Experimental Studies of Cell-Nuclei

Sinke, Namio

MEMOIRS OF THE COLLEGE OF SCIENCE, KYOTO IMPERIAL UNIVERSITY, SERIES B,
VOL. XV, No. 1, ART. 1, 1939.

Experimental Studies of Cell-Nuclei

By

Namio SINKE[1]

(Botanical Institute, Kyoto Imperial University)

With Plates I-VIII and 12 Text-figures

(Received Sept. 27, 1938)

CONTENTS

1) The author's name SHINKE is henceforth spelled as SINKE, *h* being omitted from the previous spelling.

INTRODUCTION

The purpose of the present investigation was to study the nucleus experimentally, and mainly in the living condition. The following two points were taken as the main object of the investigation, and the results obtained led the writer further to extend the investigation to the third point given below.

1) The structure of the nucleus is an important problem not only from a morphological point of view, but also from the viewpoint of genetics, and divergent views have been expressed in regard to it. As has been pointed out by ZEIGER (1935), and KUWADA (1936), however, this problem is not to be solved by the mere investigation of the nucleus itself (the "statical method of investigation"), but must be attacked by following from stage to stage the process of transformation of the telophasic chromosomes into the nucleus (the "dynamic method of investigation"). During recent decades, many cytologists have made unremitting efforts to attain a clear understanding of the chromosome structure, and the view is becoming prevalent among most cytologists that the chromosomes are composed of two parts, the spirally coiled chromonema and the matrix, and that the former is transformed to a more or less loosely convoluted form in the interphasic nucleus without undergoing any marked structural changes. It follows, then, that the chromonemata should be visible in the nucleus, but as a matter of fact, it not infrequently occurs in both plant and animal cells that the nucleus appears quite hyaline. It is the first point of inquiry in the present investigation how the nuclei can be hyaline.

2) In connection with this inquiry there arises a question: it has been reported, that living nuclei undergo reversible changes of similar kinds responding to various abnormal conditions or different agents applied artificially, such as high temperatures (van HERWERDEN, 1927: NASSONOV, 1932), mechanical injuries (CHAMBERS, 1924; BĚLAŘ, 1930), hypotonic solutions (KAMNEV, 1934), acids or alkalis (KUWADA and SAKAMURA, 1927: SAKAMURA, 1927, ZEIGER, 1935), neutral salts (STRUGGER, 1930) etc., but the processes leading to these changes have not yet been made wholly clear. Our second point of inquiry is, therefore, through what processes these changes are brought about by the artificial agents.

3) The abnormalities in behaviour of the chromosomes in division under artificial conditions, such as those due to treatment with abnormal temperatures (GEORGEVITCH, 1910, SAKAMURA, 1920, YAMAHA, 1927: BLEIER, 1930: KEMP and JUUL, 1930: KOKOTT, 1930), hypertonic solutions (NĚMEC, 1910: SAKAMURA, 1920), mechanical injuries (CHAMBERS, 1924: WADA, 1932) and narcotics (NĚMEC, 1910: SAKAMURA, 1920, SHIGENAGA, 1937: WADA, 1938) have been studied by both zoologists and botanists, and many interesting results have been reported. In a survey of literatures it is readily seen that in all these cases the characteristic abnormalities are the coalescence of chromosomes, the formation of giant di-diploid nuclei and multi-nucleate cells, and the abnormal vacuolisation in the cytoplasm. The third point of inquiry is how these abnormalities are brought about under these conditions.

The writer wishes here to acknowledge his great indebtness to Prof. Y. KUWADA, through whose kind guidance and encouragement the completion of this work has been made possible.

I. Visible Structure of the Living Nuclei

Before taking up these questions, however, it will be well to give the results of observation of the visible structure of the intact living nuclei in several plants and animals, the majority of which were used as the principal material for the experiments forming the main part of the present paper. In selecting material, special attention was paid to the following three points: 1) that the nuclei to be observed must be relatively large ones (ca. 10 μ or more in diameter), since small ones are not suitable for critical observation of structure, 2) that the tissues to be observed should be those of only a few cells in thickness at most, since in order that the cells be kept intact, sectioning the tissues by a knife must be avoided, 3) that the cells under the microscope must be those which are relatively free from formed bodies, such as chromatophores, starch grains, and other cell inclusions, as they are apt to preclude accurate observation of the nucleus. As the mounting medium of material, tap or spring water was used in the case of aquatic plants and animals, and liquid paraffin in the case of land plants.[1]

1) MERCK's liquid paraffin was used after it was washed several times with boiling di-distilled water.

Spirogyra sp. The structure of the living nucleus of this plant has been observed by several investigators, such as PRICE (1914), LEPESCHKIN (1924), SCARTH (1927) and others. The nucleus looks quite homogeneous in tap water except for a large spherical nucleolus. The observation with dark field illumination is not always successful, as it may be prevented by the brightly illuminated thick chlorophyll bands, but in some cases, it is possible to observe that the nucleus is dark except for its own outline and that of the nucleolus which are both slightly bright. In acetocarmine preparations, the nucleus often shows very distinctly the convoluted chromonema structure, but as a rule it presents a reticulate appearance. With FEULGEN's nucleal staining, the nucleus produces no positive result (cf. SINKE and SHIGENAGA, 1933), while, according to GEITLER (1935) and SUEMATSU (1936), in some species of *Spirogyra* small chromocenters which they have observed in the nucleus give a positive reaction. This difference between the two results seems to be due to the fact that the species used as material or the stage when the observations were made was different in the two cases.

Ceratopteris thalictroides. In this plant the nuclei in the aquatic leaf epidermal cells can clearly be observed without stripping off the epidermis from the body of the leaf. The nucleus is relatively large, and shows a structure of convoluted threads or the chromonema structure.

Potamogeton crispus. The young leaf is two-cells-thick. The nucleus contains one nucleolus and several minute, highly light-refractive bodies of somewhat irregular shape. In the living state the chromonema structure is not visible in the nucleus, the region of the nucleus being hyaline (homogeneous), and it first becomes visible when stained with acetocarmine. The nucleus appears, then, to be filled with a mass of delicate chromatic threads of a somewhat reticulate appearance. The highly refractive bodies are also stained, while the nucleolus remains unstained. This behaviour of the bodies towards acetocarmine suggests that they are probably chromatin masses or chromocenters.

Elodea densa. The nuclei in the spinal as well as the epidermal cells show a wide variation in visible structure from a complete homogeneous appearance (except for one or two nucleoli) to a more or less distinct presentation of the chromonema structure, intermediate fine granular appearances being also observed. In extreme cases, even the nuclear membrane is hardly visible, and only one large nucleolus is distinct in the central part of the cell. The nuclei of these different appearances are often observed in a single leaf. Sometimes several small, refractive, angular bodies which are presumably chromocenters are found. The spinal cells are suitable for observation with dark field illumination. In this illumination, the nucleus which appears to be homogeneous in the ordinary bright field illumination is quite dark except for the nuclear membrane and the outline of the nucleolus. The nucleus appearing heterogeneous in the bright field illumination is, on the contrary, diffusely bright except for the nucleolus which is again dark as in the case of the homogeneous nuclei. In acetocarmine preparations,

loosely coiled chromatin threads or chromonemata are often distinctly recognizable in the nucleus. These threads are positive to FEULGEN's nucleal staining and the nucleolus is negative. When the nucleus is fixed with osmic acid and stained with FEULGEN's method, no threads are visible, but the whole nucleus is diffusely coloured reddish-violet except for the nucleolus.

The living nucleus in *Elodea* has been observed by several investigators. While PRICE (1914) and STROHMEYER (1935) have found, in *Elodea densa*, no recognizable sign of organized structure, SCARTH (1927) has observed a fine grained structure in a species he studied. The results of observation we obtained show, on the other hand, as stated above, that in this plant the visible structure of the nucleus is different in different cells; one nucleus may present a homogeneous appearance while another shows a distinct structure, and the third may be intermediate between these two. This fact seems to suggest that the structure of the nucleus can change from one to another as a result of response to physiological conditions in the cell.

Hydrilla verticillata. In this plant, contrary to the case of *Elodea*, the nuclei in the spinal and epidermal cells are generally heterogeneous in appearance. Sinuous chromonemata are often traceable through a considerable length if a careful microscopical adjustment is made, though this is not always the case. There is one nucleolus in the nucleus. When the nucleus is stained with acetocarmine, it appears to be of a reticulate structure as is usually the case with the nuclei of *Potamogeton* stained with this dye solution, though even in this case convoluted chromonemata can often be observed in places.

Vallisneria asiatica. In this plant the nuclei in the spinal and epidermal cells of young leaves are heterogeneous as in *Hydrilla*.

Tradescantia reflexa and T. virginica. Since the early days of cytological investigation, the staminate hairs of *Tradescantia* have been used as one of the most excellent materials for the study of the living nucleus (cf. KÜSTER, 1933). In the medium of liquid paraffin the intact nuclei in the young staminate hairs of *T. reflexa* show distinct chromonemata within, which look more or less convoluted and produce a very complicated appearance of the nuclei (cf. KUWADA and NAKAMURA, 1934). In the intact state, no sign indicating the presence of the nucleolus is recognizable in the nucleus, but in an unhealthy condition one or more hyaline areas suggesting its presence are visible (cf. SCHAEDE, 1930). In old staminate hairs, the chromonema structure is often more or less indistinct. The epidermal cells in the young petals are also excellent for the study of the living nucleus, as the petals can be easily removed from the plant with little injury to the cells. In both interphasic (in the basal dividing zone of the petal) and the resting stage (in the upper stretching zone of the petal) the nuclei of the tissues show distinct chromonemata within as do the nuclei of the young staminate hair cells. In the stretching zone of the petals where no dividing nucleus is found, the nuclei are smaller and the chromonema structure appears to be coarser than in the dividing zone.

In *T. virginica* too, the nuclei of young staminate hairs show a distinct chromonema structure similar in appearance to those of *T. reflexa*, and in old staminate hairs the chromonemata are often very obscure, only several nucleoli being recognizable. In the marginal, two, or three-celled hairs of the leaf, the nuclei are usually markedly elongated, and distinctly show chromonemata within.

These results of observation of the staminate hair nuclei in the two *Tradescantia* species are in accord with the view of BĚLAŘ (1929, b), TELEŻYŃSKI (1930), and KUWADA and NAKAMURA (1934) that the nuclei are filled with numerous convoluted threads or chromonemata. The view of SCHAEDE (1928, 1929) who maintains that in *Tradescantia* the nucleus is of a "Raumgitter" or granular structure and that of PETERFI and KOJIMA (1936) who say that it presents no visible structure at all are, on the other hand, hardly acceptable, at least so far as the nuclei of the young staminate hairs and petals are concerned.

With dark field illumination, the nuclei of the young staminate hairs and petals appear to be filled with numerous minute bright granules as reported by KUWADA and NAKAMURA (1934). The "granules" present no Brownian movement at all in contrast to the case of GAIDUKOV (1910) in which he describes a movement.

In acetocarmine preparations, the chromonemata and several nucleoli are distinctly visible.

Rhoeo discolor. The nuclei in young staminate hair cells present a convoluted chromonema structure which is less distinct than in *Tradescantia* described above. In old hairs, this structure is still less distinct, and in those nuclei two or three spherical bodies are sometimes observed, of which one or two can be identified as nucleoli, and the other or the others as chromocenters, the latter being positive to FEULGEN's nucleal rection. In the living state, it is difficult to distinguish the two structures, the chromocenters and the nucleoli.

Allium fistulosum. The young petals of this plant are as good material as those of *Tradescantia*. The general appearance of the nucleus is similar to that of the resting nucleus in the young petals of *Tradescantia reflexa*. In this case too, no nucleolus is observed in the nucleus when intact. In the petals of old flower buds, the chromonemata are indistinct and the nuclei appear nearly homogeneous.

Ranunculus japonicum. A piece of a leaf mid-rib carrying thin unicellular hairs was stripped off from a young leaf of the plant, and the nucleus in the hair cell observed. The nucleus is of an elongated, ellipsoid shape, and presents a fine granular or mottled appearance. Frequently a small number of minute bodies which remind us of heteropycnotic satellites are found near the nucleolus. In the dark field illumination, the nucleus appears to be filled with slightly illuminated granules, and in acetocarmine preparations it shows a chromonema structure which appears to be reticulate and in which a nucleolus is suspended.

Stauntonia hexaphylla. The nuclei in the young petal cells are homogeneous and hyaline, each except for a nucleolus which is visible. When the nucleus is stained with acetocarmine, delicate threads forming a reticulate structure become visible, though they are not always distinct.

Nuphar japonicum var. ***crenatum.*** Since the aquatic leaf of this plant is only a-few-cells thick, observation of the epidermal nuclei can be made with the leaf in toto. In the living state the nucleus shows no chromonema structure, but exhibits certain structural variations; while in some nuclei several spherical bodies and a nucleolus are visible, in others not only these bodies but also any clear nuclear boundary, and in extreme cases, even the whole nuclei are hardly visible. In acetocarmine preparations, a deeply stained, fine reticulum of chromonemata with two or three spherical bodies is visible. With FEULGEN's nucleal staining, these bodies are identified as chromocenters (SINKE, 1937).

Vicia faba. The young petals of *Vicia faba* are also a suitable material, being detachable with little injury from the flower bud. In the epidermal nuclei, sinuous or more or less convoluted chromonemata which appear to be finer than those in *Tradescantia* are easily traceable through a certain appreciable length. Often one or two hyaline areas are observed within the nucleus. When the tissue is stained with acetocarmine, nucleoli become visible in these areas.

Primula malacoides. The leaf hair is composed of a row of cells. In the large nuclei there are often found several refractive bodies and one nucleolus. The former may possibly be regarded as the chromocenters. Neither the reticulum nor the chromonema structure is recognizable in the nucleus when intact.

Solanum lycopersicum. The nuclei in the hair cells on the leaf mid-rib appear to be homogeneous, each except for a nucleolus. In some cases, several small bodies which are probably chromocenters are also found.

Cucurbita pepo var. ***Toonas.*** The leaf hair cells in *C. pepo* was used by PRICE (1914) and found to be good material for the investigation of the cell structure by dark field illumination. The size of the nucleus differs remarkably in different cells as is generally the case with the hair cells of other plants. In the case of relatively large nuclei, there are found several small, somewhat angular bodies and one nucleolus suspended in the hyaline bulk of the nucleus. These angular bodies are positive to FEULGEN's nucleal staining, and are identifiable as chromocenters. In *C. pepo*, the presence of the chromocenters in living nuclei has been reported by GUILLIERMOND (1932) and HEITZ (1933), and illustrated by BĚLAŘ (1928) in his photomicrographs. In the dark field illumination, both nuclear membrane and outlines of the nucleolus and chromocenters are slightly bright, while all the other areas in the nucleus are dark. When stained with acetocarmine, fine chromatin threads or chromonemata become visible. In most cases the threads look confluent with one another and appear to form a reticulate structure. The chromocenters are stained somewhat deeply.

Gynura aurantiaca. The nuclei in the leaf hair cells show a wide variation in size. In relatively young cells loosely coiled chromonemata can be observed by careful focusing, while they are indistinct in old cells. Generally one nucleolus is found in the nucleus, though it is not always distinct.

Triturus pyrrhogaster. In the case of animal cells, the epithelial cells of the tail of Amphibia-larva have frequently been used as material for the investigation of the living nucleus (GROSS, 1916, ZEIGER, 1935). The observation can be made with a living larva in toto by bringing its tail under the microscope. In young larvae of *Triturus* the nuclei in these cells show no visible structure at all, and it is generally difficult to prove even the presence of the nuclei. Several refractive bodies become distinct in the nucleus only when the larvae are mechanically agitated rather violently, as has been reported by ZEIGER to be the case with *Triton* larvae. While GROSS has come to the conclusion that the "Netz-knoten" of FLEMMING and the "Oxychromiolen" of HEIDENHAIN are both visible structures in the living nuclei, ZEIGER obtained the result, which our experiments corroborate, that in a healthy condition nothing of this kind is observed, the whole nucleus appearing quite hyaline and even its contour being hardly visible. With the dark field illumination by which observation is feasible in the marginal region of the tail where the tissue is only one-cell thick, the cytoplasmic portion of the cell is found slightly illuminated in this region, and the nuclear area or the central portion of the cell is quite dark. In our material the Brownian movement of intranuclear granules which has been reported by GROSS as taking place in the epithelial cells of *Salamandra* and *Triton* larva was not observed. This negative result is in accord again with the result obtained by ZEIGER with the larvae of the same and allied animals. When the epithelial cells are stained with acetocarmine, the nuclei are found to present some distinct chromatin lumps which are identifiable with the "Netz-knoten" of FLEMMING. Fine zig-zag threads are often also observed in the nucleus. With FEULGEN's nucleal staining, the "Netz-knoten," the fine zig-zag threads, and the chromosomes in mitosis are stained distinctly.

Rhacophorus Schlegerii arborea and Rana japonica. In these animals, the nuclei in the epithelial cells of the larvae are rather small in size, but they appear to be quite similar in structure to those of *Triturus*.

As can be seen from the descriptions given above, intact nuclei show a wide variation in visible structure. While the nucleus in the epithelial cells of *Triturus* larvae does not show even the slightest trace of structure, the nucleus in the leaf epidermal cells of *Hydrilla* exhibits a more or less distinct chromonema structure with one large nucleolus and a distinct nuclear membrane which are both clearly observed. There are several other types of nucleus which may be regarded as intermediate between these two. In the intermediate types, the morphological components of the nucleus, the chromonemata, the nucleolus and the nuclear membrane are not always equally clear. In certain plants, such as *Tradescantia, Vicia* and others, the chromonemata and the nuclear membrane is very distinct, but the nucleolus

is obscure. In *Elodea*, on the other hand, the nucleolus is very distinct, but the chromonemata are generally hardly visible. When stained with aceto-carmine, these three components of the nucleus become visible with more or less distinctness in all these types of nucleus as observed. It may be noted here that the chromatin bodies—the "chromocenters" or the "Karyosomes"—the existence of which is known from the investigation of fixed materials, are a widely distributed structure visible in the living state of the nucleus (cf. HEITZ, 1933, SCHAEDE, 1935). In the present investigation, these structures were observed in the intact living nuclei in *Rhoeo, Cucurbita, Nuphar, Triturus, Rana* and some other plants and animals.

II. Effects of Hypertonic and Hypotonic Solutions

The effects of hyper- and hypotonic solutions on living nuclei have been studied by some investigators, but the results they obtained are quite divergent. While, according to KAMNEV (1934), the homogeneous nuclei of *Triton* become heterogeneous under the action of a hypotonic solution, DOYLE and METZ (1935) have reported that the homogeneous nuclei of *Sciara* become heterogeneous by a hypertonic RINGER's solution. In plants, STROHMEYER (1935) and WADA (1935) have found that in the heterogeneous nuclei of *Tradescantia* the chromonemata disappear when observed with hypertonic solutions. While most of the investigations are only fragmentary, STROHMEYER has carried out a systematic investigation, and the results he obtained with the leaf epidermis in *Elodea* are very important for the causal analysis of the divergency of the results. He found that the homogeneous nuclei in *Elodea* become heterogeneous in a hypertonic medium, and are again homogeneous in a more concentrated medium. This results shows that there are two types of homogeneous nuclei in different colloidal conditions. STROHMEYER's conclusion, however, follows experiments with the leaf epidermis of *Elodea* only, and a more extensive investigation seems necessary before a definite general conclusion is drawn on the problem. In the present investigation, a series of experiments in this line using the nuclei of several plants and animals, was begun independent of the report of STROHMEYER and the essential points of the results obtained and the conclusion drawn, taking STROHMEYER's results also into consideration, have been briefly published in FUJII *Jubilee Volume* of *Cytologia* (1937). Fuller descriptions of the results we obtained are given below in the present paper, and for the sake of convenience, the descriptions are given under five headings, according to the kind of material used. They are: 1) epidermal and hair cells in plants, 2) animal cells, 3) dividing pollen mother cells, 4) guard cells of stomata and 5) root tip cells. The method used in the investigation is given in each section.

1. Epidermal and hair cells

Two different methods were employed in the experiments with these materials. In the first method, the tissues were treated for 20-180 minutes

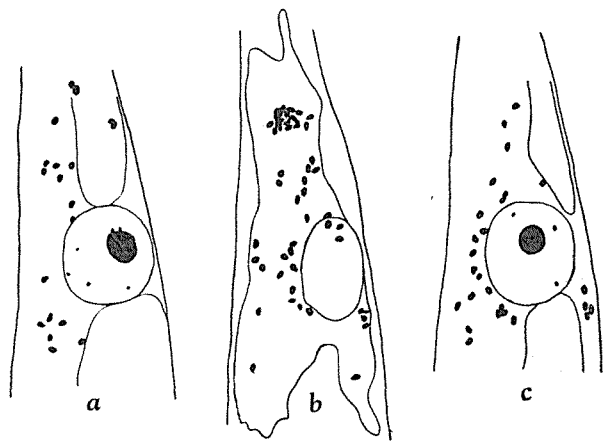
with solutions in different concentrations of the following plasmolysing agents: saccharose, glucose, urea, KNO_3, NaCl, K_2SO_4 and $CaCl_2$, and then the apparent structural changes of the nucleus were observed. This method was adopted in the case of the epidermal cells of the young petals of *Tradescantia reflexa, Allium fistulosum* and *Vicia faba,* and of those of the leaf of *Hydrilla verticillata* and *Vallisneria asiatica.* In the second method, the changes were traced under the microscope. With this second method, the epidermal cells of the leaves of *Elodea densa* and *Hydrilla verticillata,* those of the young petals of *Tradescantia reflexa* and the hair cells of the leaves of *Solanum lycopersicum, Geranium nepalense, Primula malacoides, Tradescantia virginica* and *Gynura aurantiaca* were investigated. All the organs bearing these tissues can be detached from the plants for examination without injury to the cells. The observations were made with the detached materials in toto.

a. Elodea densa. Young leaves of ca. 3-5 mm. in length were observed. As mentioned in the preceding Division I, in this plant the intact nucleus of the leaf epidermis generally presents no visible structure except for one or two nucleoli. It is a noticeable fact that there are, in this material, nuclei of two types differing in the response made to the action of hypertonic solutions. In the one type, when tap or spring water is used as medium, the chromonemata are hardly visible (Text-fig. 1, *a.* See also Fig. 1. SINKE, 1937); but when this is replaced with a concentrated solution of saccharose (0.6-1.0 M), glucose (do.), KNO_3 (0.4-1.0 M) or NaCl (do.), plasmolysis takes place more or less strongly according to the concentration of the solution used. Then the streaming of the cytoplasm stops and the nucleoli disappear, the stoppage and the disappearance being accompanied by the decrease of the nuclear volume (Text-fig. 1, b. SINKE, 1937, Fig. 2). The nucleus now appears to be a homogeneous mass with a high refractivity. The medium being replaced now, with the original tap or spring water, a rapid deplasmolysis takes place. The nucleoli reappear, the original volume of the nucleus is restored (Text-fig. 1, c. SINKE, 1937, Fig. 3), and the cytoplasmic streaming becomes clearly visible again. In this type of nucleus, during both processes toward the dehydrated and hydrated conditions of the nucleus, neither the chromonema nor the reticulate structure comes into sight. In the previous paper (1937), the nucleus of this type was designated "homogeneous nucleus I." In the second type, the chromonemata are transitorily visible during both dehydration and hydration processes.[1] They become visible somewhat distinctly during the beginning of the course of nuclear dehydration, and after a further dehydration, not only chromonemata but also nucleoli disappear accompanied by the shrinkage in volume of the nucleus, as in the case of the first type. On the replacement of the hypertonic medium with water, the original medium, the chromonemata and the nucleoli become visible again, the former being visible only for a short time

1) On usage of the terms "hydration" and "dehydration," see page 450 of the previous paper (SINKE, 1937).

in contrast to the latter which remain visible with little change after a further hydration of the nucleus. In the previous paper, the homogeneous nucleus of this second type was designated "homogeneous nucleus II," and the homogeneous nucleus due to dehydration as "homogeneous nucleus III" (SINKE, 1937). These nuclei II and III have been illustrated in Figs. 4 and 6 of the previous paper. With dark field illumination, in the three homogeneous nuclei, I, II and III, only the contour and the nucleoli in the first two are illuminated. The change of the homogeneous nucleus II towards the III by hypertonic solutions has also been observed by STROHMEYER (1935) in the same plant that we used.

In *Elodea* in cases where the nucleus belongs to the homogeneous nucleus I and where it belongs to the homogeneous nucleus II, it contains a visible nucleolus or nucleoli. For convenience we may call such a nucleus the homogeneous nucleus I or II with a visible nucleolus or nucleoli.



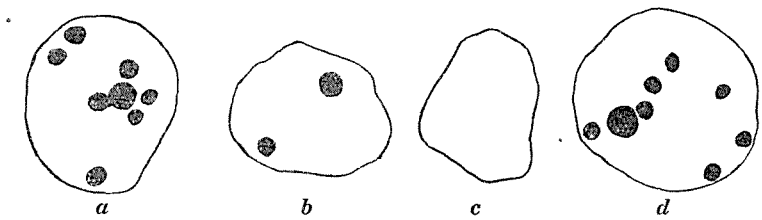
Text-fig. 1. The same nucleus in a spinal cell of *Elodea densa* (× ca. 1800). a) In tap water. b) in 0.3 M KNO_3. c) In the medium replaced with tap water again.

As mentioned in Division I, the intact nuclei of *Elodea* sometimes present an indistinct chromonema structure. Heterogeneity of this kind is often rendered clearer by treatment with hypertonic solutions of saccharose, glucose and NaCl, and the whole nuclei become homogeneous by the treatment with more concentrated solutions. This type of nucleus is found in *Vallisneria, Hydrilla* and some other plants, as will be described later.

b. Solanum lycopersicum, Primula malacoides and Pharbitis Nil. In these plants the nuclei in the leaf hair cells are homogeneous except for a nucleolus and several small chromocenters, and no change is observed in this apparent structure of the nucleus in the medium of tap water or a 0.1 M saccharose solution. When, however, the medium is replaced with hypertonic (1.0 M) solutions of saccharose, glucose, and NaCl, such changes in nuclear structure and in cytoplasmic streaming as those observed in the nuclei of *Elodea densa* belonging to the homogeneous nucleus I take place. These changes are reversible, and the reverse changes take place when the medium is replaced with the original one. In these plants, the nuclei often show a slight heterogeneity during the process of dehydration or of hy-

dration after dehydration, but the heterogeneity is so much less conspicuous than in the second type of homogeneous nucleus in *Elodea* that a correct judgment is hardly possible as to whether the underlying structure is thready or granular. This obscure presentation of the nuclear structure may perhaps be due to the thinness or smallness of the chromatic elements. In these plants, the nucleus stained with acetocarmine presents only very delicate chromonema threads. Using the abbreviated expression, the nuclei of these plants are the homogeneous nuclei I with a visible nucleolus and some small chromocenters.

c. Cucurbita pepo var. *Toonas.* In a 0.1 or a 0.2 M saccharose solution, the intact living nucleus in the leaf hair cells is seen to contain one large nucleolus and several small chromocenters suspended in the hyaline area (Text-fig. 2, *a*). When the medium is replaced with a 0.5 M or more concentrated solutions, the plasmolysis or the cell shrinkage which is expressed by the folding of the cell wall takes place, and the cytoplasm streaming stops. Then the chromocenters and nucleoli become invisible accompanied by a shrinkage and an increase in refractivity of the nucleus (Text-fig. 2, *b* and and 2, *c*). These changes are completely reversible, and the reverse changes are observed when the medium is replaced with the original dilute solution or water (Text-fig. 2, *d*). Similar changes are also observed when solutions of NaCl of different concentrations are used.



Text-fig. 2. The same nucleus in a leaf hair of *Cucurbita pepo* var. *Toonas* (× ca. 2000). a) In a 0.1 M saccharose solution. Chromocenters are distinctly visible. b) and c) In the medium replaced with a 0.5 M solution. The chromocenters are obscure. d) In the medium replaced again with di-distilled water. The chromocenters are visible again.

The nucleus of this plant may thus be said to belong to the homogeneous nucleus I with a visible nucleolus and several chromocenters as in the plants mentioned in Subsection b), but the chromocenters are larger than those in the latter.

d. Tradescantia reflexa, T. virginica, Allium fistulosum, Vicia faba and Gynura aurantiaca. In the investigation with these plants, both methods of observation mentioned above were used. In the following descriptions, the results obtained will be given under Experiments 1 and 2 according to the method used. In both cases, the epidermis of young petals in *Tradescantia reflexa* was mostly used as material.

Experiment 1. The petals were treated with solutions of saccharose, glucose, urea, NaCl, KNO_3, K_2SO_4 and $CaCl_2$ in different concentrations for 20-180 minutes.[1] The results obtained with saccharose solutions are summarised in Table 1.[2]

Table 1.

Epidermal cells of young petals in *Tradescantia reflexa*.

Concentration in mol (Saccharose)	Chromonema		Chromosome in metaphase and anaphase	Plasmolysis	Brownian movement in cytoplasm
	Resting nucleus in stretching zone	Interphasic nucleus in dividing zone			
0.00	Very distinct.	Very distinct.	Very distinct.	Not observed.	Active.
0.05	Do.	Do.	Do.	Do.	Do.
0.1	Do.	Do.	Do.	Do.	Do.
0.2	Do.	Do.	Do.	Do.	Do.
0.3	Distinct.	Distinct.	Distinct.	Do.	Weak.
0.4	Somewhat obscure.	Do.	Somewhat obscure.	Observed.	Not observed.
0.5	Invisible.	Somewhat obscure.	Invisible.	Do.	Do.
0.6	Do.	Very obscure.	Do.	Do.	Do.
0.7	Do.	Invisible.	Do.	Do.	Do.
0.8	Do.	Do.	Do.	Do.	Do.
0.9	Do.	Do.	Do.	Do.	Do.
1.0	Do.	Do.	Do.	Do.	Do.

From Table 1, it is seen that the chromonemata in the resting nucleus in the stretching zone and the chromosomes in the metaphase and anaphase become invisible at a concentration, 0.5 M, and are so in higher concentrations, while those in the interphasic nucleus in the dividing zone disappear first at the concentration 0.7 M.[3] The concentration, 0.5 or 0.7 M, at which the chromonemata or the chromosomes become first invisible will be called in this paper the "upper critical concentration." In association with the disappearance of the chromonemata in the nucleus, the plasmolysis is observed and the stoppage of the cytoplasmic streaming and Brownian movement of the microsomes is noticed. In the dividing nuclei, the migration of the

1) When the tissue is treated for a longer time with these solutions, the nuclei are penetrated by the solutions, and the changes which are regarded as due to the direct action of the solutions on karyoplasm are caused. The details of the investigation into this aspect of the problem will be given in Division VI.

2) When an experiment is repeated, a certain minor difference is found between the results obtained. In this as well as the following tables, only those results which are predominant among others obtained by repeating the same experiment several times are given.

3) The H-ion concentration of a 0.1 M and a 1.0 M solution was colorimetrically measured and found to be the same as that of di-distilled water used as solvent, which varies in the range from pH 5.4 to 5.8.

daughter chromosome groups to poles is often incomplete resulting in the formation of a dumb bell shaped chromosome mass, or it is entirely suppressed at metaphase, a completely grouped mass of chromosomes being the result. Sometimes no cell wall is formed between the two daughter nuclei. These phenomena, suspension of chromosome movement and suppression of cell wall formation, due to the action of hypertonic solutions have been observed by NĚMEC (1910) and SAKAMURA (1920) in fixed root tip cells, and by STROHMEYER (1935), and WADA (1935) and SHIGENAGA (1937) in living staminate hair cells of epidermal cells of *Tradescantia.*

The results of observation of the interphasic nuclei in the dividing zone of young petals, obtained with the other plasmolyzing agents: glucose, urea, NaCl, KNO_3, K_2SO_4 and $CaCl_2$ are given in Table 2.

Table 2.

Epidermal interphasic nuclei in the dividing zone of young petals in *Tradescantia reflexa.*

Concentration in mol	glucose	urea	KNO_3	NaCl	K_2SO_4	$CaCl_2$
0.00	++	++	++	++	++	++
0.05			++	++	++	++
0.1	++	++	++	++	++	++
0.2	++	++	+	+	± or +	+
0.25				+	± or +	± or −
0.3	++	++	+	± or −	−	−
0.35					−	−
0.4	+	+	−	−	−	−
0.45					−	−
0.5	+	+	−	−	−	−
0.6	± or +	± or +	−	−		
0.7	−	± or −	−	−		
0.8	−	−	−	−		−
0.9	−	−	−	−		−
1.0	−	−	−	−		−
1.2	−					
1.4	−					
1.8	−					

Explanation of the marks:—
++ : Chromonemata are distinctly visible.
+ : Chromonemata are somewhat obscure.
± : Chromonemata are obscure.
− : No structure at all is visible in the nucleus.

From Table 2, it is seen that the upper critical concentration is different in different plasmolyzing agents. While in glucose this concentration is ca. 0.7 M as in saccharose, it is about 0.8 M in urea. In the case of urea it was

observed, moreover, that the nucleus was heterogeneous after one hour immersion in the medium of the upper critical concentration, and that the same was also true with the same media of higher concentrations. This peculiarity in the case of urea seems to be due to a certain high penetrability of urea into the nucleus, as is well known in the case of the cell. In the cases of electrolytes, it is seen that the upper critical concentrations are markedly lower than in the cases of non-electrolytes. In the solutions of NaCl and KNO_3, the upper critical concentration is 0.4 M, and in those of the bivalent salts, K_2SO_4 and $CaCl_2$, it is 0.3 M. In some of these plasmolyzing agents, concentrated solutions often produce a granular appearance of the nucleus instead of the homogeneous appearance. The nature of this granularity is not clear at present, but it seems probable that this unusual appearance is due to the direct action of the chemicals.

From the results given above, it may be concluded that the upper critical concentration at which the chromonemata disappear varies widely in different plasmolyzing agents, especially according to the degree of their electrolytic dissociation. Variation of the same kind has been noted in the case of the critical plasmolytic concentration. In Table 3, the ratios of the upper critical concentrations of these plasmolyzing agents to that of saccharose, or conveniently speaking, the "disappearance coefficients," for the interphasic chromonemata in the dividing zone, are compared with the isotonic coefficients of the corresponding agents determined by FITTING (1917) by plasmolysis.

Table 3.

Chemicals	saccharose	glucose	KNO_3	$NaCl_2$	$CaCl_2$	K_2SO_4
Disappearance coefficient (interph. nucleus)	1.0	1.0	1.8	1.8	2.3	2.3
Isotonic coefficient (FITTING, 1917)	1.00		1.64	1.65	2.37	2.20

From this table, it is seen that the disappearance coefficients of the plasmolyzing agents are nearly equal to the isotonic coefficients of the same agents.[1] This fact may be regarded as suggesting that the disappearance of the chromonemata in concentrated solutions of plasmolyzing agents is an osmotic phenomenon.

Experiment 2. The changes in appearance of the nucleus occurring under the influence of hypertonic solutions were directly traced under the microscope. Generally speaking, nearly the same results as in Experiment 1 were obtained with different plasmolyzing agents. The nucleus decreases in volume and increases in refractivity. The chromonemata are rendered

1) As mentioned in the foot-notes on page 13, the upper critical concentrations obtained in repeated experiments with the epidermal nuclei in young petal cells of *Tradescantia* showed a variation of a certain range. An investigation with more suitable material may afford more accurate results, than those given in Table 3.

first slightly more distinct, followed by the change in which they look thicker in diameter and appear fewer in number.[1] Then they become gradually indistinct. With these changes in the appearance of the chromonemata, the nucleus shrinks and in its final state it is homogeneous. These changes are completely reversible and the reverse changes take place when the medium is replaced with the original one (SINKE, 1937, Figs. 7, 8 and 9). A single example from a series of experiments carried out is given below:

0.50 (p. m.). The epidermis of a young petal in *Tradescantia reflexa* was observed with di-distilled water as medium. The nuclei in the epidermis presented the normal appearance showing the chromonema structure within. A spherical nucleus was measured and found to be 15.6 μ in diameter. The streaming movement of the microsomes in the cytoplasm was observed though it was not very active.

2.05. Do.

2.15. The medium was replaced with a 0.2 M saccharose solution. No change was perceptible either in the nucleus or in the cytoplasm.

2.25. The medium was replaced with a 0.4 M solution.

2.45. The chremonemata were highly refractive, but the streaming of the cytoplasm was still perceptiple. The medium was replaced with a 0.5 M solution.

2.55. Chromonemata appeared slightly thicker in diameter and less in number. The nuclei shrank and showed an increased refractivity, and the chromonemeta were now somewhat indistinct (compare Fig. 1 with 3). The spherical nucleus was 14.0 μ in diameter. In dividing nuclei, the individual chromosomes in metaphase and anaphase were hardly recognizable (Fig. 1.). The streaming of the cytoplasm stopped and a strong plasmolysis was observed.

3.05. The chromonemata were very obscure, and the chromosomes disappeared. The spherical nucleus was 13.0 μ in diameter.

3.10. Do. The medium was replaced witn a 0.7 M solution.

3.20. The nuclei showed no visible structure, and appeared to be a highly refractive mass (Fig. 2). The spherical nucleus was reduced to 12.0 μ in diameter. The medium was replaced with a series of solutions, 0.4, 0.3 and 0.2 M successively, and finally with di-distilled water, the original medium.

3.40. The chromonemata were visible again, but looked thicker and seemed to be less in number than they were in the normal nuclei. The chromosomes were not distinguishable from one another.

3.50. The chromonemata were more distinct than at 3.40. The beginning of the cytoplasmic streaming was observed. No plasmolysis was perceptible. The individual chromosomes were obscurely recognizable. The spherical nucleus had increased to 14.6 μ in diameter.

4.00. The nuclei and the cytoplasm were quite normal in appearance.

4.20. The streaming of the cytoplasm was active. The spherical nucleus was 15.6 μ in diameter. The original normal appearance of the nucleus was recovered (Fig. 3).

4.40. The medium was replaced with a 0.5 M solution.

4.50. The chromonemata were again obscure. The medium was replaced with a 1.4 M solution. The chromonemata were invisible. The spherical nucleus was reduced to 10.0 μ in diameter.

---

1) This appearance of the nucleus with thicker and fewer chromonemata is usually observed in the course of the hydration of the homogeneous nucleus III.

8.20 (a. m., next morning). The nuclei were found much shrunken, and deformed chromonemata of the appearance of broken pieces of meshwork and minute granules were observed within. The determination as to whether or not the refractivity of these chromonemata and the granules is higher than that of the ground substance of the nucleus was hardly possible owing to the minuteness of these structures. The true nature of the granules was not cleared up, but it was ascertained that they disappeared on the replacement of the medium with water. The nuclei then gradually swelled and presented a coarse reticulate structure. The original appearance of the nucleus was, however, not recovered.

In the experiment given above, it is shown by the measurements of a spherical nucleus that in the media of different concentrations changes take place in nuclear volume. In the water medium, this nucleus was 15.6 μ in diameter. This diameter was reduced to 12.0 μ in the medium of a 0.7 M solution. The ratio between the original and reduced volumes in percent, obtained by calculation from the two diameters measured is 100 : 45.5. There is, therefore, a 54.5% reduction in volume in this case. Other measurements with the same material and those with the leaf epidermis nuclei in *T. virginica* show that similar changes take place in nuclear volume. The mean reduction in volume of the nucleus in the 0.7 M saccharose solution was 44.7% in the case of the leaf epidermis and 43.4% in that of the petal epidermis. These results are harmonious with the result obtained by STROHMEYER (1935) that the reduction is 50% when the nuclei in the hair cell in *Tradescantia virginica* are observed in the medium of a 0.8 M glucose solution.

The disappearance of the distinguishable chromonemata by hypertonic solutions is observed also in the living cells of the staminate hairs of *T. reflexa*, those of the epidermis and hairs of *T. virginica*, those of the leaf hairs of *Gynura aurantiaca* and those of the epidermis of the young petals of *Allium fistulosum* and *Vicia faba*. The upper critical concentration of NaCl obtained for the chromonemata in the interphasic nuclei in the dividing zone is 0.4 M in *Allium* and 0.3 M in *Vicia*.

When a nucleus which is strongly dehydrated and in which the chromonema structure is obscure, is stained with acetocarmine, the chromonemata may become perceptible. In most cases, however, they are much deformed and in extreme cases, even hardly perceptible. The fixation image of the dehydrated nuclei was studied with the leaf epidermal cells of *T. virginica*. Pieces of epidermis were treated for 3 minutes with a 1.0 M saccharose solution, and after it was confirmed under the microscope that the nuclei were completely homogeneous, the tissue was fixed with the BONN modification of FLEMMING's solution followed by staining with HEIDENHAIN's haematoxylin. In these preparations, the dehydrated nuclei show no distinct chromonema structure. In some cases, the chromonemata are observable, but they appear to be less in number than in the normal intact nuclei (compare Fig. 4 with 5). Very frequently the nuclei are found deeply stained, presenting no visible structure at all (Fig. 6). These different images are found in one and the same region of the epidermis. The same changes in nuclear structure by dehydration are also observed in the root tip cells of

Vicia faba, fixed after treatment with a hypertonic saccharose solution (cf. Section 5).

The response of the nuclei to hypotonic solutions was also observed under the microscope. In this case the nucleus undergoes little or no change. While in the nuclei in the staminate hairs in *Tradescantia virginca* the chromonemata show a swelling to a slight extent and become somewhat slender in the medium of water or a hypotonic solution, in the nuclei in the young petals in *T. reflexa* no visible change is perceptible in those media except a slight decrease in refractivity. In *Allium*, *Vicia* and *Gynura* the result is the same as in *T. reflexa*.

In the previous paper (SINKE, 1937) the nucleus presenting the heterogeneous appearance in the normal living condition was called the "Heterogeneous nucleus." In the materials mentioned above, the chromonema structure with no chromocenter is visible in the living state, but no nucleolus is visible in the healthy condition. For convenience such a nucleus may be called the heterogeneous nucleus with no visible nucleolus.

e. Hydrilla verticillata, Geranium nepalense and Vallisneria asiatica. As described in Division I, the intact living nuclei in the leaf epidermis in *Hydrilla* and *Vallisneria* and those in the leaf hairs in *Geranium* show generally a somewhat indistinct chromonema structure with a distinct nucleolus suspended in it. By the action of concentrate solutions of saccharose, glucose, NaCl and KNO_3, the nuclei undergo great shrinkage, and the chromonemata and the nucleolus become invisible.[1] The stoppage of the streaming in the cytoplasm also takes place as in the case of the other plants described above. The upper critical concentration of NaCl and KNO_3 for the chromonemata in the resting nucleus is 0.2 M in *Hydrilla* and this concentration of NaCl in *Vallisneria* is 0.3 M. In the former plant the nucleolus is visible in a 0.3 M NaCl solution, a concentration which is above the upper critical concentration and hence, a concentration in which the chromonemata are not visible. The nuclei of these plants may be regarded as belonging to the type of heterogeneous nucleus with a visible nucleolus.

In all the experiments mentioned above, unimpaired tissues only were used as material. Observation was also made on the nuclei in the inner epidermis stripped off from the bulb scale of *Allium cepa*, in which the cells were not always intact. In these nuclei, indistinctly visible chromonemata and a distinct nucleolus or nucleoli are found in most cases. On application of a hypertonic solution, the chromonemata become more or less distinct as compared with those in the normal state, but they are rendered invisible by a further treatment or by dehydration. The upper critical concentration is remarkably higher in this case than in those where the cells are intact. When saccharose is used as a dehydrating agent, it is 1.4 M, and the NaCl used is 0.6 M. So far as the present investigation is concerned, this is the

1) According to STROHMEYER, in *Vallesneria spiralis* no nuclei are rendered homogeneous by a hypertonic solution. But in the *Vallisneria* plant we used, no such a case was met with.

highest upper critical concentration we observed. The question as to whether or not this high concentration is due to an unhealthy condition or to a peculiarity of these cells is left for further investigation.

The results given in this section may be summarized as follows:—In most cases, the nucleus shows no marked change in the media of hypotonic solutions. In hypertonic solutions, on the contrary, it undergoes a decrease in volume and an increase in refractivity, and the structures, the chromonema, the chromocenter and the nucleolus, are rendered indistinguishable, the whole nucleus thus appearing homogeneous. In association with these nuclear changes, plasmolysis and the stoppage of the cytoplasmic streaming and also Brownian movement of the microsomes take place. The upper critical concentration in which the chromonemata are invisible as a result of dehydration of the nucleus, is different in different plants or tissues, or according, to different plasmolyzing agents used. In the latter case, the ratios of the upper critical concentrations of different plasmolyzing agents to that of saccharose, or the disappearance coefficients form a nearly similar series to that of the isotonic coefficients of the corresponding plasmolyzing agents given by FITTING, leading to the conclusion that the disappearance of the chromonemata in the hypertonic solution is an osmotic phenomenon, i. e. a change of the chromonemata which is due to the change in water-relation in the nucleus.

In the plants investigated, the nuclei in the natural state can be classified among the following types:

1) The homogeneous nucleus I with a visible nucleolus or nucleoli.
2) The homogeneous nucleus II with a visible nucleolus or nucleoli.
3) The homogeneous nucleus I with a visible nucleolus or nucleoli and chromocenters.
4) The heterogeneous nucleus with a visible nucleolus or nucleoli.
5) The heterogeneous nucleus with no visible nucleolus.

In all these nuclei the nuclear membrane[1] is visible.

2. *Animal cells*

In Section 1, we have seen that while in the cells of young petals and leaf hairs in plants, the visible structure of the nucleus disappears when the cells are treated with hypertonic solutions as a result of the change in water-relation in the nucleus, it shows little or no change when they are put in the medium of water or a hypotonic solution. The latter fact becomes comprehensible if we consider that in these cells a high wall pressure due to the presence of the rigid cell membrane hardly allows the penetration of sufficient water into the cells to cause the chromonemata to swell. If this interpretation is correct, it is to be expected that in animal cells which are furnished with no such rigid membrane as that of the plant cells, the chromonemata or its related structures such as chromocenters should dis-

1) Here the term is used in a broad sense.

appear when the cells are observed in a hypotonic medium. The object of the following experiments with animal cells is to see whether or not the visible structures disappear and the nuclei swell and become homogeneous in hypotonic solutions.

