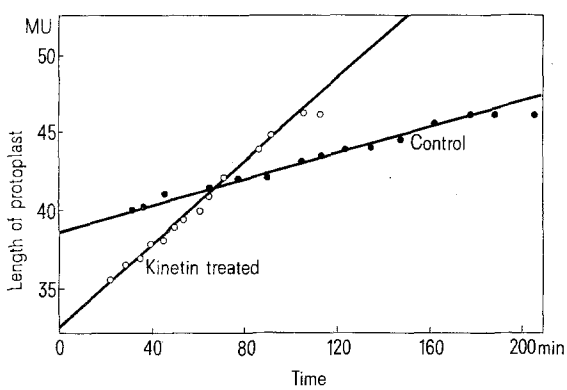
Influence of Kinetin on the Membrane Permeability of *Allium cepa* Epidermal Cells

It has been reported that kinetin 1. causes reduced capacity for absorption of phsophate in disks of beetroot tissues[1], 2. affects the uptake of monovalent cations by sunflower cotyledons[2], and 3. induces change in the selectivity of ion uptake[3]. The influence of kinetin on the membrane permeability is not clear. The purpose of this work is to study the permeability changes of onion epidermal cells that have been treated with kinetin and subsequently immersed in glycerol solutions.

Method. A plasmometric method was used for this study[4]. Treatment by kinetin was accomplished by floating upper epidermal tissue of onion (*Allium cepa*) for 10 h in kinetin solution. This solution contained 2.5 mg/l kinetin in the buffer solution (pH 6, 2.5 m*M*) with 1% sucrose. For the control, distilled water was used instead of kinetin in an otherwise identical buffer solution. The pretreated epidermis was then transferred into a perfusion chamber. A constant concentration of glycerol solution (1 *M*) was kept running through the chamber for the whole period of each experiment. A phase-contrast microscope with a time-lapse camera was used for recording the plasmolysis and the deplasmolysis of the protoplasts. The absolute permeability constant (K_s) was calculated from the deplasmolysis rate of the protoplasts in the glycerol solution. The osmotic ground value (O_g) was calculated according to the plasmolysized protoplast in the same solution. For both calculations of the K_s and O_g, the formulae were from STADELMANN and WATTENDORFF[4].



Effects of kinetin on the rate of deplasmolysis of plasmolyzed protoplasts of 2 onion epidermal cells. Onion epidermis was floated on phosphate buffer either with kinetin (2.5 mg/l), or without kinetin as a control, for 10 h, and then transferred to glycerol (1 *M*) solution. MU, means micrometer unit of an ocular micrometer.

Results and discussion. The Figure shows the time course of deplasmolysis of onion epidermal cells in glycerol (1 *M*) by plotting the changes of the protoplast lengths (MU = ocular micrometer unit) of 1 kinetin treated cell against time expressed in min. Micrometer units are converted to cm, so that the permeability constant K_s results in cm/sec^{-1} as shown in Table I. Figures for control cells of similar size to those treated with kinetin are plotted on the same graph in the Figure as the treated cells. The absolute permeability constant (K_s) of each treatment were calculated from 8 cells of the kinetin treated tissues. Controls were run similarly. The data are shown in Table I. According to this data, the experimental t-value (6.189) is greater than that from the t-table[6] (4.140; df = 14; $\alpha = 0.001$); the kinetin effect on the permeability constant is therefore very significant. On the other hand, the osmotic value of the kinetin treated cells presented in Table II, calculated from the same cells as those presented in Table I, seem to differ little from the control. Statistically, the experimental t-value (1.810) is smaller than the Table value[6] which is 2.145 (df = 14; $\alpha = 0.05$). This small variance of O_g is not significant.

Although kinetin is considered to be a compound that 1. promotes the transport, accumulation, or retention of metabolites in tissues[6], 2. induces the changes of ion uptake[2] and 3. affects certain enzyme systems[6], it does

[1] J. M. PALMER, in *Biochemistry and Physiology of Plant Growth Substances* (Eds. F. WIGHTMAN and G. SETTERFIELD, The Runge Press Ltd, Ottawa, Canada 1967), p. 401.
[2] I. ILAN, T. GILAD and L. REINHOLD, Physiologia Plant *24*, 337 (1971).
[3] I. ILAN, Physiologia Pl. *25*, 230 (1971).
[4] ED. STADELMANN and J. WATTENDORFF, Protoplasma *62*, 86 (1966).
[5] R. G. D. STELL and J. H. TORRIE, *Principles and Procedures of Statistics* (McGraw-Hill, New York 1960).
[6] D. S. LETHAM, A. Rev. Plant Physiol. *18*, 349 (1967).

