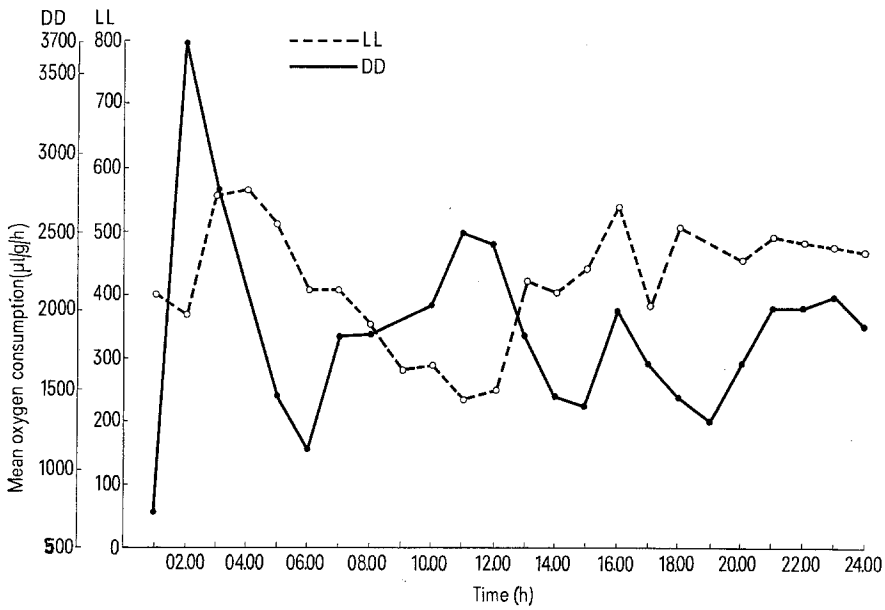

Fig. 2. Oxygen consumption of *Paragnetina media* during 24 h under continuous conditions of light (LL, 9 nymphs) and darkness (DD, 3 nymphs).

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.

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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).

Precentage inhibition data for L-leucine transport

Inhibitor (10 mM)	Inhibition of 5-sec uptake (% ± S.E.M.) Substrate (0.1 mM)
L-Methionine	65 ± 2 (9)[a]
L-Isoleucine	64 ± 2 (10)
L-Ethionine	60 ± 3 (5)
L-Norleucine	52 ± 3 (5)
L-Norvaline	51 ± 15 (4)
L-α-amino-n-butyric acid	48 ± 4 (5)
L-Valine	46 ± 4 (14)
1-Aminocyclopentane carboxylic acid (cycloleucine)	26 ± 4 (5)
Sarcosine	17 ± 7 (9)
1-Aminocyclopropane carboxylic acid	14 ± 7 (9)
L-Alanine	10 ± 5 (14)
L-Serine	9 ± 7 (5)
L-Lysine	5 ± 11 (5)
L-Proline	3 ± 8 (5)
D-Leucine (25 mM)	0 ± 4 (10)
β-Alanine	−5 ± 7 (10)
L-Glutamic acid	−9 ± 5 (5)
α-Aminoisobutyric acid (AIB)	−13 ± 8 (10)
(±) b-2-Aminobicyclo [2,2,1] heptane-2-carboxylic acid (BCH)	−15 ± 8 (10)
Glycine	−19 ± 15 (5)

[a] Number of determinations. Experimental conditions described in text.

minimum requirement for activity. N-substitution, inverting the configuration about the α-carbon atom or incorporation of formal negative or positive charge into the sidechain either abolishes or severely limits reactivity. The requirement for an α-hydrogen atom is evidenced by the low apparent affinity of cycloleucine and its cyclopropyl analog; their higher and lower homologs, BCH and AIB, are excluded. An analogy can be drawn between the mediator receptive to leucine in this study and the L system formally defined for the Ehrlich cell by OXENDER and CHRISTENSEN[3], because the reactivity of the latter agency is in proportion to the hydrocarbon mass of neutral amino acids, while AIB and N-substituted amino acids are excluded.

Zusammenfassung. Die Darmabsorption von Leucin wurde bei Hühnern in vitro im 5-Sekunden-Versuch untersucht und ein Absorptionssystem für Leucin beschrieben.

