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CYTOPHYSIOLOGICAL AND CYTOECOLOGICAL INVESTIGATIONS
OF HEAT RESISTANCE OF PLANT CELLS TOWARD THE ACTION
OF HIGH AND LOW TEMPERATURE.

V. Ya. Alexandrov

(Review of the works of the laboratory of cytophysiology
and cytoecology of V. L. Komarov Botanical Institute,
Akademia Nauk SSSR).

ABSTRACT

Thermostability of plant cells is due to the resistance of their proteins to denaturation, resistance to injurious metabolic changes, reparatory capacity, and capacity to harden. Hardiness includes the stability of several functions and increases the resistance to several injurious factors. It varies with the tissue and stage of growth. The thermostability of the proteins is constant in higher plants but changes with temperature in algae. Frost hardening increases resistance to several injurious factors including heat. The denaturation theory of injury satisfactorily explains some of the data.

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I. INTRODUCTION

The principal purpose of the laboratory of cytophysiology and cytoecology is the study of the mechanisms which determine the resistance of plant cells towards the action of various agents and also the elucidation of the role of cell resistance in adaptation of plant organisms to various external factors.

Up to the present time we had directed our attention to the reaction of the cell on ^{to} the effect of high and low temperatures. The general program of this laboratory represents the continuation of the Leningrad Cytophysiological School which had been headed by D. N. Nasonov, corresponding member of the AN SSSR.

^{As early as} ~~Already~~ ^{had} ~~beginning with the 30's,~~ ^{considerable data} several research workers of this school have accumulated ~~a large factual material~~ on the reaction of the cells - mainly animal - towards the action of various injurious and stimulating agents. This material had permitted Nasonov and me to advance a theory of ^{low} denaturing injuries and stimulation of cells which was presented in two monographs (Nasonov and Alexandrov 1940; Nasonov 1955; ^{see also} Alexandrov 1960).

On the basis of cytological and biochemical data we came to the conclusion that ^{the} stimulants of entirely different nature ^{kind's} taken in ^{specific} some definite doses produce in the proteins of protoplasm

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similar changes of denaturing type. The first stages of denaturing^{on} which are connected with the chemical activation of different groups in a protein molecule can increase the metabolism in the cell and ~~shorten the time of~~^{accelerate} the processes. They can serve as activators of ~~this or that activity~~^{specific processes characteristic of} typical for the given type of cells. ~~Very strong~~^s actions during which the denaturing^{ation} changes are considerable and include the whole protoplasm may create in the cell^a conditions which is not congenial with its normal function. In such cases the result will be not a stimulation but an injury to the cell.

When the injury has not gone too far, it usually is reversible and after removal of the acting agent the proteins of the protoplasm return to their primary conditions. With the further increase of the dose, an irreversible injury takes place, the denaturation of the cell proteins becomes final and the cell dies.

The denaturation theory easily explains such non-specific characters and injury, like lowering the dispersion of protoplasmic colloids, increase of viscosity, increase of affinity towards dyes, etc. The non-specific character of injury depends first of all upon the similarity of the signs of denaturing of protein after the action of various denaturing agents. The second source of great similarity in the behavior of the cells after different injuries is the wholeness of the cell as a system. Due to the interaction of its parts the final result of reaction may be very similar regardless of different parts where the various agents are applied. However the different types of denaturation are not identical and a wholeness of the cell system is not absolute.

This will explain^{that} the reaction of the cells towards various agents sometimes have^s particular specific features which are combined with general non-specific signs of injury (Alexandrov, 1948).

The principal points of our conception are as follows:

1. The reaction of the protoplasm of different cells towards the action of injurious agents, which are different in their physical and chemical nature, ^{to a considerable degree,} is non-specific.
2. Together with the non-specific characters of injury there exist specific peculiaritiesⁱ which are characteristic of the injurious action of each given agent.
3. The stimulation and the injury are consecutive phases of the response of the cell towards the injuring^{ous} agents.
4. At the bases of both the injury and the stimulation are denaturing changes of the protein molecules of different protoplasmic components.

During the cell injury it is necessary to distinguish the primary denaturing^{ation} which is caused by direct application of the acting agent to the proteins of the protoplasm and the secondary denaturing^{ation} when the agent causes metabolic changes and these changes in turn lead to the denaturing^{ation} of cell proteins. Such a secondary denaturing^{ation} takes place for example during^a decrease of cell respiration after the action of certain inhibitors of metabolism. These data together with some others have led us to presume that the natural state of the proteins of protoplasm is maintained actively by the normal course of cell metabolism.

After studying the physiology of animal cells we arrived at the conclusions^{ation} that the denaturing theory helps us to understand the series of phenomena which takes place in the plant cells.¹

¹See Gunar (1953) about the similarity in irritability of plant and animal cells.

II. CYTOPHYSIOLOGICAL INVESTIGATIONS OF THE REACTION OF PLANT CELLS TOWARDS THE ACTION OF HIGH TEMPERATURE

1. Materials and Methods

The principal objects of our investigations were living cells from the epidermis of leaves of several plant species. We have avoided usually the sectioning, or the ^{stripping} pulling-off of the epidermal layer. The occurrence of various changes in the protoplasm of the cells which are connected with a mechanical injury are often mentioned in the literature. Feldman (1960) had shown ^{ed} that certain plants had responded to a cutting or ^{stripping} pulling-off of the epidermal layer by increased resistance of the cell to various actions including heat. Even in the upper epidermis of the scale leaf of onion ^{bulbs} which is easily removed, the cells are very often injured although the injury may be reversible (Alexandrov and Gruzova 1960). Taking this into consideration we have studied ^{the} in ^{state} living conditions the epidermal cells of the ^{leaf} pieces of leaves without separating them from the mesophyll. For microscopic study of small pieces of leaves it is necessary to have very good illumination according to Keller, water or oil immersion objective with correction ^{collar} screw and infiltration of the tissues with the medium in which the material was mounted. The infiltration was done by means of a simplified method, using a syringe (Alexandrov 1954). The medium as a rule was ^a the top water.

In some of the plants the epidermal cells are covered with a complex sculptured cuticle. Folds and furrows and the deposits of various substances on the cuticle may prevent microscopic study; and ^{it is} quite often, through this cuticle, is impossible to see the structure of the protoplast. To overcome this obstacle a method

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was developed which in many cases gave very good results (Alexandrov 1962B). Before the ^{examination} study under the microscope, a piece of leaf which contains the ^{such a} thick cuticle is removed from the water and dried by ^{blotted with} filtered paper. Then the piece is put on a slide in the drop of liquid with ^a the index of ^{a refractive} refraction which is near to that of the cuticle, and a cover glass is applied. The preliminary measurements of the ^{refractive} index of ^{the} refraction of cuticle in a number of plants, made with the help of ^a phase contrast microscope according to ^C Grossman's method (1949), showed that the index usually varies between 1.500 to 1.540. ^{The refractive indices of} Near to this index of refraction are some of the organic oils of ^{are near this value. These substances} silicon (for example methyl polysilicon). They are not toxic and the pieces of ^{immersed in them} leaf remain alive during ^{for} several weeks. The sculptured structure of the cuticle becomes invisible when the oil is selected correctly (fig. 1a and b), and the contents of the cell ^{are} is clearly seen. The application of ordinary vaseline oil (N_D^{20} 1.481) in many cases gives good results. The substitution of water ^{for} (N_D^{20} 1.333) by ^{of} the media with ^{refractive indices} the index of reflection which is near ^{the use of} that of the cuticle has permitted to include ^{at} as objects epidermis cells from leaves of many species of plants which were absolutely unusable for microscopical examination in water.

2. A Comparison of Different Criteria of Cell Resistance to Heat

In order to evaluate the heat resistance toward the action of high temperature, it was necessary first of all to select a criterion by which it would be possible to judge the degree of injury of the cells after a certain dose of heat (Alexandrov, 1955). Such criteria as: respiration, photosynthesis, exit of substances into the medium, represent general categories which characterize the tissue as a whole. Only after application of different microscopical methods

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of investigation, ^{whether individual cells are} it is possible to judge ~~if the tissue is~~ ^{alive}.

^S The suppression of plasmolysis and deplasmolysis, the exit of pigments from the vacuoles, ^{loss} ~~the change~~ of vital staining, luminescence of the chloroplasts, and also of the fluorochromes introduced into the cell, the depression of ~~the~~ ^{streaming} protoplasmic motion, changes in the cytoplasm and the nucleus ^{observed by} during the phase contrast ^{or} and the dark field microscopy, change in viscosity which is measured by the shape of plasmolysis, ^{or by centrifugation} - all these are used as criteria of injury. Before selecting from this list the most suitable criterion, it was necessary to compare their ^{responses} indications during the different degrees of heat injury. The experiments were conducted as follows. ^V Either whole leaves or pieces of leaves were put into water heated to a desirable temperature. ~~for experiments and then placed in a thermostat for five minutes.~~ Only the heat temperature was varied. ^{such} After this preliminary treatment, ~~observations were made with this or that indicator.~~ ^{different properties were determined.} The respiration was measured in ^R Warburg's apparatus. The photosynthesis was measured by the radiometric method (Zalenskii, Semikhatova and Vosnesenskii, 1955). ^V The vital staining was done with neutral red. As a fluorochrome, acridin orange was used. The changes of luminescence of the fluorochrome and the chlorophyll were observed ^{under} in a luminescence microscope ^{with reflected} in direct light. The motion of the ^P protoplasm ^{is streaming} was judged by the motion of the spherosomes. The exit of electrolytes was determined by means of electroconductivity of the medium. ^V The viscosity was measured by the displacement of the nuclei after centrifugation. ~~To obtain the plasmolysis, the solution of KNO₃ was used.~~ ^{for plasmolysis} For vital staining and study of luminescence the material was infiltrated with the solutions of the dye and the fluorochrome.

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The ^Srosette leaves of Campanula persicifolia L. and the leaves of Tradescantia fluminensis Vell. were the main objects in these experiments. Results obtained are illustrated in figures 2 and 3. The numbers indicate the temperature of ^{during} the five minutes of heating. The behavior of each indicator of injury is shown by the corresponding ^{ed area} striping. The beginning of the ^{area} stripe for each indicator corresponds to the temperature of ^{at} heating after which first deviation from the norm is ^{first} observed. The end of ~~the~~ stripe indicates the temperature which shows the maximum injury. For example, for ^{streaming} the motion, the photosynthesis and the respiration, the beginning of the ^{area} stripe would correspond to ^{a retardation} slowing of the process and the end of ~~the~~ stripe to its final ^{cessation} stopping. During the vital staining of normal cells, the dye is accumulated in the vacuole and the nucleus and cytoplasm remains colorless. In the injured cells a reverse process takes place. The dye colors the cytoplasm and the nucleus and there is no concentration of the dye in the ^ovacules.

Fig. 2.

After heating for 5 minutes the injury to the epidermal cells of Campanula persicifolia begins to show at the temperature 56°C, with death at 60°C. This is judged by plasmolysis and staining with neutral red (fig. 2). ^{ing} If ~~to~~ judge by luminescence of acridine orange, or by respiration of the tissue, it is possible to ^Cconclude that the injury takes place at a lower temperature. ^{And} Entirely different ^{arrived at from the changes in} conclusion could be made, ^{is streaming} if the motion of cytoplasm is considered as an indicator. After heating to 39°C ^{streaming} the protoplasmic motion slows down considerably, and ^{coming to a} complete arrest of motion follows at the temperature at 44°C.