The cells of the oesophageal epithelium of *Rana* sp., those of the intestinal epithelium of *Triturus pyrrhogaster*, the spermatocytes of *Gampsocleis* sp. and *Tryxalis nasuta*, and the salivary gland cells of *Chironomus dorsalis* and *Drosophila virilis* were used as material, though there were certain disadvantages in the use of these materials. One of the disadvantages is that these cells are generally rich in cytoplasm which becomes, when treated with hypertonic solutions, highly refractive and prevents accurate observation of the nucleus. Another disadvantage is the difficulty of determining whether the cells in question are intact or not. In these cells, there takes place in most cases no such phenomenon as the streaming of the cytoplasm which indicates that the cells are intact. As mounting medium, diluted or concentrated RINGER's solutions were employed. In the following descriptions such abbreviations as 1 R for the normal RINGER's solution containing 0.65% NaCl, 10 R for the solution ten times as concentrated as the 1 R, 0.3 R for that of a three tenth dilution of the normal or 1 R, and etc. are used.[1)]

a. Gampsocleis sp. *and Tryxalis nasuta.* In these animals, the nucleus in the early prophase in the first spermatocyte shows a fine chromonema structure in which is suspended one large heteropycnotic sex-chromosome which is highly refractive in liquid paraffin or a nearly isotonic solution, 0.8 R, (Fig. 7). In the I-metaphase and anaphase the chromosomes are distinctly observable in the living state of the material. When the testis is put in di-distilled water for about ten minutes, the spermatocytes swell markedly and the whole cell becomes hyaline except for the sex-chromosome (indicated by an arrow in Fig. 8) which is often faintly observed in the swollen nucleus. In the dividing stages, neither metaphase nor anaphase chromosomes are visible, the sex-chromosome also disappearing completely in this case. These swollen cells in di-distilled water show no recovery of their original structure on the replacement of the medium with a 0.8 R solution. In a diluted RINGER's solution such as a 0.1 or a 0.2 R, the nucleus swells and no chromonemata are visible, but the existence of the sex-chromosome is distinctly recognizable (Fig. 9). First after a prolonged immersion, the latter also disappears. In the metaphase and anaphase, all the chromosomes disappear in these diluted media as in di-distilled water. The change is, however, reversible in this case. The reverse changes are

1) The H-ion concentrations of the solutions, 5 R, 1 R and 0.2 R were measured with bromo-cresol-purple with the results, pH 6.1, 5.9 and 5.8 respectively. The salt error of this indicator has been reported by PARSON and DAUGLAS (cited from KOLTHOFF, 1932) to be −0.26 for a 1 M NaCl solution, and hence, the pH values we obtained may be regarded as showing that these three RINGER's solutions of different concentrations are nearly equal in H-ion concentration (The 5 R solution contains nearly 1 M NaCl).

observed, though not completely, in all the stages, prophase, metaphase and anaphase, when the cells are treated with 0.8 R. In a 4 R and a 5 R solution, the nuclei in prophase shrink and become highly refractive accompanied by the lowering of distinctness of their chromonema structure (Fig. 10). In Figs. 7, 9 and 10 are shown the spermatocyte nuclei of *Gampsocleis* sp. observed in an 0.8 R, 0.2 R and 4 R solution respectively, and in Fig. 8 those put in di-distilled water for 20 minutes. The magnification is the same in all these photomicrographs. In Figs. 7, 8 and 9, the arrows indicate the sex-chromosome.

These results of experiments show that the nuclei studied belong to the type of heterogeneous nucleus, and that, as expected, in animal cells the heterogeneous nucleus is rendered homogeneous by treatment with hypotonic solutions.

b. Triturus pyrrhogaster and Rana sp. According to KAMNEV (1934), the nuclei in the intestinal epithelium of *Triton* and *Rana* show no visible structure in the medium of an isotonic RINGER's solution excepting that one or two nucleoli are visible. In *Triturus*, the nuclei present a somewhat different appearance from those of *Triton* observed by KAMNEV. When a piece of the intestine is observed in liquid paraffin or RINGER's solution of the normal concentration as medium, the epithelium nucleus is relatively large, and carries in it nucleoli and several large refractive bodies of chromocenters which appear to take the place of nearly the whole of the chromonema structure of the heterogeneous type of the nucleus. In the living state, it is hardly possible to distinguish between these two structures, but in acetocarmine preparations, the chromocenters are mostly stained deeply and it becomes possible to distinguish them from the nucleoli without much difficulty. The visible changes of the chromatic bodies in these nuclei, due to hyper- and hypotonic solutions were studied by the observation of pieces of intestine which had been put in either one of the solutions for about ten minutes. The results obtained are as follows:

i) Di-distilled water. The nucleus swells to a remarkable extent, and its contour is smooth and sharp. In most cases, no visible structure is recognizable in the nucleus, although in some several minute granules exhibiting an active Brownian movement are observed.

ii) 0.1 R, 0.3 R and 0.4 R. The nuclei present an appearance very similar to that of the nucleus put in di-distilled water.

iii) 0.5 R. The swelling of the nucleus does not take place so extensively as that observed in the 0.3 R solution. The refractive bodies observed in the living state are obscurely recognizable in the nucleus.

iv) 1 R. The appearance of the nucleus is similar to that observed in liquid paraffin.

v) 2 R. The refractive bodies or chromatic lumps are observed very distinctly, being highly refractive, but some of the chromatic lumps are found fused together.

vi) 3 R. The nucleus is so shrunken that the chromatic lumps are hardly distinguishable from one another, and the whole mass of the nucleus is highly refractive.

vii) 4 R, 5 R, 6 R and 7 R and 10 R. The nucleus is shrunken severely and presents the appearance of a highly refractive mass with no visible structure.

From the results given above, it is seen that the chromatic lumps are not visible in the media of concentrations higher than 4 R or those lower than 0.4 R. The concentration 4 R is, therefore, the "upper critical concentration" for the chromatic lumps, and correspondingly the 0.4 R may be called the "lower critical concentration" for the same structure. The concentrations of NaCl in the 4 R and 0.4 R solutions are ca. 0.44 M and 0.044 M respectively. The upper and lower critical concentrations of NaCl and those of KNO_3 directly determined with *Triturus* nucleus were in both cases 0.4 M and 0.05 M respectively in coincidence with these results.

The homogeneous nucleus due to hydration or dehydration is reversible to its original heterogeneous appearance, and this reversal is observed on the replacement of the medium with the original one. But when the hydration or the dehydration goes too far, the reversal is incomplete. This incomplete reversibility of the karyoplasmic elements is often observed in other animal cells also.

The nuclei studied may be regarded as a modified type of the heterogeneous nucleus with chromonemata the majority of which assume the form of chromocenters. The homogeneous *Triton* nuclei studied by KAMNEV has been reported to present a heterogeneity when the cells are treated with a hypotonic solution, and it seems highly probable that these nuclei were in a dehydrated condition. This conclusion leads us to the view that the difference observed in nuclear structure between the two related genera, *Triton* and *Triturus*, may be due to the seasonal difference of materials used. KAMNEV employed hybernating animals as material while we used summer animals. According to BODINE (1921) in insects, hybernating animals contain less water than summer animals. This seasonal difference in water content in insects seems to favour the view of regarding the difference in structure of the nucleus between the two animals, *Triton* and *Triturus* as due to a seasonal difference.

The nuclei in the œsophageal epithelium cells of *Rana* show a loosely-coiled-thread structure or the chromonema structure. The epithelial cells are furnished with numerous cilia which show an active movement in the medium of the normal RINGER's solution. When the medium is replaced with a 5 R solution or solutions of higher concentrations, the nucleus shrinks and the ciliary movement stops or becomes remarkably slow. In this case, the cytoplasm is highly refractive, and an accurate observation of the nucleus is impossible. When the medium is again replaced with the normal RINGER's, the ciliary movement begins to be rapid and the convoluted threads or the chromonemata come to visible in the nucleus again. If the nucleus is left in the 5 R solution for 2 hours or longer, the heterogeneous structure is, however, no longer capable of recovering from its obscured condition, though the ciliary movement reappears on the replacement of the medium with the normal RINGER's. In a 0.5 R solution or solutions less concentrated, the nucleus swells and is completely homogeneous except for a few refractive bodies the origin and the nature of which remain as yet undetermined.

A very active movement of the cilia is observed in these solutions. In this case, the visible change of the nuclear structure is completely reversible. In di-distilled water, the nucleus is completely homogeneous, and a gradual decrease in the velocity of the ciliary movement is noticeable. These homogeneous nuclei in di-distilled water show no trace of reversibility, although the ciliary movement recovers its activity on the replacement of the medium with the isotonic RINGER's. This and the same fact observed in the case of treatment with a hypertonic solution (5 R) show that an active movement of cilia may not indicate the healthy state of the nucleus in the cell. The *Rana* nucleus studied is in short a typical heterogeneous nucleus, and the disappearance of the structure follows treatment with either hypotonic or hypertonic solutions to the cell. The difference of nuclear structure observed in the two cases, KAMNEV's and ours, seems also due to a seasonal difference of the materials used as pointed out above in the case of the difference found between *Triton* used by KAMNEV and *Triturus* we employed.

c. Chironomus dorsalis and Drosophila virilis. The nucleus in the salivary gland cells in the Diptera-larvae is extraordinarily large, and has often been used for the study of the living nucleus, though, according to the results obtained by several investigators, this nucleus seems to be an abnormal nucleus representing a stage other than the typical resting stage (cf. ALVERDES, 1912, HEITZ and BAUER, 1933, SINOTO and YUASA, 1935). In the present investigation the salivary gland cells of the larvae of *Chironomus dorsalis* (ca. 12-15 mm in length) were used as material and also those of *Drosophila virilis* for a supplement.

As reported by GROSS (1916) and by VONWILLER and AUDOVA (1933), in *Chironomus* the salivary gland nucleus can be observed under the microscope through the body wall, and even the transverse bands or striations of the chromosomes are also observable in the intact condition of the larvae. When the gland is detached from the body of the larva and is observed in liquid paraffin or body fluid, the fine structure of the nucleus is clearer. The nucleus contains four large chromosomes which appear to consist of numerous refractive transversal bands of different thickness and the ground substance or the chromosome matrix connecting the neighbouring bands, which is less refractive than the bands (Fig. 11). The origin and the nature of these bands are as yet little known, but it seems not improbable that they are fragments of chromonema spirals, because in some early stages of chromosome development, the spiral chromonemata have been observed by ALVERDES (1912), KAUFMANN (1931), SINOTO and YUASA (1935), and KUWADA and myself (KUWADA, 1937, b) in this and other Diptera. In the present investigation the visible changes of these bands and the matrix caused by hyper- and hypotonic solutions were observed. The results obtained are briefly given in the following.

i. Di-distilled water. The nuclei present a swollen, turgescent appearance as soon as the cells are put in water. Most of the nuclei appear quite homogeneous except for one or two, more or less swollen nucleoli, and several minute granules. In most of the nuclei, both nucleoli and granules disappear from sight after a pro-

longed immersion in water. In some, however, several minute granules are still observed which show an active Brownian movement.

ii. 0.1 R, 0.2 R and 0.3 R. The chromosomes are not visible, but one or two large nucleoli are obscurely visible.

iii. 0.4 R. The chromosomes swell, and their outlines are only obscurely recognized. The transverse bands of the chromosomes also swell, and some thick bands look as if they consisted of rows of several minute, refractive, globular bodies. In this state of swelling, each band presents an aspect suggestive of a pearl necklace. Not infrequently, a weak vibration of these minute bodies can be detected. Other thinner bands in which no such necklace structure is shown are also visible, but indistinctly and only at places. In this medium, the nucleoli appear to consist of two parts, the central which is refractive and the peripheral which is swollen. A great number of minute granules are found in the swollen, peripheral part of the nucleoli, showing an active Brownian movement. Frequently the peripheral part is so strongly swollen that the territory of the nucleolus is hardly recognizable, so that the granules appear as if scattered in the karyolymph. In this case too, the granules exhibit an active Brownian movement.

iv. 0.5 R. The necklace structure of the transverse bands is frequently observed, but the contour of the chromosomes is generally somewhat distinct. By a prolonged immersion, however, the chromosomes become indistinct (Fig. 12).

v. 0.6 R. In this medium the outline of the chromosomes is distinctly perceptible.

vi. 0.7 R. The general appearance of the nucleus is similar to that observed in 0.6 R, but the chromosomes appear more distinct.

vii. 0.8 R and 0.9 R. In these media, the chromosomes and the nucleolus present an appearance similar to that we observed when body fluid or liquid paraffin was used as medium (cf. Fig. 11), both thick and thin bands being very distinct.

viii. 1 R. The bands are very distinct, but some appear to be rugged or wavy.

ix. 2 R and 3 R. The nucleus shrinks and is highly refractive. In most nuclei, the chromosomes appear to be thinner and the transverse bands are somewhat indistinct.

x. 4 R. The chromosomes are hardly recognizable in most of the nuclei, and only obscurely visible in the others. The nucleoli are invisible. In solutions of higher concentrations such as those of 5 R, 6 R and 10 R, the nuclei show no visible structure.

From the results given above, it is seen that the nucleus shrinks and that the chromosomes, their transverse bands, and the nucleoli become invisible in concentrated RINGER's solutions. They disappear in diluted RINGER's solutions as well, but in this case it is the result of swelling. The refractivity of the nucleus, therefore, increases in the former case, and decreases in the latter. The upper critical concentration observed for the transverse chromatic bands is 4 R, and the lower critical concentration is 0.3 R. The concentrations of NaCl in these two solutions are ca. 0.44 M and 0.03 M respectively. It is very interesting that the chromatic bands of the salivary chromosomes show approximately the same upper critical concentration as that we observed for the chromonemata in the interphasic nuclei in some higher plants such as *Tradescantia*. Similar changes in nuclear structure are also observed when glucose solutions are used instead of RINGER's. This fact must show that the apparent structural changes of the nucleus caused by concentrated and diluted RINGER's solutions are changes

that are due to the water content of the nuclear elements, and not to special action of the ions contained in RINGER's solution.

As in the case of the investigation with *Tradescantia* and other plants, the apparent structural change of the nucleus was traced under the microscope also. When the nucleus is observed in the body fluid, it is seen to contain distinct chromosomes with refractive bands and a nucleolus. On the replacement of the medium with 0.3 R or the solutions of lower concentrations, the nucleus swells very rapidly, the swelling being remarkable in the chromosomes and peripheral part of the nucleolus. In the beginning of the swelling, some refractive bands varying in thickness are obscurely visible, but soon come to show the necklace structure which is conspicuous in some of the thick bands. In this stage, the outline of the chromosomes is no longer visible. Then the thin bands disappear, but the necklace structure of the thick bands is still observable at this stage. The inner or central part of the nucleolus is also a visible element in the nucleus at this stage. There are often observed numerous minute granules scattered in the karyolymph. In the next stage, all the bands of both homogeneous and necklace structure disappear, accompanied by the disappearance of the nucleolus. In this final state of swelling, nothing is visible in the nucleus except several minute granules which are often observed to be in an active Brownian movement. When the medium is replaced with the isotonic RINGER's, the central portion of the nucleolus first becomes visible, and then the refractive, linearly arranged granules of the thick transverse bands come into view. In the next stage, the outline on the concave side of the curving chromosomes becomes perceptible, accompanied by a gradual reappearance of the thin refractive transverse bands. Numerous refractive granules of nuclear origin are often still visible presenting an active Brownian movement in the karyolymph. The outlines of the chromosomes not only on the concave side but also on the convex side, and the internal, refractive, transverse bands become gradually more distinct, and the peripheral portion of the nucleolus condenses and resumes its original shape, though its contour is not so distinct and smooth as in the normal state. The nucleus is now of nearly normal appearance, though some of the granules of the nucleolar origin are still left in the karyolymph and are in movement. In this series of experiments it was repeatedly ascertained with thick chromatic bands that, on the replacement of the medium with an isotonic solution, they reappear in their original positions which they occupied before their disappearance as a result of the action of hypotonic solutions. This fact is important from the viewpoint of the chromosome inividuality, as it shows that the chromatin bands retain their original positions in the chromosome even in the case where the chromosomes are swollen and the nucleus appears homogeneous. While in the case of hydration experiments, the apparent structural change is completely reversible, such a reversible change takes place in the case of dehydration experiments only seldom.

While the above descriptions are based on the results obtained with the

Chironomus larvae, those obtained with the larvae of *Drosophila virilis* differ from them in no important point in regard to the behaviour of the chromosomes toward hyper- and hypotonic media. In the latter animal, however, the nucleus retains its regular spherical shape during the whole course of hydration and dehydration. Taking advantage of this favourable condition for the measurement of the nuclear volume, the volumes of the nuclei transferred from the body fluid to di-distilled water and those of the nuclei transferred to a hypertonic solution (5 R) were measured. The two average volumes, each obtained from 10 nuclei of a gland, were 180% and 204% of the original volume in the former case—an increment; and 13.6% and 22.2% of the original in the latter—a reduction. In the case of a strong hydration or dehydration, the change in nuclear volume is not completely reversible, but it is reversible nearly completely when the hydration or dehydration is not too strong.

The results we obtained from the experiments with animal nuclei are just what were expected from the results obtained with plant nuclei. The nuclei which are heterogeneous in the body fluid or an isotonic solution are rendered homogeneous by the replacement of the medium with a hypotonic solution or water, a volume increase due to swelling occurring thereat in association. In a hypertonic solution, on the contrary, the nuclei show a severe shrinkage, and as a result of this shrinkage the chromonemata, the chromocenters and other related structures disappear. The heterogeneous structure of the nucleus is, therefore, visible only in a medium the concentration of which lies between the upper and lower critical concentrations. In animal cells the visible change of the structure of the nucleus from one to another takes place far more rapidly than in plant cells. This difference seems due to the fact that in animal cells, the protoplast is furnished with no additional cell wall as in plant cells. In short, in animal cells too, the change in apparent structure of the nucleus depends on the water-relation in the nucleus.

3. *Pollen mother cells of Trillium*

In the preceding sections, we have seen that the chromonemata or their modified structures become obscure through a change in water-relation in the nucleus. Now the question is raised as to whether or not this is also the case with the chromonemata in mitosis. According to the view of recent cytologists, the chromosomes in metaphase, anaphase and telophase consist of two parts, spirally coiled thready portions or the chromonemata, and a matrical portion. In intact cells, these chromonemata are mostly not visible in these stages in mitosis, while the chromosomes as a whole are observed more or less distinctly. Some investigators, therefore, have regarded the chromosomes as homogeneous rods, and the spirally coiled chromonemata which are only seldom visible in the living state of the cell as an unnatural structure (cf. SCHAEDE, 1929), while others are inclined to the view that the homogeneity of the chromosome is a mere apparent phenomenon due to the

lack of difference in refractivity between the spiral chromonemata and the chromosome matrix (cf. KUWADA 1937). If the latter view is correct, the chromonemata should become visible also in the metaphase and anaphase according to the tonicity of the medium, as in the case of the transversal chromatic bands of the salivary chromosomes in *Chironomus* and *Drosophila*. In this section, the results of experiments in this line obtained with the chromosomes in pollen mother cells in *Trillium Smalli* are given. The change in visibility of the chromosomes and the chromonemata was studied by means of saccharose solutions of different concentrations as medium.[1]

When the pollen mother cells are observed with a 1.0 M saccharose solution or the solutions of higher concentrations, some of them shrink and become highly refractive, the shrinkage being accompanied by a strong plasmolysis.[2] In these cells the chromosomes are hardly visible (Fig. 13). In solutions, 0.6 M-0.8 M, the chromosomes become more or less distinctly visible, but no chromonemata are recognizable within the chromosomes. In a 0.4 M or a 0.5 M solution, the chromosomes are observed most clearly, and in these solutions, they usually show distinct chromonemata coiled in spiral and more highly refractive than the matrix. In Fig. 14, these spiral chromonemata are indicated by arrows. In a 0.3 M solution, the pollen mother cells swell, and the chromosomes become very obscure (Fig. 15). In 0.2 M and 0.1 M solutions, the swelling of the pollen mother cells goes so far that distinct chromosomes are no longer visible within the cell. These changes in appearance of the chromosomes are reversible. The reversal is observed when the dilute solution is replaced with a concentrated solution. In Figs. 13-15, the pollen mother cells marked *a* are not healthy ones; they show distinct chromosomes in the saccharose solutions of various concentrations without any plasmolysis taking place.

The results enumerated above, are quite in accordance with our expectation. In concentrated solutions (0.7-1.0 M), both the chromonemata and the matrix are dehydrated and become equally refractive, thus the chromonemata are hardly visible in the chromosomes. In diluted solutions (0.1-0.3 M), on the other hand, hydration takes place. In certain concentrations (0.4 and 0.5 M), the hydration being stronger in the matrix than in the chromonema spirals, the refractive index is rendered lower in the former than in the latter, so that the chromonema spirals become visible in these concentrations. The same is also true with the disappearance and reap-

1) SHIMAKURA (1937) has reported that in the saccharose medium of a certain definite concentration the chromonemata are visible in the metaphase and anaphase chromosomes in *Trillium kamtschaticum* when in process of mitosis. BĚLAŘ (1929, a) has also reported that in an insect the visibility of the chromosomes depends on the tonicity of the medium in which the cells are observed.

2) In our material, some pollen mother cells showed no plasmolysis at all even in a 1.0 M solution. These cells seem to have been unhealthy, having lost their semi-permeability, at least to a certain extent, and thus, only those pollen mother cells which showed a distinct plasmolysis were taken up for the observation.

pearance of the whole chromosome. In a concentrate solution, the chromosomes are not distinctly visible, because the dehydration of the kinoplasmic bodies (spindle and phragmoplast) is so strong that the refractivity of the bodies approaches that of the chromosomes (Fig. 13). In a diluted solution, the chromosomes may again not be visible if they swell and their refractivity is nearly equal to that of the spindle which is also swollen. But they are visible if observed with the media of intermediate concentrations. In short, the visibility of the chromonemata in the chromosomes in division is determined by the magnitude of difference in the hydration degrees of the chromonemata and the matrical part of the chromosomes, and the visibility of the whole chromosome by that of the difference between the chromosomes and the spindle substance.

4, Guard cells of the stomata

The changes in visible structure of the guard cell nuclei of the stomata which take place in association with the stomatal movements have been described by WEBER (1927) in *Vicia faba.* According to him, in the open stomata the guard cell nuclei are very easily recognizable. They are dense and refractive, being opaque and gelatinous. In these cells the microsomes in the cytoplasm show generally no appreciable movement. In the closed stomata, on the contrary, the guard cell nuclei are thoroughly hyaline and even their contour is obscure. WEBER has regarded these nuclei in the closed stomata as being in a sol state. In this latter case, a rapid streaming movement of the microsomes is observed in the cytoplasm. He has regarded the difference in nuclear appearance between the open and closed stomata as due to the sol-gel transformation of the nuclear substances caused by the change in H-ion concentration in the guard cells. In the present investigation the same observation was repeated in *Vicia faba,* and also in *Tradescantia virginica, Zebrina pendula* and some other plants, liquid paraffin being used as medium (SINKE, 1937). The results we obtained with these plants are in accord with those of WEBER, with an additional fact that in the half-open stomata distinct chromonemata are visible in the guard cell nuclei (SINKE, 1937). These three different appearances of the guard cell nuclei occurring according to whether the stomata are open, closed, or half-open seem to be observable in a limited number of plants. In *Sagittaria sagittifolia, Podophyllum pleianthum* (?), *Ceratopteris thalictroides* and some other plants, no such regular change is observed so far as the present investigation is concerned.

In the fully-open stomata in *Tradescantia, Zebrina* and *Vicia,* the guard cell nuclei are in a shrunken state, and are highly refractive, thick chromonemata being only obscurely recognizable here and there in the nucleus, or in certain cases, even no structure at all being visible. In the half-open or the nearly closed stomata, the chromonemata are generally fine and stand out very clearly in the nucleus. When the stomata are in the state of complete closure, on the other hand, the nuclei are nearly or completely

homogeneous again as in the case of the stomata fully open, but in this case they appear hyaline and less refractive. An observation with the dark field illumination shows that in *Tradescantia* these hyaline nuclei are nearly dark, and the refractive nuclei in the fully open stomata are weakly bright at the contour. In the half-open stomata, they appear to be filled with a great number of bright particles as we have seen in the case of the staminate hair cell nuclei in *Tradescantia reflexa* (Division I).

Comparing the results of the observations just given with those obtained in the hydration and dehydration experiments described in the preceding sections, we find that there is a remarkable resemblance in external appearance between the guard cell nuclei in the fully-open stomata and the ordinary vegetative cell nuclei in *Elodea densa, Tradescantia virginica, Gampsocleis* sp. and *Tryxalis nasuta* observed in hypertonic media. In both cases, the nuclei are shrunken and highly refractive, and the chromonemata are only obscurely or hardly visible. Resemblances are also recognizable between the heterogeneous nuclei in *Tradescantia, Elodea, Gampsocleis, Tryxalis,* and some other plants and animals observed in isotonic media and the guard cell nuclei in the half-open stomata, and between the animal homogeneous nuclei in hypotonic media and the nuclei in the guard cells of the completely closed stomata. From these facts of parallelisms between each pair of cases, it may be assumed that the nuclei of the guard cells are in a dehydrated state in the fully-open stomata, and in a hydrated state in the closed stomata. To make it clear whether this assumption is correct or not, the guard cell nuclei of stomata in different apertures were subjected to dehydration and hydration experiments with sugar solutions of different concentrations and with water. In these experiments, the guard cell nuclei in thick tangential sections made along the lower surface of the leaves of *Tradescantia virginica, Vicia faba, Zebrina pendula, Sagittaria sagittifolia, Rhoeo discolor, Dahlia variabilis, Podophyllum pleianthum* (?) and some other plants were tested, the first named three plants being used as the principal material.

a. Tradescantia virginica, Zebrina pendula, Rhoeo discolor and Vicia faba. So far as the hydration and dehydration experiments are concerned, there is found among these plants no important difference in regard to the behaviour of the chromonemata in the guard cell nuclei. The following description is mainly based on the results of observations made with *Tradescantia virginica.*

In the full-open stomata, the guard cell nuclei usually appear to be highly refractive compact masses in a shrunken state, and present no distinct chromonema structure within. In an extreme case, no visible structure whatever is recognizable. In these cases the contour of the nuclei is sharp and often uneven (SINKE, 1937, Fig. 14). In the cytoplasm the streaming movement of the microsomes is generally slow or entirely invisible. With dark field illumination, the nucleus is dark except for its contour which is more or less bright. When the tissue is mounted with a 1.2 M solution or

those of higher concentrations of saccharose, the nuclei present no visible change in appearance, but when mounted with diluted solutions (e. g. 0.1 M saccharose) or water they show a marked change. In these media, the nucleus swells and becomes less refractive than in the normal state, and the chromonemata are more or less distinctly visible (SINKE, 1937, Fig. 15). When observed with dark field illumination, the whole nucleus appears like a cloudy mass of bright particles. After a prolonged immersion in the water medium, the nucleus swells to a greater extent and becomes still less refractive. The chromonemata are no longer visible (SINKE, 1937, Fig. 16), and the cytoplasmic streaming movement is now active. The appearance of the nucleus is, in this state, quite similar to that of the nuclei in the completely closed stomata. When the medium is replaced with a concentrated sugar solution, the nuclei shrink and the chromonemata become visible again. A prolonged immersion in the same medium, however, makes the chromonemata once more invisible, and the nucleus undergoes a strong shrinkage. An example from the experiments with *Zebrina pendula* is given below.

11.15 (a. m.). The tissue with open stomata was mounted with di-distilled water. The guard cell nuclei were shrunken and exhibited no visible structure within (Text-fig. 3, *a*).

11.20. The nuclei swelled and the chromonemata were visible somewhat distinctly.

11.25. The chromonemata were distinctly recognizable (Text-fig. 3, *b*).

11.30. The chromonemata were indistinct.

11.35. The nuclei appeared completely hyaline, but their contours were obscurely visible (Text-fig. 3, *c*).

11.45. The medium was replaced with a 0.4 M solution of saccharose.

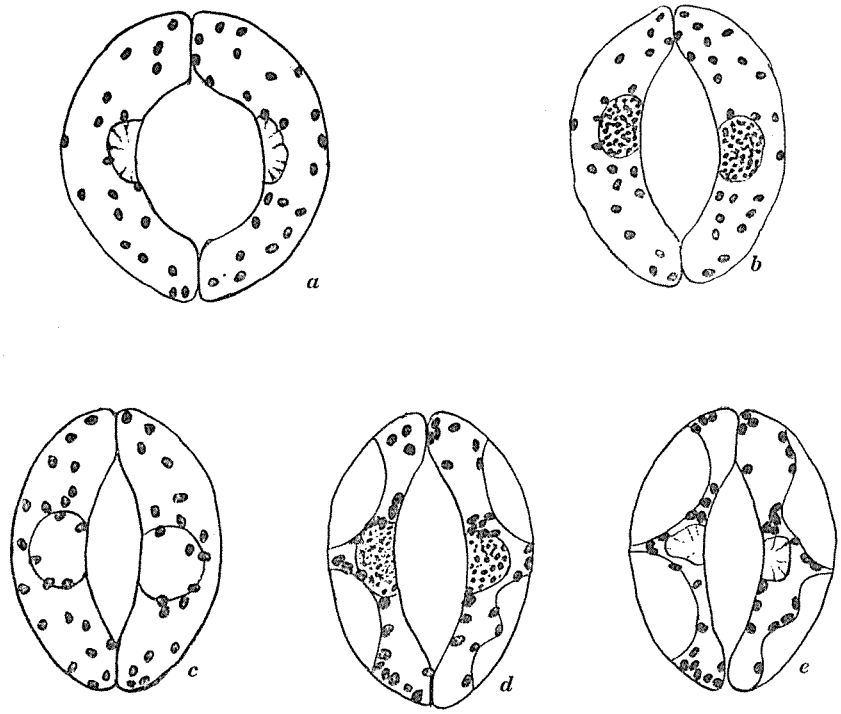
11.50. The chromonemata were hardly recognizable. The medium was replaced with a 0.8 M solution.

11.55. Do. The medium was replaced again with a 1.6 M solution.

12.00. A concave plasmolysis was noticed to have taken place. The chromonemata were very distinctly visible (Text-fig. 3, *d*).

0.25. The nuclei were shrunken and the chromonemata were obscure (Text-fig. 3, *e*).

In the moderately open stomata where a slow streaming movement of the cytoplasm is generally perceptible, the guard cell nuclei are shrunken and highly refractive. The chromonemata which often appear to be thicker in diameter and less in number than those in the guard cell nuclei in the half-open stomata, are recognizable in these nuclei. This structure of the nucleus reminds us of the structure presented by the experimentally moderately dehydratdd nuclei in the young petal cells of *Tradescantia reflexa* and some other plants. A prolonged treatment of these guard cell nuclei with a 0.1 M saccharose solution, or distilled water, makes them exhibit the fine chromonema structure very distinctly for a short time, but the nuclei soon become homogeneous. This change of the nucleus is associated with an increase in volume of the whole nucleus and an active streaming movement of the cytoplasmic microsomes. When the medium is replaced with a concentrated solution, it is found that these changes in the nucleus and the cytoplasm are completely reversible.



Text-fig. 3. The same stoma in the leaf epidermis of *Zebrina pendula* (× ca. 600). a) At 11.15 a. m. In di-distilled water. In fully open state; the nuclei are shrunken and nearly homogeneous. b) At 11.25 a. m. The chromonemata are distinctly visible. c) At 11.35 a. m. The nuclei are swollen and the chromonemata are not visible. d) At 12.00, the medium replaced with a 1.6 M saccharose solution. The chromonemata are very distinct. e) At 0.25 p. m. The nuclei are shrunken and the chromonemata are obscure.

In the half-open or somewhat closed stomata, the guard cell nuclei present the fine chromonema structure. They are refractive far less than the nuclei in the fully-open stomata (SINKE, 1937, Fig. 10). The microsomes in the cytoplasm show a more or less rapid streaming movement. When the nuclei in this state are observed in a concentrated saccharose or a glucose solution, it is found that they have become shrunken and highly refractive, and the chromonemata have disappeared (SINKE, 1937, Fig. 11). The general appearance of the nuclei is now similar to that of the nuclei in the fully-open stomata. In the cell, plasmolysis and the stoppage of the streaming movement of the microsomes are observed. When the medium is replaced with water, the chromonemata are rendered visible again, but only for a short time, and soon disappear again (SINKE, 1937, Fig. 12). The streaming movement of the microsomes in the cytoplasm also reappear, being very rapid. The nuclei are now much swollen and present an appearance similar to that of the nuclei in the completely closed stomata.

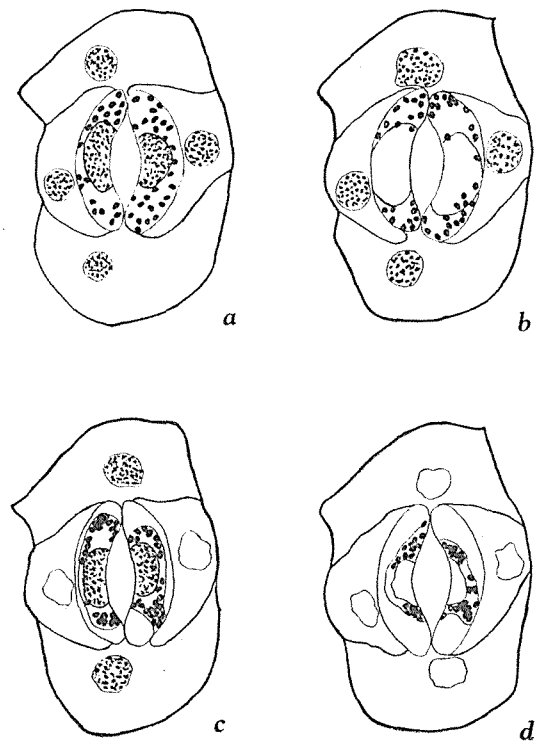
When the guard cell nuclei in the half-open stomata are observed in

water or a diluted sugar solution, the chromonemata which are visible in the nuclei become indistinct, as in the case of the animal cells, but far more gradually than in the latter, and in an extreme case they finally disappear entirely. The general appearance of the nucleus is in this state quite similar to that of the nuclei in the completely closed stomata. This change is reversible. An example from the experiment with *Tradescantia virginica* is given below.

9.50 (a. m.) The chromonemata were distinctly observed with di-distilled water as medium in the nuclei in the guard cells of half-open stomata as well as the other epidermal cells. This appearance of the nucleus showing the chromonema structure continued for about one hour with no marked change (Text-fig. 4, *a*).

10.50. The chromonemata in the guard cell nuclei were still distinctly observed.

11.10. The guard cell nuclei were hyaline, and their contours were hardly perceptible. The nuclei of the other epidermal cells showed no change, appearing quite normal with the chromonemata distinctly visible (Text-fig. 4, *b*).



Text-fig. 4. The same stoma in the leaf epidermis of *Tradescantia virginica*. (× ca. 400). a) At 9.50 a. m. In di-distilled water. The chromonemata are visible. b) At 11.10 a. m. The nuclei are quite hyaline. c) At 11.20 a. m. The medium was replaced with a 0.8 M saccharose solution. The chromonemata are very distinct. d) At 0.05 p. m. In 1.2 M solution. The nuclei are shrunken and homogeneous.

11.20. The medium was replaced with a 0.8 M saccharose solution. The chromonemata reappeared in the nucleus. They were fine and distinct as in the normal state. The convex plasmolysis of the guard cells was observed (4, *c*).

11.30. The medium replaced with water.

11.40. The nuclei of the guard cells were homogeneous again while those of the subsidiary cells exhibited the fine chromonemata.

12.00. The medium was replaced with 0.4 M saccharose solution. All the nuclei showed the chromonema structure.

12.05. The medium was replaced with 1.2 M solution.

0.05. All the nuclei shrank and appeared homogeneous (Text-fig. 4, *d*).

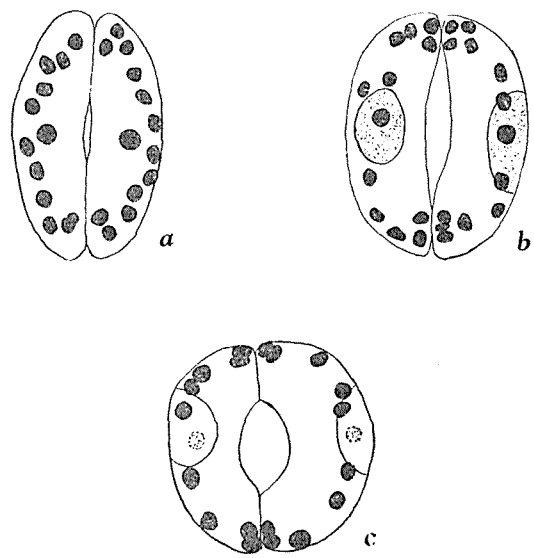
0.50. The medium was replaced with water.

1.00. The guard cell nuclei showed distinct chromonemata.

The guard cell nuclei of the completely closed stomata present generally a swollen appearance and are homogeneous. Usually even their contours are hardly perceptible, and the existence of the nuclei is indicated by the hyaline area which is surrounded by numerous chloroplasts (SINKE, 1937, Fig. 4). In most cases, the rapid streaming movement of the microsomes is observed in the cytoplasm. When these homogeneous nuclei are dehydrated with a concentrated saccharose solution, they shrink and become heterogeneous (SINKE, 1937, Fig. 5). The appearance of the nucleus in this state is similar to that of the nuclei in the half-open stomata. In a more concentrated solution, the nuclei shrink more severely and become highly refractive, and the cells show plasmolysis. In this state of shrinkage, we can hardly recognize any distinct structure in the nuclei (SINKE, 1937, Fig. 6). These nuclei remind us of those of the stomata in the fully-open state. When the medium is replaced with a diluted saccharose solution or water, the nuclei swell and again show distinct chromonemata. By a prolonged immersion in these media, the chromonemata disappear in association with the nuclear swelling. The general appearance of the cell is in this state similar to that of the cell before the dehydration. The reversible changes can be repeated several times with no visible injury to the cells.

b. Dahlia variabilis. WEBER (1926) has found that in the guard cell nuclei in *Dahlia variabilis*, the nucleolus disappears or reappears in a close association with the stomatal movements. According to him, the nucleolus is visible very distinctly in the closed stomata, while it disappears when the stomata are open. A complete disappearance of the nucleolus was not observed in the present investigation. The results we obtained are briefly described in the following.

In this plant the guard cell nuclei carry one large nucleolus each. In the closed state of the stomata, the nucleus is completely



Text-fig. 5. Three stomata from the leaf epidermis of *Dahlia variabilis* in liquid paraffin (× ca. 800). a) A closed stoma. The guard cell nuclei are not visible except for one large nucleolus in each. b) A half-open stoma. The chromonemata are visible more or less distinctly in the nuclei. c) An open stoma. The nuclei show no visible structure except the nucleolus which is slightly obscure.

hyaline except for the nucleolus. Generally, even the outline of the nucleus is hardly perceptible (Text-fig. 5, *a*). In the half-open stomata, the guard cell nuclei appear to be heterogeneous. It is here difficult to determine by direct observation whether the heterogeneity is due to the thready (chromonema) structure or the granular (Text-fig. 5, *b*). In the fully-open stomata, the guard cell nuclei are highly refractive, and are very distinctly visible. The nucleolus is somewhat indistinct in the nucleus, and no chromonemata are perceptible with precision (Text-fig. 5, *c*). When closed stomata are immersed in a concentrated saccharose solution (1.2 M or those in higher concentrations), the guard cell nuclei become heterogeneous, but only transitorily, and they soon come to be homogeneous again. The nucleolus too, is hardly visible in these nuclei. When fully-open stomata are observed with water as medium, the nuclei are rendered heterogeneous and the nucleolus becomes very distinct. In this case too, after a prolonged immersion, the nuclei become homogeneous again, except for the nucleolus.

From the results of the experiments with *Dahlia* mentioned above, it is seen that the indistinctness of the nucleolus observed by WEBER in the guard cell nuclei of fully-open stomata is caused by the dehydration of the nucleus. In the fully-open stomata, dehydration takes place in the nucleus and, therefore, the refractivity of the karyolymph approaches those of the nucleolus and the karyotin, thus resulting in the indistinctness of the nucleoli in the nuclei.

c. Sagittaria sagittifolia, Podophyllum pleianthum (?) *and Ceratopteris thalictroides.* In these plants the guard cell nuclei of the stomata are relatively large and show chromonemata more or less distinctly in all stages of the stomatal movements so far as the present observation showed. As in the cases of *Tradescantia, Zebrina* and some other plants, the chromonemata become invisible when the nuclei are treated with a concentrated saccharose solution, and visible in the medium replaced with water, and after a prolonged immersion in water, they often become invisible again, accompanied by a swelling of the whole nucleus in volume. In the medium of water, fresh nuclei or nuclei not treated before usually swell and become homogeneous as those once dehydrated, but this is not always the case. This change is reversible, if it takes place; the heterogeneity of the nucleus is restored by the replacement of water with a concentrated sugar solution.

d. Changes in starch content of the guard cells during plasmolysis. It is a well known fact that the starch content in the guard cells is smaller in amount in the open state of the stomata than in the closed state (LLOYD,[1] 1908, ILJIN, 1915 and 1922 and others). In *Vicia faba*, a marked change in starch content of the guard cells is observed in association with the functional change of the stomata. In the present investigation, an examination was made with *Vicia faba* leaves to determine whether or not the starch-sugar transformation takes place also during the process of the nuclear changes caused by hyper- and hypotonic solutions. As the histochemical reagent,

1) Cited from SCARTH (1927).

the chloral-iodine solution was used. Each experiment was repeated several times with different leaves and for different periods of time.

i. Observation with closed stomata. Two epidermal tangential sections from one and the same leaf were made, and they were observed with water as medium. In closed stomata, the nuclei of the guard cells were swollen and nearly homogeneous. The one section was stained with chloral-iodine solution, and many starch grains were found to be contained in the guard cells. The other section was immersed in a 1.0 M solution of saccharose. The nuclei became heterogeneous. The chloral-iodine solution was then applied on the section. The guard cells were found to contain as many starch grains as in the first section.

ii. Observation with fully-open stomata. As in the case of closed stomata, two sections of the same leaf were observed with water as medium, and it was confirmed that the guard cell nuclei were shrunken and homogeneous. The one section was stained with the chloral-iodine solution and it was ascertained that no appreciable amount of starch grains was contained in the guard cells. The other section was left in di-distilled water for 10 minutes, and the nuclei were found to be swollen and hyaline. In this section many starch grains were found though not so abundant as in the closed stomata.

From the results of these experiments it seems that the sugar-starch transformation takes place when open stomata are immersed in water, while the starch-sugar transformation hardly occurs or only slightly, if at all, when closed stomata are put in a hypertonic solution, or briefly speaking, that catatonosis takes place during the hydration of the nucleus while anatonosis during the dehydration is absent or very slight.

Summing up the results given in this section, the following may be noted:—In certain plants the guard cell nuclei of stomata show marked changes in visible structure in close connection with the functional changes of the stomata. While, in these plants, they are shrunken and highly refractive without presenting any distinct structure within in the fully-open state of the stomata, they are swollen and completely hyaline with a low refractivity in the completely closed stomata. In the intermediate states of opening of the stomata, the nuclear structure is recognizable more or less distinctly. These three different appearances of the guard cell nuclei, presented according to whether the stomata are fully-open, half-open, or completely closed, closely resemble those of animal nuclei observed with media of three different concentrations, i. e. a hyper-, the iso- and a hypotonic medium respectively. The characteristic features presented by the nuclei of the fully-open stomata can be artificially produced by applying a hypertonic solution to the nuclei of closed or half-open stomata, and those of the complete closed stomata by immersing the nuclei of half-open and fully-open stomata in a water medium. In the former case, the explanation of this transformation of the nuclear structure from one to another appears to be relatively simple, since the experiment shows that there takes place in the cell no anatonosis during the transformation, and the explanation would

be made from the view point of dehydration phenomenon in the same way as in the case of the nuclei in the young petal epidermal cells of *Tradescantia* and those in the leaf epidermal cells of *Elodea*, rendered homogeneous and highly refractive by hypertonic solutions. In the latter case, on the other hand, no simple explanation is sufficient, since in this case, the guard cells are in a turgescent state, and hence a further penetration of water into the cells from outside is hardly possible. The swelling of the nucleus must, therefore, be due to the decrease of tonicity in the cytoplasm or in the vacuoles. In this latter case the transformation of sugar to starch was observed, as is mentioned above. This will cause a certain decrease of the tonicity in the guard cells, and furnish an explanation. In short, the mechanism of the nuclear hydration by hypotonic solutions in the guard cell nuclei must be a different one from what we may assume in the case of animal cells, but at any rate the disappearance and reappearance of the nuclear structure are to be regarded as hydration and dehydration phenomena.

Now we are led to the conclusion that the guard cell nuclei are in a dehydrated state in the open stomata, and in a hydrated state in the closed stomata, and that in the half-open stomata they are in intermediate states between the two. The physiological cause inducing this difference in hydration is not quite clear, but it seems that the change in osmotic pressure in the guard cell occurring in association with the stomatal movement plays an important rôle here (cf. ILJIN, 1915, WIGGINS, 1921). It must be noted, however, that it seems not to be a universal phenomenon, as is stated above in the introduction to this section, that the changes in nuclear appearance take place in association with the stomatal movements.

5. *Root tip cells of Vicia*

The effect of plasmolysis on the nuclei in root tip cells has been studied by NÈMEC (1910) and SAKAMURA (1920) using a 6% KNO_3 solution as a plasmolyzing agent. According to STRUGGER (1930), however, the nuclear structure undergoes remarkable changes as direct effects of KNO_3 solutions. It is, therefore, in this case difficult to distinguish between the effect of plasmolysis and that of the ions of KNO_3. In the present investigation, therefore, saccharose solutions were used instead of KNO_3. Root tips of *Vicia faba* were immersed in 0.1 M, 0.2 M, 0.5 M, 0.7 M and 0.8 M saccharose solutions, and after fixation changes in structure of the nucleus and in migration behaviour of chromosomes in mitosis were studied. A part of the material was fixed with the BONN modification of FLEMMING's solution and stained with HEIDENHAIN's haematoxylin, and the remainder was fixed with a mixture of corrosive sublimate and acetic acid with the object of staining the sections after FEULGEN's method. The temperature of the saw-dust in which the root tips were kept growing before the experiment was 20°C–24°C, and that of the saccharose solutions used was 24°C–26°C.

i. Di-distilled water. The root tips are macroscopically normal after

immersions in di-distilled water for 1 hour, 3 hours, 8 hours, 18 hours, 24 hours and 48 hours. Microscopically, no abnormality is observed in the nuclei either in rest and in mitosis.

ii. 0.1 M solution. The root tips are quite normal both macroscopically and microscopically after immersion or treatments for 2 hours, 4 hours, 8 hours, 12 hours and 48 hours.