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[3] D. L. OXENDER and H. N. CHRISTENSEN, J. biol. Chem. *238*, 3686 (1963).

[4] This investigation was supported by a grant from the Maine Agricultural Experiment Station (Hatch Project No. 880-241) awarded to J. L. Part of this work was taken from the Ph. D. thesis of D. S. M.

Influence du glucose sur le transport intestinal de la phénylalanine en présence et en absence de dinitrophénol

Il est généralement admis[1] que le transport actif des acides aminés et des sucres par l'entérocyte n'a lieu que si un gradient de concentration de sodium est maintenu de part et d'autre de la membrane luminale de la cellule. Les mécanismes générateurs de l'énergie nécessaire au maintien de ce gradient sont peu connus. Le dinitrophénol, agent découplant la phosphorylation oxydative, abaisse l'énergie intracellulaire, et diminue le transport des acides aminés et des sucres[2]. Le but de ce travail a été d'étudier chez le rat les effets du glucose sur l'inhibition du transport intestinal de la phénylalanine provoquée par le dinitrophénol. L'emploi du dinitrophénol a permis de nouvelles observations sur l'interaction entre les sucres et les acides aminés au niveau de l'absorption intestinale[3].

Certains chercheurs[4–7], utilisant chez le rat la méthode des sacs d'intestin retourné, ont observé que le glucose stimulait le passage d'acides aminés à travers la paroi du sac tandis que le galactose l'inhibait; ils en ont conclu que les acides aminés et les sucres entrent en compétition pour l'énergie nécessaire à leurs transports respectifs. On a déjà souligné ailleurs[3,8] la faiblesse de cette théorie pour expliquer les interactions entre les sucres et les acides aminés, et les résultats obtenus dans notre travail avec le dinitrophénol suggèrent une autre explication.

Méthodes. On a prélevé et découpé en rondelles de 20–50 mg la partie terminale de l'intestin grêle de rats Wistar mâles anesthésiés à l'éther. Ces rondelles ont été incubées à 37 °C dans un tampon Krebs bicarbonate contenant de la L-phénylalanine-U-C^{14} en concentration 1 mM. En adjonction, les inhibiteurs suivants ont été utilisés: 2:4-dinitrophénol (1 mM), fluorure de sodium (10 mM), et glucose ou galactose (5,55 mM).

Tableau I. Analyse statistique des différences entre groupes expérimentaux

Différence	Valeur de F	Signification (P)
I et II	$F_{1,64} = 11{,}0$	$< 0{,}01$
III et IV	$F_{1,32} = 58{,}0$	$< 0{,}01$
IV et V	$F_{1,96} = 168{,}1$	$< 0{,}01$
V et VI	$F_{1,32} = 7{,}0$	$0{,}01 < P < 0{,}05$
V et VII	$F_{1,32} = 6{,}1$	$0{,}01 < P < 0{,}05$
VI et VII	$F_{1,32} = 0{,}3$	non significative

L'expérience a été planifiée en incubant 5 rondelles par rat pour chaque condition (le nombre de rats de chacune des conditions est précisé dans la légende de la Figure). Chaque paire de conditions a été soumise à une analyse de variance globale considérant les rats comme blocs casualisés et les rondelles individuelles comme répétitions.

[1] S. G. SCHULTZ et P. F. CURRAN, Physiol. Rev. *50*, 637 (1970).
[2] J. W. L. ROBINSON et J.-P. FELBER, Gastroenterologia *104*, 335 (1965).
[3] J. W. L. ROBINSON et F. ALVARADO, Pflügers Arch. *326*, 48 (1971).
[4] H. NEWEY et D. H. SMYTH, Nature, Lond. *202*, 400 (1964).
[5] J. K. BINGHAM, H. NEWEY et D. H. SMYTH, Biochim. biophys. Acta *130*, 281 (1966).
[6] B. G. MUNCK, Biochim. biophys. Acta. *150*, 82 (1968).
[7] S. REISER et P. A. CHRISTIANSEN, Am. J. Physiol. *216*, 915 (1969).
[8] F. ALVARADO, Am. J. clin. Nutr. *23*, 824 (1970).