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To evaluate the degree of injury, it is necessary to consider to what extent the process is reversible. With this purpose in mind, pieces, which were heated beforehand at different temperatures for 5 minutes, were left in tap water at room temperature. The examination of these pieces after different periods of time showed, that in the cells with arrested protoplasmic ^{streaming} motion, the latter can be restored even after heating to 52°C. The higher the temperature of heating, the more time is required to restore the ^{streaming} motion. Fig. 4 illustrates the results of such ^{an} experiment. Nine pieces of a leaf were heated at different temperatures between 40° and 54°. After 5 minutes heating at temperature 45.2° and higher, all cells showed ^{no movement} arrested motion of the protoplasm (^{ed line} dashing). After 40.4° and 41.5° the secession of motion took place only in a part of the cells--indicated by heavy line and dash. During the following days gradual restoration of ^{streaming} motion was noticed--heavy lines. After heating to 52.2° the ^{streaming} motion was restored only after 12 days, and not in all the cells. The ^{H.} heating to 53.7° stopped the ^{movement} motion irreversibly and led finally to complete coagulation of the protoplasm.

In the parenchymal cells the heat resistance of the protoplasmic motion is near that of the epidermal cells. The ^P photosynthesis, ^{like} similar to the ^{streaming} protoplasmic motion, is quite sensitive towards the action of high temperatures. The ^{range} limit of reversible suppression of photosynthesis is smaller, ^{than} as compared with that of the ^{is streaming} motion of protoplasm (Liutova, 1962).

After heating to 39° a definite decrease of luminescence of chloroplasts of parenchymal cells can be observed ^{under} through a luminescence microscope. With the increase ⁱⁿ of temperature the

luminescence becomes more opaque. ^T ~~The~~ temperature ^S higher than 57° causes ^C almost complete ^{luminescence} cessation of the chloroplasts' ^{loss} lamination and ^{to the} extrusion of chlorophyll ^{loss} takes place, which ~~becomes~~ absorbed by lipid globules found in these cells. The lipid globules begin to luminesce bright red (Kiknadze, 1960).

Similar results were obtained with the leaf of Tradescantia fluminensis (Fig. 3). In this material ~~the~~ plasmolysis, the respiration, and the exit of anthocyanin from the vacuoles -- were all signs of a very ^{profound} deep cell injury. These three indicators do not show deviation from the normal condition even after heating to 52-54°, which causes irreversible stoppage of protoplasmic ^{streaming} motion. ~~The motion of the~~ ^P protoplasm, ^{streaming} the photosynthesis, and the viscosity give indications of earlier stages of injury. The exit of electrolytes occupies ^{ic} an ^{te} intermediary position.

The results of these experiments, which ^{were} ~~are~~ confirmed ^{with} in some other material, show that in a wide range of temperature, equal to 20 - 30 degrees, ^{an} the increase ⁱⁿ of temperature during the 5 minutes heating is followed by the gradual increase ^a of ⁱⁿ the cell injury. The signs of injury appear gradually and follow a definite sequence. A similar sequence was observed by Glubrecht (1953) after the action of ultraviolet rays on the epidermal cells of onion scale ~~leaf~~ ^{staminal} and the hairs of ~~stamens~~ in Tradescantia virginica.

When we see a cell with coagulated protoplasm after heating to 60°, there is ~~no~~ doubt that the cell is dead. However, ^{an exact determination of} ~~to determine exactly~~ ^a at what lower temperature ^{killig point} occurs the death of the cell ^{is} impossible, because between the cell which is

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Attempt to determine the [illegible]

life of all of the cells on the basis of the [illegible] of [illegible] and [illegible]
exterior of life tested, because the [illegible] of [illegible] and [illegible]
of [illegible] is [illegible].

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alive and that which is dead, there are numerous intermediary transitions. To determine a ^{point} place in the temperature range which could be considered as ^{the} limit between the life and death of the cell proved to be impossible, because this limit depends upon the criterion of measurement selected. The limit of reversibility of suppression of photosynthesis in the parenchymal cells of Campanula persicifolia is at 48°; the limit of reversibility of ^{cessation} secession of ^{streaming} motion in these cells is at 51° (Liutova, 1962). In the epidermis of petals of Campanula persicifolia the limit of reversibility of the ^{cessation} secession of ^{streaming} motion is at 49°. However, reversible suppression of plasmolysis in these cells was obtained in the range of 51-53°. But a few days later the same cells had perished. A restoration of certain functions of the cell, and unrepairable damage to others is observed both in plant and animal cells after ^{subjection to radiation} light-action (Biebl, 1936); Alexandrov, 1948).

Thus, after the restoration of ^{individual} ~~this or that~~ function in the injured cell, it is impossible to judge about the total recuperation of the cell as a whole. Observing the temporary restoration of a certain function in a cell, which was damaged beyond repairs, one wonders how deep is a tendency towards normality in the living cell system.

After comparing the behavior of different indicators of cell viability during the injurious action of high temperature, we have selected as the main indicator of heat resistance of the cell the ^{cessation} secession of the ^{streaming} protoplasmic motion. This indicator had the following advantages: (1) being a cytological indicator,

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it gives an opportunity to judge the condition of each cell separately; (2) it belongs to the most sensitive indicators of heat injury; (3) during observation of the protoplasmic ^{streaming} motion the tissues are not subjected to additional substances, which could increase the injury to the cell (such as vital staining, ^Sfluorochromation, ^{es}plasmolysis); (4) the test can be carried on with the same cell several times, and the observations may last for a long time. The drawback of this method is the fact that not all the tissues exhibit protoplasmic motion.

3. The Curves of heat resistance of plant cells.

As the measurement of heat resistance of plant cells we have used the correlation between ^{and} the time during which the ^{streaming} motion ~~was~~ ^{persists} retained and the temperature of heating. This was calculated for the zone of injurious temperatures. These experiments have permitted us to draw a curve of heat resistance, the ^{abscissa} horizontal part of which indicates the temperature of heating, and the ^{ordinate} vertical ~~of~~ the time during which ^{streaming persists} the motion is retained. With the increase in ~~of~~ temperature ~~to some degrees~~, ^{for} the time of ^{cessation} the secession of ^{streaming} motion ~~decreases accordingly~~. If such relationship remains at all the temperatures, and the vertical line gives the logarithm of the ^{periods} of time of development of the injury, ~~it~~ ^{for} will form a straight line. In Fig. 5, 6, and 7 are given typical curves obtained in similar experiments. Accordingly, In the zone of higher temperatures, all the points received in the experiment ^{at} fall on into a straight line. A constant, reversible parabolic relationship exists along the straight section of the curve between the ^{during which the streaming persists and} time ~~the motion is retained and the degree of~~ the injurious temperature. We have obtained curves of heat resistance for

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almost 40 species of plants, and in the majority of the cases the abovementioned relationship is disturbed in the region of low temperatures and the curve makes a sharp turn downward. More about this point will be said later. The heat resistance of cells is thus characterized by the position of the curve on the temperature scale, its ^{slope} ~~incline~~, and the point of its break.

The position of the curve on the temperature scale may vary considerably in different plant species. As for example, the alga Peridinium bipes Stein stops protoplasmic motion after 5 minutes of heating at 35° ; the epidermal cells of the leaves of Zostera marina L. stop motion at 36° ; the epidermal cells of certain cacti stop motion at about $52-53^{\circ}$.

In animals, similar semi-logarithmic graphs (show also) ^{cessation} a straight line relationship between the injurious temperature and the time of loss of excitation of the muscles, or the secession of the motion of cilia and flagella. Here also, depending upon the species, great variations exist in the position of the curve in on the temperature scale. For example, after 5 minutes heating at 30° the motion of cilia stops in the ciliated epithelium of the mollusk Pecten islandicus, and in the birds (pigeon, rooster) -- at 49.2° (Alexandrov, 1952). The ability to respond by contraction to the stimulus of an electrical current is lost in the muscles of a sea mollusk Chlomyx islandicus after 5 minutes heating at 28.5° , and in the terrestrial mollusk Helicella virgata after heating to 53.2° (Djamusova 1960 a, b).

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The question is raised, what determines the resistance of cells towards the action of high temperature in the zone of the right, straight section of the curve? To answer this question it is essential first of all to look at the ^{slope} ~~incline~~-angle of the curve. ^{From slope} The ^{line portion} ~~incline~~ of the straight section of the curve ^{we determined} ~~can~~ indicate the temperature coefficient (Q_{10}) ^{for} of the heat injury of the protoplasm in the given zone of ^{the} ~~scale~~. In Table I are presented values of Q_{10} for certain plants studied. The ~~meaning~~ ^{of} Q_{10} for most ~~of the~~ chemical reactions is within the limits of 2-3, ^{but} the ^{values obtained} ~~figures~~ ^{are in the} received by us ~~have values~~ for hundreds and thousands. ^{Values are} Similar ^{for} condition ^a is found in the animal cells (Ushkov and Gasteva, 1953). In the processes observed outside the organisms, such high values of Q_{10} are known only ^{for} ~~in~~ the denaturing of proteins by heat. This fact ^{long ago} ~~had~~ ^{cause} ~~convinced~~ a number of investigators that the ^{to} ~~foundation~~ of heat injury ^{of the} ~~of the~~ cells is ~~based on~~ the denaturing ^{ation} of the protoplasmic proteins by heat (Loeb, 1919; Lepeschkin, 1923, 1935; Collander, 1924; Astaurov, 1940; Nasonov and Alexandrov, 1940 and others). Aside from the very high Q_{10} additional evidence suggests that ~~the~~ proteins extracted from the cells with high heat resistance ~~accordingly~~ are resistant towards the denaturing action of heating. ^{data} The ~~figures~~ for plant cells were obtained by Khlebnikova (1932). A large number of studies show the presence of highly heat resistant proteins in thermophilic bacteria (Koffler, Mollat, and Aoye, 1957). Brown, Nesvetaeva, and Fijenko, 1959 have isolated actomyosin from the muscles of two frogs, the southern frog Rana ridibunda, and the more northern frog Rana temporaria. In accord-

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ance with the greater heat resistance of muscle fibers of Rana
ridibunda the ^{acto} myosin extracted was more resistant to heating as
 compared with the myosin of Rana temporaria (ad^cin^esin^etriphosphatase
 activity of that protein was analyzed) (see also Vinogradov, 1961).

Thus, the thermoresistance of cells in the zone of high
temperatures is determined first of all by the resistance of proto-
plasmic proteins towards the denaturing action of heating. The
straight line section of the curves of heat resistance of cells ^{our} ^{for} ^{as a}
obtained by us should be regarded as an indicator of heat resistance
of proteins which are connected with the function of the action of
the protoplasmic streaming

The Sensitivity and the reverse quality--the cell resist-
 ance--are both measurements of the results of action upon the cell
 of this ^{an individual} or that stimulus. In a study on the injurious action of
^{radiation} light on infusoria I have shown ^{ed} already in 1952 (Alexandrov, 1952 a)
 that the result of the action of a stimulus (by which we judge the
 cell resistance) is determined not only by the primary disturbances
 caused by it but also by the secondary destructive changes which
 develop as a result of the primary disturbance and, in addition, by
 the defensive processes which are developed by the cell at the
 moment of action of the stimulus, or after its ^{cessation} secession.

4. The reparation of heat injury ^(streaming)

The cell in which the ^{ic} ^s ~~action~~ of protoplasm had been stopped
 by heating is able to restore this function if the heating was not
 excessive. In the ^{ose} cases when the conditions of ^{the} experiment permit
 the ^{repair} ~~occurrence of the reparatory action~~, the heat resistance of

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protoplasmic proteins is not the only cause which determines the of heat resistance ⁱⁿ of the cells. For example, after 5 minutes of heating the ^{cessation} ~~secession~~ of protoplasmic ^{streaming} ~~motion~~ occurs at 44.7° in the epidermal cells of Catabrosa aquatica (L) P. B. (experiments of Den'ko) and at $43.5-44.0^{\circ}$ in Campanula persicifolia. Both measurements occurred immediately after heating. Therefore, we may conclude that the cells of Catabrosa aquatica are somewhat more resistant as compared with the cells of Campanula persicifolia. However, if

streaming we were to determine the results of heating ^{from} by ~~motion~~ of the protoplasm ^{id} ~~not~~ ^{immediately} after removal of the pieces from the thermostat but after a few days, we would have come to a different conclusion because the ability of the cells of Catabrosa aquatica to repair the heat injury is less than that of Campanula persicifolia. Whereas the single cells of Campanula persicifolia restore the protoplasmic motion even after heating to 51.5° , the cells of Catabrosa aquatica can repair the motion after 5 minutes of heating to 49.0° only.