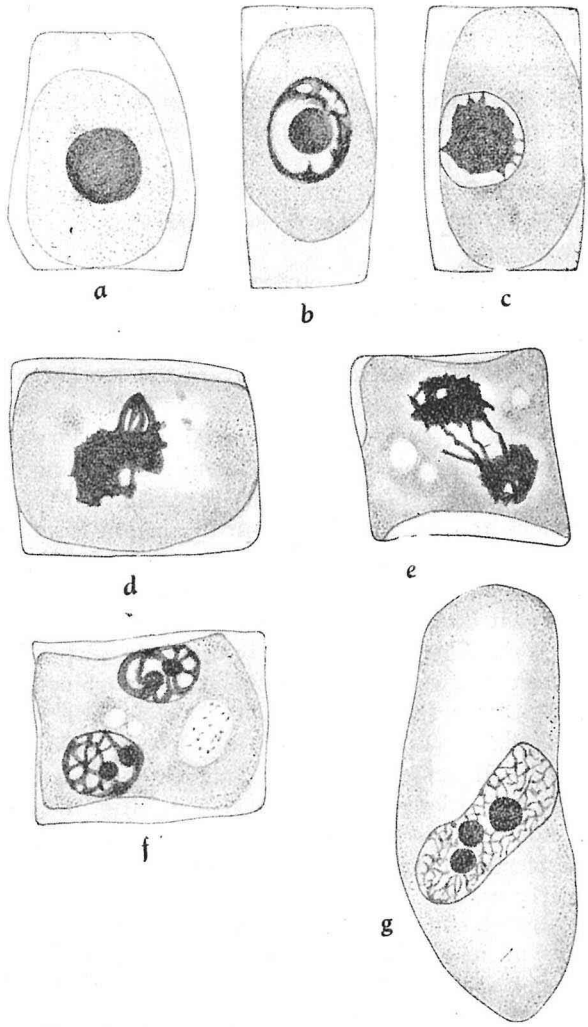
iii. 0.2 M solution. No abnormal figures are observed in the cells after treatment for 1 hour, 3 hours, 8 hours, 30 hours and 72 hours.

iv. 0.5 M solution.

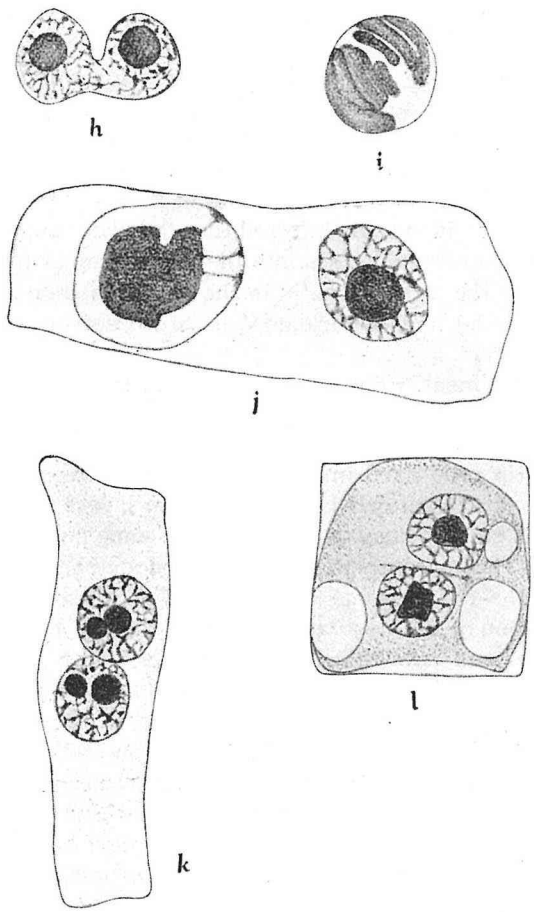
Treatments for 30 minutes, 1 hour and 2 hours. The root tips are not highly turgid, and do not reduce osmium tetraoxide contained in the fixing mixture. The cells in two or three outer layers of the peripheral part of the root tip show plasmolysis. In these layers the resting nuclei present a coarse mesh-like structure or a homogeneous structure stained evenly (Text-fig. 6, *a*). While in some nuclei there is found a large hyaline area surrounding the central, deeply stained nucleolus and pushing the chromatic substance to the periphery of the nucleus (Text-fig. 6, *b*), in others, the chromatic substance too occupies the central position forming a compact mass together with the nucleolus, which is surrounded by the hyaline area (Text-fig. 6, *c*). The chromosomes in metaphase and anaphase are more or less coalescent, and it is difficult to distinguish one chromosome from the others (Text-fig. 6, *d*). The chromosome bridge is frequently found stained very deeply between the two compact masses of the anaphase chromosomes (Text-fig. 6, *e*). POLITZER (1934) has called the coalescent state of such a chromosome mass the "Pyknose". Frequently, the spindle fibers are very distinctly observed. In the telophase, the daughter nuclei are mostly hyperchromatic and the formation of the new cell wall often fails to take place (Text-fig. 6, *f*). Sometimes, numerous granules are found in the cytoplasm (Fig. 16). In the plerome cells, the appearance of the resting and the dividing nuclei is nearly normal.

4 hours treatment. In these root tips, the homogeneously stained or coarsely reticulate nuclei are found only in the outer layers of the periblem. Most of the nuclei are not stained deeply, and show a finely reticulate appearance. In some root tips, a dense cytoplasmic layer is found surrounding the nucleus. This cytoplasm may perhaps correspond to the "Siedroplasma" which has been described by MATSUDA (1928) in *Petunia* and which is taken by BLEIER (1930) as one preventing the normal processes of mitosis. Normal mitotic figures are found frequently in the periblem as well as in the plerome.

6 hours treatment. The resting nucleus is not stained deeply, and presents the reticulate appearance or the chromonema structure. In the periblem, numerous granules are often found in the cytoplasm. They are of indefinite shape and size, and are deeply stained with HEIDENHAIN's haematoxylin, FEULGEN's staining being negative to these granules. Normal mitotic figures are observed not only in the plerome but also in the periblem cell layers.



Text-fig. 6. *a–l.* The nuclear and mitotic irregularities in the root tips of *Vicia faba* treated with hypertonic saccharose solutions, and fixed with BONN modification of FLEMMING's solution followed by the staining with HEIDENHAIN's haematoxylin (× ca. 1300). *a*) 1 hour treatment with 0.5 M solution. Showing a shrunken, homogeneous nucleus in periblem. *b*) 30 minutes treatment with 0.5 M solution. Showing the vacuolization of the nucleus or a nucleolar halo. *c*) 1 hour treatment with 0.5M solution. Showing the contraction of the chromatin in the nucleus. *d*) and *e*) 30 minutes treatment with 0.5 M solution. Showing the pycnosis or the coalescence of the chromosomes in metaphase and that in anaphase. *f*) 1 hour treatment with 0.5 M solution.



The formation of the new cell wall in telophase is suspended. *g*) and *h*) From the root tips fixed with a 7 hour interval in 0.1 M solution after the treatment with a 0.7 M solution for 80 minutes. Showing di-diploid nuclei. *i*) Do. Showing a mass of chromosomes enclosed with a nuclear membrane. *j*) Do. Showing a bi-nucleate cell with the nuclei of different structures. *k*) A bi-nucleate cell from the root tip fixed with a 24 hour interval in 0.1 M after treatment with 0.7 M for 80 minutes. *l*) Immediately fixed after treatment with a 0.8 M solution for 5 hours. A bi-nucleate cell with a rudimentary cell plate.

24 hours treatment. The cells with the nuclei of abnormal appearance and those with abnormal mitotic figures are found to be less in number than in the case of the treatment for 6 hours. In some cells, plasmolysis is not observed, and many normal mitotic figures are found. In this experiment with the 0.5 M solution, therefore, it is seen that both resting and dividing nuclei recover their normal appearance after a prolonged immersion in the solution. In this series of investigation no special experiment was carried out to explain the mechanism of taking in water which is necessary for the recovery, but it may be inferred that the water supply is due to the penetration of saccharose molecules into the protoplasm or to the occurrence of an anatonosis in the protoplasm.[1] In the root tips treated for 24 hours, giant di-diploid nuclei and bi-nucleate cells are observed not infrequently.

v. 0.7 M solution.

80 minutes treatment. The root tips treated were fixed immediately or after being transferred into a 0.1 M solution for the periods of time, 1 hour, 7 hours and 24 hours.

In the root tip cells fixed immediately after the treatment, plasmolysis is observed in the periblem cell layers. In these layers most of the nuclei are shrunken and stained homogeneously, while some nuclei appear coarsely reticulated. The chromosomes in metaphase and anaphase are more or less coalescent, and the chromosome bridge is frequently found. In the telophase, the formation of the new cell wall is frequently found, but is likely to be incomplete, and two of the daughter nuclei with high chromaticity are found in a single cell. In the plerome most of the resting nuclei show a structure of thick chromatin threads which fuse together in places to form a coarse reticulum, while others are shrunken and are homogeneously stained. The chromosomes in metaphase and anaphase are coalescent more or less strongly. In the root tip cells fixed after one hour immersion in the 0.1 M solution, a deplasmolysis is observed. Most of the nuclei appear normal, showing a chromonema or reticulate structure, and the normal mitotic figures are also visible, but pycnotic chromosome masses are found abundantly in the plerome. Sometimes, thick and short chromosomes which remind us of the meiotic chromosomes are found in the plerome. In some cases, in metaphase and anaphase, many fine granules are observed sticking to the surface of the chromosomes. This appearance of the chromosomes resembles that of those observed by SAKAMURA (1920) in the root tips treated with a solution of chloral-hydrate. In the periblem, most of the nuclei show the normal structure, but some are shrunken and pycnotic. No plasmolysis is observed in the cells in the periblem or the plerome. The mitotic figures are generally normal in the periblem, while they are pycnotic in the plerome. Cells with a giant di-diploid nucleus and those with two diploid nuclei are, however, frequently found in both periblem and plerome. In Text-figs. 6, *g* and 6, *h*, such di-diploid nuclei are shown. Sometimes it is found that in

1) The penetration of saccharose into the cell has frequently been reported by plant physiologists such as RUHLAND (1911), FITTING (1917) and HÖFLER (1926).

the bi-nucleate cell one of the two daughter nuclei presents the appearance of a resting nucleus while the other contains a contracted chromatin mass surrounded by a large hyaline area (Text-fig. 6, *j*). Not infrequently, it is observed that a group of chromosomes is surrounded by a nuclear membrane as shown in Text-fig. 6, *i*. In the root tips fixed after 24 hours immersion in the 0.1 M solution, most of the nuclei show the normal appearance, but some which are found in the periblem are somewhat deformed in contour. The mitotic figures are quite normal in most cases. Many cells may, however, be in necrosis, while the neighbouring cells appear quite normal. The nuclei in these necrotic cells are shrunken and hyperchromatic. With FEULGEN's staining method, they are deeply, but transparently coloured. Very frequently two daughter nuclei are found lying against the new cell wall so closely that they appear at first sight to be in contact directly with each other. The di-diploid nuclei and the bi-nucleate cells are also not infrequently found (Text-fig. 6, *k*).

3 hours and 8 hours treatments. A strong plasmolysis is observed not only in the periblem but also in the plerome. Most of the resting and dividing nuclei present pycnotic appearances such as those observed in the root tips treated for 80 minutes. The cell wall formation frequently fails to take place resulting in the formation of the bi-nucleate cell (Fig. 17).

24 hours treatment. A weak plasmolysis is observed, and most of the nuclei, especially those in the plerome, are found to have recovered from their ill appearance. Dead nuclei and the chromosome masses in degeneration are found strongly shrunken and deeply stained, some of them being amoeboid or discoid in shape (Fig. 18). In the periblem, in certain layers vacuolization in the cytoplasm is remarkable. The normal mitotic figures are found, though they are not abundant. Giant di-diploid nuclei (Fig. 19) and bi-nucleate cells are also found not infrequently. These cells show plasmolysis of a weaker intensity than that observed in the case of the 80 minutes treatment.

vi. 0.8 M solution.

30 minutes, 1 hour, 3 hours and 5 hours treatments. The appearance of the resting nuclei and the behaviour of the chromosomes in metaphase and anaphase are nearly the same as those observed in the root tips treated with the 0.7 M solution. In the root tips treated for 5 hours, the formation of the new cell wall is incomplete in a number of cases, as shown in Text-fig. 6, *l*.

10 hours treatment. The general appearance of the nucleus is similar to that of the nucleus observed in the cases of the treatments mentioned above. Not infrequently, there are found large nuclei of varying shapes. They usually contain several chromosomes which undergo no reconstruction process and only rarely present the usual appearance of the resting nucleus. These nuclei are probably direct products from the chromosome groups in metaphase or in an early anaphase where the chromosome separation is incomplete. Bi-nucleate cells are also found in this material.

24 hours treatment. Plasmolysis is found. In the periblem, numerous resting nuclei are found shrunken and stained deeply with haematoxylin. When stained with FEULGEN's method, these shrunken nuclei and also the chromosome masses in degeneration, are coloured evenly and appear nearly transparent. In some root tips, however, the structure of the nucleus in rest and the behaviour of the chromosomes in division are found normal in the plerome. In the preparations stained with FEULGEN's method, the chromosomes are often abnormally thick and short; they show a reticulate appearance probably as a result of deformation of the chromonemata contained.

The main results we obtained in the plasmolysis experiments with the root tip cells of *Vicia faba* are as follows:—

In the resting stage, the nucleus is shrunken with an uneven contour and is of a coarsely reticulate structure or is homogeneous and hyperchromatic. It is sometimes characterized by a large hyaline area formed in association with the contraction of the chromatin. Most of these abnormalities are those observed in the living epidermal nuclei in the young petals of *Tradescantia* treated with a concentrate sugar solution (Section 1, Division II).

In the dividing stages, a coalescence or a pycnosis of the chromosomes takes place. When the pycnosis is of a low intensity, the chromosome bridges are frequently formed, and when it is of a high intensity the chromosomes collect into a compact mass. The chromosomes in metaphase and anaphase are usually thicker and shorter in the treated root tips than in the normal. The so-called pseudo-amitotic figures or dumb-bell shaped masses of chromosomes are found frequently. Large resting nuclei of a dumb-bell, pear or other irregular shape are also found. As suggested by the results of the investigations by STROHMEYER (1935), WADA (1935) and SHIGENAGA (1937) carried out with the living cells of *Tradescantia*, it is highly probable that these structures represent di-diploid nuclei formed by the reconstruction from the pycnotic masses of chromosomes (cf. MILOVIDOV, 1938). In the present investigation, such di-diploid nuclei were found usually in the root tips treated by a successive treatment with a hypertonic solution and an isotonic solution (0.1 M), but they were often found also in the root tip cells subjected merely to prolonged immersion in a hypertonic solution (Fig. 19). According to SHIGENAGA (1937), the reconstruction of the telophasic daughter nuclei or the formation of the di-diploid nuclei is possible only when dehydration is followed by the treatment with a hypotonic solution or water. He has obtained, on the other hand, the result that, if caffeine is used, both di-diploid and bi-nucleate cells are formed by a mere treatment with the solution without any replacement of the medium with water. He has regarded this exceptional result he obtained with caffeine as due to the fact that the dehydration by means of the caffeine solutions takes place only in a low degree. In the cases we met with in the experiments with hypertonic sugar solutions also, the same fact seems to be the cause of the

reconstruction taking place without any additional treatment, and the low dehydration intensity seems to be due to a certain penetrability of sugar into the protoplasm, as suggested by the fact that strong plasmolysis is not shown by these cells. In the telophase, the formation of the new cell wall is frequently incomplete or entirely suppressed, resulting in the production of bi-nucleate cells. In some cases, the nuclear reconstruction does not take place in telophase, so that sometimes chromosomes that have not changed noticeably from the appearance they showed in metaphase are visible in the daughter nuclei with a completely formed new cell wall between them. The fragmentation or the rhexis of chromosomes of POLITZER (1934) and such irregularities in chromosome behaviour as the multipolar orientation in metaphase, the lagging in anaphase, etc. were not observed in the present investigation. The fact reported by LEWIS (1934) that the chromosomes are found scattered in the tissue culture with a hypotonic medium is very interesting when compared with the results we obtained with hypertonic solutions—the dehydration figures. It seems very likely that the chromosome scattering is a hydration figure.

In the cytoplasm, the hyperchromaticity, the plasmolysis and some other abnormalities are observed. In some cases, the hyperchromatic cytoplasm is found near or surrounding the resting nucleus or the spindle area in the dividing cell. This hyperchromatic cytoplasm reminds us of the "Siedroplasma" of BLEIER (1930), but this cytoplasm appears to have no relation to the occurrence of irregular mitoses, becanse it is not a universal occurrence in abnormal mitosis. Sometimes, small granules which are negative to the FEULGEN's nucleal-staining are found in the cytoplasm or the cytoplasmic vacuoles, or in both.

6. *Conclusion*

As stated in the introduction of this Division II, it has been reported that the apparent structure of the living nucleus changes as effect of hyper- and hypotonic solutions, but the results obtained by different investigators are not completely uniform. As to the cause of these changes, there are two opinions. KAMNEV (1934) is of the opinion that the emergence of the nuclear structure in the hypotonic solution is due to an increase in H-ion concentration of the cytoplasm. The result we obtained with the animal nucleus is, however, hardly compatible with this explanation because in this case the chromonemata disappear in the hypotonic solution. If, as assumed by KAMNEV, the H-ion concentration in the cytoplasm is increased under the influence of the hypotonic solution, the chromonemata must come into sight as demonstrated by VAN HERWERDEN (1923), KUWADA and SAKAMURA (1927), SAKAMURA (1927), STROHMEYER (1935), ZEIGER (1935) and others with solutions of known H-ion concentration. It is, moreover, noted here that when an isotonic medium is replaced with a hypotonic solution the change in nuclear appearance takes place too rapidly to be the result of the effect of the solution on the change in pH of the cytoplasm. Moreover, KAMNEV's

explanation does not fit the case of the highly refractive homogeneous nuclei in the guard cells of fully-open stomata which become heterogeneous by treatment with a hypotonic solution and swell and become homogeneous again on a prolonged treatment. If in this case, the heterogeneous appearance of the nucleus is due to an acidosis, as considered by KAMNEV, a prolonged treatment should not result in the change of the nucleus into homogeneous appearance, but should allow the appearance to remain still heterogeneous, since, as is widely accepted, acid causes a homogeneous nucleus to become heterogeneous. The effects of the hyper- and hypotonic solutions on the nuclei must, therefore, differ fundamentally from the case of the asphyxy reported by NASSONOV (1932) in which the change in nuclear appearance is due to the change in pH in the cell. STROHMEYER (1935) has, on the other hand, expressed the view that the nuclear changes caused by hypo- and hypertonic solutions are the result of the hydration and dehydration of the nuclear components due to these solutions. He has observed that in *Elodea* an application of a hypertonic solution to homogeneous nuclei causes them to become heterogeneous, and that these heterogeneous nuclei become homogeneous again by a further immersion in the same medium, now being, however, highly refractive as compared with their state before the experiment. He has explained these changes in nuclear appearance as due to an increase or decrease in magnitude of the difference in refractivity between the nuclear components. This view of STROHMEYER seems far more plausible than that maintained by KAMNEV, though the data on which STROHMEYER's view is based are those obtained in some restricted cases such as the swollen homogeneous nuclei in *Elodea* and the heterogeneous nuclei in *Tradescantia* and a few other plants, and though he has mentioned, without trying to give any explanation of it, an exceptional case, that in *Vallisneria* the heterogeneous nuclei do not become homogeneous in a hypertonic solution.

The results of experiments we obtained fully support the view of STROHMEYER, no such exceptional case as that mentioned by STROHMEYER being observed in the material of *Vallisneria* we used. The results obtained by the application of hypertonic solutions to living cells are: 1) an increase in refractivity of the nucleus, 2) a decrease in volume of the nucleus, 3) stoppage of Brownian and streaming movements in the cytoplasm, and 4) occurrence of plasmolysis. All these facts indicate that dehydration takes place in the protoplast. These characteristic changes are caused equally by different plasmolyzing agents, such as saccharose, glucose, urea, NaCl, KNO_3, $CaCl_2$ and RINGER's balanced solution, and this fact suggests that the changes are not due to the special action of the plasmolyzing agents used. We obtained, moreover, the result that the ratios of the upper critical concentrations of these plasmolyzing agents to that of saccharose or the disappearance coefficients are nearly equal to the corresponding ratios of the critical plasmolytic concentrations of the same agents or the isotonic coefficients. This result supports the view that the disappearance of the

individual chromonemata in concentrated solutions is due to an osmotic phenomenon. This view is favoured also by the fact that when the medium is replaced with the original one, the nuclear changes are usually reversible with little injury to the cells.

For the experiments with hypotonic solutions, plant cells are not suitable owing to their high wall pressure due to the cellulose membrane they carry, but the experiments with animal cells show that the disappearance of the nuclear structure is brought about also by the application of hypotonic solutions to the cells. In this case the disappearance is a hydration phenomenon in contrast to the case of the disappearance by hypertonic solutions. The heterogenization by hypotonic solution observed by KAMNEV must be due to the condition that the nuclei subjected to the experiment were in a dehydrated state as in the case of the guard cell nuclei of fully-open stomata. In the case of plant cells too, the disappearance of the nuclear structure by immersing the cell in water is observed in the case of the guard cells, but the mechanism of taking in water is quite different from the case of the animal cells, since the guard cells are furnished as are other plant cells with the cellulose membrane which causes a high wall pressure. In this case it seems highly probable that the catatonosis due to the transformation of sugar to starch plays a principal rôle in the hydration.

With STROHMEYER we may then conclude that the changes in nuclear structure caused by hyper- or hypotonic solutions are due to the hydration or the dehydration of the nuclear components, the chromonemata and the karyolymph. When the chromonemata and the karyolymph are in certain degrees of hydration or dehydration and have different refractive indices, the nuclei will appear heterogeneous, and when they are equally refractive, they will be homogeneous. It is seen from the experiments with pollen mother cells, that the same is true in the case of chromosomes. In this latter case the difference in refractivity causing the appearance or disappearance of the structure lies between the chromonemata and the chromosome matrix. In the nuclei where the chromonemata are visible, two different cases may be considered: 1) The chromonemata are in a dehydrated condition and are more refractive than the karyolymph and 2) the chromonemata are more hydrated than the karyolymph and accordingly less refractive than the latter. In the nuclei where the chromonemata are not visible we may have two other cases: 3) the chromonemata and the karyolymph are equally hydrated, so that they are similarly refractive (homogeneous nucleus I and II), and 4) both chromonemata and karyolymph are dehydrated and similarly refractive (homogeneous nucleus III). So far as the present investigations show the nucleus illustrating case 2 is not found in the natural state, nor obtainable by experiment. The hydration-dehydration experiments disclose no types, if any exist, in the heterogeneous nucleus, while, as will be seen later, by heating experiments it is possible to obtain.

According to the results we obtained with fixed root tips of *Vicia faba*, plasmolysis causes various abnormalities in mitosis. The abnormalities are

mostly the chromosome pycnosis and its derivatives as a result of recovery of the cell from a morbid condition such as the formation of di-diploid giant nuclei and bi-nucleate cells. The direct cause of the abnormality in mitosis is not thoroughly clear at present, but there seems to be little doubt left that the abnormal dehydration of the cellular components, especially that of the spindle, is the primary cause of these abnormalities. Normal mitotic processes should take place where the normal water relations are kept undisturbed in the cell.

III. Effects of High and Low Temperatures

LEPESCHKIN (1923), HEILBRUNN (1928), NASSONOV (1932), ILJIN (1934) and some others have studied the changes in the protoplasm induced by heat and cold which is reversible or may result in death of the protoplasm from the physiological point of view; but giving little attention to the changes caused thereby in the nuclei in rest and division. Many cytologists have, on the other hand, carried out investigations in this line with special attention to the changes in the nucleus and especially those in mitosis, and have observed various mitotic irregularities induced (GEORGEVITCH, 1910, SAKAMURA, 1920, YAMAHA, 1927, BLEIER, 1930, KEMP and JUUL, 1930 and others). In many of these investigations, however, since the object was purely morphological, the fixation method was employed, and hence though final results have been observed, little is known about the series of changes involved. In the present investigation, therefore, not only fixed material, but also living material was used, and study was made not only of the nuclear changes, but also of the cytoplasmic changes which may be very intimately connected with the nuclear changes. Most of the experiments were carried out with high temperatures, but some with low temperatures. The materials and methods used in the experiments will be indicated in suitable places in the following descriptions.

A. High temperatures

For the sake of convenience, the results obtained will be given below divided into four categories according to the kinds of tissues used as material, namely :— 1) Epidermal and hair cells, 2) pollen mother cells, 3) guard cells of stomata, and 4) root tip cells.

1. Epidermal and hair cells

As in the case of experiments with hypertonic solutions, the tissues were treated in two different ways. In the first, a leaf was treated by immersing it in hot water or liquid paraffin (60°C–100°C) for 1–10 minutes, (mostly at the temperature 75°C for one minute,) and then the epidermal layer was detached, if necessary, from the body of the leaf by stripping or cutting, and observed under the microscope. The observations were made with both bright and dark field illuminations. As material those plants were mostly used which were used in the experiments recorded in Section

1 in Division II, and also *Rhoeo discolor, Sagittaria sagittifolia, Alisma plantago* var. *latifolia, Caldesia parnassifolia, Zebrina pendula, Lilium longiflorum, Hosta sieboldiana, Podophyllum pleianthum* (?), *Vicia faba, Viscum album* and some others were employed. In the second method, by the aid of LEITZ's heating stage, the changes taking place were directly observed under the microscope. When the effects of relatively high temperatures were to be observed, it was necessary in order to secure a desired temperature to put the microscope in an air-thermostat. In the preliminary experiments, it was found that in the case where a desired temperature of the stage was between 35°C-45°C, and hence, where no air-thermostat needed to be used, the temperature of the mounting medium (MERCK's liquid paraffin) obtained by the aid of an electro-thermometer was practically constant 2-3 minutes after the preparation was put on the heating stage, and in the case where a desired stage-temperature was between 50°C and 65°C, and hence where the use of an air-thermostat was necessary, the temperature of the mounting medium was constant 3-4 minutes after the preparation was put on the stage. The temperatures of the medium or the medium-temperatures were of course lower than the stage-temperatures shown by mercury thermometer fitted to the heating stage, the lowering being greater in the case where the air-thermostat was not used than where it was used. The maximum deviations of the medium-temperatures at definite stage-temperatures were ca. ±0.3°C and ca. ±0.5°C respectively in the two cases where the air-thermostat was used and where it was not. In each experiment the stage-temperature was measured, but in the following descriptions the corresponding medium-temperatures are given.

In the experiments by this second method, two pieces of petals or leaves were observed under a microscope first at the room temperature (20°C-28°C) for ca. 1 hour, in order to ascertain whether or not there occurs any pathological change in the protoplasm as a result of manipulation. One of the pieces was then transferred to the heating stage, the other being left on the microscope at the room temperature as a control. As material, the epidermal cells of the young petals of *Tradescantia reflexa* were mostly used, but supplementarily those on the concave side of a bulb scale of *Allium cepa* and those of the leaves of *Elodea densa* were also used. The observation was made with petals or leaves in toto in *Tradescantia* and *Elodea* and with the epidermis stripped off from the scale in the case of *Allium*.

In the following descriptions the experiments carried out by the first and the second method are denoted briefly as "Experiment 1" and "Experiment 2" respectively. The changes in the nucleus caused by heating are markedly different in different plants, and they may be roughly classified into three types.

a. Type 1. *Elodea densa* (leaf epidermis), *Salvinia natans* (aquatic leaf), *Primula malacoides* (hairs of leaf), *Solanum lycopersicum* (do.), *Pharbitis Nil* (do.). In these plants the nuclei when intact are of the type of the homogeneous nucleus I or that of II with visible nucleoli (Fig. 20). In some of

the plants, small chromocenters are also often recognizable in the nuclei. By dark field illumination, the nuclei appear to be nearly dark (Fig. 21).

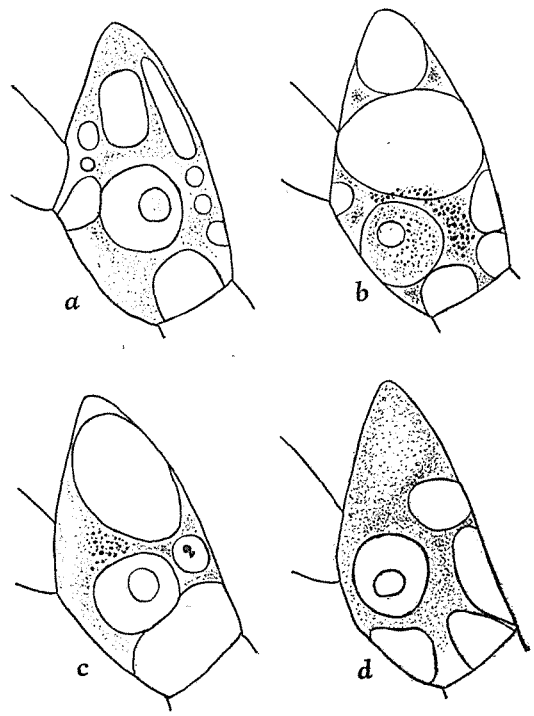
Experiment 1. When the tissues are treated with hot liquid paraffin or hot water, the nuclei are heterogeneous both in bright and dark field illumination. In the marginal spines of *Elodea*, the nuclei show an indistinct structure when heated at 60°C for one minute, and a distinct, chromonema structure or its related structure at a temperature higher than 60°C. In this latter case, the chromonemata appear in places to run zigzag, and the cytoplasm presents in general a coarsely granulated appearance. By the dark field illumination, the nucleus and the cytoplasm are both strongly illuminated. In Fig. 22, a spinal cell is shown which was treated with boiling water for 40 seconds. In this cell zigzag chromonemata are perceptible in the nucleus. Fig. 23 shows the same cell observed with the dark field illumination. In these spinal cells, the dark field illumination shows that the nuclei become turbid after 70 seconds exposure at 50°C, 40 seconds at 55°C, 20 seconds at 57.5°C, and 5 seconds at 60°C. In this series of experiments it was not determined whether this turbidness is reversible or not, but it may be said that irreversible coagulation may occur even at 50°C if the nucleus is treated long enough, since in many cases the turbidness is irreversible, as will be seen later in Experiment 2. It is noted here that, the heterogeneity of the nucleus induced by heat is markedly distinct as compared with that induced by the treatment with hypertonic solutions. The results obtained with the hair cells of *Primula, Pharbitis, Cucurbita, Solanum, Salvinia* and some other plants are similar to those described above.

The heterogeneity produced by heating an intact, homogeneous nucleus is designated in this paper as "heat coagulation, type 1." Both nuclear and cytoplasmic changes are irreversible.

Experiment 2. The leaf of *Elodea densa* mounted with tap water or liquid paraffin was heated at 48.1°C, 52.5°C, 56.7°C and 62.0°C, and its spinal cells were observed. All the nuclear changes caused by the heating at 56.7°C and 62.0°C were irreversible.

Heating at 48.1°C. The first change recognizable in the cell is an increase in velocity of the streaming movement in the cytoplasm. This change may not, however, be regarded merely as an effect of heating, since the velocity change is also observable at room temperature. Following this change, there takes place an enlargement in bulk of the vacuoles in the cytoplasm, and the disappearance of the cytoplasmic strands which connect the nucleus and the cytoplasm lining the cell wall. An increase in the nuclear volume also becomes perceptible. Then the cytoplasm which is nearly transparent in the intact state, becomes somewhat opaque and appears to be granulated. It becomes noticeable now that the streaming movement of the cytoplasm decreases in velocity. In this state, the nuclei are somewhat opaque and usually decrease in volume. Often a fine granular structure is observable in the nuclei. In the next stage, the cytoplasm becomes coarsely granulated, and is found gathering around the nucleus.

Both Brownian and streaming movements in the cytoplasm have now nearly stopped. When the cells in this state are observed with dark field illumination, the nuclei are weakly illuminated showing a slight turbidity, and the cytoplasm is very bright. The nucleus often presents a distinct structure containing delicate chromonemata, and in this case, the nucleus is somewhat brighter in the dark field illumination. These changes of the nucleus are usually irreversible, no reversal being observed when the tissue is transferred by natural cooling, back to the original room temperature. It is noted here that the nuclear and cytoplasmic changes mentioned above do not always take place in the sequence followed here, and also that some of the changes are often omitted. For example, the nuclei not infrequently show no trace of heterogeneity throughout the whole course of the change. In such a case the nuclear changes perceptible during the course to necrosis are only the decrease in volume and the increase in refractivity.



Text-fig. 7. *a-d*. The same spinal cell from a leaf of *Elodea densa* (× ca. 800). *a*) Normal nucleus. *b*) Heated at 48°C for 30 minutes. *c*) and *d*) Put at room temperature after the heating. Showing the nucleus recovering from its heterogeneous state due to heating in. *b*).

HARTMANN (1919) has reported that in *Elodea canadensis*, the nucleus decreases in volume under the temperatures 30°C-40°C, accompanied by the homogenization of its structure. This result of HARTMANN is the same that we obtained by treatment at 48.1° C. He, however, made his investigation with fixed material and made no statement as to whether the changes are reversible or not. The result we obtained shows that they are reversible, if the heating time is not too long. An example to show this fact of reversibleness is given below.

10.20 (a. m.). A leaf was observed with spring water as medium at 19°C, and a spinal cell was watched under the microscope. The nucleus of the cell was homogeneous except a nucleolus which was distinctly visible (Text-fig. 7, *a*). The streaming movement of the cytoplasm was active. The diameter of the nucleus was 19.0 μ.

11.20. The cell was heated at 48.1°C. The nucleus was homogeneous. The diameter of the nucleus was 20.2 μ.

11.25. A part of the cytoplasm had gathered together surrounding the nucleus. The cytoplasm appeared granulated.

11.30. The vacuoles in the cytoplasm became enlarged. The cytoplasmic streaming continued. No change was observed in the nucleus.

11.45. The streaming stopped.

11.50. The nucleus appeared to be filled with many minute granules. The diameter of nucleus was 17.0 μ (Text-fig. 7, *b*). The circuit of the heating stage was opened and the cell was left for cooling to the room temperature.

2.30 (p. m.). The nucleus showed no visible structure and the nucleolus was very distinct. The diameter of the nucleus was 17.9 μ. The streaming of the cytoplasm was observed in certain regions of the cell (Text-fig. 7, *c*).

4.30. Do.

9.00. (a. m., next morning). Do.

9.30. (a. m., two days after). The appearance of the nucleus was quite normal and the cytoplasmic streaming was active. The diameter of the nucleus was 17.5 μ. (Text-fig. 7, *d*).

Heating at 56.7°C. In this case the nuclear and the cytoplasmic changes are apparently nearly the same as in the case mentioned above, but here, the changes are always irreversible. As was often observed, the homogeneous nucleus may become heterogeneous by heating, but a prolonged heating causes the nucleus to be a shrunken, refractive, nearly homogeneous mass. In this shrunken state of the nucleus, the nucleolus is only obscurely visible. This change in the nuclear structure apparently resembles that of the nucleus caused by dehydration with a hypertonic solution, but this resemblance is only apparent.

A similar experiment was carried out with leaf hair cells of *Primula malacoides*, the heating being made at ca. 45°C[1] and 48.1°C. The changes which occurred in the cytoplasm were nearly the same as those observed in the case of *Elodea*, but the nucleus showed usually no distinct heterogeneity during the course of the heating.

The results of the experiments obtained with *Elodea* and *Primula* may be summarized briefly as follows. The changes in the nucleus caused by heating at 48.1°C resemble those induced by a moderate dehydration with a hypertonic solution in the fact that the nucleus becomes slightly heterogeneous as a result of the treatment, but differs from them in that the change is usually irreversible except when the treatment is made for a short time only. In the case of heating at 56.7°C the nuclear change towards heterogeneity is more distinct, presenting a marked contrast to the case of a strong dehydration with hypertonic solutions, where the nuclear structure disappears. This change is always irreversible so far as the present investi-

1) In this case the microscope was put in the air-thermostat, though the temperature, being low, was securable without using the thermostat. It seems, therefore, that the medium-temperature does not deviate much from the stage-temperature which was 45°C, and the former is here shown as ca. 45°C.

gation revealed; the nuclei are coagulated—the heat coagulation type 1—and also the cytoplasm.

b. Type II. Hydrilla verticillata (leaf epidermis), *Sagittaria sagittifolia* (do.), *Caldesia parnassifolia* (do.), *Alisma plantago* var. *latifolia* (do.), *Podophyllum plaianthum* (?) (do.), *Allium cepa* (bulb scale epidermis), *Lilium longiflorum* (young petal epidermis) and other plants. In these plants, the living nuclei are of the type of the heterogeneous nucleus. In the medium of water or liquid paraffin, they present the chromonema structure more or less distinctly.

Experiment 1. The nucleus becomes more highly refractive and more distinctly heterogeneous when immersed in hot liquid paraffin or hot water at 60°C-80°C for a few minutes. The heated nuclei show generally a somewhat coarser reticulate structure than in the intact state. Granulation of the cytoplasm is also found as in the case of *Elodea.* With dark field illumination, the nucleus and the cytoplasm are in varying degree, brighter than in the case of the normal cell. Both changes occurring in the cytoplasm and the nucleus are always irreversible, no reversal being shown by cooling the material to the room temperature. In Figs. 24, 25, 26 and 27, in which the same guard cell nuclei of a stoma in a leaf of *Alisma* are shown, the first two (Figs. 24 and 25) are the reproductions of the nuclei in the intact state, and the latter two (Figs. 26 and 27) are those of the nuclei after it had been treated with hot liquid paraffin at 75°C for one minute. In this case, the heated nuclei (Fig. 27) are strongly bright in the dark field illumination as compared with the nuclei of the intact state which are only slightly bright (Fig. 25). In the cases of *Allium*, *Lilium* and some other plants, both the heterogeneity and the brightness presented by the heated nucleus are often not markedly different from those presented by the same nucleus before the treatment, but sometimes the heterogeneous structure is coarser when the nuclei are weakly bright in the dark field illumination.

The type of coagulation by heating shown by the heterogeneous nuclei such as those of *Hydrilla*, *Sagittaria*, *Alisma*, *Allium* and the other plants mentioned under Type II in which the nuclei remain heterogeneous when heated at 60°C-80°C is called here "heat coagulation type II". The end result of this type of coagulation is the same as that of the heat coagulation type I.

At temperatures higher than 80°C the effects of the temperatures are not the same in all the plants tested as appears in Table 4.

Table 4.

Leaf epidermal nuclei

Temperature in C. / Plant.	50	60	70	80	90	100	110	120	130	140
Sagittaria sagittifolia	+	+	++	++	++	++	++	−or+	−	−
Lilium speciosum	+	+	+	+	+	+	+	+	−	−
Allium cepa	+	+	+	+	+	−	−	−	−	−

By + is indicated that the nucleus is heterogeneous, and by − that it is homogeneous.

In *Sagittaria* such a difference as that found between the nuclei of different plants, shown in Table 4, is also observed between those of the epidermal and the guard cells. While in the latter the nuclei are nearly homogeneous at 100°C, they are heterogeneous in the former.

Experiment 2. As in the case of *Elodea*, the epidermis of the bulb scale of *Allium cepa* was mounted with liquid paraffin, and was heated under the microscope, the observation being continued for periods of time from 40 minutes to 6 hours. When the cells are heated at ca. 45°C, the streaming movement of the cytoplasm becomes first very rapid but soon sluggish. The nucleus usually develops a clearer chromonema structure than in the normal state, though this change does not always take place. It is, on the other hand, rather frequently observed that the nucleus shows no perceptible change in structure throughout the whole course of the experiment. In the dark field illumination, the nucleus appears to be filled with many bright particles except for the dark areas occupied by the nucleoli. Sometimes nucleoli that are not recognizable in the normal state become visible. In the cytoplasm which becomes granulated, the streaming movement is slowed, and the cytoplasmic strands connecting the nucleus and the cytoplasm lining the cell wall disappear. In the next stage, the streaming of the cytoplasm stops completely. The coarsely granulated cytoplasm is slightly bright in the dark field illumination. When the cells are cooled to room temperature, the nucleus decreases in refractivity in most cases, and the chromonemata become somewhat indistinct, the cytoplasm also recovering its active streaming movement. An example from the protocol is given below:

8.40 (a. m.). The nucleus was found filled with very fine chromonemata in the liquid paraffin at room temperature (18°C). Both Brownian movement of the granules and cytoplasmic streaming were active.

11.05. The preparation was transferred to the heating stage and was heated at ca. 45°C.[1] No change was observed up to 11.45.

11.45. The chromonema became more distinct. The cytoplasmic streaming stopped and a weak vibration movement of the microsomes was recognized in the cytoplasm.

0.45 (p. m.). Do. The Brownian movement of the microsomes was nearly stopped.

1.45. The chromonemata were observed very distinctly.

2.45. Do.

3.05. Do.

5.05. The circuit was open and the cell was cooled to room temperature. The convoluted chromonemata were clearly observed. With dark field illumination, the nucleus was found filled with many bright granules, and the nucleolus was dark.

Next morning. This appearance of the nucleus was observed without any visible change after the cell was kept at room temperature for 16 hours, when the cytyplasmic streaming and the Brownian movement of the microsomes were active.

When cells are heated at 48.1°C, both nucleus and cytoplasm show nearly the same changes as when heated at ca. 45°C, but in this case of

1) See foot-note on p. 50.

heating at 48.1°C, the nucleus is highly refractive and shows no reverse change at all when cooled.

The results we obtained in Experiment 2 with the nuclei of *Allium cepa* of the type of the heterogeneous nucleus show that the change occurring in the nucleus at ca. 45°C is almost the same as that obtained when the nuclei are moderately dehydrated with a hypertonic solution. In both these cases, dehydrated and heated, the nucleus becomes highly refractive, and the chromonemata are somewhat more distinct than before the experiment, except in certain cases where the nucleus shows only a slight change in structure. While the effects of high temperatures and hypertonic solutions appear to be similar if the temperatures are not too high, and the solutions are not too concentrated, they are markedly different from each other, if the temperature is higher than 50°C, and the solution is a concentrated one. While in the case of high temperature the chromonemata remain more or less distinctly visible—the heat coagulation, type II,—in that of a strong dehydration they completely disappear from sight. As we have already seen, these two end-results due to heating and dehydration are the same in the heterogeneous and homogeneous nuclei. It is here noted, however, that there is another heterogeneous nucleus which behaves differently towards high temperature. This will be seen in the coming type III.

c. Type III. Tradescantia reflexa (young petals, staminate hairs), *T. virginica* (hairs on leaves), *Zebrina pendula* (leaf epidermis), *Hosta sieboldiana* (do.), *Rhodea japonica*, (do.), *Viscum album* (do), *Vicia faba* (young petals, leaf epidermis), *Allium fistulosum* (young petals) and others.

In these plants the nuclei are of the type of the heterogeneous nucleus. In the living state, convoluted chromonemata are distinctly visible as in the cases of *Sagittaria, Alisma* and other plants. With dark field illumination, the nuclei appear to be filled with numerous bright granules or particles which seem to represent optical sections of the chromonema.

Experiment 1. When the tissues are immersed in hot liquid paraffin (60°C-100°C), for 1-10 minutes, the nuclei shrink and become highly refractive. The chromonemata are only very obscurely or hardly visible, while a nucleolus or nucleoli are usually observable in the nucleus. With dark field illumination, the whole nucleus appears quite dark except for the nuclear and the nucleolar boundaries which are more or less bright. The cytoplasm is found granulated. The coagulation in which the heterogeneous nuclei become homogeneous or nearly homogeneous when heated at 60°C-80°C is called here "heat coagulation type III." Figs. 28-31, show the same nucleus in a leaf hair cell in *T. virginica* observed with the different methods of illumination before and after treatment. Figs. 28 and 29 are reproductions of the nucleus when it is intact, and Figs. 30 and 31 are those of the nucleus after it has been heated for one minute at 75°C. In Figs. 29 and 31 the nucleus is photographed being illuminated through a cardioid condenser. In the interphasic nuclei in the young petal cells in *Tradescantia reflexa*, the indistinguishableness of the individual chromonemata caused by heat is

not complete, and the nucleus usually appears to be slightly heterogeneous (an intermediate type between the heat coagulation, types I and II), while in the resting nuclei in the stretching zone of the same tissue, and also in those in the leaf epidermis and in the leaf hairs, the nuclei become quite hyaline, the individual chromonema being completely indistinguishable—the typical heat coagulation type III.

When subjected to temperatures higher than 100°C, the change induced is different in different plants. In some cases the nuclei become heterogeneous while in others they are homogeneous. In Table 5, the results obtained with the nuclei of *Tradescantia* and *Vicia* by treatment with temperatures 100°C–130°C are shown.

Table 5.

Temperature / Plants	50	60	70	80	90	100	110	120	130
Tradescantia reflexa	±	−	−	−	−	±	±	±	+
Vicia faba	±	−	−	−	−	−	−	−	−

From this table, it is seen that in *Tradescantia* the nucleus is somewhat heterogeneous at 100°C and higher temperatures, while in *Vicia* it is nearly homogeneous at the same temperatures.

