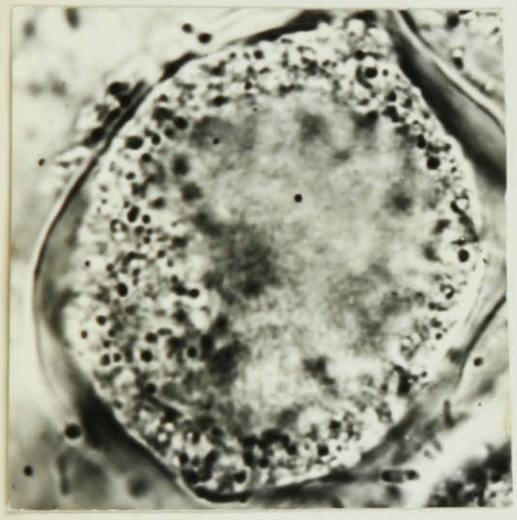

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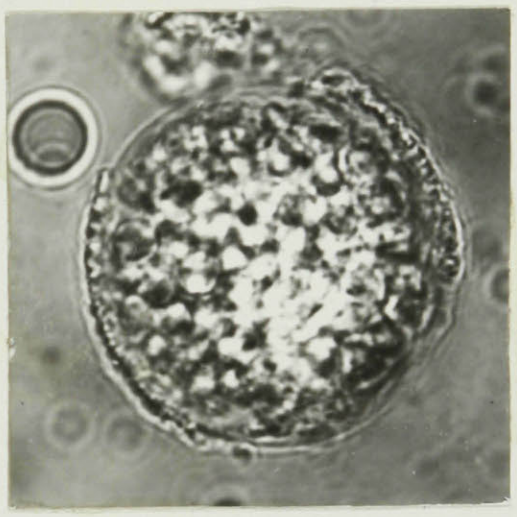

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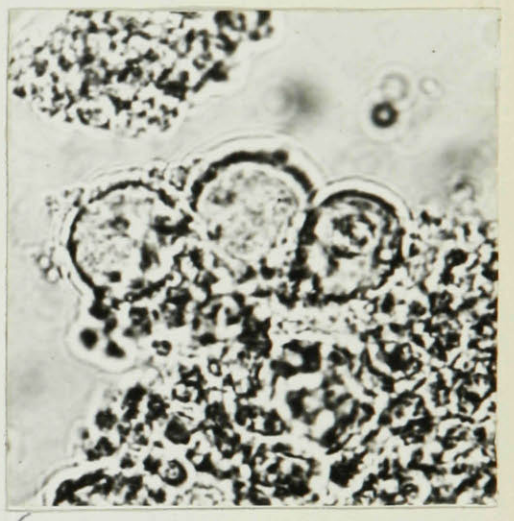

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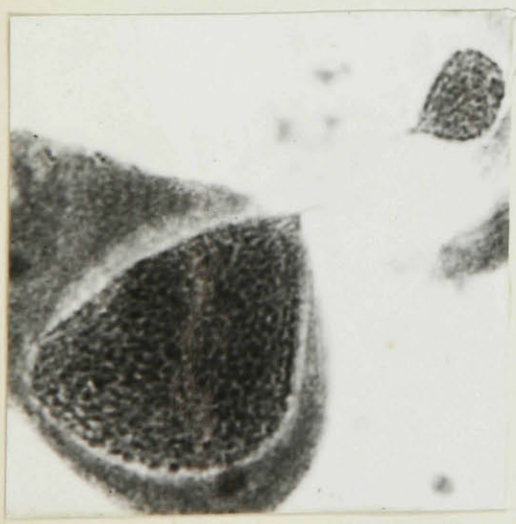