Therefore, after considering the behavior of the cells in time, we come to the opposite conclusion; namely, that the cells of Campanula persicifolia are more heat resistant ^{than} as compared with those of Catabrosa aquatica. Therefore, in this experimental set up the

difference in reparatory ^{irability} ~~capability~~ had overcome the difference in ^{thermostability} ~~heat resistance~~ of the protoplasmic proteins.

The above considerations have to be kept in mind when the sensitivity of different functions is compared within the limits of the same cell. Fig. 2 shows that in the cells of mesophyll parenchyma ^{leaves,} ~~from~~ Campanula persicifolia complete ^{cessation} ~~secession~~ of ^{streaming} ~~the motion~~ after a 5 minute heating occurs at 44° . To depress completely, the photosynthesis heating to 46° is required. Judging by this indicator,

it is possible to conclude that ~~the function of motion~~^{Streaming} is less heat resistant than ~~that of the~~ photosynthesis. However, the parenchymal cell is ~~able~~^{movement} to restore the ~~motion~~ even after ~~51.5°~~^{to 57.5°} of heating, whereas the photosynthesis is irreversibly suppressed after heating to 48° (Liutova, 1962). If we were to judge, therefore, ~~about~~^a the sensitivity of these two ~~functions~~^{Processes} several days after the action of high temperature, we would ~~have~~ come to the conclusion that ~~the motion~~^{Streaming} is more heat resistant as compared ~~than~~ with the photosynthesis.

When plant and animal cells are compared some interesting differences occur in their ability ^{RES} to restore the protoplasmic motion after the injurious action of heat. Table II shows corresponding data for certain plants. Here we see that the temperature ^{zone} varies 4 - 9° between the temperature which stops the protoplasmic ^{from} ~~motion~~^{Streaming} after 5 minutes of heating, and the maximal temperature after which the ~~motion~~^{Streaming} could still be restored. The ability to restore the motion which had been stopped by heating in animal cells is considerably smaller. After 5 minutes' heating of the ciliated epithelium of frog, the zone of ~~the~~ reversible suppression of motion was only 1.2° higher, than the temperature which stops the motion of ~~the~~ cilia (41.7°). In mollusk Unio pictorum and in Paramecium caudatum this zone was ^{the} ~~smaller~~^{less} than 1° (Alexandrov, 1955). ^{of animal cells}

The cause of such a difference apparently is as follows: Experiments illustrated in fig. 2 and 3, show that in the plant cells the dose of heating required to stop the motion is lower than that necessary for disturbing the selective permeability of the

protoplast and to increase the affinity towards dyes by the cytoplasm and the nucleus. We have shown that the increase in absorbing capacity of the protoplasm towards the dyes after the action of various injurious agents is the result of the denaturing ^{ation} changes ⁱⁿ of the protoplasmic proteins (Alexandrov and Nasonov 1939, 1943; Nasonov and Alexandrov 1940; Brown 1948 a and b, etc.). Therefore, the proteins of plant cells which are connected with the function of motion of the protoplasm are far more sensitive towards the denaturing action of heating as compared with the main masses ⁱⁿ of proteins ^{performed} of the cytoplasm and the nucleus. The preliminary investigations carried with the animal cells show that the temperatures which depress the motion, and those which increase the affinity of the protoplasm towards the dyes are much closer. This indicates that in the animal cells there is no substantial ^{difference} rift between the heat resistance of the contractile proteins and the main masses ^{that of} of the protoplasmic proteins. Consequently, the ^{cessation} secession of the protoplasmic motion (or the ability of cells to contract) occurs in the animal cells with such doses that injure the whole cell system more ^{than in the case of} as compared with the ^{cessation} secession of ^{Protoplasmic} motion of protoplasm in the plant cells. Under such conditions the ^{repair} ^{difficult} ~~reparation~~ of the cell becomes handicapped because it apparently is an expression of the living activity of the cell.

Therefore, in certain cases the renaturing of the denatured protein may take place immediately after the removal of the denaturing agent -- homodromic ~~(P.H.S.)~~ reversibility, for example, denaturing ^{ation} of tripsin and chimotripsin by heat ^K (Cunitz and Northrop, 1935). In other cases, the removal of the denaturing

agent is not sufficient, and more or less complete restoration of the original native condition could be achieved only after the additional treatment of the proteins--heterodromic reversibility (see Neurath, Greenstein, ^PPutnam, Erickson, 1944; Putnam 1956). The restoration of ~~the~~ protoplasmic motion after heating requires considerable time. In accordance with the dose used, the period of recuperation may last up to 18 days. In connection with this it is logical to assume that the restoration of the motion takes place either by means of a heterodromic reversibility or by substitution of the denatured protein molecules by newly synthesized ones. In both cases it is necessary to assume the necessary participation of the active ~~metabolism~~ of the cells. Therefore, it is logical to expect that the success of ^{the repair} ~~reparation~~ of a certain function ^{depends} upon the degree of disturbance of the whole cell system.

5. The ~~heat~~ hardening, or the increase of ⁱⁿ resistance of plant cells after ^{exposure} ~~conditioning~~ to high temperatures.

The heat resistance of cells is determined not only by the ^{thermostability} ~~heat-resistance~~ of the cell proteins and by the ability of cells to regenerate after the action of high temperatures but also by the increase of resistance in response to ^{action of} ~~the conditioning~~ by high temperatures, as our investigations have shown. The information in the literature about ^{the} capacity of plants to increase their heat resistance after heat treatment is contradictory and ^{not} based primarily on ~~the~~ cytophysiological methods of investigation (Sapper, 1953; Biebl, 1950; Laude and ^UChangule, 1953; Coffman, 1957).

An increase ⁱⁿ of the heat resistance of cells in response to the conditioning by temperature ~~temperature~~ -- heat hardening -- had been established in our laboratory for ³ more than 20 species of plants belonging to different families. The following problems were investigated:

- (1) Dependency ^e of the results of heat hardening ^{on} from
 - a) the length of time of heat hardening
 - b) the temperature of heat hardening
 - c) the initial heat resistance of the cells being hardened
- (2) Reversibility of heat hardening
- (3) Relationship of the hardened cells towards other injurious agents.

Fig. 8 shows the dependency ^e of the effects of heat hardening of the cells of Tradescantia fluminensis ^{on} from the duration of hardening at two temperatures, 36.5 and 33.0°. In these experiments every leaf was cut in two. One half of the leaf was ^{immersed} placed in water at one of the indicated temperatures, the other, control, ~~has~~ remained in water at room temperature. After different periods, indicated on the ^{abscissa} horizontal line, the temperature which stops the protoplasmic ^{streaming} motion after 5 minutes was determined in both halves of the leaf. As seen ⁱⁿ from the fig. 8, a definite increase ⁱⁿ of heat resistance at given temperatures was obtained after 30-60 min. The greatest heat resistance was achieved after 36 hours of hardening. ^{further} After this, a decrease occurred. A decrease was observed along the curve, and with still longer periods of hardening, the cells ~~had~~ perished. In the control leaves a certain

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increase of resistance is also observed after they are removed from the plant. This has been observed by us in a number of plants.

The dependency ^p of ^{the} heat resistance of cells ^A upon the temperature to which the cells had been subjected during a constant period of heating (16-18 hr) is shown in fig. 9. The heat resistance of leaves kept at 18° served as controls. Within the limits of 1° to 26° a preliminary treatment with heat does not affect the heat resistance of cells. The cells respond with ^{an} the increase ⁱⁿ of heat resistance only after the action of higher temperatures. The maximum effect of heat hardening (increase of 2.1°) occurs ^{under} in these conditions at 37.5°. Still higher temperature ^s kills ^A the cells. Bukharin (1958) obtained increase in the breaking point in the ^{coagulation} curves ^{of} ~~protein-coagulation~~ ^{for} in the protoplasm ^{is} in Lutescens wheat after keeping the plants at 30°; however, the breaking point ~~had~~ dropped considerably if plants were kept at 35°.

After ~~the~~ heat hardening, the resistance of the cells increases not only towards the 5 min heating, but the whole curve of the heat resistance of cells is displaced. In some plants after hardening the ^{slope} ~~incline~~ of the curve (Q_{10}) changes (fig. 10), whereas in others it remains the same (fig. 11).

The fact that the cells do not change their ^{heat} resistance ^{within a} ~~towards heating during~~ the wide range of temperatures but begin to respond by increasing the resistance when the temperature ^{is} ~~is~~ ^{reaching} ^{led us to conclude} the injurious zone ^{is} ~~made us presume~~ that the heat hardening is ^{the} ~~the~~ ^{specific} reaction of the cells ~~exactly~~ towards the injurious action of ~~the~~ heating. To verify this supposition a series of experiments was set up.

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In Fig. 12 ~~the~~ curve (1) shows the influence of temperature of hardening (duration of hardening is 18 hr) upon the intensity of photosynthesis ⁱⁿ of the leaf of Tradescantia fluminensis. ^{The} Other curves ^{indicate} represent the dependency ^{e. on} from the hardening temperature of heat resistance of protoplasmic motion⁽²⁾ and of photosynthesis ⁽³⁾. When these curves are compared, it becomes evident that with the increase ⁱⁿ of hardening temperature, ^{(which is by an} paralleled ^{in.} with the increase of ~~the effect of~~ hardening), there is a definite suppression of photosynthesis.

The depressive ^{ng} action of hardening temperature ^s has been noticed ^d in the experiments of Kisliuk on young plants of cereal grains. After 18 hr of hardening at 36.6°, together with the increase of heat resistance of plants, there was ^a definite ^{growth} inhibition ^{of} ~~of growth~~ equal to 15-25%, as compared with the controls. Additional proof that the heat hardening is the reaction of the cells towards the injurious action of heating can be obtained from ~~the~~ experiments which determine the connection between the hardening temperature and the general effect of hardening ^o in plants, which differ ⁱⁿ by ~~their~~ heat resistance. These experiments were carried out with the epidermal cells of ^{leaf} sheaths ~~leaves~~ of grasses. From fig. 13 we see that ~~(after 18 hr/heat-hardening)~~ ^{in order} to increase the cell resistance ^{18 hr.} per 1°, the hardening temperature required is: for Dactylis glomerata L. --30°; Phragmites communes Trin.--36°; Panicum miliacaem L. and Eleusina indica (L) Gaerth. --40-41°. The results obtained agree completely with the differences in the heat resistance of these grasses: thus, the temperature which

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stops protoplasmic motion after 5 min heating in unhardened cells of Dactylis glomerata is 44.0° ; in Phragmites communis is 46.0° ; in Panicum miliaceum is 48.5° , and in Eleusine indica is 49.0° . Therefore, the cells which are more heat resistant in the original state require ^a higher temperature ^{in order} to obtain the a effect of hardening for the given period of time.

If ^{we} to consider that during the heat hardening the increase ⁱⁿ of resistance is a result of adaptation, then not only the temperature, but also the duration of hardening is essential. However, if ^{we} to consider this process as a reaction towards the intensity of the heat injury, in such cases the main significance should be attributed ^s towards the dose of the heating, but not the duration. Lomagin (1961) has studied the possibility of decreasing the time of hardening with the increase ⁱⁿ of the temperature of hardening. He has received hardening of the epidermal cells from leaf ^{ves} epidermis of Tradescantia fluminensis, Campanula persicifolia, and Chlorophytum elatum R. Br. after ^a 1 second action of high temperature. One of his experiments is shown in Table III. During the short time of hardening, the rise ⁱⁿ of resistance ^{occurs very rapidly} is taking place very fast. It can be detected already after 5-10 seconds after ^{period} secession of the 10 seconds of hardening.

The facts, (abovementioned), led us to conclude that the increase ⁱⁿ of heat resistance of cells during the heat hardening is the response to the injurious action of heating.