Table I. Absolute permeability constant (K_s) of onion epidermal cells to glycerol (1 *M*)

Cell No.	Control $K_s \times 10^{-8}$ cm/sec^{-1}	Cell No.	Kinetin treated $K_s \times 10^{-7}$ cm/sec^{-1}
1-A	3.492	1-B	2.281
2-A	3.155	2-B	2.890
3-A	4.660	3-B	2.258
4-A	3.787	4-B	2.917
5-A	4.012	5-B	1.431
6-A	4.022	6-B	1.678
7-A	4.056	7-B	1.010
8-A	5.932	8-B	1.397
Mean	4.140 ± 0.30	Mean	1.983 ± 0.25

$t = 6.189$ df = 14

Epidermal cells were either floated on buffer solution with kinetin (2.5 mg/l), or without kinetin as a control, for 10 h.

Table II. Osmotic ground value (O_g) of the onion epidermal cells during plasmolysis in glycerol (1 *M*)

Cell No.	Control O_g m*M*	Cell No.	Kinetin treated O_g m*M*
1-A	459	1-B	426
2-A	443	2-B	400
3-A	424	3-B	394
4-A	440	4-B	477
5-A	405	5-B	518
6-A	405	6-B	433
7-A	428	7-B	454
8-A	439	8-B	510
Mean	430 ± 6.8	Mean	452 ± 17

$t = 1.181$ df = 14

Epidermal tissues were either floated on buffer solution with 2.5 mg/l kinetin, or with distilled water as a control, for 10 h.

not appear to change the osmotic value of cells (Table II). Any change in osmotic value could be an indication of fluctuations in the concentration of soluble metabolites or changes in active uptake. Since the kinetin treatment did not induce a change of O_g, changes of the absolute permeability constant of onion epidermal cells held in kinetin solution could be caused by the direct influence of this growth regulator on the membrane system. Kinetin does not appear to damage the membrane, because the rate of deplasmolysis is linear in the glycerol solution. Glycerol is known to have low oil-water coefficient. It appears likely that glycerol molecules are passing the membrane through the 'water way'[7] or by way of the 'pores'[8,9]. In the 'protein crystal model' of membrane structure[10] the 'water way' is thought to be linked to protein reaching through the membrane. Those proteins could have been changed by kinetin treatment. This, in turn, could have affected the absolute permeability constant of the treated cells.

Résumé. Nous avons employé une méthode plasmométrique pour étudier a perméabilité de la membrane d'*Allium cepa*. Après avoir fait nager l'épiderme d'un oignon sur la solution de kinetin (2,5 mg/l) pendant 10 h, on a trouvé que l'invariabilité de perméabilité a augmenté quand elle a été comparée à celles du contrôle. Le kinetin n'a pas d'influence sur la valeur osmotique reconnue des cellules de l'épiderme de l'oignon.

K. A. Feng and J. W. Unger

Department of Biology, University of Wisconsin, Oshkosh (Wisconsin 54901, USA), 17 April 1972.

[7] K. Höfler, Ber. dt. bot. Ges. *72*, 236 (1959).
[8] J. Dainty, Adv. Bot. Res. *1*, 279 (1963).
[9] J. Gutknecht, Biochim. biophys. Acta *163*, 2029 (1968).
[10] D. Green and R. F. Bucker, BioScience *22*, 13 (1972).

Oxygen Consumption of *Paragnetina media* (Walker): Light-Dark Effect on Respiratory Rates

The oxygen consumption of several stonefly (Plecoptera) species has been investigated and their oxygen consumption is related to some biological and ecological factors[1–5].

The existance of rhythms in the respiratory rate of plecopterans, however, have not been reported in the literature. If such rhythms occur then they might also be related significantly to the metabolic activity of stonefly and may very well account for different respiratory rates. The objectives of the present studies were to determine oxygen consumption rates during different time of the day and determine the effect of light and darkness on the respiration of stonefly.