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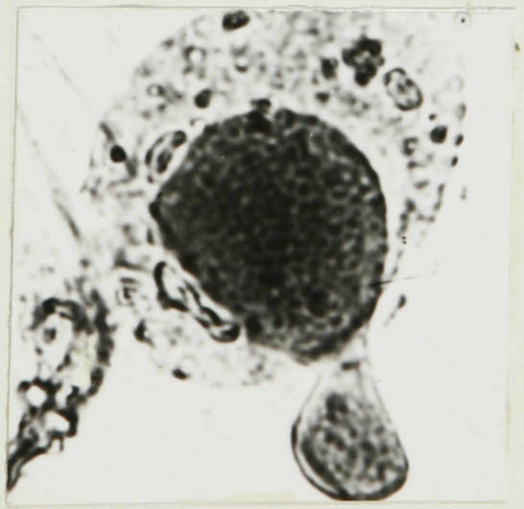

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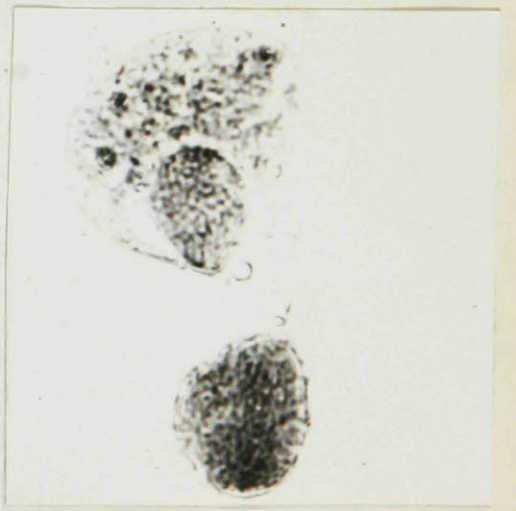

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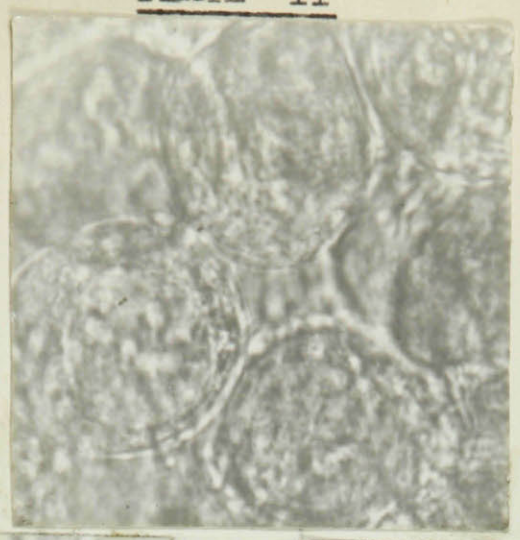
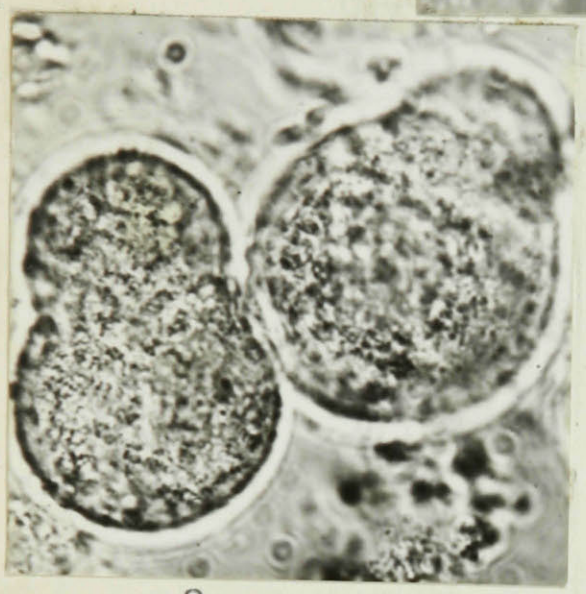
PLATE II