In the abovementioned experiments the effect of hardening was determined by the increase ⁱⁿ of resistance towards heat, as measured by the ^{protoplasmic streaming} motion of the protoplasm. However, the decrease ^{Similar results were obtained with leaves of Campanula persicifolia, Dactylis glomerata, and Leucanthemum vulgare (Zavadskaja 1963).}

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in
of sensitivity towards heating after heat hardening can^{also} be deter-
mined ~~also~~ using other indicators. As compared with the control,
the hardened cells require higher injurious temperature^S to suppress
~~the~~ plasmolysis (Alexandrov and Fel'dman 1958), for liberation of
anthocyanin from the vacuoles (Lomagin 1961), ^{for} exit of electrolytes
into the medium (Derteva), for the suppression of photosynthesis
(Liutova 1958) and respiration (Liutova 1962), for the disturbance^{ruption}
of ^{the} link between chlorophyll and chloroplasts with extrusion of the
former and its absorption by the oil globules (Kiknadze 1960).
Thus, the heat hardening is affecting entirely different components
of the cell in regard to the action of high temperatures.

An interesting problem is raised in this connection: does the
heat hardening increase the cell resistance towards the heat only,
or does it^{also} become more resistant towards other injurious agents?
We did not find in the literature any indication whether or not the
increase in heat resistance in plant cells is specific. In ~~the~~
animal cells it was indicated in a number of papers that adapta^a-
tion of cells to a certain agent can produce an increase in their
resistance towards other stimuli, which can be entirely different,
both physically and chemically (Daniel, 1909; Neuschloz, 1920;
Haffner and Wind, 1926; Orlova, 1941; Paribok, 1948; Trifonova,
1952; Barbashova and Ginetsinskii, 1956; Polianskii, 1957;
Shliakhter, 1959 and others).

The response of cells from the leaf epidermis of Tradescantia
fluminensis hardened for 18 hr at different temperatures, towards
heating, ethyl alcohol, acetic acid, and ammonia, is shown in
fig. 14. With the increase of ⁱⁿ the temperature of hardening, the
cell resistance increases not only towards the heating, but also
~~and for disruption of the link between chlorophyll and the~~
and for disruption of the link between chlorophyll and the
lipo protein complex (Liutova 1963).

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towards ~~the~~ alcohol and acetic acid. In regard to ammonia the cells did not show any definite increase of resistance. ^{The results} In Table IV ~~an experiment is shown~~ ^{an} which ⁱⁿ increased ^{the} the resistance of the cells towards ~~the~~ hydrostatic pressure. ^{An} The increase of ⁱⁿ resistance towards alcohol after heat hardening was found in the cells from the leaf epidermis of Zostera marina (exper. Fel'dman and Liutova), in the cells of parenchyma of Campanula persicifolia (Kiknadze, 1960), and in Podophyllum peltatum (exper. Liutova). ^{1962 b} ←

Our further investigations ~~had in mind to~~ ^d determine the ~~time~~ duration of the effect of hardening. We have found that this condition is labile, and during the first 24 hrs a definite decrease ⁱⁿ in resistance of cells towards heat takes place (fig. 15), as well as to some other factors.

Thus, we have found, that the plant cells may react to a large extent by nonspecific reversible increase of resistance towards the action of a high injurious temperature.

~~If to consider this fact~~ ^F from the point of view of the denaturing ^{ation} theory of injury, the results obtained are not unexpected. In order to explain the increase ⁱⁿ of resistance of the cell not only towards ~~the~~ heating, but also towards other injurious agents, often quite different in ~~their~~ nature, it is sufficient to realize that ~~at~~ ^{is} the basis of the adaptive increase of heat resistance during the heat hardening is a certain stabilization of the native state of the protoplasmic proteins (Alexander 1962c).

Lomagin, Andropova, and ^{recently} showed an increase in resistance to ethyl ether, KCN, and $CdCl_2$ of heat hardened cells of Tradescantia.

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6. The protective reaction of cells at the moment of heating.

As had been indicated above, the right straight ^{line} sections of the curves of cell heat resistance (fig. 5-7) express the dependency of the ^{streaming} motion of protoplasm ^{on} from the speed of heat denaturing ^{action} of certain proteins which are connected with the function of motion. In most of the objects this dependency ^{e persists} remains only up to ~~the~~ temperatures which stop motion during 20-80 min. At some lower temperatures this dependency ^e is ^{strongly} sharply disturbed, and the curve ^{rises} is going upwards. On the left side of the break ⁱⁿ of the curve, the protoplasmic ^{streaming} motion is preserved for a longer period ^{than} as compared ^{at} with that of the higher temperatures. In fig. 16 is given a curve of heat resistance of the grass Eleusine indica. If the period of preservation of the ^{streaming} motion in this grass were to depend upon the speed of the heat denaturation of proteins in the zone of lower temperatures, then ^{is} continuing the line ^(is extrapolated) to the left (dotted section) we would ~~have~~ expected that at a temperature of 45.0°, for example, the ^{streaming} motion of the protoplasm would ~~have~~ stopped after 47 min. In reality, the ^{streaming} motion continues for about 3000 min. at the same temperature.

What are the causes which ^e affect the break ⁱⁿ of the curve in the region of moderate heating? To elucidate this problem, I have studied the condition of the cells at different periods after the beginning of the heat ^{ing} action and at a temperature somewhat lower than that which corresponds to the ~~point of break of~~ ⁱⁿ the curve. The experiments were conducted in a special heat chamber which was installed on the stage of the microscope. A

would be expected if the relation to temperature were the same as in the high temperature range.

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piece ^{of} leaf, from Campanula persicifolia was placed in the chamber, and the same cells were observed continuously. The result of one of these experiments is given here. The motion of the spherosomes became quite slow after 40 min. of heating on the microscope stage, and the temperature of the object had reached 41.0°. ^A The further rise ⁱⁿ of temperature ^{was} ~~has been~~ stopped and ^{for} during the duration of the experiment it was maintained at 41.0-41.5°. (The break ⁱⁿ of the curve of heat resistance in this object is at 42.0°, see fig. 6). After 90 min. from the beginning of ^{the} experiment, the motion had stopped completely. ^{In spite} ^{ed} of continuing heating, after 200 min., ^{however,} several spherosomes began to move again, some exhibiting a forward motion. The motion continued to be more lively, and ^{had} ^{the} approached normal condition after 6 hr. When the heating was continued further, a secondary ^{cessation} ~~depression~~ of motion took place, and, finally the cell ~~had~~ perished. Exactly similar observations were made in the cells of Phragmites communis (Alexandrov 1956).

These experiments demonstrate the capacity of the cells to overcome the injury caused by the high temperature, not only after ^{cessation} ~~cessation~~ of heating but also during the heating, provided the heat-temperature was not too high. ^C The changes in cell metabolism must ^{resulting in} occur ^{to achieve} a repair during the ^{persisting} ~~still~~-continuing action of an injurious agent. These changes must either compensate, ^{for} or neutralize ^A the destructions which are caused by the agent. There are several investigations which deal with ~~this problem~~ (Pet'inov and Molotkovskii, 1956, 1957, 1960; Molotkovskii 1957), but the essential cause still remains obscure.

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At the temperatures which are indicated to the right of the breaking point of the curve of heat resistance, the cells do not show active resistance towards the injurious action. Thus, the breaking point of the curve should be regarded as a limit, on one side of which the cell resists heat denaturing^{ation}, and on the other side^{of which} it behaves as if it were just a part of the passive protein system.

The resistance, apparently, consists not only in the regeneration of the denatured protein but also in the increase of its resistance during the process of heat hardening of the cell.

When the cause of the cell death is compared in the two regions on both sides of the breaking point, a substantial difference can be detected in some cases. In the cells killed at higher temperature^s, which are higher than the point of resistance, neither the hood-like plasmolysis nor other signs of swelling of the cytoplasm are noticeable. ^{cap} ~~the hood-like~~ ^{On} To the contrary, at the temperatures which are lower than the limit of resistance, the swelling of the cytoplasm occurs frequently. These facts indicate that the cause^s of cell death from heat in different zones of the scale of temperature could be quite different. In the zone of higher temperatures the leading cause remains the heat denaturing^{ation} of the protoplasmic proteins. During the continuous moderate heating, the death of the cell occurs, presumably, as a result of a disruption of metabolism which leads towards^{an} accumulation of toxic products (Al'tergot 1936, 1937, 1960) and to the depletion of materials essential to life (Lundegård^h, 1930).

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Similar disturbances of metabolism can lead towards the denaturing^{ation} of the cell proteins (Nasonov and Alexandrov, 1940), but this secondary reaction is rather chemical in nature and ^a does not represent heat denaturing^{ation}.

7. ^F The factors which determine heat resistance of cells to injury caused by heat. ^{In the}

The cytophysiological analysis of the reaction of the cells to the injury^y caused by heating, shows that the result of this action by which we judge the heat resistance of cells is determined by several factors: (1) ^{the} by resistance of protoplasmic proteins towards the denaturing action of heat or, at lower temperatures, by the resistance^a of metabolic processes towards a shift in temperature; (2) ~~by~~ the reparatory ability of the cell which could occur not only after the ^{cessation} ~~secession~~ of heating, but ^{also} at lower temperatures during the heating; (3) ~~by~~ the capacity of cells to increase heat resistance, in response to the injurious action of heat, ^(heat hardening). The participation of single factors in establishing the heat resistance depends upon the temperature, the duration of heating, and the time passed after the action.

Further investigations should be directed towards: (a) the study of the physico-chemical and the biochemical mechanisms ~~which~~ are at the ^{basis} ~~foundation~~ of ^{those} single factors which determine the heat resistance of the cells, and (b) the study of the ecological significance of these factors; i. e., to determine the role which they play in adaptation of plant organism to the surrounding temperature.

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In regard to the first point, our information is very limited. We have some idea about the character of the injurious action of heating, but we hardly know anything about the processes which are connected with the reparatory action of cells and with the increase¹² of their resistance. The task of our laboratory work is directed at present to study the nature of these cell reactions. As far as the second point is concerned, the cytoecological phase of these investigations is the subject of the following chapters.

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III. CYTOECOLOGICAL STUDIES OF HEAT RESISTANCE OF PLANT CELLS.

1. Problems of Cytoecology.

A continuous and ^{reverse} manifold adaptation of organisms to the surrounding medium takes place during the process of evolution. The adaptive response towards the action of the medium occurs at different levels of ~~the~~ organization of the living matter: molecular, cellular, organismic and superorganismic ^{of} coenotic. For example, the plant may secure ~~the~~ protection against the high temperature of the medium by ~~the~~ selection of a proper coenosis, (such as growth under protection of trees), by ~~the~~ seasonal periods of development, by ~~the~~ increased transpiration, by ~~the~~ reactive increase ^{of} in heat resistance of the cells during overheating, by ~~the~~ increased stability of the cell proteins, etc.

The purpose of cytoecology consists in the study of molecular and cellular adaptations ^{individually} properly; i. e. of the peculiarities of the molecules and the cells which ^{insure} secure the ^{development} appearance of the adaptive ^{characters} results ^{even} already at these lower levels of the organization of the living matter. Without a profound investigation in the field of cytoecology a complete solution of the problem of adaptation ^f of organisms towards the surrounding medium is impossible. ^{Without it} Similarly is impossible the solution of practical ^{ly} important problems of acclimatization and resistance against frost, heat, drought and salinity of both plants and animals. The cytoecological investigations are directly connected with the cytophysiological studies of the effects of the surrounding medium upon the cells.

One of the main forms of cellular adaptation (not the only one) is the establishment of ^a relationship between the level of cell resistance to a certain factor and the degree of intensity of this factor in the medium.

