Meetings

Cytoecology of Temperature

The role of cellular reactions in adaptation of multicellular organisms to environmental temperature was the subject of an international symposium held in Leningrad, 21 May–5 June. Discussions covered three main areas of research: biological effects of freezing, hardening to cold and heat, and physiological adaptation. Papers from the laboratories of B. P. Ushakov, G. I. Poljansky, and A. V. Zhirmunsky in animal cell physiology and from V. Y. Alexandrov in plant physiology dominated the program. Fourteen of the 46 presentations were made by persons outside the Soviet Union. While there were no participants from China, two of the papers were based on work done by Russians at Chinese marine laboratories.

Despite extensive translation services, a language barrier did exist. However, after several days an understanding developed of the different meanings that the Soviet and Western biologists were applying to the same terms. Alexandrov defined the subject as: "The aim of cytoecology is to study proper molecular and cellular adaptations to environmental factors." The papers emphasized physiology, ecology, and cytology more than biochemistry and biophysics. Opportunity was provided for visits to numerous institutes in Leningrad and Moscow, and the following account comprises information gathered by the three American participants from laboratory discussions and from the formal symposium.

The mechanisms of freezing and of resistance to freezing in animal tissues are still far from established. Reports were given from one Soviet laboratory, that of Lozina-Lozinsky (Institute of Cytology, Leningrad), which is conducting such experimental work. However, the majority of papers on this topic were given by non-Soviet scientists. Meryman reviewed the more prominent hypotheses for the mechanism of freezing injury, cited experimental evidence contrary to or unexplained by each, and concluded that a mechanism common to all living organisms does not necessarily exist.

Another hypothesis for freezing injury was noted by Levitt when he proposed that the removal of water to form ice results in a folding or compression of protein molecules. Sulfhydryl groups ordinarily separated by solvent may come into apposition and oxidize to form disulfide bridges. With the rehydration upon thawing, the protein expands but, due to the strength of the disulfide linkages, ruptures occur elsewhere in the protein chain. Indirect experimental supporting evidence has been obtained in plants.

Reports on experimental freezing included a study by Lozina-Lozinsky on freezing in diapausing caterpillars of the corn borer. Isolated tissue fragments from the salivary gland and trachea showed evidence of intracellular freezing after extremely rapid freezing. In hardened caterpillars the protoplasmic reticulation caused by ice crystals was reversible with rapid thawing but not in unhardened caterpillars and not after slow thawing. Although intact caterpillars will survive rapid freezing, the freezing and thawing rates are not identical to those of the excised tissue samples. Therefore, direct evidence for the survival of cells frozen intracellularly must still be obtained.

Wolfenson *et al.* from Lozina-Lozinsky's laboratory reported that tracheal epithelium from the ground squirrel, woodchuck, and rat withstood exposure to reduced temperature for periods of time directly related to the animals' ability to hibernate. In all examples the length of time that tissues could survive at reduced temperature increased as the storage temperature decreased, reaching a maximum at about 5°C. Survival declined with temperature reduction to −5°C, and rose slightly with further temperature reduction until freezing took place.

Simatos, a collaborator of Luis Rey (France), showed a film demonstrating the protection afforded to chick embryo hearts by impregnation with glycerin prior to freezing.

The relationships between lethal freezing in sea urchin eggs and the eutectic temperature of the suspending medium was noted by Asahina (Japan). Eggs suspended in solutions of pure salts, isotonic to sea water, were uniformly killed when the eutectic temperature of the salt was reached. In a mixture of salts, death occurred at the lower eutectic temperature. In some cases, intracellular ice formed; in others, none. Death appeared related to complete solidification when the last eutectic solution froze.

There are many full-time Soviet investigators engaged in plant (especially physiological) research. The Komarov Botanical Institute at Leningrad is devoted solely to research. Among its many departments, which have a staff of about 600, is the Laboratory of Cytophysiology and Cytoecology (under the direction of Alexandrov, assisted by Feldman, Lyutova, Lomagin, and about six other investigators). The main problem investigated is resistance to heat and other injuries. Alexandrov himself has a flare for developing simple but useful techniques. In order to minimize handling injury, he uses whole leaf pieces instead of epidermal strips. But because this method may result in optical difficulties, he first infiltrates the pieces by the elegantly simple method of alternating suction and pressure in a hypodermic syringe containing water or a solution. To overcome reflection from the irregular leaf surface he covers it with silicone oil. In this way, the nucleus and cytoplasmic streaming are clearly visible under the microscope, and the effect of heat and other injurious agents on them can be easily followed. In the paleobotanical field he demonstrated the ease with which polished specimens can be observed without sectioning by means of surface-reflected fluorescent light.