The consistency of the homogeneous nucleus due to heat coagulation type III at 60°C–100°C was compared with that of the living nucleus by the aid of a micro-needle. When a stripped piece of the epidermis of the fresh leaf in *Tradescantia virginica* is mounted with liquid paraffin, and the nuclear membrane is punctured with a micro-needle, the karyoplasm which appears to be a sol extrudes out of the nucleus through the opening made by the needle, and the nucleus shrinks and appears to be a mass irregularly folded. When a nucleus is pressed slightly with a dull-pointed needle, it becomes flat, but recovers its original, spherical shape when released. In the epidermal cells heated at 75°C for 1–5 minutes, the behaviour of the nucleus against the micro-needle is quite different. The nucleus is easily punctured with the needle, but the extrusion of the nuclear content does not take place on the withdrawal of the needle. Pressed by the dull-pointed needle, the nucleus is flattened as in the case of the living nucleus, but shows here usually sharp lines on the surface suggesting cracks. By a heavy pressure, the nucleus is divided into two or more jelly-like fragments. These results of the micrurgical investigation show that the nucleus treated with a high temperature exhibits a jelly-like consistency while that of the normal cell is of a fluid consistency.

Experiment 2. In this experiment, the staminate hairs and the young petals of *Tradescantia* were used as principal material. The experiments were made at the temperatures 42.1°C, ca. 45°C,[1] 48.1°C and 56.7°C, being most frequently repeated at 48.1°C.

1) See foot-note on p. 50.

Heating at 42.1°C and ca. 45°C. The first recognizable nuclear change is the appearance of several hyaline areas or vacuoles in the nucleus. The cytoplasmic streaming becomes rapid, and the chromonemata increase in refractivity, being more distinctly visible than before the experiment. Generally, the chromonemata appear to be increased in diameter and decreased in number. Sometimes the nucleoli become visible in the hyaline vacuoles in the nucleus. Then the streaming of the cytoplasm is slowed and the vacuoles in the cytoplasm are enlarged. The nucleus characterized with these abnormalities remains without showing any marked change for as much as 7 hours or longer after the beginning of the experiment. These nuclear and cytoplasmic changes are reversible; the reversal is observed when the tissue is cooled to room temperature.

In the dividing cells, the chromosome behaviour in metaphase and anaphase is greatly disturbed. In the heating at 42.1°C and ca. 45°C, the chromosomes in metaphase and anaphase normally proceed to further stages in general and in most cases a new cell wall is formed between the telophasic chromosome groups. Sometimes the cell wall formation is incomplete, and sometimes, it hardly takes place at all (Figs. 32 and 33.) Fig. 32 shows an epidermal cell in anaphase in the young petal of *Tradescantia reflexa* at room temperature, and Fig. 33 is a reproduction of the same cell after treatment at the temperature ca. 45°C for ca. 30 minutes followed by a 16 hours' cooling to room temperature. In the latter figure the cell is bi-nucleate. Not infrequently, the metaphase chromosomes are found forming a large chromosome mass of various shapes. Usually these chromosome masses do not proceed further to nuclear reconstruction. In a single case, however, it was observed that the anaphase chromosomes which re-gathered into a mass after anaphasic separation proceeded to form a large resting nucleus. This was observed in the cell which was left at room temperature for 21 hours after exposure to 42.7°C for 25 minutes. The cytoplasmic streaming was found in the cell, and a large nucleus containing numerous fine threads was observed instead of a mass of chromosomes. This nucleus is a syndiploid or di-diploid nucleus, and was similar to those giant nuclei artificially produced by the aid of some external agents such as hypertonic solutions (STROHMEYER, 1935, WADA, 1935, and SHIGENAGA, 1937) or narcotics (SAKAMURA, 1920, SHIGENAGA, 1937). In the telophase, two daughter nuclei are reconstructed, if the cell is cooled to room temperature. These nuclei appear usually to be heterogeneous as observed in the normal nucleus, but sometimes, they are quite homogeneous and highly refractive, showing no trace of chromonemata within.

Heating at 48.1°C. As in the case mentioned above, small vacuoles often become visible in the nucleus in the first stage of heating. Then enlargement of the cytoplasmic vacuoles takes place, when the cytoplasmic streaming is rapid. The chromonemata gradually increase in refractivity and appear to be thicker in diameter than before the experiment. Often a highly refractive nucleolus becomes perceptible in the vacuole in the nucleus.

At this stage a decrease in nuclear volume is evident. Later, the cytoplasmic streaming is very inactive and the cytoplasm appears coarsely granulated. The chromonemata seem to be lessened in number, and thicker in diameter than in the normal state. With dark field illumination, these nuclei look similar to those of the normal cells; they are filled with minute bright granules while the cytoplasm appears slightly illuminated. When in this stage the cell is cooled to room temperature, both nucleus and cytoplasm recover their respective original appearance; the nucleus decreases in refractivity and increases in volume, while the nuclear vacuoles disappear, and the chromonemata become fine threads as in the normal nucleus. In Figs. 34–36, an example showing the reversibility of the changes in the nucleus and cytoplasm is given. In Fig. 34 a normal nucleus, in Fig. 35 the same nucleus after treatment at 48.1°C for one hour, and in Fig. 36, the same cooled and left at room temperature for 18 hours, are reproduced. If the nucleus is left in the heated condition longer, the chromonemata are found apparently to be less in number and their respective outline becomes somewhat indistinct. In Figs. 37–39, the nuclear change taking place in the epidermal cells of a young petal is shown. Fig. 37 shows the normal nucleus, Fig. 38 the nuclei treated at 48.1°C for 1 hour, and Fig. 39 those of 4 hours. Comparing Fig. 39 with Fig. 37 which represents the nuclei at room temperature, one would recognize that the nuclei in Fig. 39 are severely shrunken showing their chromonemata obscurely. These nuclei have no longer the power of recovering their normal condition when brought back to room temperature.

In the resting nucleus in the stretching zone of the young petal, the nuclear changes described above for the interphase nucleus in the dividing zone of the same petal occurs more rapidly. In the stretching zone, the nuclei become completely homogeneous as mentioned in Experiment 1. Fig. 40 shows the nuclei of the stretching zone of a young petal which were kept for 6 hours at 48°C. These nuclei present no visible structure except for the nuclear membrane which is refractive. With dark field illumination, they are completely dark as shown in Fig. 41. When such nuclei are stained with acetocarmine, the chromonemata usually become visible, but they are deformed and usually appear coarsely reticulated. Sometimes, the nuclei are stained only diffusely, appearing homogeneous as in the case of the dehydrated nucleus described in Division II.

When the dividing nuclei are kept at 48.1°C, the behaviour of the chromosomes is different in certain points from the case of 42.1°C and that of ca. 45°C. In the early prophase or the spiral stage, the chromonemata are thicker than in the normal state, and after 2 hours at this temperature they become obscure. In the mid- and late-prophase, the nuclear change seems to take place not so markedly as in the early prophase, but the stage does not proceed further. The chromosomes are more refractive than at the room temperature, and as in the case of the resting nucleus, vacuolization occurs in the cytoplasm shortly after the beginning of the heating. In the

metaphase, the separation of the daughter chromosomes is usually suspended and a large chromosome mass is produced. It is frequently observed that the regular arrangement of the chromosomes is often disturbed by vacuolization in the cytoplasm. The Brownian movement in the cytoplasm is slight. In the anaphase, the chromosomes can usually migrate to the spindle poles, but a new cell wall is seldom formed. Sometimes one large vacuole is formed between the two daughter chromosome groups. In such a case, no formation of cell wall is observed. When the vacuolization of the cytoplasm takes place near the poles, the separation of the daughter chromosome groups is often incomplete; they are found lying rather closely side by side. These changes are usually irreversible. No reversal is observed when the cell is brought back to room temperature.

The chromosome bridge is one of the phenomena frequently observed under high temperatures. This phenomenon is not observed usually in the natural condition. On July 16th (1933), the field temperature was 32.5°C in the shade, and only one figure of chromosome bridge was found among 116 normal metaphases and anaphases observed. In the bud kept at 50°C in an incubator for one hour, on the other hand, 7 figures of the chromosome bridge were found among 43 metaphases and anaphases.

At 48.1°C the telophasic chromosome mass is completely homogeneous. In this case no progress in stage is recognized, the high refractivity of the nuclei remaining unchanged. This was ascertained by a continuous observation for a certain long period of time, while in the control the daughter nuclei showed the normal progress to the reconstruction of the interphasic nuclei containing the fine distinct chromonemata within.

Heating at 56.7°C. Both the nuclear and the cytoplasmic changes are, generally speaking, similar to those observed in the experiment at 48.1°C. When resting nuclei are subjected to this temperature for 30 minutes or longer, the chromonemata become thicker and appear to be fewer in number, presenting a coarsely reticulate appearance. These nuclei can not recover their original appearance at room temperature though in rare exceptions the chromonemata become fine and somewhat less refractive again, accompanied by an increase in volume of the nucleus. In Figs. 42-44, a nucleus from an old staminate hair is shown. In Fig. 42, the nucleus at room temperature (before heating) and in Fig. 43, the same nucleus after being kept at 56.7°C for 40 minutes, is reproduced. In the latter figure the chromonemata are thick and much deformed. This nucleus was brought back to room temperature, and after 19 hours it was found that the nucleus increased in volume and the chromonemata were fine, but no streaming was recognized in the cytoplasm (Fig. 44). In this case, therefore, the nucleus has nearly recovered its original appearance, while, the cytoplasm has not.

By a longer treatment at 56.7°C, the nuclei both in the interphase and the reating stage shrink and become homogeneous. These nuclei seem to be coagulated; they no longer recover their original appearance when returned to room temperature.

The changes in the dividing nucleus caused by heating at 56.7°C are similar to those observed at 48.1°C.

The volume change of the nucleus caused by heating was studied with the leaf hair cells in *Tradescantia virginica.* In the hair cells heated on the heating stage at 52.5°C for an hour, in which the nuclei show generally the chromonemata indistinctly, measurements show that in these nuclei there is a volume decrease in the range between 56.2% and 15.0% of the original volume, the mean obtained from the measurements of 9 nuclei being 33.5%. This change in volume is usually irreversible.

The results obtained in experiment 2 with *Tradescantia* resemble those obtained in the dehydration experiments described in Division II. In both cases, the nucleus decreases in volume and increases in refractivity, and the chromonemata become at first somewhat distinct but soon obscure. There is, however, an important difference between these two cases that in the case of heating the nuclear change—the heat coagulation Type III,—is usually irreversible, while in dehydration they are completely reversible. The resemblance between the nuclear structure due to heat coagulation Type III and that of the dehydrated, or the homogeneous nucleus III is, therefore, only in external appearance.

Summary and conclusion. Some important results mentioned in Section 1, that is, those obtained from the experiments with intact epidermal and hair cells are briefly summarized as follows:

The following changes are observed in the cytoplasm:

1) The streaming and the Brownian movement become sluggish. In some plants (*Elodea, Hydrilla, Allium* and others), they are rendered more active in the beginning of heating, but soon cease.

2) The vacuoles become larger than in the normal state.

3) The cytoplasm is made more highly refractive and more coarsely granulated. With dark field illumination, it is slightly bright.

In the nucleus the changes are:

The nucleus shrinks. In some plants (*Elodea*), it first enlarges in bulk, but soon shrinks. The homogeneous nucleus (*Elodea*) becomes heterogeneous (Type 1), and the heterogeneous nucleus (*Allium*) is rendered more distinct than in the normal state (Type II). In the heterogeneous nuclei in *Tradescantia,* the chromonemata become thicker and fewer in number, and by a longer treatment, they are rendered indistinct (Type III). As is often observed in *Elodea, Allium* and some other plants, the nucleus may not show marked change on heating.

Both these cytoplasmic and nuclear changes may be reversible, if the temperature is relatively low, but are generally irreversible when it is high.

The above mentioned nuclear changes on heating in *Elodea, Primula, Hydrilla, Allium* and *Tradescantia* resemble those due to a moderate or incomplete dehydration of the nuclei of these plants with hypertonic solutions by which the homogeneous nuclei in *Elodea* are rendered heterogeneous and the heterogeneous nuclei in *Allium* and *Tradescantia* are made more

distinctly heterogeneous than in the natural state as a transitory stage to the final state of dehydration or the homogeneous nucleus III. While in *Elodea* (Type I) and in *Allium* (Type II) the end-results of the two changes by heating and dehydration are different from each other, being heterogeneous nuclei by the former and homogeneous nuclei by the latter, they resemble each other in the case of *Tradescantia* (Type III), both being homogeneous nuclei of nearly similar appearance. There is, on the other hand, a marked difference between the two cases of *Tradescantia* due to heating and dehydration which is common among the three types of the heated nuclei. This is the difference in reversibility of the changes. While in the case of dehydration the changes are reversible, they are in most case irreversible in the case of heating. The irreversible changes are of three types; the change found in Type I—the heat coagulation Type I, that found in Type II—the heat coagulation Type II, and that found in Type III—the heat coagulation Type III. In both cases of heat coagulation Type II and III the nuclei are of the type of the heterogeneous nuclei in the normal state. Neither mere observation nor the plasmolysis experiment can disclose the existence of these two types in the heterogeneous nuclei, but the heating experiment can distinguish these two types in those nuclei having the same appearance. Now we may call the heterogeneous nucleus showing the heat coagulation Type II the "heterogeneous nucleus I" and that showing the Type III the "heterogeneous nucleus II." While the heating method can discriminate between these two types of the heterogeneous nucleus I and II, no distinction can be made by this method between the homogeneous nuclei I and II, which is possible by the method of dehydration with hypertonic solutions.

In the dividing nuclei in *Tradescantia reflexa*, 1) the pycnosis or the coalescence of the chromosomes, 2) an incomplete or partial formation of the new cell wall between the daughter nuclei or its total suppression, and 3) a retardation in the process of nuclear division or its total suspension are induced by heating. The restoration of the normal processes at room temperature to which the material is returned is not usually observed though there are exceptions.

2. *Pollen mother cells of Tradescantia*

Pollen mother cells with swollen homogeneous nuclei were heated on the heating stage, and the nuclear changes taking place were traced under the microscope.

When the pollen mother cells suspended in the medium of a 0.1 M, 0.5 M or 1.0 M saccharose solution are heated at ca. 45°C, the spiremes which are swollen and invisible in these solutions[1] become distinctly visible in the

1) It has been reported by Fujii (1926), Kuwada and Sakamura (1927), and Yamada and Ishii (1932) that in the pollen mother cells in *Tradescantia* the chromosomes in metaphase and anaphase are swollen and not visible in the media of

nuclei. This nuclear change is usually reversible when the cells are returned to room temperature. In all cases of pollen mother cells in metaphase, anaphase, telophase and interkinesis, the results are the same. In the case of the periplasmodial nuclei too, where the nuclei are swollen and homogeneous in a diluted or a concentrated sugar solution, they show a distinct chromonema structure when heated at ca. 45°C. During the process of this change, the nuclear volume decreases to a slight extent. This change of the periplasmodial nuclei is also reversible when the nuclei are brought to the room temperature.

These results obtained with pollen mother cell nuclei and periplasmodial nuclei resemble those obtained with epidermal nuclei in *Elodea densa* in the dehydration experiments described in Division II. In both cases, the homogeneous nuclei become heterogeneous, and the change is reversible. In the former case, however, the cells have lost their semipermeability, while in the latter they are quite intact.

The cause of the nuclear changes observed in these heating experiments is not fully clear, but it is highly probable that the changes are not a simple osmotic phenomenon like that due to the semipermeability of the plasma pellicle, because in this case the cells have lost their semipermeability. In the case of the pollen mother cells, moreover, vacuoles are not clearly observed in the cytoplasm, and also no clear phenomenon is observed which may be regarded as corresponding to the vacuole enlargement in bulk that may serve to explain a certain type of dehydration in the nucleus by heating (comp. the Section 1). We are at present not in a position to state clearly the mechanism of the change, but it seems not improbable that the change represents simply a transitory stage of the change towards the heat coagulation or "Entmischung," before it has become irreversible.

3. *Guard cells of the stomata*

As mentioned in Section 4 of Division II, the guard cell nuclei are hayline and even their contour is hardly perceptible in the closed state of the stomata (Fig. 45), and in this state, the microsomes show rapid streaming movement in the cells. When these closed stomata are heated at ca. 45°C on the heating stage, the homogeneous nuclei become heterogeneous, the

---

saccharose solutions of different concentrations. According to SHIMAKURA (1934), the chromosomes swell and become invisible even in a concentrated sugar solution because of the fact that the cells are apt to lose their semipermeability by slight mechanical injury. In the present investigation, it was confirmed that this is true not only with the chromosones in metaphase and anaphase, but also with the nuclei in the pollen mother cells in early prophase and interkinesis, and also with the periplasmodial nuclei. When the pollen mother cells are observed with a 0.1 M saccharose solution, the nuclei appear quite homogeneous, and this appearance remains unchanged when the medium is replaced with a 0.5 M or a 1.0 M saccharose solution. In this case no plasmolysis takes place in the cells, and the semipermeability is lost in these pollen mother cells.

chromonema structure being more or less distinctly perceptible (Fig. 46), and the cytoplasmic streaming is somewhat inactive. When such stomata are returned to room temperature, the chromonemata usually become obscure again and the cytoplasmic streaming is found active. In this experiment, di-distilled water or liquid paraffin was used as medium, and in both cases the result was the same.

These changes of the stomatal nuclei induced by heating resemble those observed when the stomatal nuclei in the same closed state are moderately dehydrated with a hypertonic solution. This fact of resemblance seems to suggest that the dehydration phenomenon here also plays an important rôle in the inducement of these changes by heating. In this case, however, no plasmolysis takes place, and the mechanism of this dehydration is not clear, but it seems not improbable that an enlargement of the cytoplasmic vacuoles (which in this case of the stomatal cells is observable as is generally the case with living cells put in a high temperature[1], see Section 1, Division III), bears a certain intimate relation to this dehydration.

4. Root tip cells of Vicia

According to the results of experiments by GEORGEVITCH (1910), NĚMEC (1910), LUNDEGÅRDGH (1912), SAKAMURA (1920) and others, carried out with the root tip cells of some plants such as *Vicia*, *Allium* and *Galtonia*, the mitotic process is interrupted at a high temperature, and the behaviour of the chromosomes is greatly disturbed in the metaphase and anaphase. In the present investigation, the changes due to heating in the structure of the resting nucleus and those in the behaviour of the chromosomes in metaphase and anaphase were studied, and these changes were compared with the changes caused by the action of hypertonic solutions. In this series of experiments, the seeds of *Vicia faba* were shown in a sand bed put in a air thermostat at 27°C, and seedlings whose roots were 10-15 m.m. long were employed as material. The root tip cells were immersed for certain definite periods of time in HANSTEEN-CRANNER's solution[2] of a desired high temperature at which the solution had been heated in a water-thermostat. The deviation of the temperature of the solution was less than ±0.05°C. The materials were fixed immediately after experiment or with an interval of time during which the seedling was put in HANSTEEN-CRANNER's solution at 27°C, and were stained, in the following two ways: 1) Four root tips were fixed with the BONN modification of FLEMMING's solution and stained with

1) In this case of guard cells the vacuoles are larger in bulk and more irregular in shape than in the case of the petal epidermis and staminate hairs; and accordingly the enlargement is perceptible with precision only relatively seldom as compared with the latter.

2) HANSTEEN-CRANNER (1922) has shown that when root tips are put in warm water, some water soluble phosphatides come out from the cytoplasm into the surrounding medium. According to him, HANSTEEN-CRANNER's solution is a good nutrient mixture which prevents the extrusion of the phosphatides.

HEIDENHAIN's haematoxylin, and 2) three root tips were fixed with a mixture of corrosive sublimate and acetic acid, and stained by the method of FEULGEN's nucleal-staining.

To show the numerical relation between the normal and abnormal mitotic figures, the percentage of the total mitotic figures that are normal is conveniently used in this paper as a coefficient of the normal mitosis. The numbers of the normal- and abnormal-mitotic figures were obtained by counting them in each 5 microtome section of the two root tips fixed with the BONN modification and cut longitudinally across their axial region. The coefficients thus obtained in the case of immediate fixation after the exposure to different temperatures and in those with different intervals of time before fixation are graphically shown in Text-figs. 8-12.

a. Heating at 27°C. Root tips were immersed in HANSTEEN-CRANNER's solution at this temperature for 30 minutes, 90 minutes, 3 $^1/_2$ hours, 12 hours, 24 hours, 48 hours and 72 hours. In these root tips, the apparent structure of the nucleus and the behaviour of the chromosomes in migration is quite normal. In the resting stage the nucleus presents the chromonema structure or the reticulate appearance, and in division, the anaphasic chromosomes migrate regularly to the spindle poles.[1] Sometimes, however, slightly abnormal mitotic figures are found, such as chromosome coalescence or pycnosis in a low degree. The coefficient of the normal mitosis is 93.0 in the case where root tips from the sand bed are directly fixed, and 91.3 in the case where they are fixed after the immersion in HANSTEEN-CRANNER's solution at 27°C for 30 minutes; in this latter case, generally speaking, some increases are shown by longer immersions up to 97.6, which is the coefficient for the case of 72 hours immersion (comp. Text-fig. 8).

b. Heating at 39°C. The root tips were treated with a solution of this temperature for 1 hour, 2 hours, 3 hours and 5 hours. In the seedlings treated longer than 2 hours the root showed no further growth at room temperature to which they were transferred. In the following descriptions, the material fixed immediately after the treatment at 39°C for one hour is represented by the symbol "39°C-1 h" and those exposed to 39°C for one hour and fixed with a 1 hour interval of immersion in the solution at 27°C by "39°C-1 h-R-1 h," and those fixed with a 2 hours interval at 27°C by "39°C-1 h-R-2 h," and so on.

i. 39°C-1 h and 39°C-1 h-R-1 h. The apparent structure of the nucleus is nearly equal to that of the normal nucleus. In the stretching zone, several hyaline vacuoles are sometimes observed in the nucleus. Often these vacuoles are found budding or extruding from the nucleus into the cytoplasm as is observed in the normal living nuclei in the young petals of *Tradescantia reflexa.*

In the dividing cells, the chromosomes in metaphase and anaphase are more or less coalescent and in an extreme case, they are condensed into a

1) Comp. SHARP (1913), KUWADA (1921) and others.

homogeneous hyperchromatic mass. Chromosome bridges and other irregular distributions of the chromosomes in metaphase and anaphase are also observed. In the telophase, the formation of the nuclear membrane is often incompelte. A new formation of vacuoles and the enlargement of the existing vacuoles in the cytoplasm both of which have been considered by GEORGEVITCH (1910) and KOKOTT (1930) characteristic features of the cytoplasmic change induced by heat, are usually found. In some root tips, small granules which are stained deeply with HEIDENHAIN's haematoxylin are observed in the cytoplasm.

39°C-1 h-R-3 h. The normal mitotic figures are often found besides such abnormalities as just mentioned.

39°C-1 h-R-8 h and-R-24 h. Both the apparent structure of the resting nucleus and the behaviour of the chromosomes in the metaphase and anaphase are found generally to be normal. Sometimes coalescent chromosomes are found, but those grouped together into compact masses are only rarely observed. The bi-nucleate cells and the di-diploid nuclei are found both in the periblem and plerome. Fig. 47 shows a bi-nucleate cell in the plerome.

ii. 39°C-2 h. The kinds of abnormalities in structure of the nucleus and in behaviour of chromosomes are generally the same as those observed in the root tips "39°C-1 h," but the grade of abnormalities is far higher than in the latter case. In the case here in question the normal equatorial distribution of the chromosomes in metaphase is often greatly disturbed by the vacuolization in the spindle region as shown in Fig. 48.

iii. 39°C-3 h and R-1 h. In this material, many nuclei are shrunken and deeply stained in the periblem as was observed in the dehydrated root tip cells in the same plant (see Division II). In Fig. 49, such nuclei in periblem cells are shown. The vacuolization or the enlargement of the vacuoles in the cytoplasm is especially clear in the stretching zone. In the dividing cells, irregular distributions and pycnosis of the chromosomes are frequently found. In Fig. 50, an irregular distribution in anaphase in which the chromosomes are divided into three or four groups is shown. Sometimes, lagging chromosomes are found in anaphase (Fig. 51). In this case no vacuolization is obesrved among the chromosome groups, and it seems that the irregularity is not connected with vacuolization in the cytoplasm.

39°C-3 h-R-3 h and -R-6 h. Pycnotic nuclei which are stained deeply are found in abundance, and normal mitotic figures are rarely found.

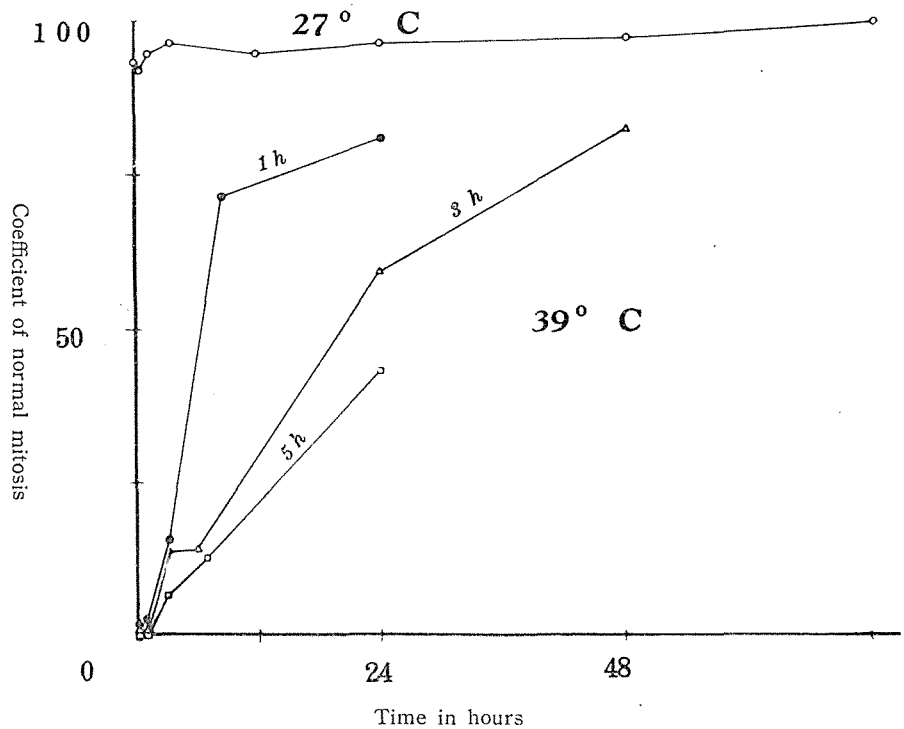
39°C-3 h-R-24 h and -R-48 h. Most of the resting nuclei show the normal structure, but in division stages coalescent chromosomes are still found. In this material the bi-nucleate cells and the di-diploid nuclei are found, but, anaphasic figures with more than 12 chromosomes in each group are only rarely observed. In Fig. 52, an example is shown in which more than 20 chromosomes were counted in each group.,

iv. 39°C-5 h. The general appearence of the nucleus is similar to that described in the case of "39°C-3 h." In some root tips, the chromosomes are very slender in the periblem, while they are of normal thickness in the plerome.

39°C-5 h-R-1 h, -R-3 h, and -R-7 h. The normal mitotic figures are few. The characteristic feature presented by the nucleus in this material is a granular structure of the nucleus. Numerous fine granules are found in the nucleus connected with one another by delicate threads. These chromatic granules are generally smaller and more numerous than the karyosomes and chromocenters observed in the normal resting nucleus in this plant. The abnormalities in mitotic figures are similar to those observed in the case of the root tips, 39°C-3 h.

39°C-5 h-R-24 h. The behaviour of the chromosomes in the metaphase and anaphase is generally normal, but sometimes, some coalescent chromosomes are found. Sometimes, one of the daughter nuclei in the telophase is smaller than the other. In Fig. 53, such a case is shown.

v. 39°C-6 $^1/_2$ h. The granular structure of the nucleus described above is frequently observed as is shown in Fig. 54.



Text-fig. 8. Graphical presentation of the effect of heating at 39°C on mitosis in the root tip cells of *Vicia faba*.

The coefficients of the normal mitosis obtained in the experiments at 39°C are graphically shown in Text-fig. 8. In this graph it is seen that while in the root tips fixed immediately after the treatment for 1 hour, 3 hours and 5 hours, the coefficients are 0.9, 0.9 and 1.0 respectively, they are

increased to 80.7, 58.4 and 42.9 respectively, 24 hours after the root tips are returned to 27°C.

c. Heating at 40°C. Root tips were heated for 5 minutes, 15 minutes, 1 hour, 2 hours, 3 hours, 5 hours and 6 hours. In this series of experiments no attempt was made to study the recovery of the cells subjected to the experiments; all the material was fixed immediately after the exposure to 40°C.

i. 40°C-5 m. The structure of the resting nucleus appears generally to be normal. Abnormal mitotic figures may be observed but only those which are abnormal in a slight degree.

ii. 40°C-15 m. In the resting nucleus, the chromonemata are found in places fusing with one another, thus forming a coarse reticulum. In dividing stages, the coalescence of the chromosomes and the vacuolisation in the cytoplasm take place in a higher degree than in the case where the heating is made for 5 minutes.

iii. 40°C-1 h, 2 h, 3 h, and 4 h. Pycnotic nuclei and compact chromosome masses which are stained homogeneously are found in abundance. In some chromosomes, many minute granules are found adhering to the surface of the chromosomes. As mentioned in Division II, this phenomenon is also found in the root tip cells treated with concentrated saccharose solutions.

iv. 40°C-5 h and 6 h. General features of the nucleus in resting stage and in mitosis are the same as mentioned above for case iii), but in addition, it is also observed that in some the cytoplasm is vacuolized, and in others the protoplasm is contracted.

d. Heating at 41°C. Root tips were heated for 5 minutes, 15 minutes, 25 minutes, 40 minutes and 1 hour. The root tips heated for 5 and 15 minutes grew normally, but those heated for 25 or 40 minutes grew far less actively than in the normal condition, and those heated for 1 hour no longer grew at all even at 27°C to which the root tips were returned. The recovery was studied with the materials heated for 15 minutes, 25 minutes, 40 minutes and 1 hour.

i. 41°C-5 m. While the structure of the resting nucleus appears nearly normal, the coalescence of the metaphasic chromosomes is frequently found in dividing stages.

ii. 41°C-15 m. In the resting nuclei the chromonemata are deformed and form a coarse reticulum. In some nuclei, hyaline spherical bodies are found extruding from the nuclear surface into the cytoplasm. In the dividing cells, pycnotic masses of chromosomes are observed. Vacuolisation in the cytoplasm is observed both in the resting and dividing stages.

41°C-15 m-R-8 h and -48 h. The resting nuclei and the mitotic figures appear to be nearly normal, but di-diploid giant nuclei, bi-nucleate cells, and various other abnormal figures are found.

iii. 41°C 25 m. Many pycnotic nuclei are found. Usually the chromosomes are thicker and shorter than those in the normal root tip.

41°C-25 m-R-7 h. Most of the resting nuclei appear to be nearly normal,

but di-diploid nuclei and bi-nucleate cells are also found. Sometimes, the reconstruction of the daughter nuclei is incomplete, and irregular groups of chromosomes are often found enclosed by a nuclear membrane. In Fig. 55, one such nucleus is shown.

41°C-25 m-R-24 h and -R-48 h. Many nuclei are shrunken and stained homogeneously with HEIDENHAIN's haematoxylin and FEULGEN's nucleal-staining method. These nuclei may be regarded as in process of degenerating. Most of the mitotic figures are normal, but compact masses of the chromosomes, di-diploid nuclei and bi-nucleate cells are often found.

iv. 41°C-40 m. Many shrunken nuclei which are stained homogeneously are found in the periblem and plerome. The chromosomes in division stages appear somewhat thick and are more or less coalescent. Normal mitotic figures are found rarely.

41°C-40 m-R-1 h and -R-3 h. The normal mitotic figures are very few. Many chromosomes are swollen in the metaphase and anaphase and are deeply stained. These chromosomes may be regarded as being in the process of degeneration.

41°C-40 m-R-10 h. The apparent structure of the resting nuclei is nearly normal in most cases, but the chromosomes in division stages are usually more or less coalescent. Compact masses of chromosomes, di-diploid nuclei and bi-nucleate cells, are also found not infrequently.

41°C-40 m-R-24 h and -R-50 h. Most of the division figures appear to be quite normal, but degenerating nuclei and compact masses of chromosomes are still often observed.

v. 41°C-1 h. The resting nuclei are coarsely reticulate or pycnotic, and the chromosomes in metaphase and anaphase are generally coalescent into a mass. Chromosomes with minute granules attached to the surface are also observed.

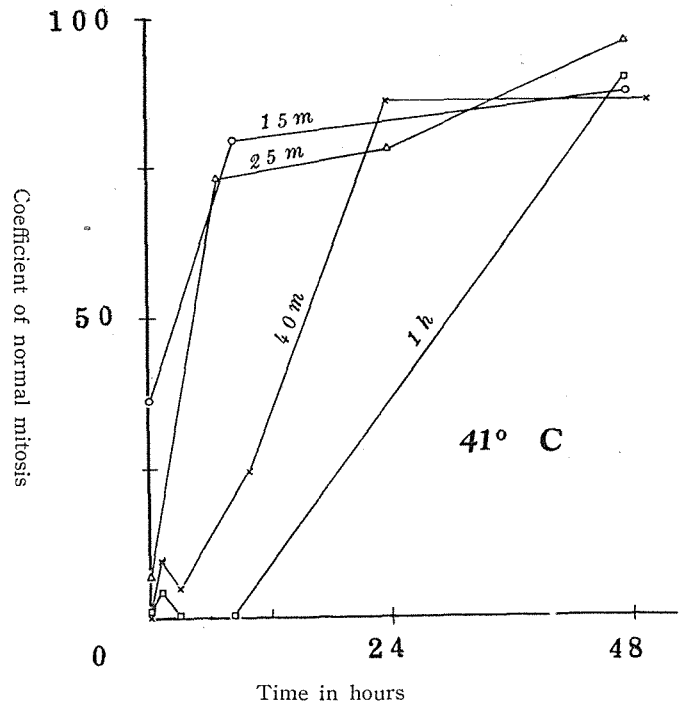
41°C-1 h-R-1 h. Many shrunken necrotic nuclei which are stained homogeneously are found in the periblem.

41°C-1 h-R-3 h and -R-8 h. The normal mitotic figures are seldom found.

41°C-1 h-R-24 h and -R-48 h. Most of the nuclei in resting stage are quite normal in appearance, but there are also degenerating nuclei which are deeply stained. In Fig. 56, in which a part of the periblem in the stretching zone is shown, it is seen that there are degenerating nuclei of irregular shape stained deeply, in the neighbourhood of which other nuclei appear to be quite normal. The regular arrangement of the cells is much disturbed in this region of the periblem.

The coefficients of the normal mitosis obtained in this series of experiments is graphically shown in Text-fig. 9. The coefficients obtained in the root tips fixed immediately after heating for 15 minutes, 25 minutes, 40 minutes and 60 minutes at 41°C are 36.3, 6.1, 0, and 1.3, respectively, and those of the same series of the root tips which were transferred to the normale tmperature (27°C) after heating and fixed after ca. 48 hours are 87.0 96.8, 86.5 and 89.5, showing marked increases as compared with those of

the cases of immediate fixation. We may, therefore, say that although the heating temperature is as high as 41°C, the cells can completely recover from their pathological conditions due to heating even if they are exposed to this high temperature as long as 60 minutes.



Text-fig. 9. Graphical presentation of the effect of heating at 41°C on mitosis in the root tip cells of *Vicia faba*.

e. Heating at 42°C. The root tips were heated at 42°C for 40 minutes, 60 minutes, 80 minutes and 100 minutes. While the root tips heated for 40 minutes showed a further growth when they were returned to the room temperature (27°C), those heated for 60-100 minutes showed no longer any growth.

i. 42°C-40 m. Most of the resting nuclei show a coarsely reticulate structure. The chromosomes in division stages are coalescent into a mass.

ii. 42°C-60 m, -80 m, and -100 m. Nuclei which are homogeneously stained are found in abundance.

f. Heating at 43°C. The root tips were heated at 43°C for 10 minutes, 20 minutes, 40 minutes and 60 minutes. The root tips heated for 10 minutes and those for 20 minutes were able to grow further, but these heated for 40 minutes and those for 60 minutes showed no growth in the room temperature (27°C) to which they were returned.

i. 43°C-10 m. In the resting stage the formation of the hyaline space

surrounding the nucleolus or the nucleolar halo and the cytoplasmic vacuolisation are observed. In an abundance of dividing nuclei, coalescence of the chromosomes is observed.

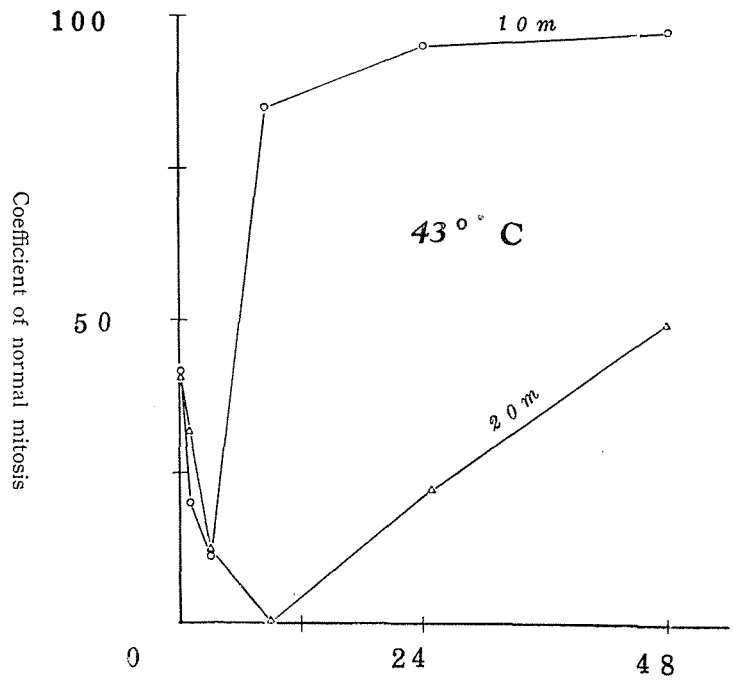
43°C-10 m-R-1 h and -R-3 h. The normal mitotic figures are smaller in number than in the above case. The pycnosis of the resting nuclei and the irregular distribution of the chromosomes in metaphase are very frequently observed. In the material R-3 h, in many cells, the chromosomes are found to form a pycnotic mass or masses.

43°C-10 m-R-8 h, -R-24 h and -R-48 h. Both resting nuclei and dividing figures show the normal appearances. Di-diploid nuclei and bi-nucleate cells are frequently found. In Fig. 57, a di-diploid nucleus of a constricted form is shown.

ii. 43°C-20 m and -R-1 h. The nuclei and the division figures present the same appearances as those in the case of 43°C-10 m.

43°C-20 m-R-3 h and -R-9 h. In most of the cells in mitosis, the chromosomes in metaphase and anaphase are found coalescent into a mass. No sign of recovery is recognizable.

43°C-20 m-R-25 h and -R-48 h. Numerous necrotic cells which are stained deeply are found. Giant cells with large vacuoles are often formed near the necrotic cells. Normal mitotic figures are also observable.



Text-fig. 10. Graphical presentation of the effect of heating at 43°C on mitosis in the root tip cells of *Vicia faba*.

The coefficients of normal mitosis in the root tips exposed to 43°C is shown graphically in Text-fig. 10. The coefficient of the case 43°C-10 m is 43.6. After 3 hours at 27°C it shows a remarkable decrease to 11.3, but increases again to 84.8 after 8 hours at 27°C. In this case of experiment of heating at 43°C for 10 minutes, therefore, an almost complete recovery of the mitotic activity is brought about by the transferring to an optimum temperature. But in the case where the heating at 43°C is made for 20 minutes, recovery is possible only to a small extent; the coefficient obtained in the root tips 43°C-20 m-R-48 h is as low as 48.6.

g. Heating at 45°C. Root tips were heated at this temperature for 5 minutes and 10 minutes. In both cases, the root tips showed no further growth when transferred to the normal temperature (27°C).

i. 45°C-5 m. Many resting nuclei appear to be normal, and mitotic figures are also found normal in many cases. In some root tips, the chromonemata are found contracted with a hyaline area between the mass of contracted chromonemata and the nuclear membrane. It is also frequently observed that a strong vacuolisation in the cytoplasm causes a disturbance in distribution of the metaphasic chromosomes or the bending of the spindle.

45°C-5 m-R-1 h and -R-5 h. The number of the normal mitotic figures is far less than in the previous case.

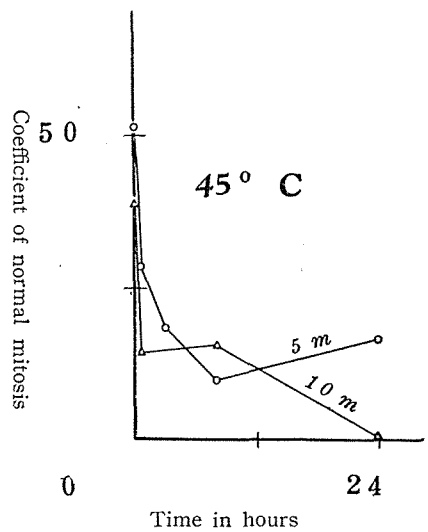
45°C-5m-R-8 h and -R-24 h. Most of the nuclei, both in rest and in mitosis, are pycnotic. Some of these pycnotic nuclei are found to be discoid, deformed by strong vacuolisation in the cytoplasm.

ii. 45°C-10 m. Many nuclei in rest and in mitosis appear quite normal, but some nuclei are pycnotic and vacuolated.

45°C-10 m-R-1 h, and -R-8 h. Coalescent chromosomes are frequently found.

45°C-10 m-R-24 h. Normal mitotic figures are not observed.

The graphical presentations of the normal mitosis coefficients obtained in this series of experiment are given in Text-fig. 11. The coefficients of the cases 45°C-5 m and 45°C-10 m are 51.3 and 38.3 respectively. When such root tips are put at 27°C for 24 hours the coefficients decrease to 16.1 and 0 respectively. In these cases, 45°C-5 m and 45°C-10 m, therefore, the root tips have lost the ability of recovering the normal mitotic activity.



Text-fig. 11. Graphical presentation of the effect of heating at 45°C on mitosis in the root tip cells of *Vicia faba.*

h. Heating at 48°C. Root tips

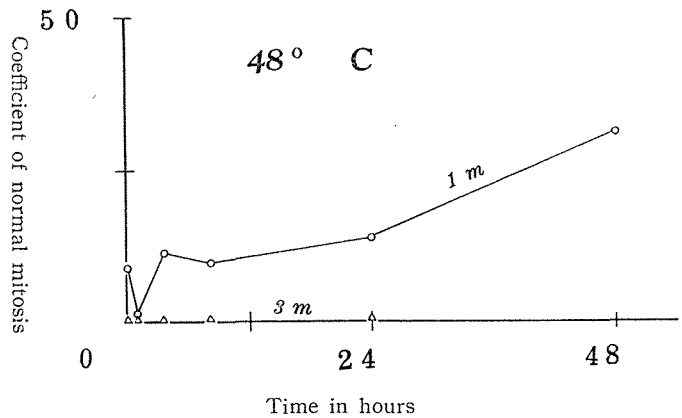
heated at this temperature for 1 minute showed a further growth to a slight extent in the normal temperature (27°C), but those treated for 3 minutes showed no longer any growth.

i. 48°C-1 m and R-1 h. Most resting nuclei present the normal chromonema structure, but necrotic nuclei which are shrunken and amoeboid in contour are also abundantly found. In division the chromosomes show coalescence in most cases.

ii. 48°C-1 m-R-4 h and -R-8 h. Normal mitotic figures are rarely observable. Sometimes the chromosomes in anaphase have a rugged contour and are thicker and shorter than those in ihe normal root tip (Fig. 58), reminding us of the chromosomes in the first metaphase in pollen mother cells of this plant.

iii. 48°C-1 m-R-24 h and -R-48 h. Normal mitotic figures are often found. In many dividing cells, the chromosomes gather into a mass and are stained deeply. In some cells, one or two chromosomes are found distributed in the region between the daughter nuclei formed by the companion chromosomes. Rarely, two anaphasic figures or four nuclei are found in a single cell, as illustrated in Figs. 59 and 60.

iv. 48°C-3 m, -R-1 h, -R-4 h, -R-8 h and -R-24 h. No normal mitotic figure is found.



Text-fig. 12. Graphical presentation of the effect of heating at 48°C on mitosis in the root tip cells of *Vicia faba*.

The normal-mitosis coefficients obtained in this series of experiments are shown graphically in Text-fig. 12. The coefficients are 8.5 and 0 in the cases of 48°C-1 m and 48°C-3 m, respectively. In the former case the coefficient shows a small increase to 13.5 when the root tips are put at 27°C for 24 hours, but in the latter no noemal mitotic figure is observed under the same conditions; the cells have completely lost their ability to recover the normal mitotic activity.

i. Heating at 70°C-100°C. Results of investigation of nuclei with such

high temperatures as 70°C–100°C have been reported by NÈMEC (1910), MILOVIDOV (1933), SINKE and SHIGENAGA (1933), NAKAMURA (1934) and other investigators. The results obtained in the present investigation are briefly given below. Exposed to these high temperatures, the resting nuclei become stained homogeneously with FEULGEN's nucleal-staining method, except for one or two nucleoli which are not stained. Usually, the nuclei are found shrunken and with amoeboid shapes. Generally, the cytoplasm presents no positive reaction colour with FEULGEN's method, but the tonoplast is not infrequently distinctly coloured. Sometimes, a clear hyaline space is observed in the nucleus, dislocating the nuclear contents which forms a chromatin mass or masses. A case from a root tip treated with boiling water for 3 minutes is illustrated in Fig. 61. In this figure, many fine threads are observable, connecting the nuclear menbrane and the chromatin mass. In mitosis, the chromosomes in metaphase and anaphase are usually swollen. The swollen chromosomes are differentiated into two portions, the axial portion which is positive to the nucleal-staining as reported by MILOVIDOV (1933), and SINKE and SHIGENAGA (1933), and the cortical portion which is negative to this staining. Sometimes the axial portion presents a zig-zag appearance and it has been regarded by SINKE and SHIGENAGA as representing the chromonema spiral of the chromosome. Often the cortical portion or presumably, the swollen matrical portion is coloured diffusely with FEULGEN's method. In some cells which are in mitosis, the cytoplasm is also stained with this method, as shown in Fig. 62. It is an interesting fact that the chromosomes in dividing stages easily swell and the thymus-nucleic acid which makes the principal chemical constituent of the chromonema spirals of the chromosome often diffuse out of them into the surrounding cytoplasm, while in the resting nucleus, such a diffusion of thymus-nucleic acid into the cytoplasm is seldom observed. The same phenomenon has been observed by treating root tips with certain lipoid solvents such as ethyl ether, chloroform and xylol (SINKE and SHIGENAGA, 1933).