2. Heat resistance of cells and the temperature conditions at which the animals exist.

As has been pointed out above, one of the postulates of the denaturing^{ation} theory of injury and excitation is the ^{assumption} admittance that the native state of the protoplasmic proteins is unstable at the temperatures compatible with the active life of the organism and is maintained in a dynamic state by the energy of cell metabolism. This postulate suggested^s the existence of a relationship between the surrounding temperature of an organism and^{the} heat resistance of its proteins. Preliminary investigations carried ^{out} on by me with the cells of the ciliated epithelium of a number of animals living at different temperatures, (Alexandrov, 1952 b), and also by some other investigators, (Battle, 1926; Runnström, 1927, 1930, 1936; Patzl, 1933; Adensamer, 1934), showed the existence of a relationship between the heat resistance of the cells and the temperature conditions under which the animals live. Later this postulate was confirmed by Ushakov and his collaborators on a ^{wide variety of} vast zoological material, (Ushakov, 1955, 1956 a, 1956 b, 1960 a, 1960 b; Svinkin, 1959; Dzhamusova, 1960 a, 1960 b; Zhirmunskii, 1960; Zhirmunskii and Pisareva, 1960; Zhirmunskii and Tsu Li-Tsun, 1960, and others). (*)

(*) These cytoecological investigations on animals ^{were} are carried ^{out} on in the laboratory of evolutionary cytology (Docent B. P. Ushakov in charge), and in the laboratory of cytology of protista (Prof. Yu. I. Polyanskii in charge), both ~~are~~ at the Institute of Cytology, Akademii Nauk, in Leningrad.

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The above-mentioned regularity appears clearly when the heat resistance of similar cells is compared in closely related species which are living at different temperatures. For example, in the frog Rana Ridibunda which lives in the south the cells of different tissues are more heat resistant ^{than} ~~as compared with~~ those from Rana temporaria (the cells compared ^{were} are those from ciliated epithelium, spinal cord ganglia, epithelium of cornea, cartilage, muscle fibers, and spermatozoids). Special studies ^{had} shown that ^{at} the ^{basis} foundation of the difference in heat resistance of cells observed was the difference in stability of the protoplasmic proteins towards ^{the} denaturing action of heat (Alexandrov and Arronet, 1956; Panteleeva and Ushakov, 1956; Brown, Nesvetaeva and Fizhenko, 1959; Kusakina, 1961).

Another important fact was established for a large number of species of animals (sea urchins, certain worms, mollusks, fishes, amphibia and reptiles), namely, that the heat resistance of the cells of the specimen of the same species is very constant and ^{is independent} ~~does not depend upon~~ ^{of whether the animal was obtained from the} ~~part of the region~~ northern or southern areas of origin (Alexandrov, 1952; Alexandrov, Ouchakov et Poljansky, 1961; Ushakov, 1955, 1956, 1958, 1960a; Ushakov and Zander, 1961; Zhirmunskii and Pisareva, 1960; Dzhamusova, 1960 c; Kusakina, 1960). There is also no difference in heat resistance of cells in poikilothermic animals collected both during the summer and the winter. This ^{indicates} ~~signifies~~ also the stability of this characteristic within the limit of the species (Alexandrov, 1952; Arronet, 1959; Shlyakhter, T. A., 1959). (*)

(*) Contrary to these data N. A. Shlyakhter (1961) reports ^{that} the heat resistance of ^{the} muscles ^{of} a frog (Rana temporaria) is somewhat higher in ^{the} summer ^{than in} ~~as compared with~~ the winter. Further works p. 32 a

from Hahn's laboratory showed increases in
the cellular heat resistance of some animals. But these changes,
however, have due not to the temperature factor but to
the age of the animals and to seasonal changes in activity of the
endocrine glands.

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A remarkable stability of temperature limits during the early stages of development of a series of marine invertebrates was observed by Runnström (1929, 1930, 1936). He considers ^{that} the limits of temperatures ^{for} of development ^{are} as a constant physiological characteristic of the species. This concerns also the heat resistance of cell proteins (Ushakov, 1959 a, b; Ushakov and Kusakina, 1960), which apparently determines the range of temperatures necessary for ~~the~~ normal embryogenesis.

In ~~the specimen~~ ^{an individual} of a certain species, the adaptation towards ~~the~~ temperature is achieved, as a rule, not through the changes in the heat resistance of ^{the} protoplasmic proteins but through some mechanisms connected with the higher levels of organization, mainly through behavior of the animal during seasonal and life cycles. In ~~the~~ phylogenesis, however, the adaptation towards the changed temperature conditions is achieved by establishment of physiological races, or new species, with ^{altered} different resistance of proteins towards the temperature factor. Therefore, the cell's heat resistance, and the limits of temperatures ^{for} of development, can serve the purposes of taxonomy, phylogenesis, and the genesis of the whole fauna (Runnström 1930, 1936; Ushakov, 1959 a, b; Zhirmunskii, 1960; Dzhamusova and Shapiro, 1960).

The ~~material on~~ ^{results with} the animal cells ^{will} shall be referred to further when a comparison with similar data on the plant cells will be made. This will give an opportunity to establish both the general biological regularities ^{and} ~~as well as~~ the differences between the plant and animal cells.

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3. Heat resistance of cells and the temperature conditions of the life of plants.

In order to evaluate
 The ecological significance of the amount of heat resistance ^{level} of the plant cells, ^{Should} is to be compared between more or less closely-related plants, ^{as well as} but which live in entirely different temperature conditions, and ~~also~~ in the same plants during different seasons of the year. During similar investigations two questions arise. immediately: (1) ^{Does} whether heat resistance remains the same in different tissues and organs; (2) ^{Does} whether it changes with the development of the plant and with the growth and differentiation of the tissues?

a) The heat resistance of cells from different ^{plant} tissues of plants.

The following two examples ~~can~~ illustrate the first question: ^{cessation} the ~~secession~~ of protoplasmic ^{streaming} motion after 5 min. of heating occurs at the temperature of about 46° in the cells from the leaf epidermis of cotton (var. 108 f), whereas in the cells of the septum of capsules at 50° (Alexandrov, 1956). This difference apparently has some ecological significance. Aisenshtadt (1952) showed that during summer ^{in fall} ~~days and under the~~ sunlight the temperature of the leaf tissues of cotton plants (usually) is 1-5° lower than that of the air. This cooling is achieved by high transpiration. At the same time, in the tissues of the capsule the temperature is 5-7 degrees higher than ^{that of the} air on the lighted side, and only 2-3 degrees on the shady side.

The second example is taken from four varieties of barley. ^{Cessation} The ~~secession~~ of protoplasmic ^{streaming} motion after 5 min. heating took place at 45.5° in the ^{epidermal} cells of epidermis from the

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leaf sheath^s leaves whereas in the cells of the spinules from the awns, it occurred at 42.5° (Alexandrov, 1956). It is quite possible that the different temperature regime^s of the tissues ^{are} is responsible for the difference. Burgerstein (1920) had described in the awns of barley highly developed stomata and a high rate of transpiration.

The data presented show that the cells from different tissues of the same plant may differ considerably in their heat resistance. Because the animal cells from different tissues show also different^{ces in} heat resistance (Ushakov, 1960 a; Rumyantsev, 1960), it is concluded that ^a the comparison of ^{the} heat resistance ^{of} in different organisms must be carried on in quite similar cells.

b) A connection between the heat resistance of plant cells and the growth.

Special studies on the relationship between the heat resistance of cells and growth ^{have} had been carried ^{out} on in our laboratory by Gorban' (1961, 1962)¹⁹⁶³. In the epidermis from Zebrina pendula Schnizl., Tradescantia fluminensis, Echeveria secunda Booth, and Dactylis glomerata she had found ^a greater sensitivity towards heating in the cells of the young growing leaves ^{than in} as compared with the cells of the leaves which had ceased to grow. There was no difference in sensitivity of the adult leaves taken from different ^{levels} layers of the same plant. For example, in Zebrina pendula the curves ^{of} for the heat resistance of the cells from the tip of the leaf of the 1st internode (counting from the top) ^{is} are ~~moved in the direction of~~ lower temperatures almost by two degrees, as compared ^{than} with the curve ^{of} of similar cells taken from the leaves of the 3d and 4th inter-

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nodes (fig. 17). The cells of the leaf from the 3d internode which had ceased to grow did not differ in their resistance from the cells of the leaves taken from the lower internodes, such as the sixth. During the winter, after general ^{cessation} of growth, the difference ^{between the} in heat resistance of the cells taken from the tips of the leaves ^{at} from different internodes disappears. The rise ⁱⁿ of the heat resistance of the cells from the leaves of ^{the} upper internodes accounts for this. In the growing leaves during the summer months, the cells at the base of the leaf are less heat resistant ^{than} as ~~compared with~~ those ^{at} from the tip. This difference ^{can} again ^{could} be explained by the higher rate of growth of the cells at the base as ^{than} ~~compared with~~ that ^{of the cells} at the tip of the leaf. In the leaves of the lower internodes which had ceased growth, there is no difference in heat resistance between the cells of the tip and those from the base. In ~~the~~ winter, after growth ceases in the upper leaves, the heat resistance of the cells at the base increases but it does not reach the level of that of the cells ^{at} from the tip.

The relationship between heat resistance and the processes of growth are still more clearly illustrated in the experiments shown in Fig. 18 where there is a difference in heat resistance ^{between} in the ^(epidermal) cells from ~~epidermis~~ of leaves of ^a Kalanchoe blossfeldiana taken from different internodes. Thus, the growing cells showed a greater sensitivity towards heating. The plant was treated with hydrazide of maleic ^{acid}. The growth had ceased and, at the same time, the difference in heat resistance of the cells from the upper and the lower leaves had disappeared. The leveling off

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of resistance ^{was due to an} ~~took place by the~~ increase ^{in the} of resistance in the upper leaves ^(until it attained) reaching the level of the lower leaves.

Therefore, ^{due to} the growth connected with cell elongation is also connected with the increased sensitivity of the cells towards heat. In the plants taken at different developmental stages there is no difference in resistance of the cells which had ceased to grow (see for example fig. 19).

The facts discussed above show that for ^a the comparison of cell resistance to heat ^{between cells of} in different plants it is necessary to take similar tissues which had ceased to grow. The difference in the developmental phases of the plant is less important.

- c) The heat resistance of protoplasmic proteins and the temperature conditions of life of plants.

The temperature conditions necessary for the existence of the plants are determined by the geographic zone ^{clear} of habitat, by the selected microclimatic ^{niche} covering, and by the seasonal periods of growth. However, the temperature of the surrounding medium does not always correspond to the temperature ^{of} at which the plant tissues live. Biebl (1950) is not quite corrected in stating that the plants are helpless in regard to the temperature factor. Thus, Lange (1959) has shown that in the desert of ^UMayritania certain plants (e. g., Citrullus colocynthis) are capable of maintaining the ¹⁷ leaf temperature almost 12° lower than that of the air because of increased transpiration. Similar data of Aisenshtadt (1952) have been given above.

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The problem of ^{the} relationship between the resistance of plants ~~towards~~ ^{the} action of high temperature and the temperature of the habitat has not been extensively investigated (we do not consider here the work on ~~the~~ thermophilic microorganisms). Sapper (1935) ~~had~~ studied the heat resistance of entire plants belonging to different families and ~~had~~ ^a come to the conclusion that in spite of certain exceptions there is a distinct connection between the ~~locality of plant~~ ^s ~~habitation~~ and its heat resistance. Biebl (1939) did not observe a correlation between the heat resistance of ~~the~~ algae and the climatic zones in which they live. However, in the algae, from the tidal zone of the Neapolitan ~~in~~ sound, Biebl ~~had~~ established a relationship between ~~the~~ heat resistance and the depth of habitat. The algae which were ~~drying~~ ^{dried} out during the ebb tide were more resistant ~~as compared with~~ ^{than} the deep water forms. In a later work Biebl (1958) ~~had~~ compared the heat resistance of a series of sublittoral ^t red algae, which have different areas of distribution, and ~~had~~ found that the cells of the southern species are ~~of~~ ^t more heat resistance ~~as compared with~~ ^{than those of} the northern ^{species}. Similar results were obtained with the algae of the Kil' Fiord (Montfort, Ried and Ried, 1955 and 1957) and with those of the White Sea (experiments of Zavadskya, Liutova, Fel'dman). Lange (1953, 1955) has shown a good correlation between resistance of lichens and mosses and the condition of their habitat. The species inhabiting wet and cool places were less resistant ~~as compared with the species~~ ^{than those} which live in ~~the~~ open, sunny locations. In ~~the~~ wet lichens ~~the~~ heat resistance was lower ~~as compared with~~ ^{than in} the dried state. It is

well known that the sensitivity of the proteins towards heating ~~in vitro~~ is becoming lower in the dry state. The differences in sensitivity towards heating between the species remained similar regardless^{of} whether the determination was made in the wet or dry state. This suggests that here also the different^{cell in} thermo-resistance of cell proteins is at the base of the differences of ~~the~~ cell resistance.