One of the most profitable aspects of the symposium was the opportunity to compare the concepts of the Soviet and non-Soviet investigators. In at least one case, apparent differences disappeared; the results of the German investigator Lange now do not seem to conflict with those of Alexandrov's group. There is, however, a difference in method. Whereas Lange determines killing of the whole plant or organ by heat, Alexandrov determines the temperature required to stop cytoplasmic streaming. Because Alexandrov realizes that this may occur below the killing temperature, he also determines the "reparation zone," that is, the difference (Δ°C) between the temperature required to stop streaming and the maximum temperature that permits resumption of streaming on subsequent cooling of the leaf. This "reparation zone" varies from 3.8° to 9°C in different plant species. For longer heating periods at more moderate temperatures, the zone decreases and may approach zero.

Feldman and coworkers reported that heat hardening may occur as a result of as little as 1-second heating if the temperature is high enough. This kind of hardening decreases photosynthesis and growth. It is accompanied by resistance to some other injurious factors but not to all (for example, frost) and therefore is in agreement with Lange's summer heat hardening which also does not involve frost hardening. Lyutova and coworkers showed that algae differ from higher plants in respect to adaptation to moderately high temperatures.

Another department at the Institute is concerned with frost resistance. Konovalov has succeeded in inducing sufficient resistance in many trees to enable them to survive the Leningrad winters that completely kill the untreated controls. The methods involve exposure to short photoperiods during the first years, lowering of the soil moisture, and so forth. Shkolnik has detected marked effects of trace essential elements on frost resistance.

The Institute of Plant Industry at Pushkin, in the environs of Leningrad, is a classical one, well known since the times of the former famous directors, Maximov and Vavilov. While the original freezing machine used in Maximov's early work is still functional, several additional modern chambers have replaced it. Now under the direction of Razumov, this Institute is continuing many lines of investigation. One of the experiments has resulted in barley selections that act either as spring or as winter types. When planted in the fall they form rosettes and are hardy; when planted in the spring they elongate and are nonhardy. The same results can be obtained by controlling the photoperiod. Oleinikova is using the conductivity method for determining varietal frost resistance. An interesting direct correlation with such resistance was shown for the ratio of the uptake of P^{32} at 5° to 7°C to that at 14°C.

The best known Soviet research group investigating frost hardiness is that of Tumanov. It is part of the Timiriazev Institute which is located just outside of Moscow and which contains the controlled chambers of the phytotron. Tumanov considers the hardening process as occurring in three stages: (i) the dormancy stage, during which starch is assimilated; (ii) the first hardening stage at 0°C, during which sugars accumulate and protoplasmic and auxin changes occur; and (iii) the second hardening stage which occurs at freezing temperatures down to as low as −60°C and is caused by the low temperature and the dehydration on freezing. Calorimetric measurements indicated that ice formation continues down to −60°C. Tumanov believes that frost injury is due to mechanical rather than biochemical changes and therefore found it difficult to accept Levitt's new SH hypothesis.

Many observations of freezing are being made under the microscope. Samygin has confirmed the protective effect of sugar solutions against freezing injury and Krasavtzev has observed the fluorescence of polyphenols in the frozen cells of woody plants. These substances moved from the vacuole into the protoplasm of frozen cells, thus indicating that the injury occurred during freezing. In other cases, however, injury occurred during thawing. The extracts of these substances altered the hardiness of the cells when applied externally to them.

Among the non-Soviet investigators, Teltscherova (Czechoslovakia) observed increases in soluble proteins and in proline in the shoot apexes of wheat after hardening. SH increased later in winter when development began and also during vernalization.

Heber (Germany), in describing results of his work in California, showed that freezing had pronounced effects on the activity of lipoproteins (actually the chlorophyll-protein complex) from wheat leaves. The most striking effect was an uncoupling of phosphorylation, which could be prevented by the addition of sugars before freezing. A summary of many fundamental results in the field of heat resistance of plants was made by Lange (Germany).

In the discussions which dealt with animal adaptations to heat and cold it appeared at the outset that the Soviet group differed sharply with the views of Precht and Schlieper (Germany), Rao (India), and Prosser. However, as the language barrier was gradually overcome, a deeper mutual understanding developed and general agreement resulted. The Soviet group is concerned mainly with resistance adaptation, while the Westerners are involved more with capacity adaptation. The essence of the Ushakov position is that plants and protozoans (and Hydra) show cellular acclimation to high and low temperature and that higher animals show resistance acclimation of the whole organism but not of individual cells. Poljansky has found that *Paramecium caudatum* can be acclimated to temperatures of from 0° to 30°C, and that clones vary genetically in capacity for heat tolerance. The high Q_{10} of acclimation and its reversibility indicate that acclimation within a clone is not the result of selection. He finds also that *Opalina* from a stream frog (*Rana temporaria*) has a lower heat tolerance than *Opalina* from a lake frog (*R. ridibunda*). Opalinids from the two frogs, kept at the same temperature for some days, retained their differences in thermostability, thus indicating that the differences are genetic.