The noticeable changes in the cellular and nuclear structures and the chromosome behaviour in division observed in the root tips heated at 39°C–48°C are summarized as follows:

a) Cytoplasmic changes.
   1) Vacuolisation of the cytoplasm (Fig. 48) or enlargement of the vacuoles.
   2) Contraction of the cytoplasm (Fig. 48).
   3) Hyperchromaticity of the cytoplasm.
   4) Local aggregations of the cytoplasm.
   5) Formation of small FEULGEN-negative granules in the cytoplasm or in the vacuoles.

b) Nuclear changes.
   6) Unevenness of contour of the nucleus (Fig. 49).
   7) Deformation and flattening of the nucleus due to the formation of large vacuoles in the cytoplasm.

8) Shrinkage of the nucleus (Fig. 49).
9) Contraction of the chromatic substance.
10) Reticulation of the chromonema structure.
11) Granular structure of the nucleus (Fig. 54).
12) Hyperchromaticity of the nucleus (Fig. 49).
13) Formation of the nucleolar halo (Fig. 50).
14) Nuclear vacuolisation.
15) Necrotic deformation of the nuclei (Fig. 56).

c) Changes observed in nuclear division.

16) Thickening and shortening of the chromosomes in diameter and length (Fig. 58).
17) Pycnosis or coalescence of the chromosomes in metaphase and anaphase.
18) Formation of the pycnotic compact mass of chromosomes in degeneration.
19) Irregular distribution of chromosomes accompanied with the formation of cytoplasmic vacuoles (Fig. 48).
20) The same not accompanied by the formation of the vacuoles (Fig. 50).
21) Chromosome lagging (Fig. 51).
22) Bending of the spindle by the cytoplasmic vacuolisation.
23) Dislocation of the daughter nuclei in telophase by the vacuolization in the cytoplasm.
24) Suppression of the loosening of the spiral chromonemata in the telophase.
25) Formation of the multi-nucleate cell (Figs. 47, 53 and 60).
26) Formation of the micro-nuclei (Fig. 60).
27) Formation of the di-diploid nuclei (Fig. 57).
28) Formation of two spindles in a single cell (Fig. 59).

A comparison of the abnormalities or changes mentioned above with those observed in the root tip cells treated with hypertonic solutions of saccharose shows that there are many resemblances between these two cases. Resemblances in change or abnormality are those numbered above 2), 3), 5), 6), 8), 9), 10), 12), 13), 15), 16), 17), 18), 24), 25), 26), 27), and 28). They are observed in both cases and they may be regarded as caused mainly by the dehydration of the protoplasm. The heat causes vacuolization of the cytoplasm or enlargement of vacuoles, 1), and it seems highly probable that this vacuolization or the enlargement of the vacuoles may play some important rôle in the dehydration phenomenon in the heated protoplast (comp. Section 1 and 3). The other abnormalities are found only in the case of heat treatment. Some of these abnormalities such as 20) and perhaps also 21) seem to show that heat may have a certain direct causal relation to the swelling of the protoplast as will be discussed later. The abnormalities 7), 19), 22), and 23) are the results of the mechanical disturbance of the normal localization by the vacuolization of the cytoplasm, 1), and may be regarded

as indirect effects of heat, and the remaining three abnormalities, 4), 11) and 14) are probably the direct effects on the karyoplasm and the cytoplasm. A tentative conclusion derivable from this comparison is, therefore, that the abnormalities caused by heat are partly direct effects of heat on the cytoplasm and the karyoplasm and partly indirect effects derived from a more direct effect, the cytoplasmic vacuolization which causes the dehydration of the nucleus or the mechanical disturbance of the normal localization of the protoplasmic components.

5. *Conclusion*

i. Reversible changes in the resting nucleus caused by heaing. Reversible changes of the resting nucleus due to heat have been reported by some investigators. Van HERWERDEN (1927) and NASSONOV (1932) have carried out the experiments with animal cells. In the intestinal epithelial cells in *Rana temporaria*, NASSONOV has observed that several chromatic lumps which are invisible in the normal state become perceptible in the nucleus when the tissue is heated. According to this author, the increase in the acidity of the cytoplasm in the range pH 6.53 to pH 6.13 occurs accompanied by this change in the nucleus, and from this parallel occurrence of these two phenomena he has concluded that the appearance of the chromatic lumps in the nucleus after heating is a direct effect of the increased acidity on the nucleus. The result obtained by HURD-KARRER[1] (1929) in *Triticum* that the sap of the tissue of a plant cultivated at a high temperature is more acid than that of the same plant cultivated at a low temperature, is in accord with the result obtained by NASSONOV and in favour of the view he maintains.

HARTMANN (1919) has, on the other hand, expressed another view. He has heated the leaves of *Elodea* and the bulbs of *Allium cepa* at 40°C and studied the results by fixing the materials with a mixture of picric acid, corrosive sublimate and alcohol. In the epidermal cells he has observed a decrease in nuclear volume, the disappearance of the nuclear structure, and the vacuolisation of the cytoplasm, and has come to the conclusion: "Der Volumverminderung parallel geht ein Kompakter- und Homogenerwerden der Kernstruktur offenbar infolge Wasserabgabe." The decrease in volume of the nucleus is regarded by him as due to the extrusion of chromatin and water from the nucleus into the cytoplasm. This view of the changes of the nucleus as caused by dehydration seems to us reasonable, but he has carried out no investigation as to the question whether the nuclear changes are reversible or not.

According to the results we obtained, the nuclear change in visible structure caused by heating shows a wide variation according to the kind of plant subjected to experiment. In *Elodea* and *Primula*, we observed that the nucleus is homogeneous in the intact state, and becomes often slightly

1) Cited from BĚLEHRÁDEK (1935).

heterogeneous when heated at ca. 45°C or 48.1°C. This nuclear change is reversible when the tissue is returned to the ordinary temperature. In *Allium*, the nucleus presents the chromonema structure somewhat distinctly in the intact state and becomes more or less highly distinct when heated at ca. 45°C or 48.1°C. This change is also reversible. These nuclear changes observed in *Elodea*, *Primula* and *Allium* may be explained by the hypothesis of NASSONOV that the change is due to the increase in the acidity of the cytoplasm, but the change observed in the case of *Tradescantia* is incompatible with this hypothesis. In this plant, the intact nucleus contains distinct convoluted chromonemata at the room temperature, and when heated at 42.1°C, ca. 45°C or 48.1°C, the chromonemata which become at first more distinct than before heating become gradually indistinct, accompanied by the shrinkage of the nucleus. This nuclear change is reversible when the cells are returned to room temperature. If in this case, too, the nuclear change caused by heat were the direct effect of increased acidity in the heated cytoplasm as explained by NASSONOV in his case, the fact that the chromonemata become gradually indistinct at a high temperature is incomprehensible, because the chromonemata and the chromosomes become distinct by the direct action of acids while they swell and become obscure in alkaline solutions (LEWIS and LEWIS, 1924, KUWADA and SAKAMURA, 1927, ZEIGER, 1935, STROHMEYER, 1935, MORITA, 1935). Thus we must seek another factor to explain the whol matter, at least for the case of *Tradescantia*.

The reversible heat changes of the nucleus observed in *Elodea*, *Primula*, *Allium* and *Tradescantia* resemble the changes observed in the nuclei of the same plants when subjected to a moderate dehydration by means of moderately concentrated solutions. In both cases, the nucleus decreases in volume and the chromonemata become refractive and more or less distinctly visible at the first stage of the change. The clearest resemblance is found in the case of the guard cell nuclei in *Tradescantia* stomata. This parallelism between the case where the cells are treated with hypertonic solutions and the case where they are subjected to high temperatures suggests that the dehydration phenomenon must take place in the latter case too. There are of course certain phenomena which are not common to both cases, a fact which suggests that the abnormalities are not caused merely by the dehydration. They are the formation of the vacuoles in both nucleus and cytoplasm and the restricted ability of recovery from the abnormalities, and both are found in the case of heating treatment. Moreover, in the heated cytoplasm, the existing vacuoles are enlarged. From this fact of the vacuole enlargement it may be inferred that water is absorbed by the vacuoles from the cytoplasm and the nuclei through a certain mechanism which we are not at present able to describe definitely. It seems highly probable that the characteristics in the heated cytoplasm, the vacuolization and the vacuole enlargement are closely related to the occurrence of the dehydration due to heating. The view that increasing acidity in the cell is a direct cause of the nuclear change seems, as discussed above, to be hardly plausible in our case, especially in the case of *Tradescantia*.

There seems to be another type of reversible nuclear change due to heating. This is the case of the heterogeneity by heat of the swollen homogeneous nuclei of pollen mother cells in which the semipermeability is greatly reduced (Section 2). In this case neither vacuolization nor vacuole enlargement is found. This change may be regarded as "Entmischung" at a certain stage of the change toward the irreversible coagulation while the change is still reversible.

We may now conclude as follows:—There are, so far as the present investigation is coneerned, two different cases with respect to reversible nuelear changes caused by heat. In one case, the change is regarded as due to dehydration of the nucleus which is probably directly connected with the formation or the enlargement of the vacuoles in the cytoplasm, and in the second case the change appears to be a more direct effect of heat than in the first case; it is probably due to the "Entmischung" of the karyoplasm (the chromonema substance!).

ii. Effect of heat on the dividing nuclei. As seen from the summary of Section 4 and comparing it with the results obtained by dehydration, most of the abnormalities in mitosis induced by heating are connected with dehydration which may be in causal dependency on the abnormal formation or enlargement of vacuoles in the cytoplasm. The normal migration movement of chromosomes is thus prevented not only directly in a mechanical way, but also indirectly through the increase of the viscosity of the cytoplasm, by the vacuole formation or enlargement.

KOKOTT (1930) has regarded irregular mitotic figures caused by heat as due to the "Phasenverschiebung" of the protoplasm colloid. He states that the phenomenon is indicated microscopically by the vacuolization of the cytoplasm. Thus it seems that KOKOTT's "Phasenverschiebung" may be replaced by the term "Entmischung." The "Siedroplasma" too, which has been emphasized by BLEIER (1930) as a disturbing factor of the chromosome behaviour in mitosis seems to be a product of the "Entmischung" in the cytoplasm. In the present investigation, such a structure as "Siedroplasma" was only rarely observed, but an uneven granulation or local aggregations of the cytoplasm was the phenomenon generally observed when root tip cells or epidermal cells were heated to a high temperature. This granulation or aggregation of the cytoplasm probably indicates the "Entmischung" in the cytoplasm, and it seems probable that such an "Entmischung" may also play a rôle in mechanically disturbing the normal chromosome behaviour in the metaphase and anaphase.

With the leaf epidermal cells of *Elodea densa* we observed that the heated nuclei shrink after first swelling, and it has been reported by several investigators that one of the first visible effects of high temperatures is the increase of cell permeability (cf. STILES and JÖRGENSEN, 1916, LEPESCHKIN, 1923, PORT, 1927). From these facts, it may be inferred that under the condition of high temperature, hydration of the protoplast occurs and this may cause irregular distribution of the anaphasic chromosomes. On this point further discussion will be made at the end of this paper.

The conclusion derived from the above considerations may be briefly stated as follows:—The important irregularities of the mitosis induced by heat are mainly due to hydration, dehydration and to the "Entmischung" of the protoplast (see Section 4).

iii. Irreversible changes of the nucleus induced by heat. In the heat coagulation type I and II, the nucleus when heated at 60°C-100°C shows a heterogeneous structure more or less distinctly than before heating, while in the heat coagulation type III the nucleus becomes homogeneous by the same treatment. In the former two cases, the mechanism of the change is easily comprehensible, since most proteins are coagulated and precipitated at these high temperatures. In the third case, on the other hand, a further explanation is needed. According to the results from the histochemical study of the nucleus, the main chemical components of the chromonemata are considered to be thymus nucleic acid, proteins and lipoids (SINKE and SHIGENAGA, 1933). While proteins are coagulated at a high temperature, thymus nucleic acid is liquidified. It may be assumed, therefore, that the difference in behaviour of the nucleus toward heat coagulation between the first and second case and the third is due to the difference in relative amount of proteins and thymus nucleic acid contained in these nuclei. A test shows that in the nuclei which show the heat coagulation type I and II the isoelectric point is found in a range from 4.2 to 5.4, while in those showing the type III it lies in a range from pH 2.2 to 4.6.[1] This result is in accord with our expectation, since the isoelectric point of the nucleic acid is on the far more acid side than that of the proteins (cf. MICHAELIS and DAVIDSOHN, 1912).

B. Low temperatures

The effects of low temperatures on the behaviour of the chromosomes in mitosis have been studied by GEORGEVITCH (1910) and others with fixed root tip cells, but the effect on the structure of the nucleus in the living state has been neglected. In the present investigation, living cells were observed under low temperatures, and parrallel with this, fixed material of root tips treated was also observed.

1. Epidermal cells of Tradescantia

Young petals were mounted with liquid paraffin, and the preparations were put in a thermos of a practically constant definite low temperature for a certain period of time, and then observed under the microscope. Most of these cells died, and it was not possible to observe a sufficient number of cases from which to draw a definite general conclusion, but the characteristic changes occurring in the nucleus and the cytoplasm could be observed somewhat clearly. These changes are the stoppage of the cytoplasmic streaming, a decrease in nuclear volume, and an increase in refractivity of

1) The details to be published later.

the chromonemata which is accompanied by the gradual disappearance of their individual distinguishing qualities. As stated in Subdivision A), these characteristic changes are also observed in the cells exposed to high temperatures. In Fig. 63, normal epidermal cell nuclei of a young petal at room temperature and in Fig. 64, the same after a 3 hours' treatment at ca. −5°C are shown. In Fig. 64, the nuclei are shrunken and the chromonemata which are distinctly observable in Fig. 63 are very obscure. This change is irreversible. Frequently it is clearly observed that the cytoplasmic vacuoles increase in volume markedly and the nucleus decreases in volume when the cells are exposed to low temperatures.

In Division II, it was stated that the guard cell nuclei of closed stomata are swollen and homogeneous. It is sometimes observed that when such closed stomata of *Tradescantia virginica* are cooled at ca. −5°C for 20 minutes, the chromonemata become more or less distinctly visible being accompanied by the stoppage of the cytoplasmic streaming. This change is reversible when the cells are returned to the room temperature. In the guard cells of stomata of *Tradescantia* and *Zebrina*, it is also often observed that the cytoplasm shrinks in a low temperature.

2. *Root tip cells of Vicia*

Root tips of *Vicia faba* were immersed for 30 minutes in liquid paraffin of ca. −3°C. Then the root tips were fixed in part immediately with the BONN modification of FLEMMING's solution with the pre-treatment with CARNOY's fluid, and in the remaining part with the same fixative after they were put back in saw-dust of the room temperature with intervals of 2 hours, 24 hours and 48 hours. The results of observation are, speaking in general, in agreement with those of GEORGEVITCH (1910).

In the root tip cells fixed immediately after the treatment, the resting nuclei are coarsely reticulated or vacuolated, and in some cases, they shrink and are stained homogeneously as observed in the root tips treated with a hypertonic sugar solution (see Division II). In metaphase and anaphase, the chromosomes are coalescent, in some cases accompanied by a contraction of the cytoplasm. As observed by GEORGEVITCH (1910), the distribution of the chromosomes in metaphase and anaphase is often disturbed by the vacuolization of the cytoplasm. In the root tips fixed after an interval of two hours the contraction of the cytoplasm is often very marked. Shrunken nuclei which are homogeneously stained are frequently found, and the binucleate cells and the di-diploid nuclei are also observed though rarely. Most of the mitotic figures appear quite normal. In the root tips fixed after the interval of 24 hours or 48 hours, cells in necrosis are found which are shrunken and stained deeply as in the case of the experiment with high temperatures. Giant or di-diploid nuclei and bi-nucleate cells are frequently observed also in these root tips. In Fig. 65, a di-diploid, dumb-bell shaped nucleus is shown. The behaviour of the chromosomes in division is generally normal.

3. *Conclusion*

While the mechanism of the death by cold of the plant cells has been discussed by many physiologists (ÅKERMANN, 1927, ILJIN. 1934, BĚLEHARÁDEK, 1935 and others), the nuclear change caused by cold has been overlooked. In our experiments, it was observed that decrease in volume and increase in refractivity of the nucleus, disappearance of the distinguishable chromonemata, stoppage of the cytoplasmic streaming, occurrence of the giant di-diploid nuclei and the bi-nucleate cells, and coalescence of the chromosomes are caused by low temperatures. These abnormalities are those observed on the dehydration of the nucleus with hypertonic solutions. This fact of parallelism between the two cases suggests that the nuclear changes caused by low temperatures may at least in part be due to the dehydration of the nucleus. The mechanism of this nuclear dehydration caused by low temperatures is not fully clear, but it seems not improbable that the enlargement of the cytoplasmic vacuoles observed in the root tip cells during freezing bears a certain relation to the dehydration.

IV. Effects of Mechanical Injury

The effects of mechanical injuries on the structure of the resting nucleus have been studied by various investigators with various methods, cutting the cells with a sharp knife (BÜNNING, 1926), puncturing the cells with micro-needles (CHAMBERS, 1924, WADA, 1930, MARTENS and CHAMBERS 1932 and others), pressing the cells by pressure applied on the cover glass (BĚLAŘ, 1930, YAMAHA and ISHII, 1933) etc. The results obtained with these methods agree in showing that the homogeneous nuclei become heterogeneous and the heterogeneous nuclei become coarsely reticulated or swollen and hyaline. In the case of the latter change, it has been also observed that the heterogeneous nuclei become homogeneous being accompanied not by a swelling of the nucleus but by a decrease in volume (BĚLAŘ, 1930).

In the present investigation, the experiments were made by cutting tissues with a sharp knife, both methods of observation in fresh and fixed states of material being employed. In the case of the fresh material the observations were begun immediately after the experiment, and in that of the fixed material the fixation was made immediately or with different intervals of time after cutting.

1. *Epidermal cells*

a. Young petals of Tradescantia reflexa. When young petals of *Tradescantia reflexa* are cut in a 0.1 M saccharose solution which is nearly isotonic or in liquid paraffin as medium, many heterogeneous nuclei in the neighbourhood of the wounded cells show visible changes in structure, while those remote from them present no such change. The changes may be classified practically into five different types.

In the first type, the chromonemata become thicker and more highly

refractive than in the normal nuclei. This change is usually reversible, and the reversed change is observed after a period of time. In the second type, the whole nucleus appears to be coarsely reticulated as shown in Fig. 66, *a*. These nuclei show no recovery of the original chromonema structure after 24 hours at room temperature. In some cases, the nuclei swell and the nuclear membrane bursts and only the chromatin masses of hemi-sperical or crescent shapes remain visible. This phenomenon is far more frequently visible in the resting nuclei than in the interphase nuclei. A similar case has been observed by STRUGGER (1929) in unhealthy nuclei, and by MARTENS and CHAMBERS (1932) in a micro-dissection experiment. In the third type, the nuclei become shrunken and highly refractive accompanied by the disappearance of the individual threads of chromonemata. This nuclear change is irreversible. In Fig. 66, *b*, an example of such nuclei is shown. In the fourth type, the nuclei become also homogeneous and decrease in volume, but only to a certain limited extent, and they are refractive only a little more than in the normal nucleus. This change is usually reversible, and such nuclei are commonly found in the dividing zone and not in the stretching zone. In Fig. 67, a nucleus which belongs to this fourth type is shown, and in 68, the same nucleus after it has recovered its chromonema structure is reproduced. The nuclei shown in BĚLAŘ's Figs. 18-21 (1930) may probably be those belonging to this type. In the fifth type, the nuclei swell accompanied by a lowering of their refractivity, and no structure is visible in the nuclei as shown in Fig. 69. Such nuclei usually recover their original chromonema structure. Fig. 70 is a photomicrograph of the same nucleus taken about 1 hour after the experiment. In this figure, the recovery has been almost accomplished, and the chromonemata are clearly visible. This type of nuclear change is usually found in the case of sugar solution used as medium and rarely in the case of liquid paraffin, while the other four types are frequently visible in liquid paraffin too.

In dividing cells, WADA (1930) has succeeded in obtaining bi-nucleate cells and di-diploid giant nuclei by puncturing the cells with the micro-needle. In the present investigation, the same results were obtained by cutting the cells with a sharp knife. In Fig. 71, which was photographed at 9.40 (a. m.), a dividing cell in metaphase found near the wounded cells is reproduced. In the upper part of the cell vacuolization is clearly visible in the cytoplasm. Fig. 72 shows the same cell photographed at 0.50 (p. m.). In this figure, thick chromonemata are visible instead of the chromosomes. Fig. 73 is a microphotograph of the same cell taken at 9.00 a. m. in the next morning. In this figure a large resting nucleus which contains convoluted chromonemata is seen. There is little doubt that this nucleus is a di-diploid nucleus formed by the direct reconstruction of the metaphasic chromosome group shown in Fig. 71. In this preparation liquid paraffin was used as mounting medium.

In all the types except the fifth, the nuclei show a more or less strong shrinkage which is accompanied by the disappearance of the cytoplasmic

strands connecting the cell wall and the nucleus. This as well as other cytoplasmic changes such as the stoppage of the streaming is also in most cases reversible.

With the leaf epidermis of *Tradescantia virginica* the same results were obtained in general as those described above in the case of the stretching zone of the young petal.

b. Leaf of Elodea densa. As was mentioned in Division I, the epidermal nuclei of this plant are of the type of the homogeneous nucleus I or II with a nucleolus or nucleoli (Fig. 74, *a*). When the leaf is cut in the medium of pond water (pH 6.8), many nuclei devleop the reticulate or chromonema structure as shown in Fig. 74, *b*. Frequently the nuclei shrink and increase in refractivity. In this latter case, the structure is hardly visible except for the nucleolus which is observable more or less distinctly. This latter type of nuclear change is usually irreversible.

2. *Root-tip cells of Vicia*

The root tips were cut at the region 2-3 m.m. from the tip and were fixed with BONN modification of FLEMMING's solution immediately (R-0 h), or after 30 minutes (R-30 m), 1 hour (R-1 h), 3 hours (R-3 h), 8 hours (R-8 h), 24 hours (R-24 h) and 72 hours (R-72 h) preservation in saw-dust. The results obtained are given in a summarized form in the following:

i. R-0 h. Both resting nuclei and mitotic figures found near the wounded region appear generally normal, but the nuclei show a strong tendency to displacement toward the side of the cell towards the wounded region, as reported by KARLING (1926 and 1928), LOOS (1932) and others ("Traumatotaxis"). In some cases, it is observed that the anaphasic distribution of the chromosomes is markedly disturbed owing to an enormous enlargement of the cytoplasmic vacuoles.

ii. R-30 m, R-1 h and R-3 h. The resting or the interphasic nuclei in the neighbourhood of the wounded region are strongly shrunken, and are deeply stained as is often observed in the case of root tip cells treated with hypertonic solutions or high temperatures. The vacuolization of the cytoplasm is often remarkable. The chromosomes in metaphase and anaphase usually show a more or less strong coalescence.

iii. R-8 h, R-24 h and R-72 h. The mitotic figures found near the wounded region usually appear to be normal. Bi-nucleate cells are frequently observed while the di-diploid giant nuclei are relatively rarely met with. In Fig. 75, a bi-nucleate cell and in Fig. 76, a di-diploid giant nucleus is shown. These irregularities have been observed by WADA (1930) in his micro-dissection experiment with the staminate hairs of *Tradescantia.*

3. *Conclusion*

From the viewpoint of the mechanism through which the change is brought about, the five types of nuclear changes found near the wounded cells may be classified roughly into three classes, though the mechanism is

not satisfactorily clear in each case. In the first case where the heterogeneous nuclei of *Tradescantia* become homogeneous accompanied by shrinkage (the 3rd and 4th type), there seems to be little question that the nuclei are dehydrated, since they decrease in volume and increase in refractivity. This change is expressed by BĚLAŘ (1930) by the term "Entquellung." The enlargement of the cytoplasmic vacuoles which we observed in the case of heating too, seems to suggest that this dehydration of the nucleus is due to the absorption of water by the cytoplasmic vacuoles from the surrounding cytoplasm and the nucleus. The reports of BÜNNING (1926) and others that the viscosity of the cytoplasm increases when the cell is mechanically injured favours this view. The reversible nuclear change belonging to the 2nd type may also be regarded as due to dehydration. On the other hand, in the second case where the chromonemata increase in refractivity but present a coarsely reticulate appearance of the nucleus (the 2nd type), we cannot explain the mechanism simply as dehydration, since such a change has never been observed in nuclear dehydration with hypertonic solutions. According to BĚLAŘ (1930), such a nuclear change is due to the "Entmischung" of the nuclear colloid. In the third case, the nucleus often swells and its structure becomes very obscure (the 5th type). In this case, the hydration of the nucleus must play a main rôle. It is important here to note that this change very frequently occurs in a watery medium, such as a water solution of sugar or water from any source, while it is observed only rarely in the medium of liquid paraffin. This fact suggests that the hydration of the nucleus is partly due to the penetration of water from outside (LEPESCHKIN, 1935). FREUNDLICH (1935) has put forward the view that the liquifaction of the protoplasm by mechanical injury is a thixotropical phenomenon of the protoplasmic colloid. LEPESCHKIN states, on the other hand, that it may be possible to explain the liquifaction of the protoplasm by mechanical injuries as due to an increased permeability of water, which in many cases has been explained as thixotropy. This alternative of LEPESCHKIN of seeking the source of water outside of the cell is favoured by the fact we observed that the disappearance of the chromonemata accompanied by nuclear swelling does not occur with equal frequency in water and paraffin media.

In conclusion, it may be said that in the nuclear changes caused by mechanical injuries, too, both hydration and dehydration play important rôles.

V. Effects of Acids and Alkalis

By various investigators the effects of acid and alkaline solutions upon the visible structure of the nucleus and the chromosomes havbe een studied. In plant cells, KUWADA and SAKAMURA (1927), SAKAMURA (1927), and YAMAHA and ISHII (1932) have studied the relation between the H-ion concentration in the mounting media and the visibility of the chromosomes in metaphase and anaphase, and STRUGGER (1928), STROHMEYER (1935) and some others

have studied the nuclear changes caused by acids. In animal cells, VAN HERWERDEN (1923), LEWIS and LEWIS (1924), ZEIGER (1935) and MORITA (1935) have investigated the effects of these solutions on the structure of the resting nuclei. The results obtained by these investigators are quite consistent. While in alkaline media, both nuclei and chromosomes swell and become hyaline, in acid media they show their respective structures distinctly. In the present investigation, it was intended to observe not only the direct effects of acid and alkaline solutions on the nucleus, but also indirect effects of these solutions or the effects which are indirectly produced on the nucleus through the changes occurring in the cytosome. For the purpose of investigating the indirect effects, epidermis cells of young petals and guard cells of stomata were used as material, and for that of the direct effects pollen mother cells and periplasmodial nuclei were employed.

1. Indirect effects

In this series of experiments solutions in different concentrations of acetic acid and ammonium hydroxide were used[1] notwithstanding their having no buffer action, for the following reasons:—1) In these solutions the cation or anion is far more rapidly penetratable into the cell than those of the ordinary buffer solutions (cf. GELHORN, 1931), so that the effects of the H-ion and OH-ion on the nuclei or chromosomes can more readily be observed. 2) As has been shown by YAMAHA and ISHII (1932), in the use of the ordinary buffer solutions the effects on nuclear structure of the ions of the solution components are so large that the nuclear changes caused by the buffer solutions cannot be regarded as mere changes due to the action of the H-ion or OH-ion. In the present investigation, the changes taking place were traced under the microscope, the observations being made with both ordinary and dark field illuminations.

a. Epidermis cells in young petals in Tradescantia reflexa. As mentioned in Division I, the nuclei in the epidermal cells in *Tradescantia* show a distinct chromonema structure in the medium of di-distilled water (usually pH 5.4-5.8). When the medium is replaced with a 1/200 M acetic acid (pH 3.5) or less concentrated solutions, the chromonemata are rendered far more highly refractive and become far more distinctly visible than in di-distilled water. In this medium of acetic acid, the streaming of the cytoplasm takes place rapidly. In a 1/100 M (pH 3.3) or more concentrated solutions of acetic acid, the chromonemata are highly refractive and very distinct and appear thicker in diameter than those in the normal nucleus. In these cells, not only the chromonemata in the resting nucleus but also the chromosomes in metaphase and anaphase are highly refractive and the cytoplasmic streaming is completely stopped and thus the nuclei appear to be coagulated. The changes, both nuclear and cytoplasmic, are, however, usually reversible when the material is washed with di-distilled water.

1) The pH value of these solutions was colorimetrically determined.

The nuclear changes caused by diluted alkaline solutions are different from those due to acid solutions described above. When the epidermal cell is observed with a 1/100 M NH_4OH solution (pH 10.4), the streaming of the cytoplasm stops, accompanied by the enlargement of the vacuoles, and the chromonemata in the nucleus become slightly indistinct. In a 1/20 M NH_4OH solution (pH 11.0), the vacuoles greatly enlarge and press upon the nucleus, often so much as to cause its deformation. The nucleus is now highly refractive, and the chromonemata appear thicker in diameter and less in number than in the untreated natural nucleus. The chromonemata, then, become gradually indistinct as was observed in the nucleus dehydrated with hypertonic solutions (see Division II). In the cytoplasm neither the streaming nor the Brownian movement is perceptible. These changes in the nucleus and cytoplasm are reversible when the medium is replaced with di-distilled water. The following examples are the results obtained with acetic acid and NH_4OH respectively.

The case of acid solution.

9.30 (a. m.). Mounted with di-distilled water (pH 5.8). The nucleus appeared to be filled with many convoluted chromonemata. The Brownian movement and streaming were not perceptible in the cytoplasm (Fig. 77).

10.25. The medium was replaced with a 1/10000 M acetic acid (pH 4.5). The appearance of the nucleus was the same as in di-distilled water.

10.35. The medium was replaced with 1/1000 M acetic acid (pH 3.8). The cytoplasmic streaming was somewhat rapid. The chromonemata were more distinct than before.

10.50 (p. m.). Do. The medium was replaced with a 1/200 M acetic acid (pH 3.5).

11.10. The chromonemata were more distinct than before. The cytoplasmic streaming was slowed.

11.35. Do. The medium was replaced with a 1/100 M acetic acid (pH 3.3). Two or three vacuoles were visible in the nucleus.

11.45. The chromonemata were very distinctly observable, and appeared to be somewhat thicker than those in the normal nucleus. A nucleolus became visible in each vacuole. The general appearance of the nucleus was similar to that of the nucleus stained with acetocarmine. The cytoplasmic streaming and the Brownian movement was stopped (Fig. 78).

11.50. Do. The material was washed several times with a 1/500 M NH_4OH and then was put in di-distilled water.

12.00. The chromonemata became lowered in refractivity and appeared to be normal. The nucleoli disappeared.[1] The streaming of the cytoplasm began to take place.

0.50. The general appearance of the nucleus and the cytoplasm was quite normal (Fig. 79).

The case of alkaline solution.

9.15 (a. m.). Mounted in di-distilled water (pH 5.8). The chromonema structure was clearly recognized in the nucleus. The cytoplasmic streaming was perceptible, but was not active (Fig. 80).

---

1) In this material, the nucleoli are not visible in the healthy condition of the cell.

10.15. The medium was replaced with a 1/500 M NH_4OH solution (pH 9.8).

10.25. The nucleus showed no marked change. The cytoplasmic streaming was active. The diameter of the nucleus was 15 μ.

11.10. Do. The medium was replaced with a 1/100 M NH_4OH solution (pH 10.4).

11.20. Do. The cytoplasm appeared to be very fluid.

11.55. The refractivity of the nucleus increased and the chromonemata were slightly less distinct. The streaming of the cytoplasm was somewhat inactive. The diameter of the nucleus was 14 μ.

0.05 (p. m.). Enlargement of the cytoplasmic vacuoles was observed. The nucleus was shrunken and highly refractive. (Fig. 81). The diameter of the nucleus was 13.5 μ. The cytoplasmic streaming was completely stopped.

0.20. Do. The diameter of the nucleus was 12.5 μ.

1.00. The chromonemata appeared thicker in diameter and less in number than those of the normal nucleus.

1.10. The medium was replaced with a 1/20 M NH_4OH solution (pH 11.0).

1.20. The nucleus was highly refractive and the chromonemata were somewhat indistinct. No cytoplasmic streaming was observed. The diameter of the nucleus was 12.0 μ.

1.30. Do. The photomicrograph reproduced in Fig. 82 was taken. Then the material was washed several times with di-distilled water.

1.55. The medium was replaced with a 1/1000 M acetic acid (pH 3.8).

4.00. The nucleus decreased in refractivity and increased in volume. The chromonemata were distinctly visible.

The results given above concern the cells which retain their semipermeability intact.

In the dark field illumination, many illuminated particles in the nucleus which are weakly bright in its natural state become very strongly bright in a 1.0 M acetic acid (pH 2.5). In a more or less acid solution, this brightness decreases. In a 10 M (pH 1.9) and a 1/10 M acetic acid (pH 2.8), the nucleus is clearly less bright than in the case of the 1.0 M acetic acid. In this case, therefore, the maximum turbidity or the isoelectric point of the nucleus or the chromonemata is found at about pH 2.5, a value which is nearly equal to pHi 2.2 determined by PISCHINGER's (1926) staining method (see p. 92). The turbidity caused by acetic acid in different concentrations is shown in Table 6.[1]

Table 6.

Conc. in mol	10	1	1/10	1/100	1/1000
pH	1.9	2.5	2.8	3.3	3.8
Turbidity	±	++	+	+ or ±	+

The results we obtained with unimpaired epidermal cells are not harmonious with those obtained by other investigators. While in ours, the nucleus shrinks and increases in refractivity in a dilute solution of NH_4OH,

1) In this and the following tables, the mark ++ and + show a strong and a weak turbidity of the nucleus respectively, and the mark ± a slight turbidity, the absence of the visible turbidity being denoted by the mark −.

it swells in alkaline solutions in those of the others. In our case, the chromonemata appear thicker in diameter and less in number, and finally have lost their sharp outlines as in the case of dehydration with hypertonic solution.[1] It is characteristic in this case that these nuclear changes are accompanied by an enlargement of the vacuoles and the stoppage of the streaming in the cytoplasm.

In case the cells have lost their semipermeability, the nuclei swell and the chromonemata become invisible by dilute alkaline solutions as in the case of pollen mother cells and periplasmodial nuclei to be described later. In this case the results are the same as those obtained hitherto by other investigators.

In the case of acid solutions, there is no difference in result between the two cases where the cells are unimpared and where they have lost their semipermeability, and in both cases the chromonemata become highly refractive and far more distinctly visible than in di-distilles water, excepting the case where unimpaired cells have previously been treated with dilute alkaline solutions. In this latter case, it is often observed that the dehydrated nuclei swell and become hyaline on the replacement of the alkaline solution with a dilute acid solution. The same phenomenon is also observed in the case of the guard cells of stomata as will be seen later.

b. Guard cells of stomata in Tradescantia virginica. In the study of the mechanism of the stomatal movement, SCARTH (1927, b) states that in open stomata the reaction of the cytoplasmic vacuoles is alkaline, and that accordingly, the colloids in the vacuoles are strongly swollen. If this swelling of the cytoplasmic vacuoles causes dehydration of the nuclei and the cytoplasm, this statement of SCARTH seems harmonious with the fact we observed, that in open stomata the guard cell nuclei are dehydrated (Division II).

In the present series of experiments it was aimed first to see whether or not the swollen homogeneous nuclei in the guard cells of closed stomata are dehydrated and rendered heterogeneous by treatment with dilute alkaline solutions and second to see the effect of acid on these nuclei. *Tradescantia virginica, Zebrina pendula, Vicia faba and Rhoeo discolor* were used as material, and nearly uniform results were obtained with these plants. In the following the results obtained on these points with *Tradescantia virginica* are given as representative.

When a piece of the epidermis from the lower side of the leaf is observed in di-distilled water, the guard cell nuclei in the closed stomata are swollen and homogeneous as already mentioned in Division II. In these cells the cytoplasmic streaming is usually active. When the medium is repleced with a 1/10000 M (pH 4.5) or a 1/1000 M (pH 3.8) acetic acid, the streaming is still active, but the chromonemata become indistinctly perceptible. In a

1) We have been told that Mr. SHIGENAGA succeeded in producing di-diploid nuclei in young leaf epidermal cells by treating dividing cells in metaphase with alkaline solutions in the same way he had succeeded earlier with the treatment with hypertonic saccharose solutions (SHIGENAGA, 1937).

1/200 M (pH 3.5) or a 1/100 M (pH 3.3) acetic acid, the chromonemata are highly refractive, and are observed very distinctly, the cytoplasmic streaming being no longer recognizable. Both these nuclear and cytoplasmic changes are reversible when the medium is replaced with di-distilled water. In the following one example out of a series of experiments is given.

1.05 (p. m.). In di-distilled water (pH 6.4), the guard cell nuclei were swollen and hyaline, and the streaming of the cytoplasm was rapid (Fig. 83).

1.25. Do. The medium was replaced with a 1/10000 M acetic acid (pH 4.5).

1.30. Do. The medium was replaced with a 1/1000 M acetic acid (pH 3.8).

1.35. The cytoplasmic streaming decreased in velocity.

1.40. The medium was replaced with a 1/200 M acetic acid (pH 3.2). The chromonemata became gradually distinct, and appeared to be highly refractive. The cytoplasmic streaming was not observed.

1.45. The chromonemata were very distinct, and appeared thicker in diameter than they were at 1.40 (Fig. 84).

1.50. The tissue was washed several times with 1/1000 M NH_4OH (pH 9.6) and then was put in a 1/10000 M acetic acid solution (pH 4.5). The cytoplasmic streaming became active again.

3.00. The nuclei swelled and the chromonemata were indistinct. The streaming was active.

3.40. Do. The medium was replaced with di-distilled water (pH 6.4). The nuclei appeared swollen and were quite homogeneous as shown in Fig. 85.

As exemplified above, the hyaline nuclei in the guard cells of closed stomata become reversibly heterogeneous when the stomata are observed with an acid solution.

In alkaline solutions, such as a 1/1000 M (pH 9.6) or a 1/500 M (pH 9.8) NH_4OH the chromonemata which are not visible in the closed state of the stomata become somewhat distinct, and the cytoplasmic vacuoles are diffusely coloured yellow.[1] In a 1/100 M (pH 10.4) or a 1/20 M (pH 11.0) solution, the nuclei shrink and increase in refractivity, and the chromonemata appear thicker in diameter than in the natural condition. When the refractivity is increased still further, the chromonemata become somewhat indistinct. The general appearance of the nucleus in this state is, as we expected, nearly the same as that of the same nuclei of the closed stomata when treated with a hypertonic solution and that of the natural nuclei of open stomata. This resemblance in general appearance suggests that the nuclei of the closed stomata are dehydrated when the cells are treated with an alkaline solution. The stoppage of the cytoplasmic streaming indicates also that in alkaline solutions the cytoplasm is dehydrated. These nuclear changes due to the alkaline solutions are reversible when the medium is replaced with di-distilled water. As described above in the case of the petal epidermis,

1) This pigment is found in the guard cells as well as the auxiliary cells of stomata, and the reaction colour is very deep when the cells are immersed in strong alkaline solutions. The nature of this pigment is left undetermined in the present investigation, but it is suggested by the reaction colour that the pigment may be a form of flavons (LINSBAUER, 1932).

it is also often observed in this case of the guard cells that the dehydrated, hence heterogeneous nuclei of closed stomata caused by alkaline solutions, swell in dilute solutions of acetic acid (1/1000 M-1/500 M), and the chromonemata become indistinct; and these changes generally take place more quickly than do those of the natural heterogeneous nuclei of half-open stomata in distilled water, and no such swelling takes place by a mere treatment of the latter (heterogeneous nuclei of half-open stomata) with acetic acid of the same concentrations. An example of the dehydration by NH_4OH and this peculiar swelling phenomenon by the acetic acid treatment that follows the treatment with NH_4OH is shown below:

8.30 (a. m.). The nuclei of closed stomata were swollen and homogeneous in di-distilled water (pH 6.4). The streaming of the cytoplasm was active.

8.47. Do. The photomicrograph reproduced in Fig. 86 was taken.

8.48. Do. The medium was replaced with a solution a 1/1000 M NH_4OH (pH 9.6).

8.55. The nuclei appeared nearly homogeneous. The medium was replaced with 1/500 M NH_4OH (pH 9.8).

9.00. The chromonemata were visible, but not distinct. The guard cells were coloured diffusely yellow.

9.07. Do. The medium was replaced with 1/100 M NH_4OH (pH 10.4). The chromonemata were distinctly observed and the cytoplasmic streaming was stopped. The yellow colour was deeper than before.

9.10. Do. A photomicrograph (Fig. 87) was taken.

9.12. The medium was replaced with 1/10000 M acetic acid (pH 4.5).

9.20. The nuclei were swollen and homogeneous. The cytoplasmic streaming was rapid again, and the yellowish colour in the guard cells disappeared. The general appearance of the nuclei was similar to that observed at 8.30.

9.30. Do. The medium was replaced with 1/500 M NH_4OH (pH 9.8).

9.35. The chromonemata were distinctly visible, and the cytoplasmic streaming was stopped. The medium was replaced with 1/20 M NH_4OH (pH 11.0). The chromonemata soon became thicker in diameter and appeared less in number than they were at 9.35. The nuclei were shrunken and highly refractive (Fig. 88).

9.50. The medium was replaced with 1/10000 M acetic acid (pH 4.5).

10.00. The nuclei were swollen and homogeneous, and appeared quite normal. The streaming of the cytoplasm was recovered and rapid (Fig. 89).

These results show that, as we should expect, the swollen homogeneous nuclei of natural closed stomata become dehydrated and heterogeneous by treatment with a dilute alkaline solution. SAYRE (1926), SCARTH (1926, 1927) and PEKAREK (1933) have reported that in the guard cells of open stomata where the nuclei show a strong shrinkage, the cytoplasmic vacuoles are more alkaline in reaction than are the vacuoles in the closed stomata when the nuclei markedly swell. Our own observations seem to be in consistence with these conclusions. It will be discussed later, however, whether or not in the natural state the dehydration condition in open stomata of the guard cell nuclei is due simply to the lowering in acidity of the cytoplasmic vacuoles.

2. *Direct effects*

Pollen mother cells and periplasmodial nuclei of *Tradescantia reflexa.* The periplasmodial nuclei and the pollen mother cells are very liable to loss

of semipermeability when cells are squeezed out of the anther. The loss of the semipermeability is indicated by the fact that in these nuclei and the cells nuclear swelling is caused by concentrated sugar solutions or that the cells show no plasmolysis from hypertonic solutions.

The effects of different H-ion concentrations on the pollen mother cells of *Tradescantia virginica* has been studied in detail by KUWADA and SAKAMURA (1927). In this study by these authors, the observations have been made generally with the bright field illumination. In the present investigation, it was the principal object to study the effects with the dark field illumination. We employed as media, McILVAINE's solution, KOLTHOFF's citrate mixture, WALPOLES's acetate mixture, SÖRENSEN's HCl mixture and several other mixtures. The H-ion concentrations were measured with a quinhydrone electrode.

a. Experiment with McILVAINE's *mixture of 1/10 M citric acid and 1/5 M* Na_2HPO_4.

i. pH 2.3. In the early prophase, the nucleus appears to be filled with many very bright particles, while in the cytoplasm a slight turbidity is recognizable. In the mid-prophase, the spiremes are found contracted into a mass in this mixture and are also strongly bright. The karyolymph is quite dark occupying the area between the nuclear membrane and the mass of the spiremes. In the late prophase, metaphase, and anaphase, the chromosomes are less bright than in the early prophase. In the metaphase and anaphase they often show spiral chromonemata which are brighter than the matrix. In these stages the brightness of the cytoplasm is very weak except for the starch grains contained. The nuclei in the telophase and interkinesis and those of the periplasmodium are illuminated brightly.

ii. pH 2.5 and 2.7. In these mixtures the brightness of the chromonemata or the chromosomes and of the periplasmodial nuclei is nearly equal in intensity to the corresponding case with pH 2.3, while the turbidity in the cytoplasm is stronger.

iii. pH 2.9 and 3.1. The cytoplasm is considerably bright, and the nuclear components, especially the chromosomes, are often hardly distinguishable from the cytoplasm. The karyolymph shows no turbidity. When the chromosomes are recognizable to a certain extent by their faintly illuminated outlines, they are found slightly swollen. Generally speaking, the turbidity shown by the spiremes and chromosomes is slightly lower than in case ii.

iv. pH 3.6. In this mixture, the turbidity of the cytoplasm is greater than in case iii, and as a consequence of this high turbidity of the cytoplasm it is somewhat difficult to obtain sharp images of chromosomes in metaphase and anaphase. The outlines of the chromosomes are usually indistinct in this mixture.

v. pH 3.9 and 4.1. The cytoplasm is strongly bright, and the chromosomes are hardly recognizable. In the early and the mid-prophase, the area occupied by the karyolymph is completely dark.

vi. pH 4.3 and 4.4. The turbidity of the cytoplasm being too high, the cells appear to be mere bright masses.

vii. pH 4.6. In the prophase, metaphase and anaphase, the nucleus and the chromosomes present nearly the same general appearance as in case vi, while the turbidity of the cytoplasm appears to be less.

viii. pH 5.0. The cells are swollen, and the chromonemata, the spiremes, the chromosomes and the cytoplasm are all nearly dark.

ix. pH 5.2, 5.4, 5.8, 7.4 and 8.0. The cells appear dark except that the outlines of the starch grains contained are slightly illuminated.

