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.

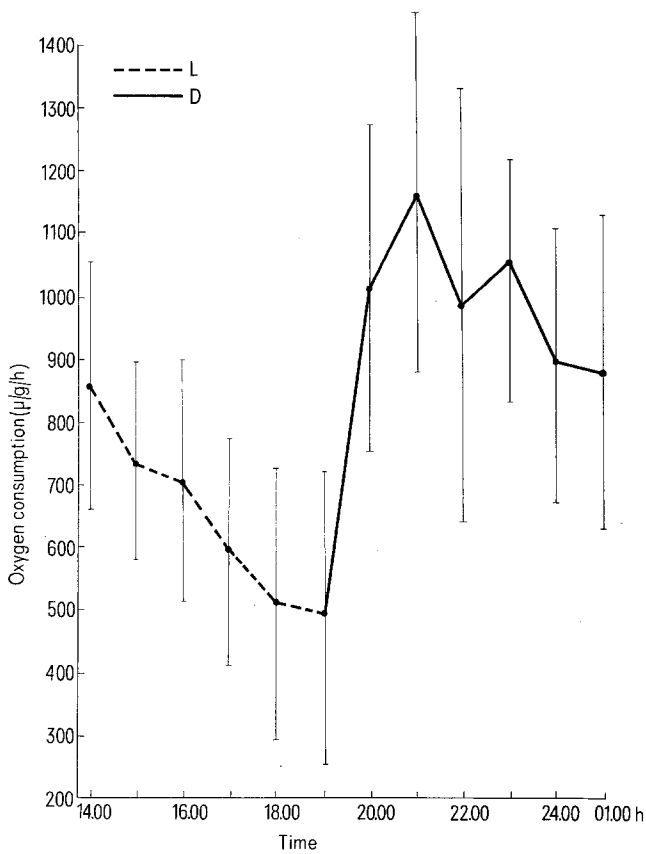

Fig. 1. Oxygen consumption of 8 nymphs during 12 h under 6 h of light (L) and 6 h of darkness (D).

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).

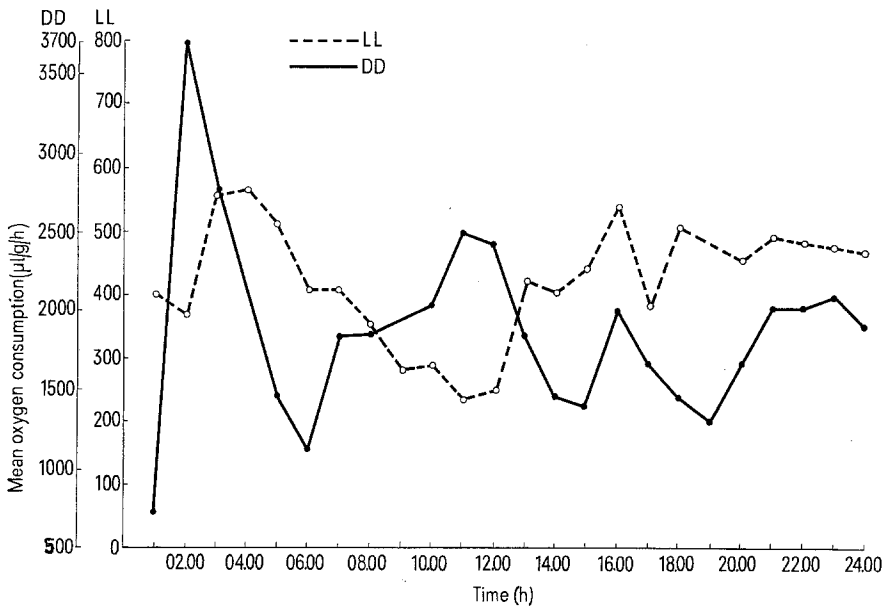

Fig. 2. Oxygen consumption of *Paragnetina media* during 24 h under continuous conditions of light (LL, 9 nymphs) and darkness (DD, 3 nymphs).