We have been studying the heat resistance of the epidermal cells from the ^{leaf} sheath ^S leaves of a large number of grasses belonging to the subfamilies of Panicoideae R. Br. and Pooideae (R. Br.) Asch. (Alexandrov, 1956). Table V shows the temperatures which stop ~~the motion of the protoplasm~~^{protoplasmic streaming} after 5 min. heating, and ~~the~~ fig. 20 indicates ^{gives the} curves of heat resistance ^{for} of a series of grasses. (*) ^{was}

As ~~has been~~^{was} indicated previously, the determination of heat resistance by ~~the motion of the protoplasm~~^{protoplasmic streaming} immediately after a ~~short-time~~^{brief} heating, ^{es} is characterizing the stability in regard to the denaturing action of heating of those proteins which are connected with ~~the function of~~^{streaming} protoplasmic motion.

The species of ^{the} subfamily Panicoideae, which include mostly tropical and subtropical plants, have more heat resistant proteins ^{than those of} ~~as compared with~~ the subfamily Pooideae which, as a rule, inhabit more temperate and cold regions. However, those species of Pooideae, which inhabit the subtropics, are either short-lived and complete their cycle of development during the cool period of the year or are found only in the mountains. One exception to this is Phragmites

(*) The figures in Table V and in Fig. 20 belong to the periods when the maximum temperature of the medium had ^{not} reached the level corresponding to that of ~~the~~ heat hardening (see further).

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which is a cosmopolitan species. Because of its relatively low heat resistance, it is capable of growing in the far north. At the same time, because of its water habitat and, presumably, high transpiration it also grows in the hot climate without becoming an ephemeral species.

Lange (1959 a, b) did not find a direct correlation between the heat resistance of the leaves (30 min. heating) and the conditions of habitat of a large number of plant species growing in the Arabian desert. However, there was a ^{direct} complete correlation between the heat resistance and the temperature of the leaves. All the plants which were capable ^{of} ^{ing} ~~to~~ lower substantially the ^r leaf temperature by transpiration showed ^a lower heat resistance of the leaves as compared with the plants which did not have these qualities. Fel'dman and Liutova (1962) have clearly demonstrated in two species of marine plants ^a the correlation between the temperature conditions ~~of the life~~ of the cells and their heat resistance. The cells of Zostera marina L., which do not dry up during the ebb tide are far more sensitive towards heating as compared ^{than} with the cells of Zostera nana Roth., which inhabits higher levels and dries up during the ebb tide.

The facts presented above, and also some data on ~~the~~ thermophilic microorganisms, ^{led} brought us to the following conclusion: the establishment of a definite level of heat resistance of the proteins is one of the ways of adaptation of species, both plant and animal, towards the temperature of the surrounding medium. The heat resistance of both plants and animals is the factor which ^{in many cases,} determines and limits the geographical distribution

of the species and also the seasonal periods and the rate of development of the organisms. If the low heat resistance limits the spreading of the organisms into the regions with a hot climate, the extremely high heat resistance serves as a limiting factor for habitation in the northern regions.

2. The stability of heat resistance ⁱⁿ of the cells of higher plants.

The heat resistance of animal cells is a constant characteristic of the species, as has been mentioned above. Do or do not ~~the~~ plants exhibit ^a similar stability of heat resistance?

Biebl (1950) considers it impossible to change the heat resistance of plants by adapting them towards high temperature. Lange (1953, 1955) ~~had~~ observed very slight differences in the heat resistance of lichens and mosses of the same species living in different temperature conditions. He considers this characteristic ~~as~~ specific for the given species.

As was stated above (fig. 9), the ^{maintenance} keeping of ^(leaf) pieces of ~~leaves~~ ^{for} 18 hrs. ^{over} at a wide range of temperatures does not influence their heat resistance except for the zone of high injurious temperatures where it is possible to increase ~~the~~ heat resistance or to obtain heat hardening. These experiments, of course, do not solve the problem ^{of} about the possibility of changing the sensitivity of ~~the~~ cells towards heating after a prolonged treatment with different temperatures. A comparison of the curves of heat resistance of cells of ~~the~~ representatives of the same species growing in different climatic conditions is of some

interest in this respect. Fig. 22 shows curves of Dactylis glomerata studied in the polar-alpine botanical garden near Kirovsk, Kola peninsula; in Leningrad, and in Diushanbe, Tadzhikistan. ^{The} Median, ^m minimum, and maximum 24-hr. temperatures for the last 30 days before the ^(points on the) curves were measured were; in the polar-alpine botanical garden 4° and 18°; in Leningrad 11.2° and 19.4°, and in Diushanbe 13° and 31°. ^{In spite} Regardless of a substantial difference in the surrounding temperatures, the curves related to ^{for} the heat resistance of ^{the} protoplasmic proteins are ^{identical} coinciding. The difference does not exceed the ^(experimental) error of experiment. A coincidence of curves was also obtained for Phragmites communis grown in Leningrad, Diushanbe and Ashkhabad, Turkmenia (fig. 23). In the Pamir mountains, plants of Swertia marginata Schrenk and Hymenolaena nana Rup., grown at elevations of 4800 met. and 3860 met., were studied simultaneously. Plants grown at ^{an} the altitude of 1000 met. higher and at a considerably lower temperature did not show any lowering of heat resistance. Year-round observations on Echeveria secunda, grown in the greenhouse of our laboratory, did not show ^a change in heat resistance. The surrounding temperature varied in ~~the~~ winter from 8-10° and in ~~the~~ summer, as a rule, was higher than 20°.

Thus, the literature, as well as our own observations indicate that in the temperate zone the heat resistance of ^{the} protoplasmic proteins of the higher plants shows ^a great stability during the change of the surrounding temperature. A shift of heat resistance of the cells of higher plants can be obtained as a result of ^{the} action of high temperatures (heat hardening) and, as we shall see further, of low temperature; both are on the verge of being injurious.

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The degree of heat resistance of plant cells which had ceased to grow and which were not subjected to the extreme temperatures represents a stable characteristic of the species. It may be used as a criterion in solving taxonomical problems. For example, some authors classify Aeluropus in the tribe of Festuceae (Bentham and Hooker, 1883, Pilger, 1954) and ^{the} a genus Aristida in the tribe of Agrostideae (Bentham and Hooker), or in the tribe Stipeae (Stapf, 1934). The Table V shows ^{that} if we were to consider the high heat resistance of the protoplasm of ~~the~~ representatives of the genera Aeluropus and Aristida, their classification among the Pooideae is not well founded. From the cytophysiological point of view the opinion of Rozhevits is correct; he classifies these genera in the tribes of ^{the} subfamily Panicoideae.

3. The "adjustment" of heat resistance of cells in lower organisms.

The cells of algae and of ^{lower} ~~the simple~~ animals exhibit a different relationship to the temperature factor as compared with the cells of higher plants and animals. Their heat resistance changes ~~in~~ accordance with the change ^{in the} of temperature ^{at} in which they live and this is true for the whole range of ~~the~~ temperature ^s scale, including the zone of the ^{optimal} temperatures. In the infusoria such an "adjustment" to ^{its} ~~the~~ temperature of ~~existence~~ is shown in the works of: Serebrovskii (1916); Polyanskii and Orlova (1948); Polyanskii (1957, 1959); Mikhal'chenko (1958); Irlina (1959); Sukhanova (1959). The changes in heat resistance of the lower organisms can be observed ^{as early as} already several hours after the change of temperature.

conditions. The changes ⁱⁿ of the level in cell thermoresistance has ^{ve} been described also for the ciliated epithelium of ctenophores (Kamilov, 1960), and in the muscles of the worm Arenicola marina (Gorodilov, 1961).

The ability ~~towards~~ heat adjustment in the zone of optimal temperatures was observed in our laboratory ¹ on the unicellular plants (Peridinium cinctum (O.F.M.) Ehr., P. bipes Stein, ^a Chlamydomonas eugametos Moewus., ^{Loznitskaya} Spirogyra sp. (Bakhteeva 1960), as well as ¹ on the higher algae (Fucus inflatus Vahl., ³ Enteromorpha compressa (L) Grev., Porphyra sp. (Liutova and Fel'dman 1960) ^{Feldman, Zavadskaya and Liutova 1963}). The level of heat resistance changes in this material ^{within} in several hours after they are removed from one temperature and ~~placed~~ into another. This shift occurs more rapid ^{ly} when the plants are ^{transferred} brought from the lower temperature ¹ into the higher ^{than} as ~~compared~~ with the reverse procedure. In fig. 24 ^F ^S shows the course of ^{the} change in heat resistance of Chlamydomonas eugametos after it has been ^{transferred} placed from the room temperature (control) ~~into~~ a lower one. This alga can lower its heat resistance almost by 4°. Similar results are obtained with the higher algae (fig. 25).

It was important to determine whether the algae in their natural habitat utilize ^{is} the ability ^{to} of adjusting ^{it} the heat resistance in accordance with the surrounding temperature. The observations of ^{Loznitskaya} Bakhteeva ¹ on Peridinium bipes have shown that ~~the~~ heat resistance does change with the change ⁱⁿ of the temperature ~~of the pond~~. In the higher algae of the White Sea, ~~the~~ heat resistance was higher during the summer months ^{than} as ~~compared~~ with that of the ^{and} in early spring ~~period~~, (experiments of Zavadskaya, ^{and} Liutova, ¹ Fel'dman, with Fucus vesiculosus L., Fucus serratus L., Ascophyllum nodosum L.)

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At present it is difficult to evaluate the significance of the shift in heat resistance ^(for the life of) in infusoria and algae. Generally, as far as the temperature factor is concerned, the reaction of the cells of the lower organisms is different as compared with the cells from the tissues of higher, organized plants and animals. Therefore, if we were to consider the heat resistance as a stable taxonomical characteristic for the species of both higher plants and animals, then we could not agree with Moewus (1940), who considers heat resistance as a taxonomical characteristic for the species of Chlamydomonas.

It is important to establish at what stage of the ^y phylogenetic development the plant and animal cells have lost their ability to adjust the ^{or} heat resistance ^{according to} by the temperature of the surrounding medium in the wide range of the temperature scale which includes the zone of the physiological optimum. The fact that during the process of evolution both the plant and the animal kingdoms have experienced this loss suggests, apparently, an important biological significance in the change towards ^{the} more constant state of the cellular proteins. Possibly this loss occurred as a by-product of some other stabilizing mechanisms. ^{This} A similar direction in the evolution of the relation of cells towards the temperature factor in the two independent kingdoms of living organisms should be regarded as an expression of the parallelism discovered by Zavarzin (1950, 1953) in the study of the evolutionary dynamics of animal tissues.