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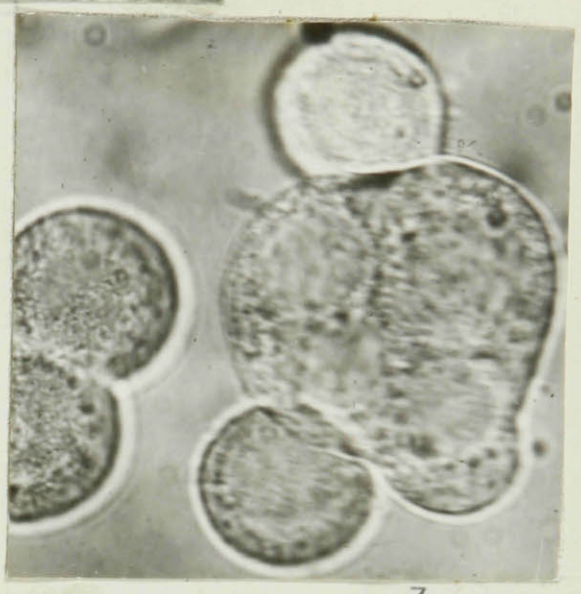

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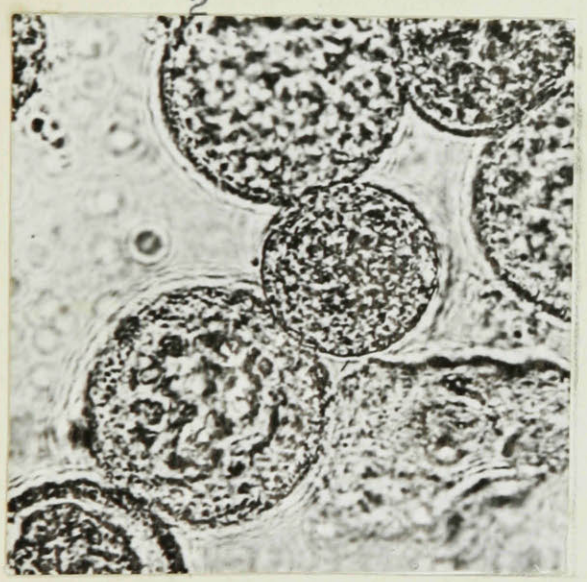

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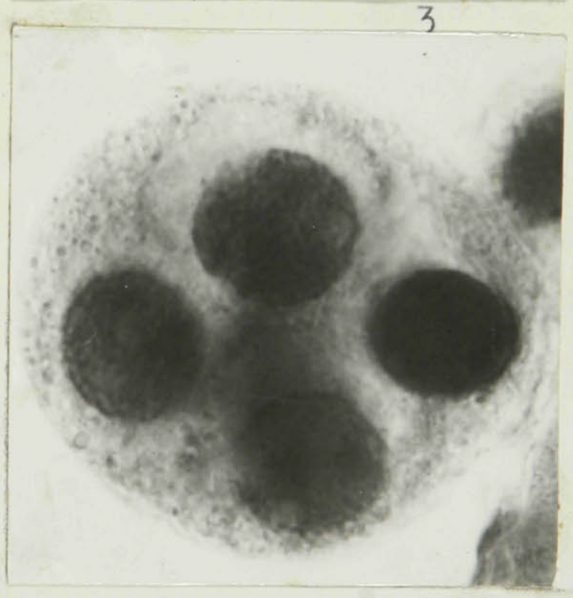

4

5

Formation of multi-nucleate cells:

Fig. 1: PMC after 6 days in 0.8M sucrose. The cells are of the "B" type and are spherical; the walls, if present are loosed from the protoplasts.

Figs. 2,3: Fusion of cells in 1.5M sucrose.

Fig. 4: Multi-nucleate cells at pachytene-diplotene stage showing single aggregation of chromosomes: cells are in 0.5M sucrose, the cell in the center of the photograph being uni-nucleate.

Fig. 5: Fixed multi-nucleate cell stained with aceto-carmine in 0.8M sucrose + .01M $CaCl_2$.