Most of Ushakov's cellular work has been on heat tolerance of muscle and ciliated epithelium. Except for slight adaptive changes in excitability of summer frogs, no acclimatory changes in thermostability of sartorius muscles, of myosin adenosine triphosphatase, or of muscle cholinesterase were found. However there are seasonal changes which are associated with hormonal cycles and not necessarily related to temperature.

In many studies on both invertebrates and cold-blooded vertebrates, the muscles of various animals from the Bering Sea, the Japan Sea, the Black Sea, and from freshwater lakes show adaptive differences in thermostability which are genetic. These correlate well with ecology, and evidence was presented by Zhirmunsky that thermostability of muscles can be used to trace evolutionary trends within groups of species and to indicate paleotemperatures. Littorina from the low intertidal have less thermostable muscles than Littorina high on the shore, according to one investigator. Heat-resistant sperm from frogs was used to fertilize eggs. The tadpole offspring were compared with those from an unselected population; the thermoresistance curves showed a narrowing of the resistance-temperature curve on the lower temperature side. Thermoresistance of cholinesterase and adenosine triphosphatase from muscles of a series of fish species correlates well with temperatures of loss of excitability but not with saturation of the muscle lipids.

The series correlated well with the highest temperatures of the normal environment.

Ushakov described an interesting geographic study on populations of a frog collected over a range from northwestern Russia to central Asia. The populations at the western and eastern ends of the cline are clearly different in thermostability of muscles, those in the central region are intermediate. It is argued that cellular adaptations are important for natural selection only in gametes and that integrative adaptations are dominant in adult animals.

There was general agreement that the tolerance limits of intact animals are narrower than for cells studied in vitro. Also concurred upon was the concept shown by one of Ushakov's experiments with leeches. He postulated that there may be meaningful capacity adaptation of cholinesterase in muscle with no change in thermal tolerance of the enzyme after acclimation to different temperatures. Ushakov generalized that acclimatory changes result from "integrative" effects in whole animals, and that thermoresistance is a "conservative" character, not adaptive for cells. Prosser suggested that perhaps similar tests made on nerve cells might give results close to those with whole animals.

Because the survival-temperature curves for heat death are very steep (Q_{10} approaches 4), it is suggested that heat death of muscle may be due to protein denaturation. A possible explanation of the apparent lack of resistance adaptation in muscle may be that protein primary structure is strictly determined by the genotype, hence there can be no qualitative effects on protein structure induced by the environment. The effects induced by environment which do occur in cells of animals may be in capacity adaptation and may result from quantitative changes in various enzymes, that is, by induction, repression, and inhibition of alternate pathways, or from changes in cofactors, lipids, and so forth. There seems to be no clear case of a direct acclimation effect on primary structure of any cellular protein, and this is to be expected if all protein synthesis is genetically determined. The possibility of effects on tertiary structure is less clear.

That cellular capacity adaptation can occur was indicated by Precht (Germany) in experiments in which the anterior and posterior portions of an eel were cooled or warmed separately. Capacity adaptation in the sense of changes in oxygen consumption of muscle measured at intermediate temperatures occurred. Nervous acclimation was discussed by Prosser. In a report on hormonal factors in fish and earthworms Rao (India) noted a histological increase in height of cells secreting thyroid in Tilapia and inactivity of neurosecretory cells of a tropical earthworm when cold-acclimated. He also reported that body fluids from these cold-adapted animals stimulate oxygen consumption by isolated tissues of warm-adapted ones. The nature of such stimulating agents is unknown.

There were expectations that some vestige of Lysenkoism, in respect to cold adaptation, would be found, but Leningrad and Moscow groups seem to be going to extremes to prove that resistance adaptation in animals is genetic and that variation is fixed only by natural selection. When the question was raised in group discussions, a most emphatic denial of belief in Lysenko's theories was voiced. In fact, the Lysenkoists who are most active in the field of plant hardiness were not even included in the symposium. However, as recently as December 1962, a conference was held in Moscow on the problems of controlling heredity of farm crops.

It is apparent that in several fields Western biologists cannot neglect Soviet research. As an example, though reprints have been exchanged by the plant group for several years, a total of some three dozen new reprints and six books were presented to an American participant during the sessions. There is also an urgent need for more extensive translation of Russian articles.

The symposium was organized by the Institute of Cytology under the auspices of the Academy of Science of the U.S.S.R. with some support from UNESCO. Hospitality of the Soviet scientists to the foreign visitors was most generous and it was evident that there was a real thirst for information about the Western interpretation of research in the fields discussed. The general consensus was that more interchange between the U.S.S.R. and the United States is desirable.

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