From the results mentioned above, it is seen that in the early and the mid-prophase, in the metaphase, and in the anaphase, the maximum turbidity of the chromonemata, the spiremes and the chromosomes is found at pH 2.3, or at a point more acid, and on the side less acid than pH 2.3 the turbidity decreases. At pH 2.9 and 3.1, the chromonemata and the spiremes are illuminated brighter than the chromosomes in metaphase. This behaviour toward the H-ion concentration is the same in the chromonemata of the periplasmodial nuclei as in the early prophase of the first sporocyte. In the cytoplasm the maximum turbidity is found in a zone between pH 3.9 and 4.4, and no turbidity is shown by the karyolymph at any point between pH 2.3 and 8.0.

b. Experiment with KOLTHOFF*'s mixture of 1/10 M citric acid and 1/10 M K-mono-citrate* (KOLTHOFF, *1932*). In this and the following series of experiments, only the chromosomes in the first metaphase and anaphase were observed. The results obtained with KOLTHOFF's mixture are shown in Table 7.

Table 7.

pH	2.3	2.7	3.3	3.6	4.1	4.9	5.8
Turbidity of chromosomes	++	+	+	+	±	−	−
Turbidity of cytoplasm	−	+	++	++	++	+	−

From this table, it is seen that in the chromosomes the maximum turbidity is found at pH 2.3, and in the cytoplasm, in a zone from pH 3.3 to 4.1. These results are the same or nearly the same as those obtained in the experiments with MCILVAINE's mixture.

c. Experiment with acetic acid and WALPOLE*'s solution (1/5 M acetic acid and 1/5 M Na-acetate).* In the following descriptions, the concentrations of the acid are given in parentheses in case acetic acid is used singly.

i. pH 2.0 (5.0 M acetic acid). The chromosomes are fairly bright, and are easily distinguishable from the cytoplasm which is nearly dark (Fig. 90). The spiral chromonemata are brighter than the chromosome matrix. The periplasmodial nuclei are also bright.

ii. pH 2.4 (1.0 M acetic acid) and pH 2.7 (1/5 M acetic acid). Both chromosomes and cytoplasm are brighter than in case i. The chromosomes show the maximum turbidity in these media (Fig. 91).

iii. pH 3.2. The chromosomes become somewhat obscure, and reversely the cytoplasm increases in brightness. The periplasmodial nuclei are strongly bright.

iv. pH 3.8 and pH 4.1. The chromosomes are hardly perceptible, clear observation being prevented by the too strong brightness of the cytoplasm (Fig. 92). Often the chromosomes are recognizable, and in these cases they are found to be slightly swollen, not being so bright as in the case iii. The periplasmodial nuclei are also less bright than in that case.

v. pH 4.4. The chromosomes swell to a certain extent, though their contour is still perceptible. The periplasmodial nuclei are also swollen and slightly bright. The cytoplasm is very bright.

vi. pH 4.9. In this medium the cytoplasm shows a very weak turbidity. The chromosomes are swollen and hardly perceptible (Fig. 93).

vii. pH 5.2 and pH 5.6. *Both chromosomes and cytoplasm are nearly* dark except for the starch grains contained (Fig. 94). In the bright field illumination, the chromosomes are still visible. The periplasmodial nuclei are swollen and only their contour is slightly bright.

viii. pH 7.0 and pH 9.0. The chromosomes swell and are hardly perceptible either with the bright field illumination or the dark field illumination (Fig. 95).

Briefly speaking, the results mentioned above show that in the case of the periplasmodial nuclei and the chromosomes in the first metaphase and anaphase the maximum turbidity is found in a zone from pH 2.4 to 2.7, and in the case of the cytoplasm it is in the zone pH 3.8 to 4.4. At pH 5.2 and 5.6 the chromosomes become hardly visible by the dark field illumination, while they are still perceptible by the ordinary illumination.

d. Experiment with a mixture of 1/10 M acetic acid and 1/10 M Na-acetate (a modification of MICHAELIS' *solution).*

In *c*, the Na-ion concentration of the mixture used was different in different mixtures. In this experiment, *d*, the mixtures were so prepared as to have a definite concentration of the Na-ions and variable concentrations of the H-ions by mixing 5 c.c. of the 1/10 M sodium acetate with variable quantities of 1/10 M or 1.0 M acetic acid and water, they amounting to 25 c.c. in total. The results obtained with these solutions are shown in Table 8.

Table 8.

1/10 M Na-acetate in c.c.	1/10 M acetic acid in c.c.	1.0 M acetic acid in c.c.	H_2O in c.c.	pH	Turbidity of	
					chromosome	cytoplasm
5	0	20	0	3.1	+ or ++	− or ±
5	0	10	10	3.4	+	± or +
5	0	5	15	3.7	+	++
5	20	0	0	4.1	± or +	++
5	10	0	10	4.4	± or +	++
5	5	0	15	4.7	−	+
5	1	0	19	5.4	−	± or +

From Table 8, it is seen that in the cytoplasm the maximum turbidity is found in a zone between pH 3.7-4.4 and in the chromosomes at 3.1 or

points on the more acid side. These results are largely in accord with those obtained in Experiment c, and we may conclude that the latter results have not been appreciably influenced by the difference in Na-ion concentration.

e. Experiment with SORENSEN's *mixture of 1/10 N HCl and 1/10 M glycocoll.*

The results obtained with this buffer solution are given in Table 9.

Table 9.

pH	1.0	1.2	1.4	1.6	1.9	2.3	2.6	2.9	3.3	3.7
Turbidity of chromosomes	++	++	++	+or++	+or++	+	±or+	±or+	±or+	±or+
Turbidity of cytoplasm	−or±	−or±	−or±	±	±	+	++	++	++	+

As is seen from this table, the concentrations of H-ions at which the chromosomes show the maximum turbidity are found in a zone between pH 1.0 and 1.4 and those for the cytoplasm are between pH 2.6 and 3.3. These H-ion concentrations are higher than those obtained by the other buffer solutions mentioned above. The cause of this difference is not fully clear at present, but it seems probable that this is due to the fact that in this mixture HCl is used as the source of H-ions, since in the use of another mixture containing HCl the same phenomenon is observed as shown by the following Experiment f (see Table 10).

f. Experiment with a mixture of 1/10 N HCl and 1/20 M K-phthalate and a mixture of 1/10 M phthalate and 1/20 M borax.

The results obtained are shown in Table 10.

Table 10.

pH	1.5	2.2	3.1	4.0	5.1	6.5
Turbidity of chromosomes	++	+or++	+	±or+	+	−
Turbidity of cytoplasm	−	+	+or++	+or++	±	−

In this table, nearly the same results are shown in respect to the relation between pH value and turbidity as those shown in the preceding tables.

In the Experiments a-*f*, the results are given which were obtained with the pollen mother cells in *Tradescantia reflexa*, but similar results were also obtained with the pollen mother cells in *Lilium tigrinum*. In the latter plant, using MCILVAINE's solution, the maximum turbidity of the chromosomes was found at pH 2.9 or points on the more acid side, and that of the cytoplasm in the zone pH 3.4-3.6.

It must be added here that the nuclear changes caused by the buffer solutions are generally reversible.

The results of the experiments obtained with the pollen mother cells may be summarized as follows:—

The maximum turbidity or maximum brightness of the chromonemata (in the early prophase), the chromatin spiremes (in the mid-prophase) and

the chromosomes (in the metaphase and anaphase) is reached at a definite H-ion concentration of the medium. Similar phenomena are also observed in the cytoplasm. In McILVAINE's solution, the maximum turbidity of the chromosomes is at pH 2.3 (or on the more acid side) and that of the cytoplasm is in the zone between pH 3.9 and 4.4. The H-ion concentration for the maximum turbidity is, however, different in different buffer solutions. This is shown in Table 11.

Table 11.

Buffer solutions	McILVAINE	KOLTHOFF	WALPOLE	MICHAELIS	SÖRENSEN
Maximum turbidity of chromosome	<2.3	<2.3	2.4-2.7	<3.1	1.0-1.4
Maximum turbidity of cytoplasm	3.9-4.4	3.3-4.1	3.8-4.4	3.7-4.4	2.6-3.3

The cause of these differences in H-ion concentrations is not fully clear at present, but it must be borne in mind that the effects of ions in the solutions other than the H-ion play some rôle in the inducement of the turbidity (STRUGGER, 1929, 1930; YAMAHA and ISHII, 1932).

Nearly the same behaviour as exhibited by the chromosomes is shown also by the periplasmodial nuclei toward different buffer solutions. The chromonema spirals in metaphase and anaphase are more highly turbid in a 5.0 M solution of acetic acid than is the matrical part of the chromosomes.

3. Conclusion

The effects of alkalis on the structure of the nucleus are either direct or indirect. According to the results we obtained direct effects are observed in the case of the periplasmodial nuclei and the nuclei in injured cells which have lost their semipermeability. In alkaline solutions, these nuclei increase in volume and decrease in refractivity, and the chromonemata and the chromosomes disappear from sight. In acid solutions the nuclei shrink, and the coromonemata and the chromosomes become more distinct than in the unimpaired state of the cells. With dark field illumination, the chromonemata and the chromosomes are more or less strongly bright in acid solutions, and completely dark in alkaline solutions. These results coincide, generally speaking, with those obtained by other investigators such as VAN HERWERDEN (1923), LEWIS and LEWIS (1924), NASSONOV (1932), ZEIGER (1935) and MORITA (1935) in animal cells and those by KUWADA and SAKAMURA (1927), SAKAMURA (1927) and others in pollen mother cells.

We found that the maximum turbidity of the nucleus and chromosomes in which condition the chromonemata and the chromosomes are most clearly observable by both bright and dark field illuminations, is found in the range between pH 1.0-2.7. It is to be expected, therefore, that this range represents the pHi of the nuclear and the chromosome substance, and this pHi value was determined by PISCHINGER's staining method (PISCHINGER, 1927) to be 2.2 in the case of the nucleus in *Tradescantia*. The value of pHi of

the nuclear and the chromosome substance is to be determined by their chemical components, such as thymus nucleic acid, proteins and lipoids (SINKE and SHIGENAGA, 1933), and it is naturally expected that the value is larger than that of nucleic acid as one of the components (cf. MICHAELIS and DAVIDSOHN, 1912).

Indirect effects are observed in the case of the cells which are unimpaired and show normal semipermeability. In these cells, when suspended in the medium of a dilute NH_4OH solution, the nuclei are shrunken and highly refractive, and the chromonemata have lost their sharp outline; these changes are accompanied by the enlargement of the vacuoles and the stoppage of the streaming in the cytoplasm. These nuclear changes must indicate that the nuclei are dehydrated. The nuclear dehydration is clearly observed in the case of the guard cell nuclei of closed stomata, where the nuclei are swollen and are completely homogeneous in the natural state. In alkaline media, these nuclei present a marked resemblance in appearance to those of the half-open stomata, the chromonemata becoming distinctly visible in the nuclei. On prolonged immersion, they shrink and the chromonemata become somewhat indistinct. This sequence of the changes caused by alkaline solutions very much resembles that of the changes observed when the cells are treated with hypertonic solutions (see Division II). The possible mechanism of this dehydration by alkalis or the indirect effect of the solutions may be explained again on the basis of the fact that the dehydration phenomena are accompanied, as is clearly seen in the case of the stomata of *Zebrina*, by the enlargement of vacuoles; we have earlier explained in this way the dehydration mechanism in the cases of high temperatures and mechanical injuries. The vacuolization in the cytoplasm as caused by alkaline solutions has been reported by KLEMM (1895), DEGEN (1905), YAMAHA (1927 a) and others. The swelling of vacuoles by alkaline solutions has been observed by SCARTH (1926) in the guard cells of stomata, and the viscosity increase of the cytoplasm due to these solutions has been shown by BOKORNY (1888), PRÁT (1926)[1] and others. The mechanism which we can postulate is thus as follows:—In alkaline media, the vacuoles in the cell swell and absorb water from the surrounding cytoplasm and the nuclei, so that the cytoplasmic streaming stops and the nuclei are dehydrated.

In the case of acetic acids, only the direct effect is observable except in the complicated case where an acid solution is used after treatment with an alkaline solution. This difference found between NH_4OH and acetic acid may perhaps be explained as due to the different ability of these agents to penetrate into the nucleus, though we have at present no experimental data in support of this view.

VI. Effects of Neutral Salts

In the pollen mother cells of *Tradescantia virginica* and the epidermal cells in the bulb scale of *Allium cepa*, STRUGGER (1930) has found that while

1) Cifed from HEILBRUNN (1928).

the nuclei appear quite homogeneous in both 0.05 M and 0.3 M KNO_3 solutions they show a heterogeneity in an intermediate concentration, 0.17 M of the same solution. This phenomenon was reobserved by SHIGENAGA,[1] and by YAMAHA and ISHII (1932) with the chromosomes in the pollen mother cells of *Tradescantia reflexa* using with different neutral salts. According to STRUGGER (1935), the phenomenon is observed with cells which have lost their semipermeability, and hence it may be regarded as a direct effect of the salts on the nuclear colloids.[2]

In carrying out the plasmolysis experiments with the scale epidermal cells of *Allium cepa* with the neutral salts used by the authors mentioned above, we often observed that in unhealthy cells, the nuclei showed heterogeneity not only in the 0.2 M solution in which they were observed by STRUGGER to be heterogeneous, but also in the medium of 1.0 M or those of higher concentrations. In the present investigation, such direct effects on the karyoplasm of the salts, KCl, KNO_3, KI and KCNS in high concentrations were studied with the pollen mother cells of *Trillium Smalli* which have lost their semipermeability through the mechanical agitation caused by their being gently pressed out of the anther cut transversally.

1. KCl and KNO_3

The solutions of KCl and KNO_3 in 0.01, 0.05, 0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 2.0 and 3.0 M concentrations were employed. The chromonemata are distinctly visible in 0.1 and 0.2 M solutions (nuclear heterogeneity concentrations) while they are swollen and invisible in 0.01, and 0.05 M solutions (first nuclear homogeneity concentrations) as well as 0.3, 0.5, 0.8, 1.0, 2.0 and 3.0 M solutions (second nuclear homogeneity concentrations). In the former case where the cells are observed with a 0.1 or a 0.2 M solution, the nuclei contain highly refractive chromonemata which are very distinctly visible but gradually become coarsely vacuolated. In some cases, the chromonemata contract into a compact mass and are clearly distinguishable from the nuclear ground substance which appears fluid as indicated by the rapid Brownian movement of a few minute granules in it. Often three different parts, two homogeneous of different consistencies and one heterogeneous, mostly in lateral arrangement, are distinguishable in the nucleus as shown in Fig. 98, a condition which STRUGGER has also observed and regards as a result of "Entmischung" of the nuclear colloids. These and other phenomena have been described by him in detail, and it seems superfluous here to repeat. It must be noted, however, that in the homogeneous nuclei in the 0.01 and 0.05 M solutions, the increase in volume does not take place so extensively as in the case of the heterogeneous nuclei in the higher concentrations, and moreover that, in the former the nucleolus is visible, while in the latter it is usually obscure

1) Cited from KUWADA (1932).

2) In the case of the cells which retain their semipermeability intact, concentrated salt solutions cause plasmolysis and in these cases the nuclear changes—dehydration figures—are indirect effects of these solutions (see Division II).

(cf. STRUGGER, 1935). This difference is more clearly demonstrated in the cases where solutions of KI and KCNS are used.

2. *KI and KCNS*

The effects of the solutions of these salts in lower concentrations than 0.8 M differ in no point of especial importance from those of the solutions of KCl and KNO_3, both concentrations of the first nuclear homogeneity (Fig. 96) and the nuclear heterogeneity (Figs. 97 and 98) being found in the same range of concentrations as in the latter. The second nuclear homogeneity concentrations are, however, limited within a smaller range up to 0.8 M (Fig. 99), and in higher concentrations, 1.0 and 2.0 and also 3.0 M (in the case of KCNS), the nuclei are heterogeneous again notwithstanding the fact that they swell and increase in volume far more extensively than those in the first nuclear homogeneity concentrations. In these high concentrations the chromonemata appear to consist of a row of a large number of refractive granules or the whole nucleus looks as if it were filled with such granules (Fig. 100). In this case, therefore, we have the second nuclear heterogeneity concentrations. Sometimes, the granules in the nucleus show a weak vibration movement. While in the case of KI the nuclei are heterogeneous in the second nuclear heterogeneity concentrations only after a few minutes later, in the case of KCN they show their heterogeneity immediately after treatment with the solutions. The comparison of these two results suggests that the shrinking effect on the chromonemata is weaker in the case of KI than in that of KCNS. The nuclei observed with a 3.0 M KCNS solution are reproduced in Fig. 101. In these nuclei something like a thread structure of ambiguous nature is perceptible though the threads are somewhat swollen and indistinct.

The swelling grades of the nuclear ground substance in different concentrations and those in the corresponding concentrations of the chromonemata as indicated by their visibility are shown in Table 12.

Table 12.

Concentration of KI and KCNS in mol	Chromonemata	Nuclear ground substance
0.01	Invisible.	Slightly swollen.
0.05	Nearly invisible.	Slightly shrunken.
0.1	Visible.	Do.
0.2	Do.	Shrunken.
0.3	Nearly invisible.	Slightly swollen.
0.5	Invisible.	Markedly swollen.
0.8	Nearly invisible.	Do.
1.0	Visible.	Do.
1.5	Do.	Do.
2.0	Do.	Do.
3.0 (KCNS)	Indistinct.	Do.

3. Conclusion

In the Experiments 1 and 2, we saw that we have two groups of concentrations exerting different influences on the nuclei, i. e. the nuclear homogeneity concentrations and the nuclear heterogeneity concentrations. In both concentrations we find again two different groups, the first aud second nuclear homogeneity or heterogeneity concentrations. While the first nuclear homogeneity concentrations (0.01-0.05 M), and the first nuclear heterogeneity concentrations (0.1-0.2 M) are the same in the range of concentrations in the case of both KCl and KNO_3 (Experiment 1) and KI and KCNS (Experiment 2), the second homogeneity and heterogeneity concentrations differ in these two cases. Namely, in the case of KI and KCNS the second homogeneity concentrations are found in the range between 0.3-0.8 M and the second heterogeneity concentration in the range between 1.0-3.0 M, and in the KCl and KNO_3, the second homogeneity concentrations are found in the range between 0.3-3.0 M, covering the range between 1.0-3.0 M which belongs to the second heterogeneity concentration in the case of KI and KCNS. In short, the effects of the salts KCl, KNO_3, KI and KCNS are the same while the concentrations are in the range between 0.01-0.8 M, but they differ in the range between 1.0-3.0 M according to the kind of salt. In this latter range we find that the shrinking effect of the anions on the chromonemata differs in the order, Cl, NO_3<I<CNS. No such difference is observed in effects on the nuclear ground substance, because the nuclear swelling in volume is practically the same in all the cases of these salts.

When we compare the nuclei in the first nuclear homogeneity concentrations and those in the second nuclear heterogeneity concentrations, we notice that the former nuclei show no marked swelling while the latter swell with a marked increase in volume, and nevertheless, the former appear homogeneous and the latter heterogeneous. We must then assume that the behaviour toward the concentration of the salts is different in the chromonemata and the nuclear ground substance. In the first nuclear homogeneity concentrations the chromonemata swell, but not the nuclear ground substance, and consequently the nucleus is homogeneous, but shows no marked increase in volume. In the second nuclear heterogeneity concentrations, the nuclear ground substance swells but the chromonemata do not, and the nucleus is heterogeneous but swollen in volume. In the first nuclear heterogeneity concentrations both chromonemata and the nuclear ground supstance shrink, and consequently the chromonemata are visible and the nuclear volume is decreased. In the second homogeneity concentrations both chromonemata and the nuclear ground substance swell, and consequently the chromonemata is not visible and the nuclear volume is increased. In conclusion, we may say that, so far as the salts we used are concerned, there is a range of concentration in which both chromonemata and the nuclear ground substance shrink, and that while this range is the only range in which the ground substance shrinks, in the case of the chromonema there is another shrinkage range, in which, however, different influences of the different anions on shrinkage are shown.

VII. Effects of Chloral-hydrate and Coal tar Extract

As to the influences of chloral-hydrate and coal tar extract on mitosis, there is information from NÈMEC (1910), KEMP (1910), SAKAMURA (1920), KOMURO (1932), SHIGENAGA (1937) and others, but little is known regarding the influences of these solutions upon the structure of the living nuclei. In the present series of experiments, the nuclear changes caused by these solutions were studied with the living cells of the staminate hairs and the young petals in *Tradescantia reflexa* and the living guard cells of stomata in *Tradescantia virginica,* and with the root tip cells in *Vicia faba* fixed after experiment.

1. Chloral-hydrate

a. Staminate hair cells and young petal epidermal cells in Tradescantia reflexa. The resting or the interphasic nuclei of the staminate hairs and the epidermal cells in young petals were treated with 1.0% and 2.0% solutions of chloral-hydrate. As described in Division I, the resting nuclei in the epidermal cells show a distinct chromonema structure when observed with di-distilled water or a 0.1 M solution of saccharose as medium. The streaming in the cytoplasm is usually more or less distinctly visible. When the medium is replaced with the chloral-hydrate solutions the streaming of the cytoplasm becomes first very active but gradually inactive. The chromonemata in this state are more highly refractive than those in the natural unimpaired state. Usually, several hyaline globules become visible in the nucleus. Meanwhile, the cytoplasmic vacuoles become large, accompanied by the disappearance of the cytoplasmic strands connecting the cell wall and the nucleus across the vacuoles. In this state of the cell, the nuclei are smaller in size and more highly refractive than in the natural state. In dark field illumination, these nuclei are brighter than in the natural state where they are more or less bright. The nuclear and cytoplasmic changes mentioned above are reversible when the medium is replaced with the original one. In a prolonged treatment with the chloral-hydrate solutions, however, the nuclei show an increase in both refractivity and turbidity, and the chromonemata become somewhat indistinct. Often the nuclei are found deformed by the pressure caused by the swelling of vacuoles. The cytoplasm appears to be somewhat turbid, and many refractive granules are visible in the vacuoles. The nuclei in this state are weakly bright or nearly dark in the dark field illumination. These nuclei and the cytoplasm do not show any sign of recovery of their original appearance on the replacement of the medium with water or the isotonic saccharose solution. These results of the experiments show that the changes of the nuclear structure induced by chloral-hydrate closely resemble those caused by hypertonic solutions, those induced by high or low temperatures, and those by weak alkaline solutions in all of which the shrinkage and refractivity increase of the nucleus and the obscureness or indistinctness of the chromonemata are the characteristic features (see Divisions II, III and V). This fact of resemblance

suggests that the effect of chloral-hydrate on the nucleus is mainly dehydration.

b. Guard cells of stomata in Tradescantia virginica. As stated in Division II, the guard cell nuclei are swollen and hyaline, when closed stomata are observed with water or dilute saccharose solutions as medium. When the medium is replaced with a 1.0% or a 2.0% chloral-hydrate solution, the active streaming movement of the cytoplasm becomes gradually inactive, and the cytoplasmic strands which connect the nucleus and the cell wall disappear. This latter fact probably indicates that an enlargement takes place here in the vacuoles. The nuclei shrink and increase in refractivity, and the fine chromonemata become distinctly visible. In the auxiliary cells the chromonemata which are distinct in the water medium show an indistinctness as a result of nuclear shrinkage. During these nuclear and cytoplasmic changes, no plasmolysis takes place either in the guard cells or in the auxiliary cells. All these changes in the guard cells and the auxiliary cells are reversible; the reversal is observed when the stomata are washed several times with di-distilled water. The nuclear change induced by chloral-hydrate solutions by which the homogeneous nuclei become heterogeneous, is identical with what is observed in the experiments with hypertonic solutions, and it is suggested that in the case of the guard cells too the nuclear change caused by chloral-hydrate is mainly a dehydration phenomenon.

c. Root tip cells in Vicia faba. Following the pioneer works of NÈMEC and STRASBURGER, SAKAMURA (1920) has obtained results which indicate that in the root tip cells in *Vicia faba* the mitosis is much disturbed by the action of a 0.75% chloral-hydrate solution resulting in the formation of di-diploid or syndiploid giant nuclei, multi-nucleate cells and cells with various other irregularities. Recently, SHIGENAGA (1937) has traced the processes of the formation of the di-diploid nuclei and the bi-nucleate cells by the chloral-hydrate solution under the microscope with the living leaf epidermal cells in *Tradescantia reflexa.* As mentioned above in Divisions II and III, these abnormalities are also observed in the root tip cells treated with hypertonic solutions or with abnormally high or low temperatures. In the present series of experiments a comparison is attempted between the abnormalities caused by the hypertonic solutions and abnormal temperatures both of which are agencies causing nuclear dehydration, with those induced by chloral-hydrate which is also shown by the preceding experiments *a* and *b* to be a nuclear dehydration agency.

The root tips of *Vicia faba* grown in sawdust[1] were treated for one hour with a 1.0% chloral-hydrate solution which was kept at 20°C in a water thermostat, the treatment being followed by a one hour rinsing with running water. The root tips were fixed immediately after rinsing with running water, or after 1 hour, 3 hours, 23 hours, 47 hours and 71 hours, and placed again in the sawdust after the rinsing. One part of the root tips

1) Sawdust was washed several times with boiling water before it was used.

was fixed with the BONN modification of FLEMMING's solution and stained with HEIDENHAIN's haematoxylin, and the remaining part was fixed with a mixture of corrosive sublimate and acetic acid, and stained with FEULGEN's nucleal-staining method.

i. Fixed immediately after rinsing in running water. The general appearance of the nuclei bears a striking resemblance to that of the nuclei exposed to high temperatures. Most of the resting nuclei appear to be coarsely reticulated, and hyaline areas or nucleolar halos are observed surrounding the nuclei. It is often observed that the nuclei are shrunken and are deeply stained as is frequently the case with the nuclei in the root tip cells treated with hypertonic solutions or high temperatures. Sometimes, the chromatic substance of the nucleus contracts into a mass and a large space is left empty between the mass and the nuclear membrane (Fig. 102). In the cytoplasm an enlargement of the vacuoles is often observed, but not so strong as in the case of the root tip cells treated with high temperatures. In some of the root tips, the chromosomes in the prophase, metaphase and anaphase are found more or less coalescent and in extreme cases they appear as a compact mass. Chromosome bridges, lagging chromosomes, a spindle with its axis bent, di-diploid nuclei, bi-nucleate cells and other various irregularities are frequently found. In this material the normal mitotic figures are only rarely observed.

ii. Fixed after 1 hour and 3 hours in sawdust. The appearance of the nuclei is generally the same as in the case mentioned above, but the chromosome coalescence is much stronger.

iii. Fixed after 23 hours in sawdust. Many mitotic figures appear to be quite normal, but various abnormal figures such as chromosome pycnosis, irregular chromosome arrangement, chromosome lagging, and bi-nucleate cells are also observed. In Fig. 103, a bi-nucleate cell in prophase is reproduced. A giant di-diploid nucleus is shown in Fig. 104. In the periblem, many necrotic nuclei are found (Fig. 105).

iv. Fixed after 47 hours and 71 hours in sawdust. Most of the resting and the dividing nuclei appear to be quite normal, the characteristic features being only the presence of di-diploid nuclei and multinucleate cells. In the case of the multinucleate cell, incomplete cell wall is often perceptible. Sometimes two mitotic figures are visible in a single cell (Fig. 106).

As described above, the structure of the resting nucleus and the behaviour of chromosomes in mitosis is made abnormal by one hour treatment with a 1.0% chloral-hydrate solution. Abnormal structure and behaviour are both found recovered after 23 hours. The normal-mitosis coefficient is 1.3 in the root tips fixed after one hour's rinsing, and increases gradually. In the material fixed after 71 hour's rinsing it is 72.2.

The abnormalities caused by chloral-hydrate closely resemble those observed in the root tip cells treated with high temperatures in that such irregularities as irregular chromosome distribution, chromosome pycnosis and the formation of di-diploid giant nuclei and multinucleate cells are found.

These irregularities are also found in the root tips treated with hypertonic solutions except the irregular chromosome distribution in metaphase and anaphase.

2. *Coal tar extract*

It is known that in animals an artificial tumor is produced by the influence of coal tar. In his investigation with root tips, KOMURO (1932) has reported that the "Phytoteertumor" is formed as the effect of coal tar, and in these root tips he has found various abnormal figures of which chromosome pycnosis, bi-nucleate cells etc. were also observed in the experiments we carried out with hypertonic solutions, abnormal temperatures and other agencies as described in the preceding pages. We concluded that in these cases the abnormalities are mainly due to dehydration taking place in certain morphological units of the protoplast which play important rôles for the normal progress of mitosis. As discussed above this dehydration may be direct or indirect, and in the former case plasmolysis takes place, and in the latter vacuole enlargement is a characteristic feature. The object of present investigation was to learn whether or not the abnormalities caused by coal tar are also accompanied by the presence of plasmolysis or vacuole enlargement, that is, to see whether or not they are the result of dehydration which we may designate as direct or indirect.

a. Young petals of Tradescantia reflexa. From the protocols an example is given below.

10.00 (a. m.). In a 1/8 M saccharose solution, the appearance of the nuclei were quite normal and the streaming of the cytoplasm was very active (Fig. 107).

10.40. Do. The medium was replaced with coal tar extract (stock solution).[1)]

10.43. The cytoplasmic vacuoles were strongly enlarged accompanied by the disappearance of the cytoplasmic strands across the vacuoles connecting the cell wall and the nucleus. The cytoplasmic streaming was less active than in the saccharose solution. The chromonemata were thicker and more distinctly visible than those in the normal nuclei (Fig. 108).

11.00. The nuclei were highly refractive, and the chromonemata were somewhat indistinct. The cytoplasmic streaming was very inactive. No plasmolysis was observed.

11.05. The medium was replaced with a 1/8 M saccharose solution.

11.10. The cytoplasmic streaming was very active again.

11.20. The refractivity of the nucleus lowered and fine chromonemata was observed. The cytoplasmic streaming was active and the connective cytoplasmic strands reappeared. The cells appeared nearly normal.

In this material, the nucleoli are not clearly visible and did not come to sight throughout the whole course of the experiment, though often their existence was recognizable. By a prolonged treatment with the coal tar extract, the nucleus becomes very highly refractive and the chromonemata

1) The stock coal tar extract was prepared by mixing equal volume of coal tar and di-distilled water and then filtering the mixture after it was kept at ca. 40°C for 24 hours.

are very indistinct. In most cases, the nucleus in this state shows no longer any recovery on the replacement of its medium with sugar solution or di-distilled water.

b. Root tip cells of Vicia faba. The root tips were treated with coal tar extract[1] for 30 minutes at 18°C, and then washed with running water for 1 hour. They were then kept in sawdust for variable lengths of time before fixation as in the case of the experiments with chloral-hydrate.

i. Those fixed immediately after rinsing with running water and those fixed after 1 hour and 3 hours in sawdust. The mitotic irregularities observed in these materials closely resemble those caused by the chloral-hydrate solutions, but they are not of such strong intensity as in the latter. The nuclear and chromosome pycnosis, the irregular chromosome distribution, and the contraction and the vacuolization of a certain intensity of the cytoplasm are frequently observed.

ii. Those fixed after 6 hours, 23 hours, 47 hours and 71 hours in sawdust. In these materials, the pycnotic nuclei are deeply and homogeneously stained as in the case of chloral-hydrate. Bi-nucleate cells (Fig. 110) and di-diploid giant nuclei (Fig. 109) are also found. Figs. 109 and 110 show these abnormalities from the root tips placed in sawdust for 23 hours after the treatment and rinsing.

3. *Conclusion*

Under the influence of the chloral-hydrate and coal tar solutions, the following three changes were observed in the living resting nuclei in *Tradescantia reflexa*:—1) decrease in volume, 2) increase in refractivity, and 3) decrease in sharpness of the outlines of the chromonemata. There are characteristic changes which are observed under the action of hypertonic solutions. They indicate nuclear dehydration, as already discussed above. It is, therefore, highly probable that the dehydration takes place also in the nuclei under the influences of the chloral-hydrate and the coal tar extract. In his experiments with the epidermis of a young leaf in *Tradescantia reflexa*, SHIGENAGA (1937) has also come to the conclusion that the mitotic spindle is dehydrated by the action of chloral-hydrate and other narcotics, and of certain alkaloids.

In the root tip cells in *Vicia faba* treated with chloral-hydrate and coal tar extract, chromosome pycnosis, cytoplasmic vacuolization, irregular chromosome distribution, the formation of di-diploid nuclei and bi-nucleate cells, and various other irregularities are observed. The irregularities that we observed have already been reported by NÈMEC (1910), SAKAMURA (1920), KOMURO (1932) and others in their experimental investigations with root tips, but in the present investigation, the "Phytoteertumor" of KOMURO was not found. All these irregularities except the irregular chromosome distribution are observed under the influence of hypertonic solutions (see Division II),

1) Stock solution was used.

and it is concluded that they are the result of dehydration, but the occurrence of the exceptional irregularity or the irregularity in the chromosome distribution indicates that the effects of chloral-hydrate and coal tar extract is not the result of mere dehydration. On this point discussion will be offered at the end of this paper.

VIII. Effects of Lower Organisms by Infection

The abnormal features of the nuclei in galls and mycorrhiza or in tumors in plants and animals have been investigated cytologically by SAKAMURA (1920), WINGE (1927, 1932), BURGEFF (1932), KOSTOFF and KENDALL (1929, 1933) and others, and the results obtained by these authors are summarized by TISCHLER in his "*Allgemeine Pflanzen Karyologie*" (1934). These investigations are all carried out exclusively with the ordinary staining methods in histology. In the present investigation, FEULGEN's nucleal-staining method was also used parallel with the staining method with HEIDENHAIN's haematoxylin, and not only a natural mycorrhiza, but also galls experimentally produced were studied.

1. Mycorrhiza of Spiranthes australis

Most of the roots collected were fixed with the BONN modification of FLEMMING's solution and stained with HEIDENHAIN's haematoxylin, the remainder being fixed with a mixture of corrosive sublimate and acetic acid followed by the staining with FEULGEN's method. In these roots, the resting nuclei which appear normal are small in size and carry several chromocenters which are stained deeply by the two methods named above. In Fig. 111, the nucleus carries several chromocenters in it. Abnormal nuclei are found not only in the infected cells, but also in the neighbouring cells. These cells show a remarkable hypertrophy and the nuclei a marked increase in volume. Frequently, these giant nuclei are hyper- or hypochromatic. As shown in Figs. 112, 113 and 114, these giant nuclei show a wide variation in structure. In Fig. 112, the chromonemata are thicker in diameter than in the normal nuclei, while in Fig. 114, many small granules which are positive to FEULGEN's nucleal-staining are found suspended in the karyolymph. In the latter case, the nucleus appears to be granular in structure under a low magnification, but a high magnification reveals that the granules are connected with one another by very fine filaments faintly stained. The nucleoli are far larger in these giant nuclei than those in the normal nuclei. In some cells, the large nuclei are found nearly divided into two parts, a thin portion bridging over between the parts. In Fig. 113 one such nucleus is shown. In this figure the thin portion is out of focus.

2. Bacterial galls experimentally produced on the stem of Ricinus, Solanum and Helianthus

The galls were experimentally produced by injecting an emulsion of *Bacterium tumefaciens* into the stem of *Ricinus communis, Solanum lycoper-*

sicum and *Helianthus annus*. The galls thus formed were fixed 40 days after the injection, in the same way as in the case of mycorrhiza. In these galls the parenchymatous cells exhibit a remarkable hypertrophy and the nuclei are usually far larger than those in the normal cells. In some cells, the large nuclei are only faintly stained by either haematoxylin or FEULGEN's method (Fig. 115). Bi-nucleate cells are also found frequently, in which the nuclei present the normal appearance and show the normal stainability (Fig. 116). In the gall in *Helianthus*, the number of chromosomes was counted and found to be about 30 in mitotic figures which are often observed. This number corresponds to the diploid number of this plant (cf. TISCHLER, 1922–23).

3. Conclusion

The hypo- and hyperchromaticity and the marked enlargement of the nucleus and the deformations in nuclear structure are the principal abnormalities observed in the mycorrhiza and gall tissue, bi-nucleate cells also being observed frequently in the bacterial gall. According to KOSTOFF and KENDALL (1933), the doubling of chromosomes is also observed in gall tissues. Similar abnormalities are found in the case of root tips treated with hypertonic solutions, abnormal temperatures, chloral-hydrate and coal tar extract, in all of which the abnormalities are, as discussed already in each case, regarded as mainly due to the direct or indirect dehydration in the protoplast, especially the dehydration in the nucleus. It seems, thus, not improbable that in the mycorrhiza and gall tissues too, the disturbance in the normal water distribution in the protoplast bears an important relation to the occurrence of these abnormalities, though we have, at present, no further evidence to support this view.

IX. Discussions

In this discussion, the following three problems are considered on the basis of the results obtained in the present investigation.

1. Structure of the living nucleus

As seen from the papers by LEWIS and LEWIS (1924), MARTENS (1928), SCHAEDE (1929), NÈMEC (1929), SEIFRIZ (1930), GRAY (1931), STRUGGER (1930), KUWADA and NAKAMURA (1934) and others, different investigators have obtained different results on the structure of the living nucleus in interphase or resting stage. This divergency in the results among these authors must in part be due to differences in the kind of 1) species or 2) tissues observed, or 3) those in the physiological function of the cell as for instance that which we find between the guard cells of stomata closed and open. A fourth factor become evident, further, when we come to notice the fact that different results have been obtained by different investigators in the same tissue cells of the same or allied species. While GROSS (1916), for example, has reported many chromatin lumps found in the living nuclei in the epithelial cells of *Salamandra* and *Triton* larva, ZEIGER (1935) has observed no such structure

at all in these nuclei of the same or allied animals. It seems here highly probable that the material employed by GROSS was in an unhealthy condition, since it is reported by ZEIGER that such a granular structure is often visible in nuclei mechanically agitated. The changes due to a certain mechanical agitation during manipulation seem to have led GROSS to incorrect perception. In plants there are cases where such a mechanical agitation is inevitable during preparation. The nuclear change occurring in the pollen mother cells taken out of the anthers is an instance of this. The method of sectioning root tips used by LUNDEGÅRDH (1912), SCHAEDE (1925, 1928) and others can, thus, not be regarded as satisfactory. KARLING (1926) points out: "It is questionable whether any living root tip can be sectioned thin enough for study under the high power of the microscope" (comp. also BÜNNING, 1926 and BĚLAŘ, 1930). Finally it must be mentioned, as the fifth factor, that, as remarked by KUWADA and SAKAMURA (1927), YAMAHA and ISHII (1932), SHIMAKURA (1934), ZEIGER (1935) and others, the tonicity of the mounting medium as well as the concentration of the ions contained in the medium play a conspicuous rôle in changing the apparent structure of the nucleus. While the first three factors mentioned are natural factors, the fourth and fifth are artificial.

In both cases, where the change is due to natural and where to artificial factors, there are two types in the nucleus in respect to its general appearance, and the existence of these types in the former case demands discussion. In one type it is heterogeneous in appearance and in the other it appears to be homogeneous. As to the structure of the microscopically homogeneous nuclei, LEWIS and LEWIS (1924), STRUGGER (1930), GRAY (1930) and others are inclined to the view that in these nuclei the nuclear substance is a sol in which the karyotin and the karyolymph are the dispersed phase and the dispersing medium respectively. This view stands on the basis of the low viscosity of the karyoplasm indicated by the fact of the Brownian movement of granules (GAIDUKOV, 1910, GROSS, 1916, LEPESCHKIN, 1925, STRUGGER, 1930), or the displacement of the nucleolus (GRAY, 1930, LINSBAUER, 1932) observed in these nuclei, as well as by the results obtained from the micrurugical studies of these nuclei (CHAMBERS, 1924). The lowering of viscosity as indicated by these phenomena may, however, be of a local nature and may not necessarily indicate the fluid state of the whole nucleus. For instance, in the salivary gland nucleus of *Chironomus* it becomes hyaline in a hypotonic solution as medium, and active Brownian movements of granules are visible in it (see Division II), but when the hypotonic solution is replaced with an isotonic solution, the banded structure of the chromosomes which disappeared in the first medium reappears, it being traceable in thick bands as they reappear in their original positions on the chromosomes. If the whole nucleus is homogeneously a sol of the same state in the hypotonic solution, it would not be expected that the bands would reappear in the original positions.

In his hydration-dehydration experiments on living plant nuclei, STROH-

MEYER (1935) has, on the other hand, arrived at the conclusion that the apparent homogeneity of the nucleus is only a seeming one, and that the individuality of the chromonemata must be retained even in the homogeneous nuclei. This conclusion finds further support in the results of the experiments we obtained (Division II) which are of both confirmation and extention of STROHMEYER's results. Briefly to repeat some important points:— A heterogeneous nucleus is rendered homogeneous by hydration with a hypotonic solution[1] (the homogeneous nucleus II), or dehydration with a hypertonic solution (the homogeneous nucleus, III). These changes are completely reversible. In stomata, they are observed in the natural state; the homogeneous nuclei in the guard cells become heterogeneous when the stomata open or when they close, according to whether the homogeneity shown in the original state of the nuclei is due to the stomatal closure (swelling) or opening (shrinkage), and vice versa. This fact of experimental as well as physiological reversibility of the nuclear heterogeneity or homogeneity due to the mere change in water-relation and the fact which we observed of maintenance of the original positions of the chromatic bands of the salivary chromosomes when they reappear on the change in tonicity of the medium, must show that there is no fundamental difference in structure between the heterogeneous and the homogeneous nuclei. The difference in type of these nuclei depends only on the difference in the grade of hydration or dehydration of the nuclear components. If the one component is less hydrated and more highly refractive than the other, the nucleus will be heterogeneous, and if both are hydrated or dehydrated to such an extent as to have nearly equal refractive indices, the nucleus will be homogeneous in appearance. In any case, however, the nucleus is in reality heterogeneous.

On the structure of the microscopically heterogeneous nucleus, there are again different opinions. SCHAEDE (1929, 1930) has expressed the view that in the nuclei of the staminate hairs of *Tradescantia virginica*, the nuclear substance is an emulsion consisting of gelatinous karyotin and fluid karyolymph. While similar views have been held by STRUGGER (1930), SEIFRIZ (1930), LEPESCHKIN (1924), KARLING (1926) and others, SCHIWAGO (1926), BĚLAŘ (1929), TELEŻYŃSKI (1930), KUWADA and NAKAMURA (1934) have come to the view that in the staminate hair cells of *Tradescantia* and in certain animal cells, the nuclei are of a heterogeneous structure consisting in the main of irregularly convoluted chromonemata and the karyolymph. Comparing the photomicrographs taken by these and the other investigators, KUWADA (1936, 1937 b) has pointed out that there is no essential difference in appearance among the nuclei reproduced in their respective figures (comp., for example, their respective figures, 1, 1, 17 of SCHEADE, 1930, SCHIWAGO, 1926 and SEIFRIZ, 1930). The different views expressed by these and the other authors are in the main due to the difference in interpretation of the same images which they observed. As emphasized by ZEIGER (1935) and KUWADA (1936), the true

1) In plant cells this nuclear hydration is mostly prevented by the cell wall pressure, except in the cases of stomata and young staminate hairs.

grasp of the structure of the interphasic or the resting nuclei is not to be attained by direct observation of these nuclei themselves, without consulting the process taking place in the transformation of the telophasic chromosome group to the interphasic nucleus. Thus as one of several examples, it is plausible to assume that the nucleus is of the chromonema structure, since it has been increasingly confirmed by recent investigators that the chromosomes themselves, from which the nucleus is formed, are of chromonema structure, and that the regular spirals of the chromonema threads in the metaphase and anaphase are loosened and irregular in later stages and assume irregularly convoluted forms in the interphase.

In his experimental study of the nucleus with KNO_3, STRUGGER (1929, 1930) has observed that numerous minute chromatin granules come to sight or disappear in the nucleus according to the concentration of KNO_3 used as medium. The changes are reversible. On the basis of the result of this investigation he argues that the resting nucleus is a dispersed system of karyotin and karyolymph. The changes are, however, observed in unhealthy cells which have lost their semipermeability as he himself admits. In the case of unimpaired cells, plasmolysis takes place and the nuclei show no such change, but a shrinkage, and they appear homogeneous (cf. Division II). In short, the granulation observed by STRUGGER is a direct effect of ions on the nuclear colloid. It would be dangerous to draw a definite conclusion on the nuclear structure from results obtained from experiments with unhealthy cells.

While the granular structure of STRUGGER is regarded as an artifact, there is another granular structure which must represent the natural structure. We often observed, that in the nuclei in the living guard cells of half-open stomata, which appear to be filled in with minute granules, an adequate dehydration may result in the transformation of this granular structure into a thready or the chromonema structure. Such a structural transformation as seen in the guard cell nuclei was also observed in the salivary chromosomes of *Chironomus* larvae (see Division II). In these chromosomes, the chromatin bands which are clearly visible in the isotonic solution develop a pearl-necklace appearance in a hypotonic solution, and are transformed back to the original linear structure in the isotonic solution. These facts observed in the guard cell nuclei and the salivary chromosomes may be taken as suggesting that the granular structure observed in a certain condition of these nuclei and chromosomes is an outward expression of the discontinuous structure of the chromonemata. The chromonemata would probably consist of discontinuous chromatic segments and a continuous ground substance in which the former are imbedded. If in hydrated nuclei the ground substance is more highly hydrated and less refractive than the chromatic segments or granules, the convoluted chromonemata would appear to be an irregular group of fine granules. Only in those less hydrated, they should appear as continuous threads. The granular structure of the nuclei may thus be regarded as a modification of the thready chromonema structure.

The large chromatin lumps or chromocenters which have also been observed in the living condition of the nucleus, by NASSONOV (1932), ZEIGER (1935) and others in epithelial cells of *Triton* and *Rana*, are structures quite different in the order of constitution from the granules discussed above. As reported in the previous paper (SINKE, 1937), these chromatin lumps are transformed into a loose mass of convoluted chromonemata of the ordinary thready form by the treatment of the nucleus with a NH_4Cl solution before staining with acetocarmine. They must, therefore, represent a convoluted chromonema or chromonemata which in succession represent a group or groups of the granules discussed above. While in the case of the latter granules, they are conspicuous because of the fact that the ground substance in which they are imbedded is highly hydrated, in the case of the former the conspicuousness of the structure is due to the fact that not only this ground substance but also that in which the chromonemata themselves are imbedded are in a condensed state,—the heteropycnosis (cf. KUWADA, 1937, a).