CHASTON[7,8] studied the activity and drift patterns of 2 plecopterans, *Isoperla* and, *Protonemeura*, and found them most active between sunset and sunrise. Such activity pattern was considered to contain both endogenous and exogenous components of rhythms. The light and dark period showed a definite effect on the respiratory rates of *Paragnetina media* and these variations in oxygen consumption may be related to their nocturnal habit. When the oxygen consumption was compared for light and dark, the uptake of oxygen was significantly higher in the dark ($p < 0.05$). The fact that the oxygen consumption rates showed a pattern under different photoperiods, must be considered for future studies on the respiration of plecopterans[9].

Résumé. Le taux de consommation d'oxygène des Plécoptères (stonefly) fut étudié à différentes heures de la journée. On a déterminé en outre l'effet de la lumière et de l'obscurité sur la respiration de ces insects. La consommation d'oxygène fut significativement plus élevée dans l'obscurité.

N. N. KAPOOR

Department of Biology, University of Waterloo, Waterloo (Ontario, Canada), 18 April 1972.

7 I. CHASTON, Arch. Hydrobiol. *64*, 324 (1968).
8 I. CHASTON, Am. Midl. Nat. *82*, 302 (1969).
9 This work was supported by a grant from National Research Council of Canada.

Specificity Limits of L-Leucine Transport in Chicken Small Intestine

In an effort to explore the apparent initial reaction of amino acids with the brush-border epithelium in chicken ileum, we recently developed a technique which has allowed measurement of substrate uptake in 5 sec[1]. In that work we showed leucine to be capable of entering the tissue via a saturable process which is subject to inhibition by isoleucine, but not by homoarginine in 5-sec incubations. The present study deals with the specificity characteristics of leucine absorption in short-term experiments.

Chickens, 7 to 14 weeks of age, were fasted 24 h prior to sacrifice and were then killed by cervical dislocation. A portion of small intestine 3 cm long on either side of the yolk stalk was excised and immersed in previously gassed (O_2:CO_2, 95:5% by vol) physiological saline enriched with 0.3% glucose. This solution was maintained at 37 °C. The intestine was manually stripped of mesentery and fatty tissue and then cut into segments weighing about 100 mg each. The latter were then cut lengthwise and allowed to contact towel paper to remove excess fluid. Tissue was incubated for 5 sec on a Hirsch funnel which was inserted into a vacuum flask. The reaction was monitored with a clock system that was coupled electronically to a relay, which opened or closed a vacuum line communicating with the flask. The incubation was started by pouring the preheated (37 °C) and pregassed (O_2:CO_2, 95:5% by vol) 5 ml portion of glucose-enriched Krebs-Henseleit buffer containing ^{14}C-labeled L-leucine onto the filter, and was terminated when the relay allowed a strong vacuum to evacuate substrate solution from the funnel. The tissues were simultaneously irrigated with buffer, dried of adherent fluids, weighed and then ground in a Potter-Elvehjem tissue grinder in a portion of 2.5% trichloroacetic acid, using 5 ml of this solution per g of tissue. The tissue extracts were centrifuged at 49,500 × *g* for 30 min and the ^{14}C-amino acid assayed by scintillation techniques[2].

The specificity limits of leucine uptake are presented in the Table. The site(s) reactive with this substrate appears to have preference for both straight-chain and branched-chain neutral, α-amino acids of the L-configuration which possess appreciable hydrocarbon bulk. Despite the tolerance of a wide variety of hydrocarbon conformations, virtual exclusion from the site occurs by the deletion of a single methylene group from α-amino-n-butyric acid; thus a 4-carbon backbone would seem to be a

1 W. C. LABELLE, D. S. MILLER and J. LERNER, Biochem. biophys. Res. Commun. *45*, 131 (1971).
2 G. A. BRAY, Analyt. Biochem. *1*, 279 (1960).