4. Heat hardening of plants growing in natural conditions. ^{under}

The Biological evolution ^{assures} secures the adaptation of the organisms towards the environment in two ways. First, the development

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of the adaptable character is guaranteed by ~~the~~ ontogenesis regardless of the ^{intensity} ~~rate of action~~ ^{the} of corresponding factor in the medium during the process of ontogenesis. In an adult organism such ^a character is stable and does not react ^{adaptively} ~~towards~~ the fluctuations in the surrounding medium. Secondly, through ^{by} the means of selection, it is not the adaptive character itself ^{that} becomes stabilized but ^{rather} the ability of the organism to react ~~accordingly~~ at the moment when the ^{intensity} ~~rate~~ ^{the} of corresponding factor has reached a certain level. ^{An} ~~As~~ ^{is the} ~~an~~ example, of the first type of adaptation may serve higher heat resistance of the protoplasmic proteins in plants of tropical origin ^{than in} ~~as compared with~~ the plants of northern and temperate zones. ^{An} ~~The~~ example of the second type is found in the ability of plants to increase their cold resistance at the beginning of the early fall frosts. There are intermediary ^{te} stages which can be combined with one another between the stationary and the reactive adaptations.

^{An} ~~The~~ increased ⁱⁿ transpiration ^{rate} is one of the strong reactive adaptations against ~~the~~ overheating of leaves of plants growing in the ~~conditions~~ of a hot, dry climate. This form of protection represents an example ^{at} ~~of~~ a supracellular level ^{of} ~~an~~ adaptive mechanism. To the cellular, or more exact^{ly}, the molecular level of adaptations belongs the ability of a number of plants (e.g., mosses and lichens) to tolerate high temperature as a result of loss of water. However, many plants do not ^{tolerate} stand a similar method of protection against overheating because the drying itself kills them. It was interesting to find out whether the ability of cells to respond to supraoptimal temperature by hardening, observed by us under ~~the~~ experimental laboratory conditions, takes place in natural habitats.

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Under the conditions of ^{the} extremely hot continental climate of Turkmenia during the spring-summer periods, Alexandrov and Yazkulyev (1961) carried out observations on heat resistance of certain plants. ^{Serving a} As the main object ^{was} served a perennial grass, which grows during the whole summer on the sands of Midasiatic deserts ~~42~~ (Aristida Karelini (Trin. et Rupr) Roshev.) It has a very high heat resistance. During the periods when the maximum temperature of the air varied from 22° to 36°, the heat resistance of the epidermal cells from the sheath leaf of Aristida Karelini remained constant. To stop ~~the motion of~~ ^{ie streaming} protoplasm after 5 min. heating, a temperature of about 49.5° was required. Slight variations ⁱⁿ of heat resistance from 49° to 50° were not connected with the change of temperature ⁱⁿ of the medium and were easily within the limits of an experimental error. ^{and individual variations} However, when the air temperature had reached 38-44°, a strong increase in ^{the} heat resistance of ^{the} cells was observed, and the temperature, which stops ^{ped} ~~the motion of~~ the protoplasm, had reached 53°. This increase in heat resistance, as well as the heat hardening which had been obtained ^{under} in the laboratory conditions, are both reversible. During ~~the~~ cooler weather, the heat resistance of the cells became ^{dropped} lower, but it had never reached the ^{original value} starting point. Moreover, during ~~the~~ hot days the heat resistance of the cells ^{in the afternoon} during ~~day~~ hours (4-5 p.m.) was higher ^{than} as compared with that of ~~in~~ the morning hours (8-9 a.m.) which ~~was~~ after a cool night (Table VI). These diurnal differences were absent, when the day temperature did not reach over 36-37° (Table VII).

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The heat hardening apparently occurs in many plants under the natural conditions of growth in ^a hot climate. Aside from Aristida Karelini, we have found it also in the grass Arundo donax and in Catalpa speciosa. In Africa during ^{the period} hot time of the year, Lange (1959) ^s has observed difference in heat resistance ^{in the} of leaves in (Acacia torulis and Citrullus colocynthis) ^{of different} in the individuals of each ^{of two} species grown under different temperature regimes and in different habitats. It is quite possible that these differences, which did not surpass 3° , were caused by different ^{hardening} heat hardening ^{in some of} of the plants.

The increase ⁱⁿ of heat resistance ^{during} in the process of natural hardening by $(2-3^{\circ})$ may have a substantial protective significance for the plant. For example, in unhardened Aristida Karelini a temperature of 49.7° stops the ~~motion~~ ^{is streaming in} of the protoplasm during 5 min., whereas in naturally hardened specimen ^s the secession ^{cessation} of ^{streaming} motion occurs after 40 minutes at 49.7° . Thus, ^{at} in regard to this temperature, ^s the natural heat hardening ^s has increased the heat resistance of ^{the} cells 8 times.

Notwithstanding the fact that the plants have diverse, and often very effective ^a adaptations against the dangerous overheating in the ~~condition~~ ^a of hot climate, many of them suffer from the injurious action of high temperature. In such circumstances, the total effect of injury depends upon the reparatory capability of the cells. We have presented above a series of facts showing that under experimental conditions in the laboratory, the cells have the ~~capability~~ ^s to restore the functions which were interrupted

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by heating. We do not know as yet to what extent this capability occurs during the natural life of the plants, and what is its significance. Further studies ^{are} needed ~~in the future~~. However, the streaming of the protoplasm, which is a very sensitive criterium^{on} of the heat injury of the cells, can be successfully used in similar investigations.

IV. COLD HARDENING AND SEASONAL CHANGES IN RESISTANCE OF PLANT CELLS TOWARDS VARIOUS AGENTS.

It has been known for a long time that ^{the} cold resistance of most ~~of the~~ evergreen- and wintergreen plants increases in the fall, reaches its maximum during ~~the~~ winter, and decreases ~~in the~~ spring again, reaching its minimum during ~~the~~ summer. The seasonal changes in ^{the} cold resistance of plants are of primary importance both scientifically and economically. The capability of plants to increase ~~their~~ ^{the level of} cold resistance during ~~the~~ winter season determines to a great extent ^{the} geographical distribution and the possibility of extending ~~the~~ valuable crops into ~~the~~ regions with a severe winter. ~~The~~ understanding of the mechanism, which is at the base of the adaptive increase ⁱⁿ of cold resistance, may serve to discover effective ways to introduce it in ~~the~~ plants useful ^{to} for man. The cytologist has a decisive role in solving this problem because the seasonal changes ⁱⁿ of cold resistance ^{of} in plants are determined to a great extent by the corresponding changes in the resistance of the cells towards the action of low temperatures. It is not our task to review here the tremendous literature on this topic (see Maximov 1913-1952; Tumanov 1940; Levitt, 1955, 1958), but merely to record the findings of our laboratory. One problem, however, needs special attention.

The degree of specificity of an agent is one of the first problems which requires solution during the study of the mechanisms of ^{the} adaptive increase ⁱⁿ of resistance of an object towards a given agent. It is necessary to determine in the first place to what extent the adaptive increase in resistance ^{of}, caused by a given agent, influences the resistance of the object towards ^{an} the action of a different kind. The

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answer to this question determines to a great extent on the path of the further investigations of the whole problem.

In the literature on cold hardening, it is possible to find some experimental data and numerous speculations indicating that with the increase ⁱⁿ of cold resistance ^{P41} the cells become less sensitive ^{also} towards some other agents. There are indications of higher resistance towards plasmolysis and deplasmolysis in ~~the~~ cold hardened cells (Scarth and Levitt, 1937; Scarth, 1944; Sulakadze, 1949), towards ice pressure (Tumanov, 1951), inundation, (Timofeeva, 1935), drying (Pisek and Larcher, 1954), drought (Siminovitch, 1940-41). Illert (1924) had mentioned the increase ⁱⁿ of heat resistance of the leaves ^{of} in Oxalis acetosella during the fall. Sapper (1935) placed flowering plants of Eranthis hiemalis for two days at the temperatures of 10°, 14° and at minus 4°. The plants kept in the cold were more heat resistant ^{than} ~~as compared with~~ the others. Cut shoots of Prunus laurocerasus, Hedera helix, Linaria cymbalaria were more heat resistant immediately after ^a winter period ~~as compared with~~ ^{than} similar shoots cut after advent of warm, rainy weather (Sapper, 1935). Tysdal (1934) found that the diastase of the alfalfa extract is more resistant ~~towards~~ ^{than in} ~~heating~~ in the winter ~~as compared with the~~ summer. Tumanov (1940) considers: "hardening... is a universal means for protection of winter crops from almost all kinds of winter-spring killing of plants." Levitt (1951, 1956 a and 1958) is inclined to find common causes at the base of drought, heat, and resistance towards osmotic water loss. However, ~~the~~ cold hardened cells do not show ^a great resistance towards ^{the} action of acetic acid and high temperature (Scarth and Levitt, 1937).

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Thus, there are certain indications in the literature that the cold hardening is nonspecific. However, in spite of considerable literature which is devoted to the problem of cold hardening in general, this ^{important} specific question has been studied very little. It is quite natural, therefore, ~~that~~ after being convinced that the heat hardening is widely nonspecific, to inquire if ^a similar condition exists for cold hardening. The plan of the investigation was quite clear. It was necessary to compare in the winter- or evergreen plants the path of the seasonal change in the cold resistance of the cells in connection with the change ⁱⁿ of their resistance to other agents.

Three grasses were selected for the study: Dactylis glomerata L., Elymus arenarius L., Elymus angustus Trin., ^{and} Hepatica nobilis Schreb. (Alexandrov, Lyutova, Fel'dman, 1959). The seasonal changes in resistance towards cold, heat and high hydrostatic pressure of the cells from the sheath leaf epidermis of the two grasses is shown in fig. 26 and 27. (Elymus angustus gave similar results). To determine ~~the~~ cold resistance, pieces of ^{leaf} sheath ~~leaves~~ were placed for 5 min. in silicon oil cooled to a given temperature. The cooling was conducted in a special semiconductive cooler. The minimum temperature after which it was still possible to detect ~~the~~ protoplasmic movement served as a measurement of cold resistance. ^R The resistances towards heating and pressure ^{was} were determined, as in the majority of above-described experiments, after 5 min. heating ^{action} and ^{was} were measured by the maximum temperature or pressure during which ^{streaming} the protoplasmic motion was still preserved.

The curves ^{for} of cold resistance of the cells had the usual

appearance in all the objects studied: there was a minimum during the summer and a maximum during the cold time of the year. Our experiments have established a fact that the seasonal changes in cold resistance are concomitant^{ed} with similar changes in resistance towards heat and high hydrostatic pressure. The curves of resistance towards these entirely different agents almost coincide for each of the grasses studied. A seasonal change in sensitivity towards ethyl alcohol is shown in fig. 18 for the cells of Dactylis glomerata. The sensitivity of the cells towards this agent is decreasing^{ed} with the advent of the winter. The results obtained by us were completely confirmed by the ~~recently published work by~~ Lange (1961). In three species of plants Erica tetralix, Asarum europeum, and Taxus boccato he obtained an increase in the heat resistance of leaves which was parallel with the increase^{ed} of cold resistance. We have also concluded observations showing concomitant seasonal changes in resistance towards cold, heating, and high hydrostatic pressure in Geum rivale L., Vinca minor L., and Calluna vulgaris Salisb. (sin. Erica tetralix) (Alexandrov, Den'ko and Shukhtina^{ina}) and Kislyuk). The same was established by Shukhtina (1962) for Carex rostrata Stokes and Carex rotundata Wahlb.

Thus, in the plants belonging to different families, a non-specific increase^{ed} of resistance of cells takes place after the cold hardening. As a result, the cells become more resistant towards the injurious action of agents of^{an} entirely different nature.

To determine whether the nonspecific increase^{ed} of cell resistance during the fall is a direct reaction to the cold, the following experiments were conducted. Dactylis glomerata was grown in

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pots which were sunk into the ground during the whole summer of 1957. On September 3 one-half of the pots ^{were} placed in a greenhouse, the temperature of which did not go beyond ^{low} 8°. The second half ~~of the lot~~ ^{the} remained in open air. At the beginning of October the resistance of the cells towards high temperature and hydrostatic pressure began to rise in both of the groups studied. This increase, however, ceased rapidly in the plants grown in ^{the} greenhouse; whereas in those remaining outside, it ~~did not~~ ^{the} ~~ceased~~ ^{continued} to increase and ~~had~~ reached the limits typical for ~~the~~ winter (fig. ~~30~~ ²⁹ and ~~31~~ ³⁰). This permits us to conclude that the nonspecific increase ⁱⁿ of cell resistance in the fall is the direct result of the action of low temperature. A somewhat insignificant initial rise of resistance is apparently connected with some other undetermined (as yet, causes.