Now we may conclude as follows: The heterogeneous nuclei are composed of two components, the convoluted chromonemata of a jelly consistency and the karyolymph which is a much less viscous colloid. The former component is more highly refractive than the latter, so that the whole nucleus presents a heterogeneous appearance. The chromonemata may appear as a linear series of granules, and in this case the nucleus appears to be of granular structure. In certain chromonemata or certain parts of them, they remain compact as in the dividing stages, and in such cases they are called the chromocenters, net-knots or karyosomes. The homogeneous nuclei are differentiated from the heterogeneous nuclei only by the refractive indices of the two components being equalized by hydration or by dehydration. While this comprehension of the heterogeneous and the homogeneous nuclei is in harmony with the theory of chromosome individuality, the view of regarding them as the structure of a homogeneous sol is not, as pointed out by FUJII (1931), KUWADA (1936) and others, in full harmony with the theory which forms the physical basis of the modern theory of heredity. It must be mentioned here that there are numerous intermediate types between the heterogeneous and homogeneous nuclei according to the grades of hydration or dehydration of the nuclear components.

According to the results we obtained three types of the homogeneous nucleus are distinguished: 1) Homogeneous nucleus I (a hydrated nucleus). In this type, the nucleus is swollen and less refractive. It shrinks and becomes homogeneous when treated with hypertonic solutions without showing any heterogeneity during the course of dehydration. Nuclei of this type are often found in the leaf epidermis of *Elodea densa*. 2) Homogeneous nucleus II (another hydrated nucleus). In this type too, the nucleus is swollen and less refractive, but a more or less distinct heterogeneity is shown during the course of the nuclear dehydration. The nuclei in the guard cells of the closed stomata of *Tradescantia*, *Vicia* and some other plants, and those in the epidermal cells of the leaf of *Elodea densa* belong to this type. 3)

Homogeneous nucleus III (a dehydrated nucleus). In this type, the nucleus assumes a shrunken form and is highly refractive. In a hypotonic medium, it is hydrated and becomes homogeneous through a stage where it appears heterogeneous. The guard cell nuclei of fully open stomata of *Tradescantia, Vicia, Zebrina* and some other plants belong to this type.

The heterogeneous nuclei are of two types: 1) The heterogeneous nucleus I. In this type, the nucleus retains its heterogeneity when heated above 60°C. To this type belong the nuclei in the leaf epidermal cells of *Tradescantia reflexa, T. virginica, Vicia faba* and some other plants. 2) The heterogeneous nucleus II. In this type the nucleus becomes homogeneous when heated above 60°C. The nuclei belonging to this type are found in the leaf epidermal cells of *Sagittaria, Caldesia, Alisma, Elodea,* etc.

While the distinction between the homogeneous and heterogeneous nuclei and among the three types of the homogeneous nuclei is a matter of the degree of hydration, the distinction between the heterogeneous nuclei I and II seems to be the difference in the relative amount of the chemical components of the chromonemata (see Division III).

The interphasic nucleus and the resting or the metabolic nucleus are hardly distinguishable from each other by the external appearance, but certain differences are recognizable between these kinds of nucleus in the behaviour towards hypertonic solutions, heat or mechanical injuries. While in the basal part of the petal epidermis of *Tradescantia reflexa,* the upper critical concentration of the interphasic nuclei is 0.7 M, that of the resting nuclei in the upper part of the same petal epidermis is 0.5 M (see Division II), and while these heterogeneous resting nuclei become completely homogeneous in the medium of higher temperatures than 60°C (the heterogeneous nucleus II), the heterogeneous interphasic nuclei show only a slight heterogeneity in the same temperatures. By mechanical injuries, while the resting nuclei are liable to swell and to form hemispherical extrusions on the surface of the nuclear membrane, such are only rarely observable in the case of the interphasic nuclei. The causes inducing these differences between the resting and the interphasic nuclei are not clear, but it may be inferred that the difference in critical concentration between these nuclei is probably due to the difference in osmotic condition of the cells containing these nuclei, and that the difference in the behaviour toward heating rests on a certain causal relation in the difference in relative amount of the chemical components of these nuclei (cf. Division III).

2. *Artificial and physiological reversible nuclear changes*

In the present investigation it was observed that reversible nuclear changes are caused by several artificial means and that the nuclei in unimpaired cells behave differently toward these means from those in unhealthy cells. With hypertonic solutions of KNO_3, for instance, the unimpaired cells become more or less strongly dehydrated as indicated by the plasmolysis taking place in the cell and by a shrinkage and an increase in refractivity

occurring in the nucleus. Thus, heterogeneous nuclei become homogeneous (homogeneous nuclei III), and swollen homogeneous nuclei (homogeneous nuclei II) become heterogeneous finally (homogeneous nuclei III). If, on the other hand, the cells are unhealthy and have lost their semi-permeability, the characteristic changes occurring are quite different from those above, indicating that the changes are direct effects of the ions of the salts on the nuclei penetrating into the cells. In this case the heterogeneous nuclei become swollen and homogeneous (see Division VI).

With hypertonic solutions of saccharose and KCNS, the results obtained with unimpaired cells differ in no important points from those with the hypertonic KNO_3 solutions. However, if the cell is unhealthy, the results are not the same in these three cases. In the case of saccharose, the nucleus is hydrated and homogeneous in any concentration practically in use; in the case of KNO_3, it is heterogeneous in a certain range of concentrations, and both above and below this range it is hydrated and homogeneous as in the case of saccharose of corresponding concentrations; and in that of KCNS, there is another range of concentrations where the nucleus is again heterogeneous presenting a granular appearance (see Division VI). In short, while the effect on the nuclei of unhealthy cells or the direct effect on the nuclei is specific to the plasmolyticum used, the indirect effect or the effect on the nuclei of healthy cells which are capable of being plasmolyzed is in all cases simply dehydration.

In the above cases of indirect effect, nuclear dehydration always accompanies the plasmolysis, and this case of nuclear dehydration may be regarded as direct dehydration. Another case of the indirect effect on nuclear dehydration which may be called indirect dehydration is found in the case where healthy cells are treated with NH_4OH solutions. In this case the result on the nucleus is the same as in the case of hypertonic solutions used, swollen homogeneous nuclei (homogeneous nuclei II) becoming heterogeneous, but here no plasmolysis is induced owing to the high penetrability of the ions of NH_4OH, and instead of plasmolysis a remarkable enlargement of the cytoplasmic vacuoles takes place. It is highly probable that the vacuoles swell and enlarge because of the fact that they are made alkaline in reaction, and withdraw water from the surrounding medium, thus resting in the dehydration of the nucleus. The NH_4OH solutions as dehydrating means act, therefore, on the nucleus not directly but indirectly (see SCARTH, 1927). This action of NH_4OH on the nucleus of healthy cells differs markedly from that of acetic acid. In the latter case no enlargement of the cytoplasmic vacuoles is observed; the acid acts directly on the nucleus and causes the coagulation or the gelatinization of the nucleus. This difference between NH_4OH and acetic acid seems to suggest that the penetrability through the nuclear membrane is different in the two cases.

In all the other cases of artificial agents we studied, that is, in those of abnormal temperatures, mechanical injuries, chloral-hydrate and a coal tar extract, nuclear changes similar to those induced by the hypertonic solutions

and NH_4OH solutions are also observed, if the cells are healthy, and in these cases the changes are always accompanied with the enlargement of the cytoplasmic vacuoles as in the case of NH_4OH. This associated occurrence of the dehydration change of the nucleus and the vacuolar enlargement strongly suggests that there is a causal relation between these two phenomena, but no suggestion as to the mechanism of this vacuolar enlargement can be made at present in any clear form.

The hydration of the nucleus also occurs when the cells are exposed to high temperatures, or mechanically injured as described in Division III and IV respectively. It is worth noting here that in the case of temperature it is clear that the dehydration mentioned above is preceded by this hydration.

There are some nuclear changes which seem to have no intimate relation to hydration or dehydration of the nucleus. These are the formation of a gross and rough frame-work of irregular structure and the nuclear vacuolization, and they are found in cells which are treated with high temperatures, narcotics and mechanical injuries. The mechanisms of these changes are not clear at present, but it seems probable that the changes are due to the "Entmischung" of the karyoplasm.

The above discussions concern the reversible nuclear changes caused by artificial means. In the case of stomata the same nuclear changes as those which are regarded as the indirect effects of artificial means take place in the natural state of the cell too, being accompanied by functional changes of the guard cells. They are the changes which we observed in the guard cell nuclei of stomata in *Tradescantia, Zebrina, Vicia* and some other plants, and the changes take place according to the stomatal movements. With regard to the mechanism of these changes, WEBER (1926) has suggested that the changes are due to the fluctuation of the H-ion concentrations in the guard cells, but without making any statement as to whether they are due to an increase or a decrease of the H-ion concentration. According to SAYRE (1926), SCARTH (1926, 1927) and PEKAREK (1933), the H-ion concentration in the guard cell vacuoles is higher when the stomata are closed than when open. This statement leads us to the conclusion that the nucleus is in a more shrunken state when the stoma is open than when it is closed, because we know from the result of experiment with NH_4OH that in unimpaired cells the nuclei become dehydrated when the cell vacuoles are made alkaline. This conclusion is in accord with the observed facts and thus it becomes probable that the shrunken and highly refractive state of the nuclei in the open stomata is due to the decrease in H-ion concentration in the cell vacuoles. In *Tradescantia*, however, we have observed that while the guard cells are colored yellowish as the effect of alkaline solutions of the concentration at which the nuclear changes are caused, no such coloration is observed in any natural state of stomata open or closed. Therefore, we must conclude that in the open stomata the lowering of the H-ion concentration does not take place so strongly as to cause the disappearance of the chromonemata in the nucleus. The results we obtained in the hydration-dehydration ex-

periments with stomata by hypertonic and hypotonic solutions (Division II) and the fact of the osmotic pressure of the guard cells being far higher in open stomata than in closed stomata (ILJIN, 1915, WIGGANS, 1921), on the other hand, strongly indicate that the nuclear changes in the guard cells occurring parallel with the stomatal movement are due to the hydration and the dehydration of the nuclei caused by catatonosis and anatonosis as discussed more fully in Section 4 of Division II. It is, however, here noted that the lowering of H-ion concentration in the guard cells in open stomata is significant in the sense that this lowering may be favourable to initiating the starch-sugar transformation in the guard cells (cf. SCARTH, 1926).

In view of the fact that the reversible nuclear changes as indirect effects of artificial means are the same in result as those taking place in the living guard cells, we may say that these artificial nuclear changes are reversible in both biological and colloid-chemical senses and those as the direct effects of ions which are observed in the case where the cells are unhealthy are reversible simply in the sense of colloid-chemistry.

From the above discussion, it is seen that the mechanism of the nuclear changes occurring in unimpaired cells is not of a similar kind in all cases, but may be different in different cases. We may imagine the mechanism of the nuclear changes in the guard cells of stomata taking place in association with stomatal movement as suggested above, but as to the question by what reason the nuclei are hydrated and appear to be homogeneous in certain tissues or plants and heterogeneous in others nothing can be stated at present.

3. *Hydration and dehydration as the causes of abnormal mitoses and their significance for the origin of polyploidy*

According to the results we obtained, the mitotic abnormalities caused by various artificial means are roughly classified into two groups: In the one group, the abnormalities are those which are obtainable by the mere treatment with the hypertonic solutions of saccharose as a plasmolyticum, and their occurrence may be regarded as connected directly with dehydration taking place in certain regions of the cell where the dehydration gives rise to the abnormalities, or an abnormal water distribution in the protoplast. To this group belong the following abnormalities;—chromosome bridges, chromosome coalescence, pseudo-amitosis, the di-diploid nucleus, failure of the telophasic reconstruction, etc. These abnormalities are also induced by the treatment with a chloral-hydrate solution, a coal tar extract, an abnormal temperature, as might be expected from the fact that these agents cause the dehydration of the nucleus and the cytoplasm. These abnormal figures may be called dehydration figures.

It must be stated here that the di-diploid giant nuclei are not always caused by dehydration. When the dilute solutions of colchicine are added, for instance, to the medium with which the dividing cells in the young petal epidermis of *Tradescantia reflexa* are mounted, di-diploid nuclei are formed

without the replacement of the medium with water, and neither the severe shrinkage of the dividing nuclei nor the marked enlargement of the cytoplasmic vacuoles is observed (cf. NEBEL and RUTTLE, 1938).

To the other group of abnormalities belong the chromosome scattering or the irregular chromosome distribution in anaphase and the formation of micro-nuclei as the direct consequence of the irregular distribution. They are observed in the root tip cells subjected to abnormal high temperatures or those treated with chloral-hydrate or a coal tar extract, but never, so far as the present investigation is concerned, in those treated with hypertonic saccharose solutions. According to LEWIS (1934), the irregular distribution of the anaphasic chromosomes is observed when the culture medium is replaced with a hypotonic one. In *Elodea*, as was stated in Division III, the nuclear swelling occurs as one of the first visible effects of high temperatures. It may be inferred, therefore, that under the high temperature condition, there may be cases where the chromosomes are scattered irregularly as the result of the spindle substance being hydrated as the first effect of high temperature. The same may be the case with the irregular distribution of the chromosomes induced by the action of chloral-hydrate which causes, as described in Division VII and stated by HEILBRUNN (1928) in his monograph, first an increase in velocity of the cytoplasmic streaming, followed by a decrease after longer treatment. In the case of high temperature the hydration of the nucleus as the first sign of the nuclear change is usually not perceptible except in the case of *Elodea*, but the fact that the capacity of the cell for taking in water is higher in the dividing stage than in the resting stage of the nucleus is very interesting if the chromosome scattering induced by the high temperature is caused by the hydration of the spindle substance (cf. FAURÉ-FREMIET, 1925). The abnormal figures of the second group may be called presumably the hydration figures as opposed to the dehydration figures of the first group, though it is left for a further investigation whether these abnormalities are due merely to hydration or not.

The "Rhexis" or the fragmentation of chromosomes and the "Lysis" of the chromosome both of which are also observed as characteristic of the abnormal mitosis are phenomena that concern the morphology of the chromosomes themselves. The chromosome lysis which was observed by NĚMEC (1910) in the root tip cells treated with 6% KNO_3 solution (cf. POLITZER, 1934) seems to be due to the direct action on chromosomes of the ions of KNO_3 penetrating into the cell (cf. STRUGGER, 1930, YAMAHA and ISHII, 1932), rather than being an effect of plasmolysis as considered by NĚMEC.

The dehydration figures such as the amitosis-like figures, the bi-nucleate figures and the giant di-diploid nuclei are found in certain tissues in normal development (COOPER, 1933). In the tapetum the occurrence of these dehydration figures may perhaps be explained as caused by the pollen mother cells withdrawing water from the surrounding tapetal cells, since it is characteristic of the pollen mother cells that in the early stage of the microsporogenesis they rapidly increase in volume. The multinucleate state

found in the jacket cells in gymnospermous plants (LAWSON, 1904, see also TISCHLER, 1934) and the bi-nucleate state in animal follicle cells (WILSON, 1925) may similarly be regarded as occurring through the dehydration connected with the function of these cells supplying water and nutrient substances to the rapidly growing egg cells (COULTER and CHAMBERLAIN, 1910). It is an interesting fact that some dehydration figures such as the bi-nucleate cell or multi-nucleate cells are often found in glandular cells such as those of the pancreas, the salivary gland, and etc. in animals (BÖHM, 1931), and those in various secretory or excretory organs (MOLISCH, 1918, SCHÜRHOFF, 1918) and the antipodal cells in plants (HUSS, 1906, KUWADA, 1911, see also TISCHLER, 1934). The occurrence in high frequency of the bi-nucleate cells in the liver in some higher animals (MÜNTZER, 1923) may also be explained as due to the osmotic dehydration caused by the blood or body fluids of high osmotic pressure with rich nutrient substances such as glucose, transported from the digesting organ into the liver.

The nature of the di-diploid nucleus as a dehydration figure suggests that the origin of polyploidy is connected with the dehydration phenomenon in the cell, and that polyploidy may be a common phenomenon in those districts where the soil is dry or rich in salts, or the climate is extremely hot or cold. In this connection, it is very interesting that according to SHIMOTOMAI (1933), RHOWEDER (1936), TISCHLER (1937), STRELKOVA (1938) and SOKOLOVSKAYA (1938) many of the plants are polyploid and more strongly resisting than the diploid plants in a sea-side district and in the arctic and high mountain regions.

It is noted also that the occurrence of polyploid shoots in grafting (WINKLER, 1916, JÖRGENSEN, 1926, LINDSTRÖM and KOOS, 1931) may also be regarded as the dehydration phenomenon caused by cutting.

It is a well known fact that while in higher plants polyploidy is a prominent feature, it is less marked in higher animals. The cause of this difference in prominency of polyploidy between animals and plants has been discussed by various investigators (cf. HEILBORN, 1934) and most of the opinions concern the question of the preservation of the polyploid individuals raised. It seems to us, however, that the difference lies also on the question of the possibility as to whether they are raised or not. It seems highly probable that the difference in the ability of osmo-regulation plays here a great rôle. According to SCHLÖSSER (1937) and KISCH (1937), in some higher plants, the osmotic pressure of the press sap of a tissue is different according to different environmental conditions to which the plants have been subjected, while in higher animals, the osmotic value of the blood is kept nearly constant independent of the environmental condition (homoiosmotic).

X. Summary

1. The structure of the nuclei in the unimpaired state of various plants and animals was studied with bright and dark field illuminations. These

nuclei show wide variations in visible structure according to the species or tissues used, and the physiological conditions in the cells.

2. The guard cell nuclei of stomata of some plants were found to be an interesting example of the influence of physiological conditions. In these plants the nuclei are swollen and homogeneous in the closed state of stomata, and are shrunken in the fully open state showing no distinct structure, and in the half-open state, they present a distinct chromonema structure.

3. It was experimentally demonstrated that the variations mentioned in 1 and 2 in apparent structure of the nucleus are chiefly due to the grade of hydration or dehydration of the two nuclear components, the chromonemata and the karyolymph.

4. By hydration-dehydration experiments with hypotonic and hypertonic solutions it was possible to classify unimpaired nuclei into four types as follows:—

i. The homogeneous nucleus I. In this type, the chromonemata are strongly hydrated with a refractivity approaching that of the karyolymph. When artificially dehydrated, the nucleus of this type is transformed to the shrunken condition in which it is homogeneous but highly refractive, without presenting any distinct visible structure during the dehydration.

ii. The homogeneous nucleus II. In this type, the nucleus presents the same appearance as that of the homogeneous nucleus I, but differs from the latter in the point that it for a time shows a heterogeneity in the course of the artificial dehydration.

iii. The homogeneous nucleus III. In this type, the nucleus is shrunken and appears homogeneous, with the karyolymph strongly dehydrated and having a refractivity approaching that of the chromonemata. In both homogeneous nuclei I and II the end result of dehydration is the homogeneous nucleus III.

iv. The heterogeneous nucleus. In this type of nucleus, the chromonemata are more highly refractive than the karyolymph, so that they are visible more or less distinctly. In animal cells, the heterogeneous nucleus is rendered homogeneous both by hydration (homogeneous nucleus II) and dehydration (homogeneous nucleus III) due to the action of hypotonic and hypertonic solutions on the cells, but in plants, except such a special case as the stomatal guard cells, the hydration of these nuclei hypotonic solutions is prevented from taking place by the pressure of the cell wall.

5. These results of the hydration and dehydration experiments show that the apparent homogeneity of the nuclei is only a seeming one, and that, if the individuality of the chromosomes is maintained in the heterogeneous nucleus, it must be maintained also in the homogeneous nuclei mentioned above.

6. By heating experiments two different types are distinguished in the heterogeneous nuclei.

i. The heterogeneous nucleus I. In this type, the nucleus retains its heterogeneity on being heated above 60°C.

ii. The heterogeneous nucleus II. The nucleus becomes homogeneous or the chromonemata become very obscure, when heated above 60°C.

7. It was experimentally demonstrated that, in the case of the chromosomes in metaphase and anaphase too, the visibility of the chromonemata depends on the magnitude of difference in hydration degree between the chromonemata and the matrical part of the chromosomes.

8. Reversible nuclear changes caused by abnormal temperatures, mechanical injuries, acids, alkalis, neutral salts, chloral-hydrate and a coal tar water extract were studied with living cells observed directly under the microscope. It was ascertained that these agents cause a decrease in volume and an increase in refractivity of the nucleus, changes which suggest that in these nuclei dehydration takes place.

9. The fact that, although an alkali causes a swelling of the nuclei if the cells are unhealthy (direct effect), it causes dehydration or a shrinkage of the nuclei in the case of intact cells (indirect effect), is interpreted as due to the cytoplasmic vacuoles being swollen as an alkaline effect and absorbing water from the surrounding media, the nucleus and the cytoplasm, on the basis of the fact that the vacuoles become markedly enlarged in association with the nuclear shrinkage.

10. The disturbance in the normal behaviour of the chromosomes in mitosis, due to the influences of external agents such as hypertonic solutions, abnormal temperatures, mechanical injuries, chloral-hydrate and a coal tar water extract, was studied with the root tip cells of *Vicia faba*. The important karyokinetic abnormalities observed are the following:—

i. The pycnosis or the coalescence of the chromosomes and the formation of giant di-diploid nuclei and bi-nucleate cells. These abnormalities are observed on the mere treatment of the cells with a hypertonic saccharose solution, and are to be regarded as due chiefly to the dehydration taking place in the spindle area. They are also observed in the root tips mechanically injured or treated with abnormal temperatures, chloral-hydrate or a coal tar water extract. In these cases we are not able to tell how the dehydration is caused by these agents or conditions, but it seems highly probable that the vacuolar enlargement in the cytoplasm taking place in all these cases has an important causal relation to the dehydration.

ii. The irregular chromosome distribution in metaphase and anaphase. This irregularity is not caused by a hypertonic solution, As to the cause, no clear inference is to be drawn at present, but it seems not improbable that the irregularity is closely connected with the hydration phenomenon in the spindle area.

11. The nuclear abnormalities found in mycorrhiza and bacterial galls were studied with FEULGEN's nucleal-staining method. The important abnormalities observed were the occurrence of giant hyper- and hypochromatic nuclei which are negative to FEULGEN's staining, and of multinucleate cells.

12. The origin of the polyploidy was discussed from the viewpoint of the fact that chromosome doubling is brought about by dehydration due to various causes.

LITERATURE CITED

ÅKERMAN, Å. (1927) Studien über den Kältetod und die Kälteresistenz der Pflanzen. Lund.

ALVERDES, F. (1912) Die Kerne in den Speicheldrüsen der *Chironomus*-Larve. Arch. Zellforschg., Bd. 9.

BĚLAŘ, K. (1928) Die Zytologische Grundlagen der Vererbung. Berlin.

—— (1929, a) Beiträge zur Kausalanalyse der Mitose. II. Untersuchungen an den Spermatocyten von *Chorthippus* (*Stenobothrus*) Lineatus Panz. Arch. Entwick. Mech., Bd. 118.

—— (1929, b) Beiträge zur Kausalanalyse der Mitose. III. Untersuchungen in dem Staubfadenhaarezellen und Blattmeristemzellen von *Tradescantia virginica*. Zeitschr. f. Zellforschg. u. mikr. Anat., Bd. 10.

—— (1930) Über die reversible Entmischung des lebenden Protoplasmas. Protoplasma, Bd. 9.

BĚLEHRÁDEK, J. (1935) Temperature and living matter. Berlin.

BLEIER, H. (1930) Experimentell-Cytologische Untersuchungen. I. Einfluss abnormaler Temperatur auf die Reduktionsteilung. Zeitschr. f. Zellforschg. u. mikr. Anat., Bd. 11.

BODINE, J. H. (1921) Factors influencing the water content and the rate of metabolism of certain orthoptera. Jour. Exp. Zool., Vol. 32.

BÖHM, T. (1931) Untersuchungen über zweikernige Zellen. II. Mitteilung. Die Auszahlung und Berechnung der zweikernigen Leberzellen. Zeitschr. f. mikrosk.-anat. Forschg., Bd. 24.

BOKORNY, Th. (1888) Über die Einwirkung basischer Stoffe auf das lebende Protoplasma. Jahrb. wiss. Bot., Bd. 19.

BÜNNING, E. (1926) Untersuchungen über der Koagulation des Protoplasmas bei Wundreizen. Bot. Arch., Bd. 14.

BURGEFF, H. (1932) Saprophytismus und Symbiose. Studien an tropischen Orchideen. Jena.

CHAMBERS, R. (1924) The physical structure of protoplasm as determined by microdissection and injection. COWDRY's General Cytology.

COOPER, D. C. (1933) Nuclear divisions in the tapetal cells of certain angiosperms. Amer. Jour. Bot., Vol. 20.

COULTER, J. M. and C. J. CHAMBERLAIN (1910) Morphology of Gymnosperms. Chicago.

DEGEN, A. (1905) Untersuchungen über die kontraktile Vakuole und die Wabenstruktur des Protoplasmas. Bot. Ztg., Bd. 63.

DOYLE, W. L. and C. W. METZ (1935) Structure of the Chromosomes in the Salivary Gland Cells in *Sciara* (Diptera). Biol. Bull., Vol. 64.

FAURÉ-FREMIET, E. (1925) La Cinétique du Développement. Paris.

FITTING, H. (1917) Untersuchungen über isotonische Koeffizienten und ihren Nutzen für Permeabilitätsbestimmungen. Jahrb. f. wiss. Bot., Bd. 57.

FREUNDLICH, H. (1935) Thixotropy. Paris.

FUJII, K. (1926) The Recent Progress in Cytology, and Methods of Its Investigation. (Japanese). Rep. Japan. Assoc. Adv. Sci., Vol. 2.

—— (1931) Cytology in the Past and Present. (Japanese). Iwanami-Koza. (Iwanami's Monographs in Biology).

GAIDUKOV, N. (1910) Dunkelfeldbeleuchtung und Ultramikroskopie in der Biologie und Medizin. Jena.

GEITLER, L. (1935) Untersuchungen über den Kernbau von *Spirogyra* mittels FEULGEN's Nuclealfärbung. Ber. d. D. Bot. Ges., Bd. 53.

GELLHORN, E. (1931) Lehrbuch der Allgemeine Physiologie. Leipzig.

GEORGEVITCH, P. M. (1910) Über den Einfluss von extremen Temperaturen auf die Zellen der Wurzelspitze von *Galtonia candicans*. Beih. Bot. Centralbl. (I), Bd. 25.

GRAY, J. (1931) Experimental Cytology. Cambridge.

GROSS, R. (1926) Beobachtungen und Versuche an lebenden Zellkernen. Arch. Zellforschg. Bd., 14.

GUILLIERMOND, A. (1932) La structure des cellules végétales à l'ultramicroscope. Protoplasma, Bd. 16.

HANSTEEN-CRANNER, B. (1922) Zur Biochemie und Physiologie der Grenzschichten lebender Pflanzenzellen. Meldinger fra Norges Landbruks., Vol. 2.

HARTMANN, O. (1919) Über den Einfluss der Temperatur auf Plasma, Kern und Nucleolus und Cytologische Gleichgewichtszustände. Arch. Zellforschg., Bd. 15.

HEILBORN, O. (1934) On the Origin and Preservation of Polyploidy. Hereditas, Bd. 19.

HEILBRUNN, L. V. (1928) The colloid chemistry of protoplasm. Berlin.

HEITZ, E. (1933) Die Herkunft der Chromozentren. Planta, Bd. 18.

HEITZ, E. und H. BAUER (1933) Beweise für die Chromosomennatur der Kernschleifen in den Knäuelkernen von *Bibio hortulans* L. Zeitschr. f. Zellforschg. u. mikro. Anat., Bd. 17.

HERWERDEN, VAN, M. A. (1923) Reversible Gelbildung in Epithelzellen der Froschlarve und ihre Anwendung zur Prüfung auf Permeabilitätsunterschiede in der lebenden Zelle. Arch. Exp. Zellforschg., Bd. 1.

—— (1927) Umkehrbare Gelatinierung durch Temperaturerhöhung bei einer Süsswasseramöbe. Protoplasma, Bd. 2.

HÖFLER, K. (1926) Über die Zuckerpermeabilität plasmolysierter Protoplaste. Planta, Bd. 2.

HUSS, H. A. (1906) Beiträge zur Morphologie und Physiologie der Antipoden. Dissertation (Univ. Zürich).

ILJIN, W. S. (1915) Die Regulierung der Spaltöffnungen im Zusammenhang mit der Veränderung des osmotischen Druckes. Beih. Bot. Zentralbl. (I), Bd. 32.

—— (1922) Über den Einfluss des Welkens der Pflanzen auf die Regulierung der Spaltöffnungen. Jahrb. wiss. Bot., Bd. 61.

—— (1934) Über den Kältetod der Pflanzen und seine Ursachen. Protoplasma, Bd. 20.

JÖRGENSEN., C. A. (1928) The experimental Formation of Heteroploid Plants in the Genus *Solanum*. Jour. Genet., Vol. 19.

KAMNEV, J. E. (1934) Der Einfluss von hypo- und hypertonischen Lösungen auf die Struktur und Vitalfärbung der Epithelzellen des Amphibiendarmes (*Rana temporaria, Triton taeniatus*). Protoplasma, Bd. 21.

KARLING, J. S. (1926) Nuclear and cell division in *Nitella* and *Chara*. Bull. Torrey Bot. Club., Vol. 53.

—— (1928) Nuclear and cell division in the antheridial filaments of the Characeae. Ibid., Vol. 55.

KAUFMANN, B. P. (1931) Chromosome structure in *Drosophila*. Amer. Nat., Vol. 65.

KEMP, H. P. (1910) On the Question of the Occurrence of "Heterotypical Reduction" in Somatic Cells. Ann. Bot., Vol. 24.

KEMP, T. and J. JUUL (1930) Influence of various agents (X-rays, Radium, Heat, Ether) upon mitosis in tissue cultures. Acta Pathol. et Microbiol. Scand., Vol. 7.

KISCH, R. (1937) Die Bedeutung der Wasserversorgung für den Ablauf der Meiosis. Jahrb. wiss. Bot. Bd., 85.

KLEMM, P. (1895) Desorganisationserscheinungen der Zelle. Jahrb. wiss. Bot., Bd. 28.

KOKOTT, (1930) Zur Frage des Einfluss erhöhter Temperatur auf die Mitosen in Gewebskulturen. Zeitschr. f. Zellforschg. u. mikr. Anat., Bd. 11.

KOLTHOFF, I. M. (1932) Säure-Basen-Indicatoren. Berlin.
KOMURO, H. (1932) Betrachtungen über die zytologischen Veränderungen in den in Kohlenteerlösung getauchten Wurzelspitzen junger Pflanzen. Cellule, Tome. 41.
KOSTOFF, D. and H. KENDALL (1929) Irregular meiosis in *Lycium halimifolium* MILL. produced by gall mite (*Eriophyes*). Jour. Genet., Vol. 21.
—— and —— (1933) Studies on Plant Tumors and Polyploidy produced by Bacteria and other Agents. Arch. Mikrobiolog., Bd. 4.
KÜSTER, E. (1933) Hundert Jahre *Tradescantia*. Jena.
KUWADA, Y. (1911) A Cytological Study of *Oryza sativa* L. Bot. Mag., Tokyo., Vol. 24.
—— (1921) On the So-called Longitudinal Split of Chromosomes in the Telophase. Ibid., Vol. 35.
—— (1932) The Life-History of Chromosomes. (Japanese). Kagaku, Vol. 2.
—— (1936) On the Structure of the Cell Nucleus. (Japanese) Zeitschr., Jap. mikrobiol. Ges., Bd. 30.
—— (1937, a) The Hydration and Dehydration Phenomena in Mitosis. Cytologia, FUJII Jub. Vol.
—— (1937, b) Chromosome Structure. (Japanese). Tokyo.
KUWADA, Y. and T. SAKAMURA (1927) A contribution to the colloid chemical and morphological study of chromosomes. Protoplasma, Bd. 1.
KUWADA, Y. and T. NAKAMURA (1934) Behaviour of Chromonemata in Mitosis. III. Observation of Living Staminate Hair Cells in *Tradescantia reflexa*. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B., Vol. 9.
LAWSON, A. A. (1904) The Gametophytes, Fertilisation and Embryo of *Cryptomeria japonica*. Ann. Bot., Vol. 18.
LEPESCHKIN, U. W. (1923) The Constancy of the Living Substance. Stud. Plant Physiolog. Lab. Charles Univ., Prague., Vol. 1.
—— (1925) Morphologische Eigentümlichkeiten der roten Blutkörperchen in Lichte der Kolloidchemie. Biolog. General., Bd. 1.
—— (1924) Kolloidchemie des Protoplasmas. Berlin.
—— (1935) Fortschritte der Kolloidchemie des Protoplasmas in den letzten zehn Jahren. Protoplasma, Bd. 24.
LEWIS, M. R. (1934) Reversible solation of the mitotic spindle of living chick embryo cells studied in vitro. Arch. Exp. Zellforschg., Bd. 16.
LEWIS, W. H. and M. R. LEWIS (1924) Behaviour of Cells in Tissue Cultures. COWDRY's General Cytology.
LINDSTÖM, E. W. and K. KOOS (1931) Cytogenetic investigations of a haploid tomato and its diploid and tetraploid progeny. Amer. Jour. Bot., Vol. 18.
LINSBAUER, K. (1932) Kerne, Nucleolen und Plasmabewegungen in den Blasenzellen von *Mesembryanthemum cristallium*. Sitz. Ber. Math. Naturwiss. Kl. Abt. 1, 141.
—— (1930) Die Epidermis. Handb. Pflanzenanat. 4. Berlin.
LOOS, W. (1932) Zur Kenntnis Wundreaktion des pflanzlichen Zellkerns. Protoplasma, Bd. 14.
LUNDEGÅRDH, H. (1912) Die Kernteilung bei höheren Organismen nach Untersuchungen an lebendem Material. Jahrb. wiss. Bot., Bd. 51.
—— (1914) Zur Mechanik der Kernteilung. Svensk. Bot. Tidskr., Bd. 8.
MARTENS, P. (1928) Le Cycle du Chromosome somatique dans les Phanérogames. III. Recherches expérimentales sur la cinèse dans la cellule vivante. La Cellule, Bd. 38.
MARTENS, P. and R. CHAMBERS (1932) Etudes de Microdissection. V. Les poils staminaux de *Tradescantia*. Ibid., Tom. 41.
MATSUDA, H. (1928) On the origin of big pollen grains with an abnormal number of chromosomes. Ibid., Bd. 38.

MICHAELIS, L. and H. DAVIDSOHN (1912) Über das Flockungsoptimum von Kolloidgemischen. Bioch. Zeitschr., Bd. 39.

MILOVIDOV, P. F. (1933) Einfluss von Wasser hoher Temperatur auf den Kern der Pflanzenzellen im Lichte der Nuklealreaktion. Protoplasma, Bd. 17.

—— (1938) Durch Welken und Austrocken kunstlich hervorgerufende Myxoploidie bei Pflanzen. Ein Beitrag zur Experimentalcytologie. Protoplasma, Bd. 30.

MOLISCH, H. (1918) Beiträge zur Mikrochemie der Pflanze. Nr. 10: Über Kieselkörper in der Epidermis von *Campelia Zanonia* Rich. Ber. d. D. Bot. Ges., Bd. 36.

MORITA, J. (1935) On the Structure of the Resting Nucleus (Japanese). Proc. Jap. Associat. Acad. Sci., Vol. 7.

MÜNTZER, F. T. (1923) Über die Zweikernigkeit der Leberzellen. Arch. Mikrosk.-Anat. u. Entwick. Mech., Bd. 98.

NAKAMURA, T. (1934) Structure of Chromosomes Variously Treated before Fixation (Japanese). Bull. Kagoshima Imp. Coll. Agr. and Forest. 25th. anniversary. Vol. 1.

NASSONOV, D. (1932) Über die Ursachen der reversiblen Gelatinierung des Zellkerns. Protoplasma, Bd. 15.

NEBEL, B. R. and M. L. RUTTLE (1938) The cytological and genetical significance of colchicine. Jour. Hend., 29.

NĚMEC, B. (1910) Das Problem der Befruchtungsvorgänge und andere zytologische Fragen. Berlin.

—— (1929) Über Struktur und Aggregatzustand des Zellkerns. Protoplasma, Bd. 7.

PEKAREK, J. (1933) Über die Aziditätsverhältnisse in den Epidermis- und Schliesszellen bei *Rumex acetosa* im Licht und im Dunkeln. Planta, Bd. 21.

PETERFI, and H. KOJIMA (1936) Die Wirkung mikrurgischer Eingriffe auf den ruhe Kern der Pflanzenzellen. I. Anstiche Versuche. Protoplasma, Bd. 25.

PISCHINGER, A. (1926) Die Lage des isoelectrischen Punktes histologischer Elemente als Ursache ihrer verschiedenen Farbbarkeit. Zeitschr. f. Zellforschg. u. mikr. Anat., Bd. 3.

POLITZER, G. (1934) Pathologie der Mitose. Berlin.

PORT, J. (1927) Beiträge zur Kenntnis der Temperaturwirkung auf die Pulsation der Vakuolen bei *Paramaecium caudatum*. Protoplasma, Bd. 1.

PRICE, S. R. (1914) Some Studies on the Structure of the Plant Cell by the Method of Darkground Illumination. Ann. Bot., Vol. 28.

RHOWEDER, H. (1936) Die Bedeutung der Polyploide für die Anpassung der Angiospermen an die Kalkgebiete Schleswig-Holsteins. Beih. Bot. Centralbl. (I), Bd. 54.

RUHLAND, W. (1912) Untersuchungen über den Kohlenhydratstoffwechsel von *Beta vulgaris* (Zuckerrübe) Jahrb. w. Bot. Bd., 50.

SAKAMURA, T. (1920) Experimentelle Studien uber die Zell- und Kernteilung mit besonderer Rücksicht auf Form, Grosse und Zahl der Chromosomen. Jour. Coll. Sci. Tokyo Imp. Univ., Vol. 39.

—— (1927) Chromosomenforschung an frischem Material. Protoplasma, Bd. 1.

SAYRE, J. D. (1926) Physiology of Stomata of *Rumex patientia*. Dissertation. (Ohio Univ.).

SCARTH, G. W. (1926) The Influence of H-ion Concentration on the Turgor and Movement of Plant Cells with Special Reference to Stomatal Behaviour. Proc. Internat. Congress Plant Sci., Vol. 2.

—— (1927, a) The structural organisation of plant protoplasm in the light of micrurgy. Protoplasma, Bd. 2.

—— (1927, b) Stomatal movement. Ibid., Bd. 2.

SCHAEDE, R. (1928) Vergleichende Untersuchungen über Cytoplasma, Kern und Kernteilung in lebenden und im fixierten Zustand. Protoplasma, Bd. 3.

—— (1929) Die Kolloidchemie des pflanzlichen Zellkernes in der Ruhe und in der Teilung. Ergeb. d. Biolo., Bd. 5.
—— (1930) Über die Struktur des ruhenden Kernes. Ber. deut. Bot. Ges. Bd., 48.
—— (1935) Beiträge zum Artefaktproblem. Protoplasma, Bd. 23.
SCHIWAGO, P. (1926) Über die Beweglichkeit der Fadenstruktur im lebenden „Ruhekerne" der Froschleukozyten. Biol. Centralbl., Bd. 46.
SCHLÖSSER, L. (1937) Ein neuer Weg zur Auslösung von Mutationen. Zeitschr. f. Ind. Abst. Vererb., Bd. 72.
SCHÜRHOFF, P. (1918) Die Drussenzellen des Griffelkanals von *Lilium Martagon*. Biolog. Zentralbl., Bd. 38.
SEIFRIZ, W. (1930) The alveolar structure of protoplasm. Protoplasma, Bd. 9.
SHARP, L. W. (1913) Somatic Chromosomes in *Vicia faba*. Cellule, Tome. 29.
SHIGENAGA, M. (1937) An Experimental Study of the Abnormal Nuclear and Cell Divisions in Living Cells. Cytologia, FUJII Jub. Vol.
SHIMAKURA, K. (1934) The Capacity of Continuing Divisions of the *Tradescantia* Pollen Mother-cell in Saccharose Solution. Cytologia, Vol. 5.
—— (1937) The Chromonemata Observed in the Fresh Pollen Mother-cells of *Trillium kamtschaticum*. Pall. mounted with Saccharose Solution. Cytologia, FUJII Jub. Vol.
SHIMOTOMAI, N. (1933) Zur Karyogenetik der Gattung *Chrysanthemum*. Jour. Sci. Hiroshima Univ., B. Vol. 2.
SINKE, N. (1937) An experimental Study on the Structure of Living Nuclei in the Resting Stage. Cytologia, FUJII Jub. Vol.
SINKE, N. and M. SHIGENAGA (1933) A Histochemical Study of Plant Nuclei in Rest and Mitosis. Cytologia, Vol. 4.
SINOTÔ, Y. and A. YUASA. (1935) Spiral structure of salivary chromosomes in *Lycoria* (*Sciara*) and *Drosophila* (a preliminary Note). (Japanese) Jap. Jour. Genet., Vol. 10.
SOKOLOVSKAYA, A. P. (1938) A Caryo-geographical Study of the Genus *Agrastis*. Cytologia, Vol. 8.
STILES, W. and I. JÖRGENSEN (1915) Studies in Permeability. I. The Exosmosis of Electrolytes as a Criterion of Antagonic Ion-action. Ann. Bot. Vol., 29.
STRELKOVA, O. (1938) Polyploidy and Geographo-Systematic Groups in the Genus *Alopecurus* L. Cytologia, Vol. 8.
STROHMEYER, G. (1935) Beiträge zur experimentellen Zytologie. Planta, Bd. 24.
STRUGGER, S. (1928) Untersuchungen über den Einfluss der Wasserstoffionen auf das Protoplasma der Wurzelhaare von *Hordeum vulgare*, L. II. Sitz. Ber. Akad. Wiss. Wien Mathem.-Naturwiss. Abt. I., Bd. 137.
—— (1929) Untersuchungen über Plasma und Plasmaströmung an Characeen. III. Beobachtungen am ausgeflossen Protoplasma durchschnittener *Chara*-Internodialzellen. Protoplasma, Bd. 7.
—— (1930) Beitrag zur Kolloidchemie des pflanzlichen Ruhekernes. Protoplasma, Bd. 10.
—— (1935) Praktikum der Zell- und Gewebephysiologie der Pflanze. Berlin.
SUEMATSU, S. (1936) Karyological Study of *Spirogyra* by means of nucleal-reaction. Sci. Rep. Tokyo Bunrika Daigaku, Sec. B., No. 47.
TELEŻYŃSKI, H. (1930) Observation vitals sur la structure du chromosomes dans les poils staminaux de *Tradescantia*. Compt. Rend. Soc. Biol., Vol. 104.
TISCHLER, G. (1922-23) Allgemeine Pflanzenkaryologie. I. Aufl. Berlin.
—— (1934) Allgemeine Pflanzenkaryologie. II. Aufl. Berlin.
—— (1937) On some problems of cytotaxonomy and cytoecology. Jour. Ind. Bot. Soc., Vol. 16.

VONWILLER, P. and A. AUDOVA (1933) Mikrodissektion an der Speicheldrüse von *Chironomus*. Protoplasma, Bd. 19.

WADA, B. (1930) Anstichversuche an der Zellen der Staubfadenhaare von *Tradescantia virginica*. Cytologia, Vol. 1.

—— (1932) Mikrurgische Untersuchungen lebender Zellen in der Teilung. I. Die Bildung von Tetraploidkernen, zweikernigen Zellen und anderen abnormen Teilungsbildern. Ibid., Vol. 4.

—— (1935) III. Die Einwirkung der Plasmolyse auf die Mitose bei den Staubfadenhaarzellen von *Tradescantia reflexa*. Ibid., Vol. 7.

—— (1937) V. Die Einwirkung des Ammonia-Dampfes auf die Mitose bei den Staubfadenhaarzellen von *Tradescantia reflexa*. Ibid., FUJII Jub. Vol.

—— (1938) Experimentelle Untersuchungen lebender Zellen in der Teilung. II. Die Einwirkung des Normalbutylalkoholdampfes auf die Mitose bei den *Tradescantia*-Haarzellen. Ibid., Vol. 9.

WEBER, F. (1926) Der Zellkern der Schliesszellen. Planta, Bd. 1.

—— (1927) Cytoplasma- und Kern-Zustandänderungen bei Schliesszellen. Protoplasma, Bd. 2.

WIGGANS, R. G. (1921) Variations in the osmotic concentration of the guard cells during the opening and closing of stomata. Amer. Jour. Bot., Vol. 8.

WILSON, E. B. (1925) The Cell in Development and Heredity. New York.

WINGE, Ö (1927) Zytologische Untersuhungen über die Natur maligner Tumoren I. „Crown-gall" der Zuckerrübe. Zeitschr. f. Zellforschg. u. mikro. Anat., Bd. 6.

—— (1932) On the Origin of Constant Species-Hybrids. Svensk Bot. Tidsk., Bd. 26.

WINKLER, H, (1916) Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Zeitschr. f. Bot. 8.

YAMAHA, G. (1927, a) Experimentelle zytologische Beiträge. I. Mitt. Orientierungsversuche an den Wurzelspitzenzellen einiger Pflanzen. Jour. Fac. Sci. Imp. Univ. Tokyo., Sec. 3, Vol. 2.

YAMAHA, G. (1927, a) Experimentelle Zytologische Beiträge II. Über die Wirkung des destillierten Wassers auf die Wurzelspitzenzellen von *Vicia faba* bei verschiedenen Temperaturen. Ibid., Vol. 2.

YAMAHA, G. and T. ISHII (1932) Über die Ionenwirkung auf die Chromosomen der Pollenmutterzellen von *Tradescantia reflexa* I., Cytologia Vol. 3.

ZEIGER, K. (1935) Zum Problem der vitalen Struktur der Zellkernes. Zeitschr. f. Zellforschg. u. mikro. Anat. Bd., 22.