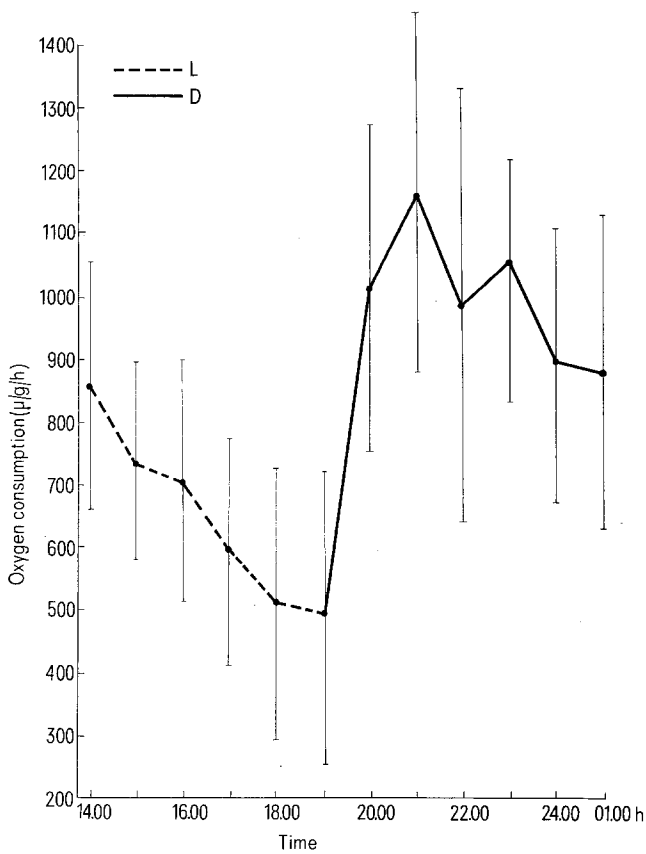


Fig. 1. Oxygen consumption of 8 nymphs during 12 h under 6 h of light (L) and 6 h of darkness (D).

Material and methods. The nymphs of *Paragnetina media* (Walker), used in the present study, were collected from the Speed River, Ontario, and were maintained in the laboratory streams[6]. Three series of experiments were run: series I. 8 animals were maintained at a photoperiod of 16L: 8D and their oxygen consumption was measured for 6 h in light (350 Lux) and 6 h in the dark; series II. 9 experiments (9 animals) were run in continuous light for 24; and series III. 3 experiments were carried out in the dark for 24 h.

The oxygen consumption of individual animals was measured with a continuous flow polarographic oxygen electrode technique. All experiments were run at a constant water flow rate of 0.36 ± 0.01 cm/sec in a constant temperature room maintained at 10 ± 1°C.

Results and discussion. The nymphs were not fed for several hours before experimentation. The animals were confined to a small area in the respiratory chamber and were unable to make any locomotory movements. Therefore, measurement of any respiratory changes in these insects was not complicated by increases in metabolism resulting from locomotory movements or from digestion and assimilation of food.

It is evident from Figure 1 that the onset of darkness caused an increase in the mean oxygen consumption and higher respiratory rates were maintained in the dark. In continuous 24 h light period, a constant decline in the respiratory rate was observed from 05.00 h to 12.00 h and higher level of oxygen uptake was maintained between 13.00–05.00 h (Figure 2). Under the continuous period of darkness, nymphs showed a decrease in oxygen consumption at 06.00, 14.00–16.00 and 23.00 h. This pattern of oxygen consumption in the dark does not correspond to the pattern in light. However, it appears that the metabolic state of animals is different under the 2 photoperiods.

[1] L. Istenič, Raspr. slov. Akad. Znan. Umet. *7*, 201 (1963).
[2] A. W. Knight and A. R. Gaufin, J. Insect Physiol. *12*, 347 (1966).
[3] T. A. Olson and M. E. Reuger, J. Wat. Pollut. Contuol Fed. *40*, R188 (1968).
[4] L. Benedetto, Int. Revue ges. Hydrobiol. Hydrogr. *55*, 505 (1970).
[5] H. B. N. Hynes, *The Ecology of Running Waters* (University of Toronto Press, Toronto 1970).
[6] N. N. Kapoor, Hydrobiologia *40*, 51 (1972).