The increase ⁱⁿ of resistance towards heating and hydrostatic pressure as a result of the action of cold was obtained experimentally also ^{at} on the summer plants of Dactylis glomerata. It was sufficient to place the plants grown in light at room temperature into the ^{cold room} ~~cooler~~ at 2° and at -4° for 2 nights (Alexandrov, Lyutova, Fel'dman, 1959). An increase ^d of heat resistance ^a in the sprouts of wheat (var. Borovicheskaya) after cold hardening in under laboratory conditions was obtained by Kislyuk (1962). In the middle of August, 1956, in the region of Kirovsk (67°30'n.1) we have observed a sudden rise ⁱⁿ of heat resistance in the cells of Dactylis glomerata, Antoxanthum alpinum and Carex bigelowii growing under natural conditions. This was caused by a night frost. Several days after the frost, the heat resistance of the cells ^{dropped} ~~has lowered~~ again to normal (Alexandrov and Fel'dman, 1958).

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It is well known that cold hardening is a reversible process. For example, according to Tumanov (1940) ^{plants of} the winter wheat variety *Lutescens* 329 taken immediately from the open ground in the winter ^{were} ~~was~~ killed at ^a the temperature of -20° ; after being kept in the greenhouse for two days ^(they were killed) at -15° ; and after 3 days, ^(they were killed) at -13° . In the cells of *Dactylis glomerata* ^{during} brought ^{the} from an open ground into warm surroundings, the resistance ~~is~~ decreased not only towards the low temperature but also towards heating and high hydrostatic pressure (fig. 32). ³¹ ¹⁵ The type of adaptation towards the severe conditions of the winter period, described for the winter-green plants, is not the only one. In the epidermal cells from the leaves of *Hepatica nobilis* we could not find neither the a winter increase ⁱⁿ of cold resistance, ^{to show from tracing} nor any tangible changes in the sensitivity of the cells towards heating or hydrostatic pressure. The young leaves ^{of} appear ⁱⁿ *Hepatica nobilis* at the beginning of May. Their epidermal cells are very sensitive to all the three agents. With the development of the leaf, the general resistance of the cells increases and reaches ^a very high level about July. This level is sufficient to secure the survival of the cells during winter. These results contradict the finding ^s of Till (1956) who had recorded an increase ⁱⁿ of cold resistance ^{of} in *Hepatica nobilis* (*Anemone hepatica*) in winter. He had estimated the cold resistance macroscopically by the appearance of the whole organs after a prolonged action of low temperature. It is possible that this condition depended upon tissues which had ^{hardened to} cold hardening in contrast to the epidermis.

We have ~~a~~ reason to believe that the presence of the initial

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high level of cold resistance (^{as} ~~like~~ in Hepatica nobilis) and the capacity ^{for} ~~towards~~ nonspecific cold hardening, which ~~exhibits also~~ ^{an} ~~the~~ increase ⁱⁿ of cell resistance to various agents, do not represent the only two ways of adaptation of the cells ~~towards~~ cold. Further investigations must discover still other adaptive mechanisms of the cell which ^{result in} secure the cold resistance of plant organisms. There are some interesting facts observed in our laboratory which may ^{and Kamentseva (1943)} speak in favor of such a possibility. Fel'dman (unpublished) have studied the resistance towards different agents in the epidermal leaves of an early spring plant Gagea lutea. In the young leaves, which appear before the ^{melts} ~~melting of the snow~~, the cells have very high cold resistance and very low heat resistance. With the development of the leaves and the increase ⁱⁿ of the ^{air} temperature of ~~the air~~, the cold resistance decreases, and the heat resistance increases. It is quite obvious that here we have reverse conditions as compared with the development and the loss of cold hardening: the two types of resistance instead of exhibiting parallel changes, ^{change} move in opposite directions.

A highly-developed capacity of the plant cells to restore the injuries caused by heating was observed in our laboratory. In connection with this we have presumed that this reparatory capacity may have a great significance for the plants subjected to overheating ^{under} ~~in the~~ natural conditions. According to our data, the comparatively easy ^{reparation} ~~reparation~~ of the heat injury is connected with the characteristic peculiarity of the injurious action of heating: (with the increase of the dose the breaking down of the cell system proceeds gradually. Therefore, the restoration of the disturbances ^{of the} of more sensitive functions proceed ^s under ~~the~~ conditions ^{of comparatively good preservation of the} when most vital components of the cell, ~~still remain comparatively well preserved.~~

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^{An} The examination of the tissues injured by cold reveals that with the increase of the dose of cooling ^{there is} occurs a different sequence of injury ^{as} compared with the heating. During the rapid cooling the change from the normal state to ~~a~~ ^{definitely} ~~killed~~ ^{death} proceeds not gradually but almost ^{in impulses}. Therefore, it is only very rarely possible to catch that state of injury after which ^{cessation} ~~the~~ ^{streaming} ~~secession~~ of protoplasmic motion can be reversed. From ^{it} ~~this~~ ^{the} follows that in cold resistance of plants the capability of cells to repair ~~the~~ cold injury plays a less significant role.

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CONCLUSIONS

The main content of ^{the} present article is concerned with the problem of cell resistance towards the action of high and low temperatures. From the study of ^{the} resistance of cells towards the action of different factors, it is easy to be convinced that there exists a whole series of common properties in the behavior of the cells towards the injuries of an entirely different nature. This makes us consider the problem of sensitivity of cells not only in respect to ^{one} that or other concrete agents but forces us to consider the general problem of resistance. Its theoretical significance is determined by the fact that ~~the~~ resistance is the main characteristic of the cell as a whole living system (Alexandrov, 1962a). The importance for practical purposes is determined by the significance of ~~the~~ cell resistance in the life of micro- and macro-organism^s. Besides ~~the~~ ecology, the problem of cell resistance is directly connected with ^{the} such divisions of medicine as veterinary, ~~and~~ agronomy, as well as in the use of pharmaceutical and toxic~~a~~ substances, insecticides, herbicides, frost-heat-drought resistance, and salt resistance, acclimatization, introduction, etc.

We have seen that ^{the} resistance of plants is a complex quality of the cell system. It is determined to a great extent by the stability of cell components and of ^{individual} the singular links of metabolism in ~~the~~ relation to the direct action of the injurious agent. The occurrence of the primary site of injury can lead towards more or less considerable destructive after-action. The character of the latter depends upon the site of the primary injury and also upon the properties and metabolic condition of the cell system. The cell, however, does not accept passively the blows, and at the moment of the action of the injurious

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agent it is possible to observe the occurrence of a protective reaction such as reparation ability and ⁱⁿ the increase of resistance.

According to ^{the} denaturing ^{action} theory of injury and stimulation, the native proteins of the protoplasm are in ^a the condition of stationary or stable non-equilibrium (Bauer, 1935). Such condition of ^{the} proteins is maintained by the energy of metabolism. The injurious agent can denature, that is, destroy the secondary and the tertiary structure of the protein molecules not only by direct action but also by way of disturbing those links of metabolism which insure the maintenance of the native state of protein. Therefore the determination of the place of application of the agent in a course of metabolic processes is not sufficient for characterization of its injurious action. It is necessary to consider the influence of the change caused by the agent upon the native state of all cell proteins. At the same time during the primary denaturing action of the agent, it is necessary to find out what relation it has to the general metabolism of the cell. As an example of ~~the~~ heat resistance we saw that a degree of stability of ^{the} native structure of the protein in relation to the ecological factor has great significance in the adaptation of plant and animal organisms towards it.

The contemporary cytologists being led by remarkable successes ⁱⁿ of the analytical direction of study of ^{the} cell and by achievements of the study of problems of auto-reproduction pay very little attention to the study of the cell as the whole system and in particular to the reparatory and adaptive phenomena (except the problems of adaptive enzymes and recovery ^{radiation} after injury).

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Reparatory action of the cells is very little studied even in the purely phenomenological relation. ~~The~~ ^I information is very scarce as to what extent the reparatory ability depends upon the nature of the injurious agent or upon the morphological ^{of} ~~type~~ ^{the} of cells. We do not know what metabolic processes cause the ^{reparation} ~~reparation~~; what role is played by the substitution of the injured components of the protoplasm by the newly-synthesized ones, and what place is occupied by the processes of homo- and heterodromic reversibility. The study of these processes requires detailed investigations of the reversibility of the ^{denaturation} ~~denaturing~~ of different proteins after the action of different denaturing agents.

In relation to ^{the} ecological meaning of cell ^{reparation} ~~reparation~~, we also possess very limited material. It is obvious only that in an experiment the cells can exhibit very high repairability.

The problem of increased resistance of cells is studied somewhat more. As concrete examples of this ability are the heat and cold hardening considered in the present papers. Their significance for plant ecology is unquestionable. The combination of the level of natural temperature resistance of the cells, stabilized ^{philogenetically} ~~philogenetically~~, with their ability to increase the resistance during the action of high temperature in the process of conditioning, ^{greatly increases the ability} ~~forms large possibility~~ for ^{to adapt} ~~adaptation~~ of plants ^{the} ~~towards~~ surrounding temperature.

A remarkable peculiarity of such hardening is that it is not specific. However, there is reason to believe that this ~~condition~~ is not ^{always true} ~~ubiquitous~~. For example, after hardening ^{in the} ~~towards~~ heat we have established an increase ^{of} ~~of~~ resistance of the cells ^{towards} ~~towards~~ heat, hydrostatic pressure,

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alcohol and acetic acid but at the same time we could not detect an increase ⁱⁿ of resistance ^{to} towards cold. Thus, it's more correct to speak about the different ^{kind of} hardening as belonging to ^{the} one type of reactions rather than about their identity (see Lange, 1961).

Besides ^{resistance to} the extreme temperatures, ^{the} non-specific increase ⁱⁿ of resistance was found by Fel'dman (1960) after wound injury. The cells adjacent to the cut in the leaves of Campanula persicifolia and Gagea lutea had ^{an} increased the resistance towards high temperature and ethyl alcohol.

Because of the non-specific nature of ^{the} increase ^{of} of resistance of cells, of the possibility to obtain it after entirely different action, ^S and also of the fact that the increase ⁱⁿ of resistance appears in a cell as a whole but not in any separate component of it, all these ^{facts lead} ~~make~~ us to postulate that at the foundation of the reaction is the increase ⁱⁿ of ~~the~~ stability of the macro-structure of the protein molecules of the protoplasm. The ~~latter~~ could be ^{due to}:

1. ~~The result of~~ ^A increase in the cell of the concentration of substances which have anti-denaturing action (in particular the sugars belong to this group).
2. ~~The occurrence of~~ ^A the change in the structure of ^{the} protein molecules which increases ^{their} ~~its~~ stability.
3. It is also possible to postulate that in some cases the heat conditioning is determined by ^{an} the increase ⁱⁿ of the processes which lead towards reconstruction ^{of} or towards ~~the~~ substitution ^{for} of the injured molecules of protein (Allen, 1950).

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The last explanation cannot be applied ~~towards~~ the cold hardening because the increased resistance in this case appears at the low temperatures which depress ~~the~~ metabolism. To judge which of the possibilities indicated above takes place in reality, is the problem to be studied in the future. However, in order to achieve substantial progress in this field it is necessary to increase considerably the study of the anti-denaturing agents ~~upon~~ the proteins in vitro and also to investigate in detail other mechanisms which stabilize the secondary and the tertiary structures of the protein molecules.

It is quite obvious that a fruitful study of the problem of cell resistance requires ^{the} a combined work of ~~the~~ cytologists and biochemists. Contrary to other ^{branches} compartments of cytology, such combined efforts do not take place in this important field.

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