EXPLANATION OF PLATES

The photomicrographs which are not otherwise indicated are those taken with a LEITZ MAKAM camera with a LEITZ 1/12 achrom. imm. and the LEITZ periplan oc. 8. In those where other objectives and oculars or other cameras are used they are indicated at the end of each explanation by the following abbreviations:

Z. 1/12 . . . . . for ZEISS 1/12 achrom. imm. obj.
L. 7 . . . . . for LEITZ obj. 7
L. 8 . . . . . for LEITZ oc. 8
15 K. . . . . for ZEISS comp. oc. ×15.
L.O.C. . . . . for LEITZ ordinary camera.

PLATE I

Figs. 1, 2 and 3. The epidermis of young petals of *Tradescantia reflexa*. The same cells in different media.

Fig. 1. Mounted with a 0.5 M saccharose solution. Chromonemata are visible more or less distinctly. The nucleus indicated by an arrow is in metaphase.

Fig. 2. In the medium replaced with a 0.7 M solution. Chromonemata are not visible.

Fig. 3. In the medium replaced with di-distilled water. Chromonemata are very distinctly observed.

Figs. 4, 5 and 6. Nuclei in the leaf epidermal cells of *T. virginica* fixed with BONN modification of FLEMMING's solution followed by the staining with HEIDENHAIN's haematoxylin.

Fig. 4. A nucleus without any treatment before fixation (1/12 L × 15 K. L.O.C.).

Figs. 5 and 6. Nuclei treated for 3 minutes with a 1.0 M saccharose solution. Fig. 5, (1/12 L. × 15 K. L.O.C).

Figs. 7, 8, 9 and 10. Spermatocyte nuclei in the early prophase of *Gampsocleis* sp. Arrows indicate sex-chromosomes.

Fig. 7. In the medium of a 0.8 R solution. The chromonemata are clearly visible.

Fig. 8. Showing swollen nuclei in di-distilled water. The sex-chromosomes are obscurely visible in the nuclei, while the chromonemata of the autosomes are no longer recognizable.

Fig. 9. In a 0.2 R solution. The sex-chromosomes are distinctly visible while the chromonemata are swollen and are hardly perceptible.

Fig. 10. In a 4.0 R solution. The nuclei are shrunken and no internal structure is recognizable.

Figs. 11 and 12. Salivary gland nuclei in the larva of *Chironomus dorsalis*.

Fig. 11. In the body fluid as a medium. The transversal bands are distinctly visible in the chromosomes.

Fig. 12. In the medium of a 0.5 R solution. The nuclei is swollen and the chromosomes have disappeared.

PLATE II

Figs. 13, 14 and 15. The same pollen mother cell of *Trillium Smalli* (L. 7×L. 10).

Fig. 13. Mounted with a 1.0 M saccharose solution. The pollen mother cell is plasmolysed and shrunken, and the chromosomes are invisible.

Fig. 14. In the medium replaced with a 0.5 M solution. The presence of spiral chromonemata is recognizable in the chromosomes which are indicated by arrows.

Fig. 15. In the medium replaced with a 0.3 M solution. The chromosomes are swollen and are very obscure.

Figs. 16, 17, 18 and 19. Various abnormal nuclei in the root tips treated with hypertonic saccharose solutions, and fixed with BONN modification of FLEMMING's solution.

Fig. 16. Showing cytoplasmic granules in the cells of the root tips treated with a 0.5 M solutions for 2 hours.

Fig. 17. Showing a bi-nucleate cell in the root tip treated with a 0.7 M saccharose solution for 3 hours.

Fig. 18. Showing two necrotic cells in the periblems of the root tips treated with a 0.7 M solution for 24 hours (L. 7×8).

Fig. 19. Showing a giant di-diploid nucleus. Treatment is the same as above (Fig. 18).

Figs. 20 and 21. The same spinal cell in the living state of the leaf of *Elodea densa* (Z. 1/12×L. 8).

Fig. 20. Showing the nucleus observed with bright field illumination.

Fig. 21. The same observed with dark field illumination. The nucleus is hardly visible.

Figs. 22 and 23. The same spinal cell of the leaf of *Elodea densa* boiled for 40 seconds (Z. 1/12×L. 8).

Fig. 22. Showing the nucleus observed with bright field illumination. The nucleus shows a distinct heterogeneous structure.

Fig. 23. Showing the nucleus appearing quite dark in dark field illumination.

Figs. 24, 25, 26 and 27. The same stoma in the leaf epidermis of *Alisma plantago* var. *latifolia,* observed in liquid paraffin at room temperature (Figs. 24 and 25) and at 75°C (Figs. 26 and 27).

Fig. 24. Chromonemata are distinctly visible in the nuclei.

Fig. 25. Observed with the dark field illumination. The nuclei are weakly bright.

Fig. 26. Nuclei appear heterogeneous.

Fig. 27. The nuclei are strongly bright with dark field illumination.

PLATE III

Figs. 28, 29, 30 and 31. The same nucleus of the leaf hair cell of *Tradescantia virginica* (Z. 1/12×L. 8), observed in liquid paraffin at room temperature (Figs. 28 and 29) and at 75°C (Figs. 30 and 31).

Fig. 28. The chromonemata are distinctly visible.

Fig. 29. Observed with dark field illumination. The nucleus is weakly bright.

Fig. 30. The nucleus appears to be homogeneous.

Fig. 31. Observed with dark field illumination. The nucleus appears completely dark.

Figs. 32 and 33. The same cells of the epidermis of the young petal of *Tradescantia reflexa* in liquid paraffin.

Fig. 32. At room temperature. The middle cell is in anaphase.

Fig. 33. After 30 minutes at 45°C. The middle cell has become bi-nucleate.

Fig. 34. Showing normal nuclei.

Fig. 35. Treated at 48.1°C for one hour. The nuclei are smaller and the chromonemata are thicker than in Fig. 34.

Fig. 36. Put at room temperature for 18 hours after treatment, showing the nuclei recovering their normal appearance.

Figs. 37, 33 and 39. The same cells of the epidermis of the young petals of *Tradescantia reflexa* in liquid paraffin.

Fig. 37. Normal nuclei at room temperature. The chromonemata are distinctly visible in the nuclei.

Fig. 38. Heated at 48.1°C for one hour. The chromonemata are slightly indistinct.

Fig. 39. After 4 hours' heating. The chromonemata are somewhat indistinct.

Figs. 40 and 41. The same nuclei of old epidermal cells in stretching zone of the young petal of *Tradescantia reflexa* heated at 48°C for 6 hours. Fig. 40 in the ordinary illumination and Fig. 41 in dark field illumination (Z. 1/12×L. 8).

Figs. 42, 43 and 44. The same old staminate hair cell of *Tradescantia reflexa.*

Fig. 42. Nucleus in intact state at room temperature.

Fig. 43. Heated at 56.7°C for 40 minutes. The nucleus shows a coarsely reticulate appearance.

Fig. 44. Put at room temperature for 19 hours after the heating treatment. The nucleus has recovered its normal appearance.

PLATE IV

Figs. 45 and 46. The same closed stoma in water medium.

Fig. 45. At room temperature. The guard cell nuclei are homogeneous.

Fig. 46. Heated at 50°C for 20 minutes. The nuclei show chromonemata distinctly.

Figs. 47-60. Showing nuclear and mitotic irregularities in heated root tip cells of *Vicia faba.*

Fig. 47. A bi-nucleate cell in inner periblem (39° C-1 h-R-24 h).

Fig. 48. An irregular distribution of the chromosomes in metaphase. Note formation of enormous vacuoles in cytoplasm disturbing the normal arrangement of the chromosomes.

Fig. 49. Pycnotic nuclei in periblem (39°C-3 h-R-0 h).

Fig. 50. An irregular distribution of the chromosomes in metaphase (39°C-3 h-R-0 h).

Fig. 51. A pair of lagging chromosomes in anaphase (39°C-3 h-R-6 h).

Fig. 52. A di-diploid spindle in anaphase. In each daughter chromosome group 20 or more than 20 chromosomes were countable (39°C-3 h-R-48 h).

Fig. 53. A bi-nucleate cell in periblem. The lower nucleus is larger than the upper one (39°C-5 h-R-24 h).

Fig. 54. Showing a granular structure observable in the nucleus on the left hand side of the figure. Outer layer of periblem (39°C-6½ h-R-0 h).

Fig. 55. Telophasic nuclei showing incomplete reconstruction (41°C-25 m-R-7 h).

Fig. 56. Showing many degenerated cells in periblem (41°C-1 h-R-48 h).

Fig. 57. Showing a giant di-diploid nucleus of the shape of a constricted sphere of two parts of unequal size.

Fig. 58. Showing chromosomes in metaphase thicker and shorter than those in the normal state (48°C-1 m-R-8 h).

Fig. 59. Showing two mitotic figures in a single cell (48°C-1 m-R-24 h).

Fig. 60. Showing a four nucleate cell (48°C-1 m-R-24 h).

PLATE V

Figs. 61 and 62. Root tip cells of *Vicia faba* treated with boiling water for 3 minutes and stained with FEULGEN'S method.

Fig. 61. Showing contraction of the chromatin substance in the nucleus.

Fig. 62. Showing swollen chromosomes in anaphase. Chromonemata are visible occupying the axial region of the swollen matrix of chromosomes.

Figs. 63 and 64. The same cell of the epidermis of the young petal of *Tradescantia reflexa* in liquid paraffin.

Fig. 63. At room temperature.

Fig. 64. After the exposure to ca. −5°C for 3 hours. Here the chromonemata and the chromosomes are obscure.

Fig. 65. Root tip cells of *Vicia faba* put at room temperature for 24 hours after exposure to −3°C for 30 minutes. Showing a dumbbell shaped di-diploid nucleus.

Figs. 66-73. The epidermal cells of the young petal of *Tradescantia reflexa.* Nuclear and mitotic irregularities caused by mechanical injuries given by cutting.

Fig. 66. Showing two nuclei *a* and *b* of different appearance. In *a* the structure is of coarse meshes and in *b* the nucleus is shrunken and appears homogeneous.

Fig. 67. Showing a shrunken homogeneous nucleus (in the middle of the figure).

Fig. 68. The same nucleus as that shown in Fig. 67, 24 hours and 30 minutes after the injuries given. The nucleus has recovered its normal appearance.

Fig. 69. Showing a swollen homogeneous nucleus.

Fig. 70. The same nucleus as that shown in Fig. 69, one hour after the injuries given. The nucleus shows here distinct chromonema structure.

Fig. 71. Showing the chromosome group in metaphase, shrunken into a mass.

Fig. 72. The same nucleus 3 hours and 10 minutes after the injuries given. Chromosomes are very obscurely seen.

Fig. 73. The same nucleus, 24 hours and 40 minutes after the injuries given. The di-diploid nucleus was formed, in which the distinct chromonema structure is shown.

Fig. 74. Showing a nucleus of the leaf epidermis of *Elodea densa* mechanically injured by cutting.

Figs. 75 and 76. Root tip cells of *Vicia faba* fixed with BONN modification of FLEMMING's solution, 48 hours after mechanical injuries by cutting given, and stained with HEIDENHAIN's haematoxylin.

Fig. 75. Showing a bi-nucleate cell found in the region near the wounded cells.

Fig. 76. Showing a di-diploid giant nucleus found in the same region.

PLATE VI

Figs. 77, 78 and 79. The same cells of epidermis of young petal of *Tradescantia reflexa*.

Fig. 77. In di-distilled water (pH 5.8). The chromonemata are very fine. The second nucleus from right is in prophase.

Fig. 78. In 1/100 M acetic acid (pH 3.3). The chromonemata are thicker than those in the case of di-distilled water as medium.

Fig. 79. In the medium replaced with di-distilled water. The nuclei show fine chromonemata as in Fig. 77. The second nucleus is now in division.

Figs. 80, 81 and 82. The same cells of epidermis of young petals of *Tradescantia reflexa*.

Fig. 80. In di-distilled water (pH 5.8). The chromonemata are fine and distinct.

Fig. 81. Replaced with a 1/100 M NH_4OH solution (pH 10.4). The chromonemata are thicker and slightly indistinct.

Fig. 82. Replaced with 1/20 M NH_4OH solution. The nuclei are shrunken and the chromonemata are indistinct.

Figs. 83, 84 and 85. The same closed stoma in leaf epidermis of *T. virginica*.

Fig. 83. In di-distilled water (pH. 6.4). The guard cell nuclei are swollen and hyaline.

Fig. 84. Thick chromonemata are visible in the guard cell nuclei. Replaced with 1/200 M acetic acid (pH 3.2).

Fig. 85. Washed repeatedly and replaced with di-distilled water. The guard cell nuclei are again hyaline.

Figs. 86, 87, 88 and 89. The same stoma in leaf epidermis of *Tradescantia virginica*.

Fig. 86. In di-distilled water (pH 6.4). The guard cell nuclei are swollen and hyaline.

Fig. 87. Replaced with 1/100 M NH_4OH (pH 10.4). The chromonemata are distinctly visible.

Fig. 88. Replaced with 1/20 M NH_4OH (pH 11.0). The chromonemata are thicker than in the case of 1/100 M solution as medium.

Fig. 89. Replaced with di-distilled water. The nuclei are hyaline. (pH 6.4).

PLATE VII

Figs. 90-95. The pollen mother cells of *Tradescantia reflexa* observed with acetic acid (pH 2.0), WALPOLES's buffer solutions (pH 2.7-5.6) and sodium acetate (pH 9.0) of different H-ion concentrations. Fig. 90, pH 2.0; Fig. 91, pH 2.7; Fig. 92, pH 4.1; Fig. 93, pH 4.9; Fig. 94, pH 5.6 and Fig. 95, pH 9.0 (Z. D×15 K.).

Figs. 96–101. The pollen mother cells of *Trillium Smalli* in prophase observed with with 0.05 M (Fig. 96), 0.2 M (Fig. 97), 0.1 M (Fig. 98), 0.5 M (Fig. 99), 10 M (Fig. 100) and 4.0 M (Fig. 101) solutions of KCNS.

PLATE VIII

Figs. 102–106. Root tip cells of *Vicia faba* treated with a 1% solution of chloral-hydrate for one hour, and fixed after treatment with different intervals of time in sawdust.

Fig. 102. Fixed after one hour conservation in sawdust. Showing pycnotic nuclei.

Fig. 103. Fixed after 23 hours in sawdust. Showing a bi-nucleate cell.

Fig. 104. Showing a giant di-diploid nucleus. The same root tip.

Fig. 105. The same root tip. Showing a necrotic nucleus with contracted cytoplasm surrounding it (middle of the figure).

Fig. 106. Fixed after 71 hours in sawdust after the treatment. Showing the mitotic figures in a cell.

Figs. 107 and 108. The same cells of epidermis of young petal of *Tradescantia reflexa*.

Fig. 107. In di-distilled water.

Fig. 108. Replaced with coal tar water extract, 20 minutes after the replacement. The cytoplasm is vacuolated and the nuclei shrunken.

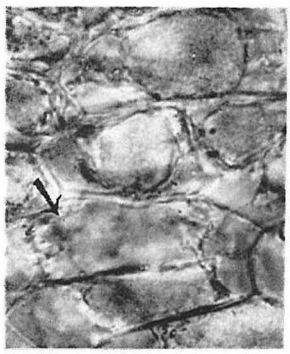
Figs. 109 and 110. The root tip cells of *Vicia faba* fixed with 24 hours interval in sawdust after the treatment with coal tar extract for 30 minutes.

Fig. 109. Showing a di-diploid glant nucleus.

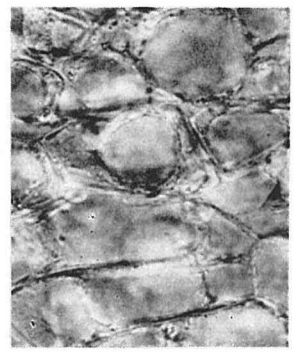
Fig. 110. Showing a bi-nucleate cells.

Figs. 111, 112, 113 and 114. Normal (Fig. 111) and abnormal (Figs. 112–114) nuclei found in the mycorrhiza of *Spiranthes australis*.

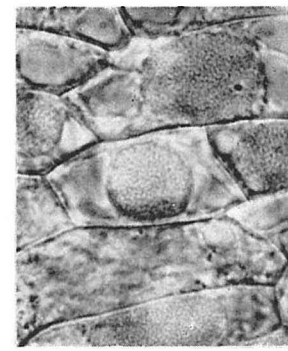
Figs. 115 and 116. Showing a swollen nucleus (Fig. 115) and a bi-nucleate cell (Fig. 116) in the tissue of bacterial-gall by infection of *Bacterium tumefaciens* on the stem of *Ricinus communis*.



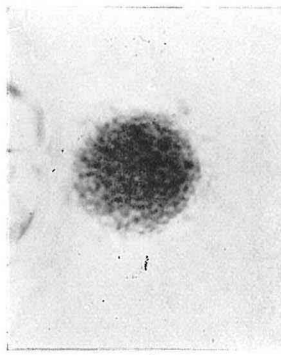
1



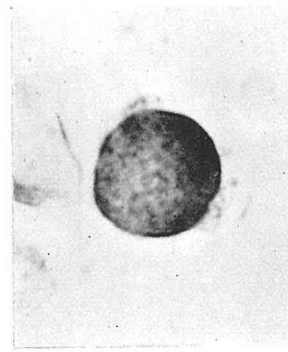
2



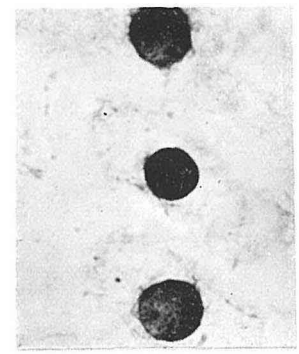
3



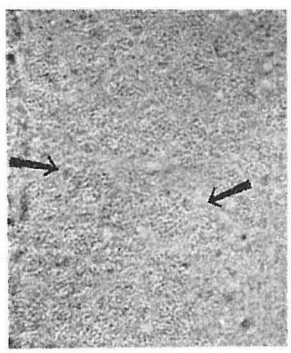
4



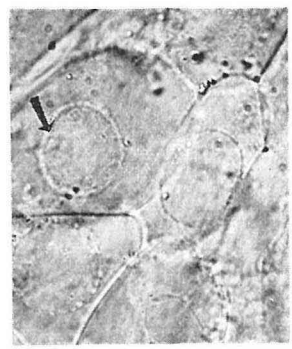
5



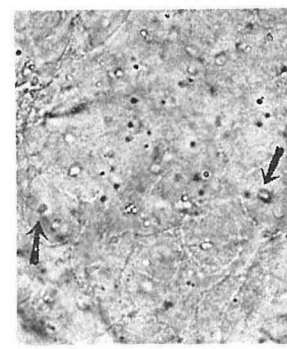
6



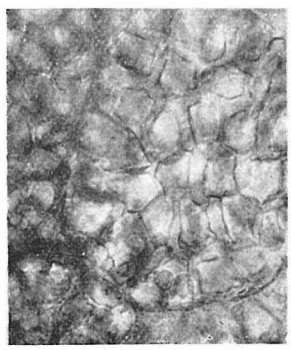
7



8

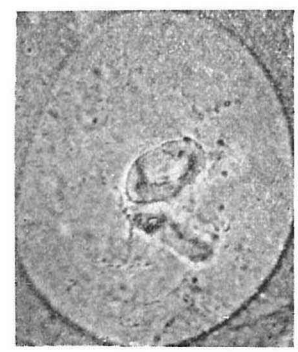


9

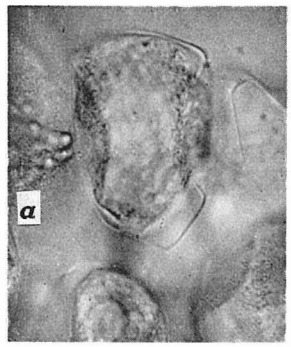


10

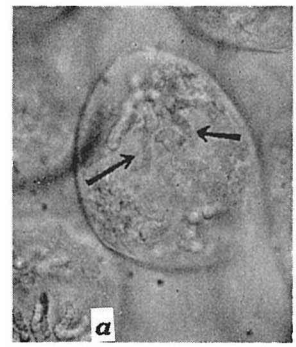
11



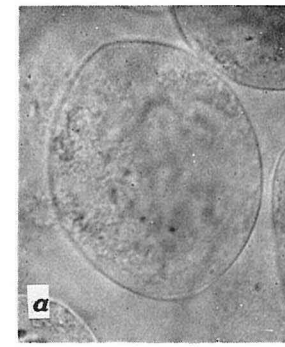
12



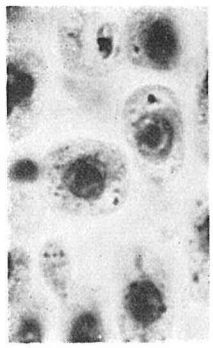
13



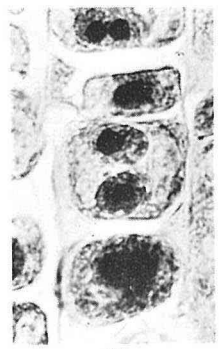
14



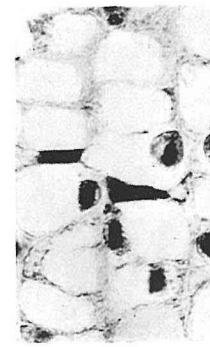
15



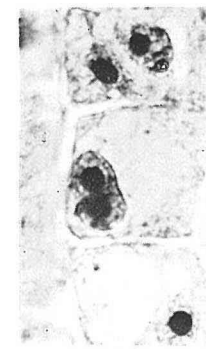
16



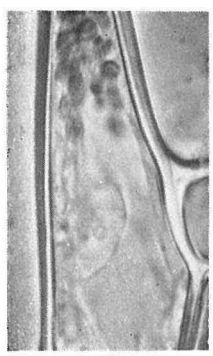
17



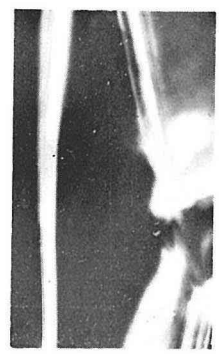
18



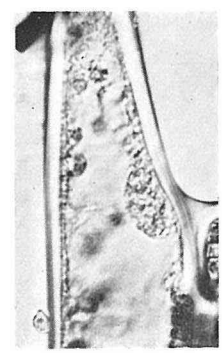
19



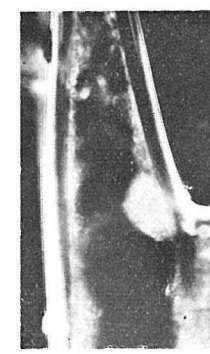
20



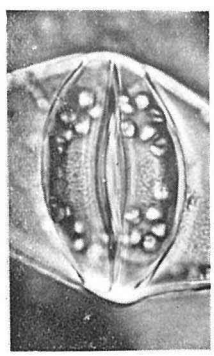
21



22

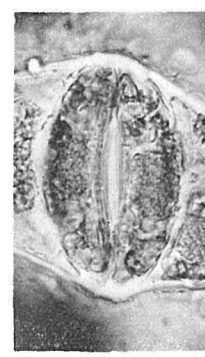


23



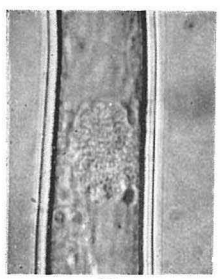
24

25

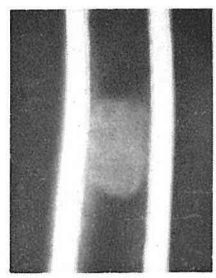


26

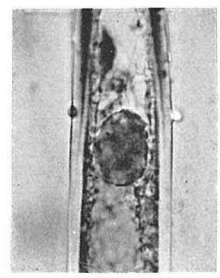
27



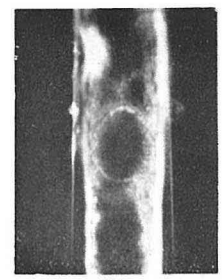
28



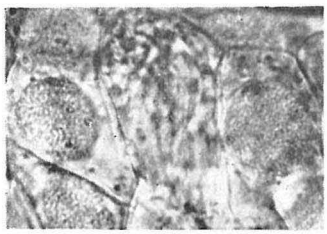
29



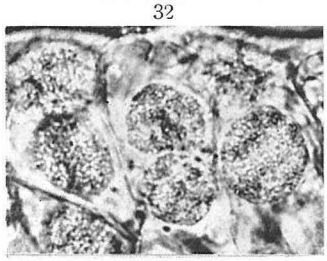
30



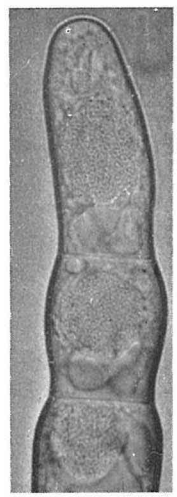
31



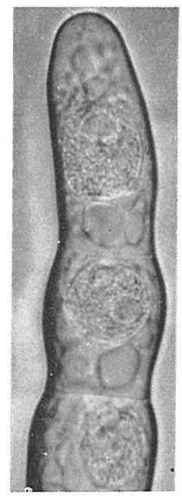
32



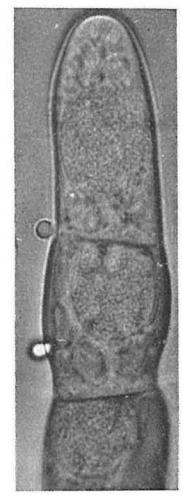
33



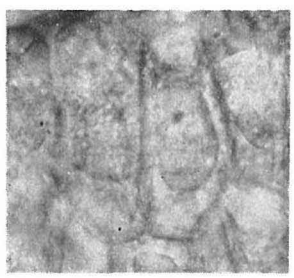
34



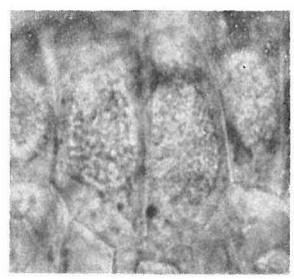
35



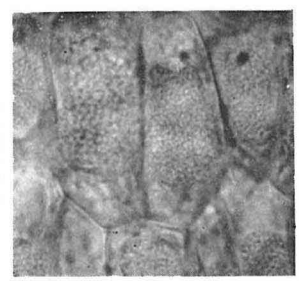
36



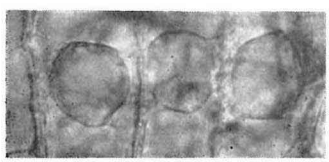
37



38

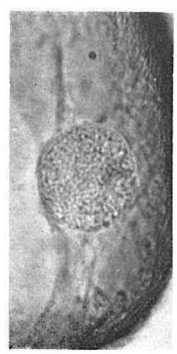


39

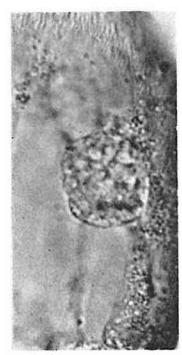


40

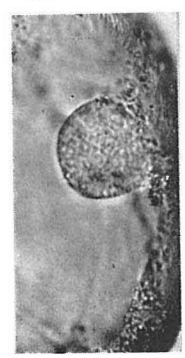
41



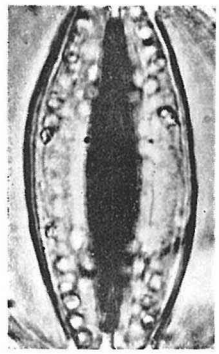
42

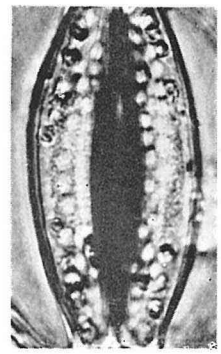


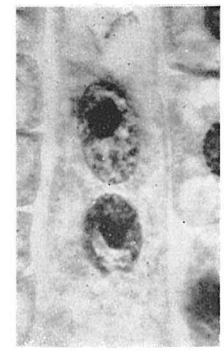
43

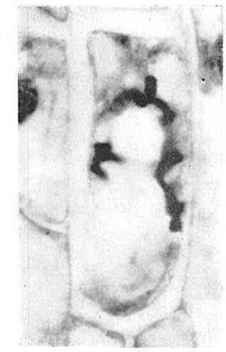


44

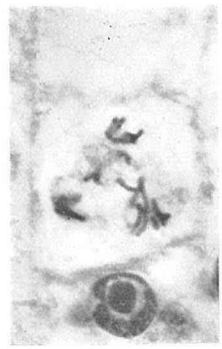

45

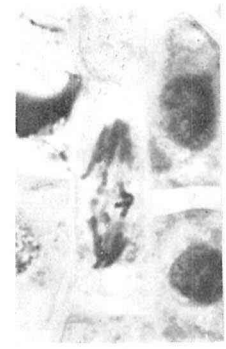

46

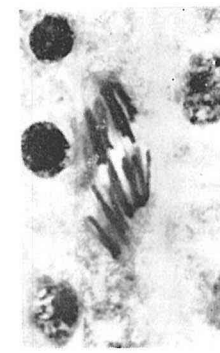

47


48

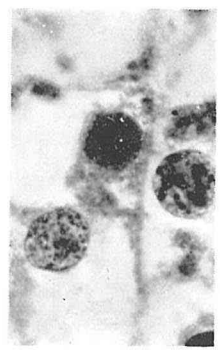

49

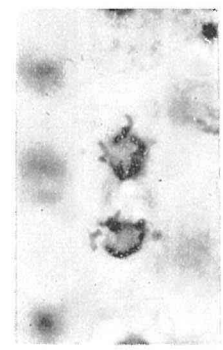

50

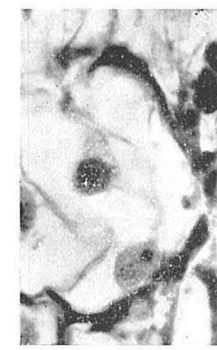

51

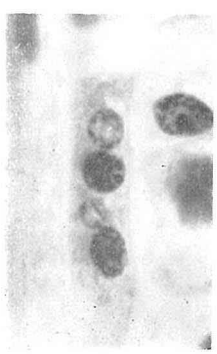

52

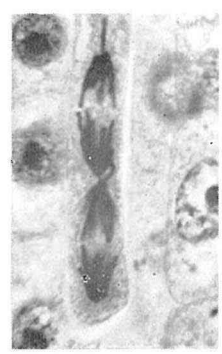

53

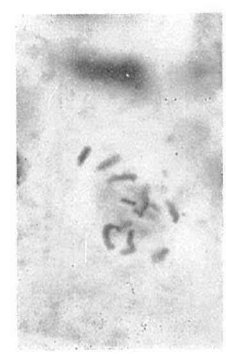

54

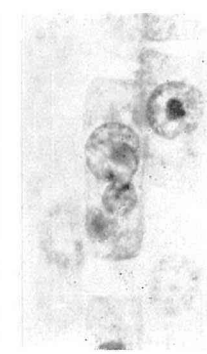

55

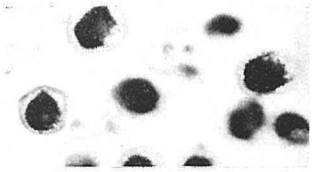

56


57

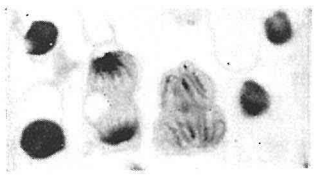

58


59


60



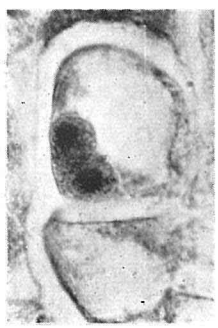
61



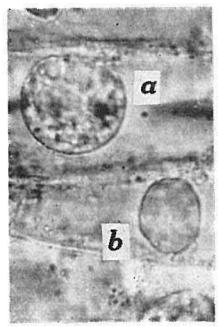
62

63

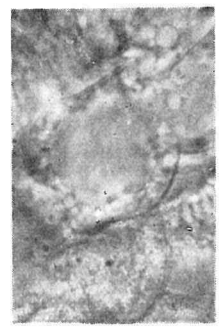
64



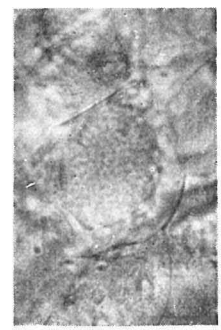
65



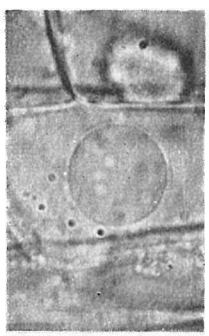
66



67

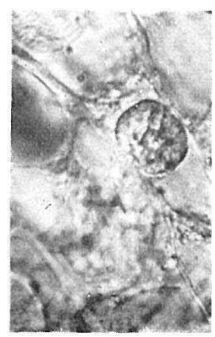


68

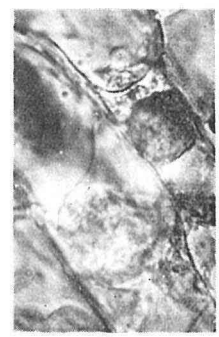


69

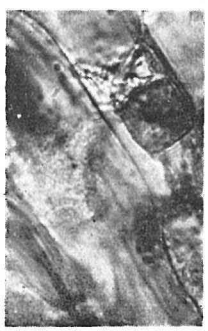
70



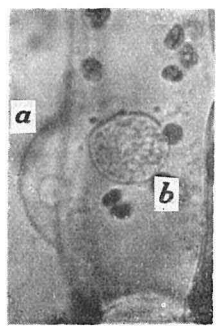
71



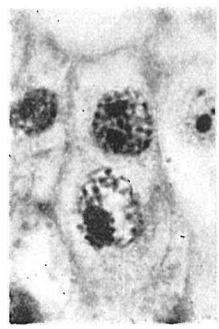
72



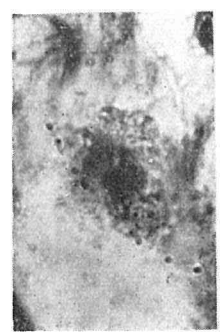
73



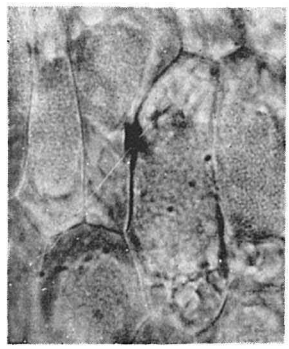
74



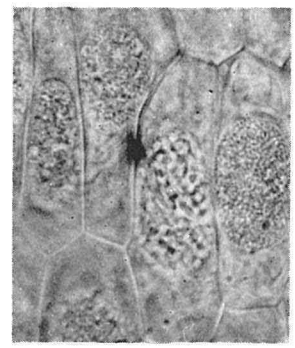
75



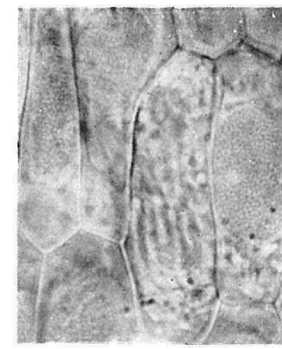
76



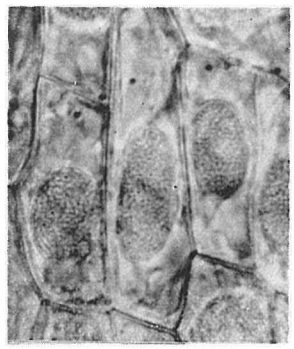
77



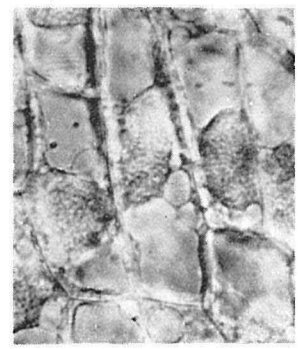
78



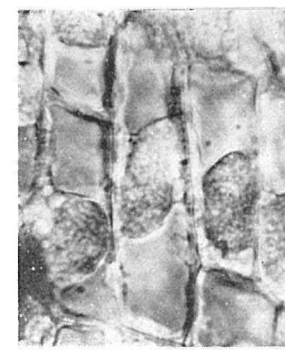
79



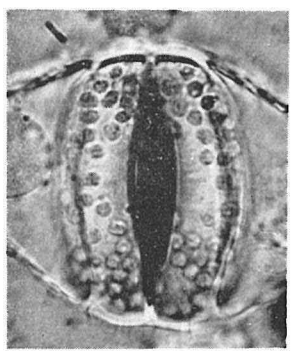
80



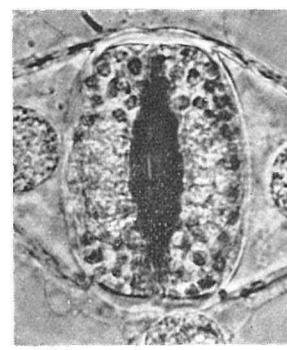
81



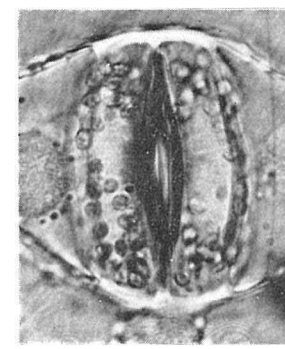
82



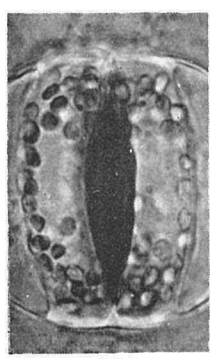
83



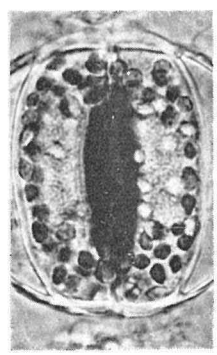
84



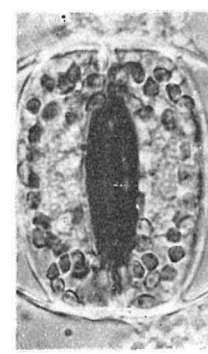
85



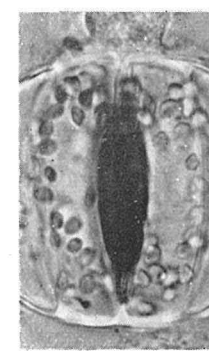
86



87

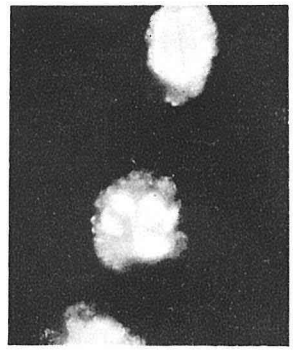


88

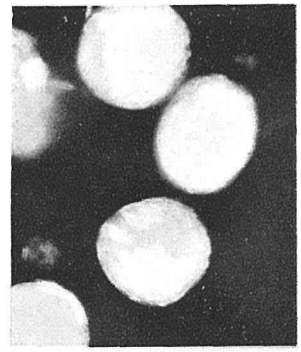


89

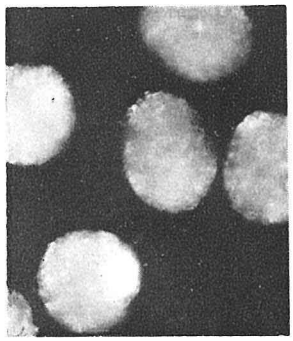
90



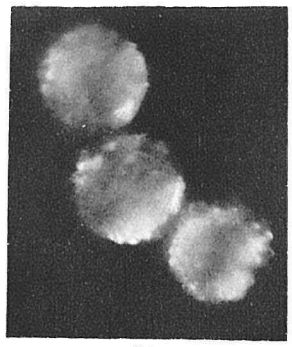
91



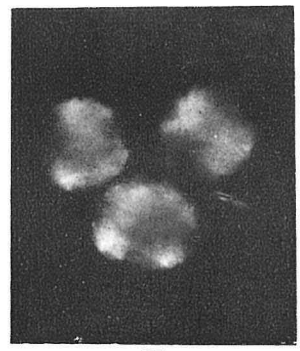
92



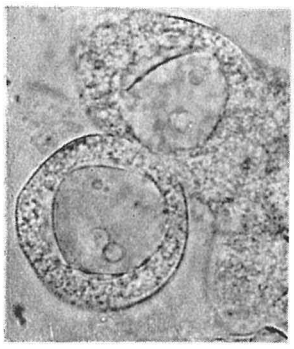
93



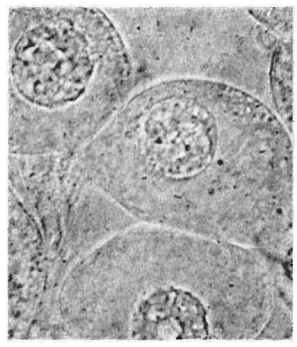
94



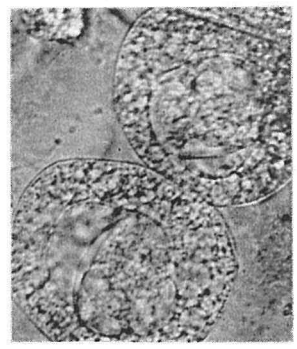
95



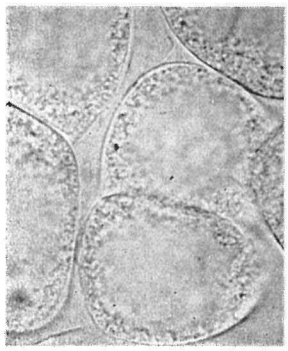
96



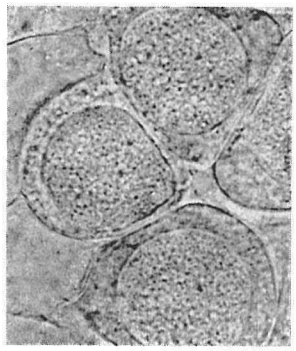
97



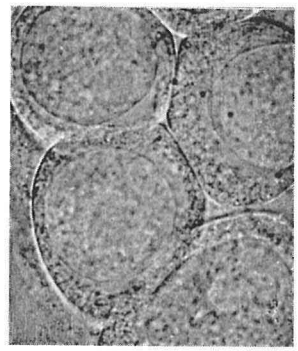
98



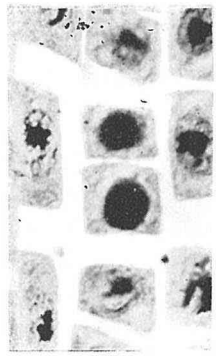
99

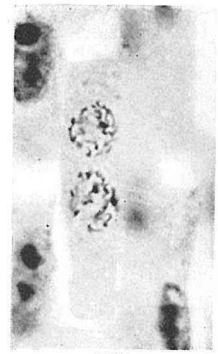


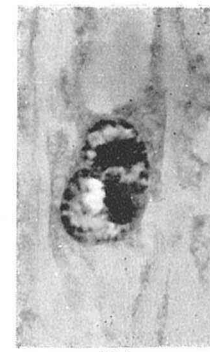
100

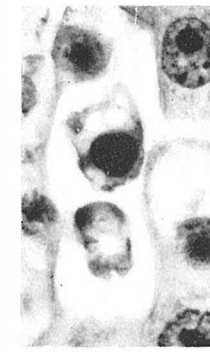


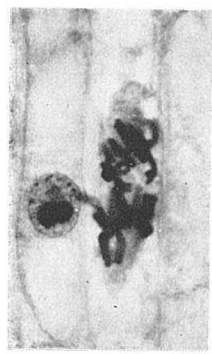
101

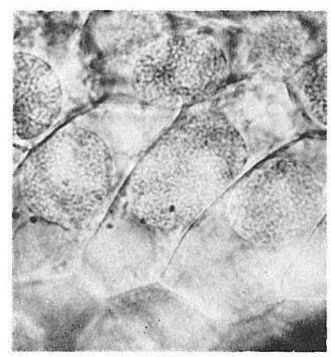

102

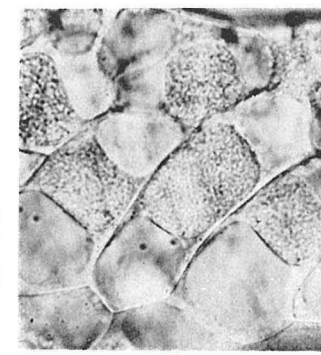

103

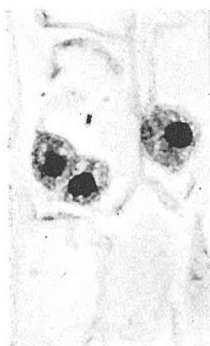

104

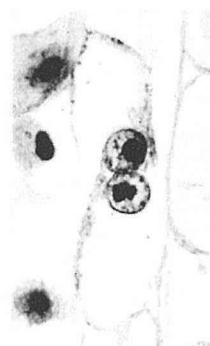

105

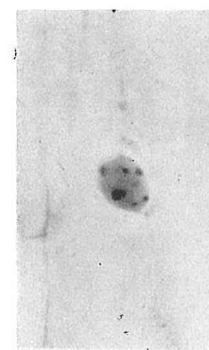

106

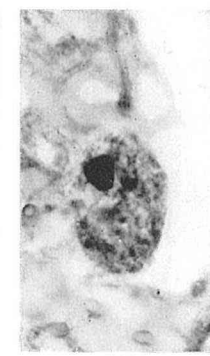

107

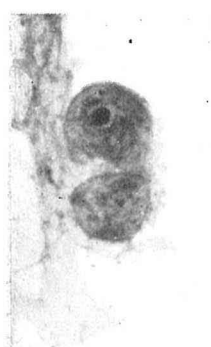

108

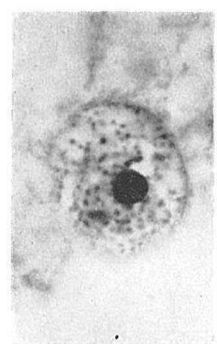

109

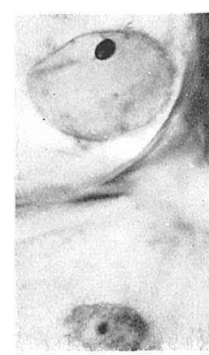

110

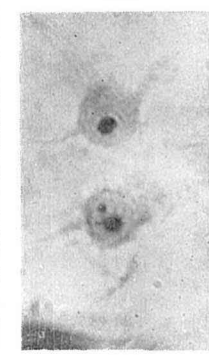

111


112


113


114


115


116