voie glycolytique pour permettre un certain transport d'acides aminés même en présence de dinitrophénol. Cette hypothèse est confirmée par le fait que le fluorure, inhibiteur spécifique de la glycolyse, abolit l'effet du glucose. L'intestin de rat est particulièrement sensible à la présence de glucose dans le milieu d'incubation[10]; ceci pourrait expliquer la stimulation du transport des acides aminés par le glucose, qu'ont observée d'autres expérimentateurs[4–7] lors de longues incubations. Le glucose, comme le galactose et d'autres sucres[3], a un effet inhibiteur sur l'entrée initiale des acides aminés. Cet effet n'est visible, en présence de dinitrophénol, que lors de courtes incubations, puisque dans des incubations de longue durée, il est masqué par l'action énergétique du glucose.

Une explication possible de ces résultats serait celle de SEMENZA[11], selon laquelle l'inhibition du flux net des acides aminés serait due à une augmentation locale de sodium à l'intérieur des microvilli. Si ceci était vérifié dans notre expérience, on pourrait s'attendre à ce que l'inhibition entre glucose et phénylalanine soit plus grande en présence de dinitrophénol, car celui-ci favorise une accumulation intracellulaire de sodium[9]. Nous voyons que l'interaction est plus importante en absence de dinitrophénol. L'ensemble des résultats est en fait mieux expliqué par l'hypothèse d'une interaction allostérique entre les deux groupes de substrats (sucres et acides aminés) au niveau d'un transporteur multifonctionnel dans la membrane luminale de la cellule épithéliale. Cette théorie a été exposée en détail dans d'autres publications récentes[3,12].

Summary. Glucose inhibits the accumulation of L-phenylalanine by rat intestinal slices during 45-minute incubations. In the presence of DNP (which alone abolishes all active transport), glucose, but not galactose, significantly stimulates amino-acid uptake. However, during short incubations, glucose inhibits the entry of phenylalanine into the tissue, both in the presence and absence of DNP.

A.-L. LUISIER et J. W. L. ROBINSON[13]

Département de Chirurgie Expérimentale, Hôpital Cantonal Universitaire, CH-1011 Lausanne (Suisse), 12 avril 1972.

[10] J. W. L. ROBINSON, Pflügers Arch. ges. Physiol. *294*, 182 (1967).
[11] G. SEMENZA, Biochim. biophys. Acta *241*, 637 (1971).
[12] F. ALVARADO, Boln. R. Soc. esp. Hist. Nat. (Biol.) *68*, 33 (1970).
[13] Ce travail a été subventionné par le Fonds National Suisse. Nous tenons à remercier Mlles C. BRANDT et U. PÄSSLER pour leur aide technique efficace.

Gas Liquid Chromatographic Analysis of Amino Acids in the Hemolymph of *Oncopeltus fasciatus*

Although it is well known that metabolite level in the blood of mammals controls feeding[1–3], this aspect of feeding control in insects has not been studied because of the lack of techniques for quantifying solutes in very small untreated biological samples. We have used gas liquid chromatography to quantify the amino acids in the hemolymph of the large milkweed bug and to demonstrate the affect of starvation on amino acids in the hemolymph.

A Varian 1520B gas chromatograph and Model 20 recorder with disc integrator were used. The $3' \times 1/8''$ coiled glass columns were packed with 1.5% w/w OV17 on H.P. acid washed Chromosorb G[4]. The detector temperature was 250 °C and the injector was 150 °C. The program was 2 °C/min from 75° to 225 °C. N_2 gas flow was 55 ml/min and H_2 was 30 ml/min. A mixture of amino acids (2.5 mM each) was run repeatedly to determine retention temperature and to confirm the integrity of the column. The N-trifluoroacetyl n-butyl ester derivatives of the amino acids were injected on column.

Newly ecdysed 5th instar bugs were collected from the stock colony between 10.00 h and 13.00 h. On each day of the 5th instar, 2 µl of hemolymph was collected from 4 of these insects and the individual samples were analyzed for amino acids. The starved insects were deprived of food but not water for 24 h before bleeding.

The relative mole percent[4] was calculated for each peak of the chromatogram. The relative mole percent of an amino acid equals the

$$\frac{\dfrac{\text{area of amino acid peak}}{\text{amino acid standard molar response / glutamic acid molar response}}}{\dfrac{\text{areas of amino acid peaks}}{\text{amino acid standard molar response / glutamic acid molar response.}}} \times 100$$

If a peak contained 2 or 3 unresolved amino acids, the upper denominator in the above formula was multiplied by 2 or 3, respectively.

Results. The values for hydroxyproline-proline, glutamic acid, and tryptophan were lower in the fed than in the starved insects (Table). The values for the fed and starved conditions were approximately the same for threonine-glycine, isoleucine-leucine, methionine, phenylalanine-aspartic acid-histidine, tyrosine, lysine, and arginine. The values for alanine and cystine were clearly higher in the fed insects than in the starved ones. There was little variation in the quantities of individual amino acids from day to day except for alanine, glutamic acid, and cystine. In the fed bugs, alanine showed 2 periods of high relative mole percent: at the beginning of the instar and again around day 5. The second peak was not present in the starved insects. Glutamic acid was highest at the beginning of the instar and then gradually decreased. The starved bugs surprisingly had higher relative mole percents than did the fed bugs on almost all days of the instar. Cystine in the fed insects was lowest at the beginning of the instar and then gradually increased to reach its highest concentration on the last day of the instar. The concentra-

[1] J. D. DAVIS, R. L. GALLAGHER and R. LADOVE, Science *156*, 1247 (1967).
[2] J. D. DAVIS, R. L. GALLAGHER, R. F. LADOVE and A. J. TURAUSKY, J. comp. Physiol. Psych. *67*, 407 (1969).
[3] A. E. HARPER, H. J. BENEVENGA and R. M. WOHLHUETER, Physiol. Rev. *50*, 428 (1970).
[4] C. W. GEHRKE, D. ROACH, R. W. ZUMWALT, D. L. STALLING and L. L. WALL, *Quantitative Gas Liquid Chromatography of Amino Acids in Proteins and Biological Substances, Macro, Semimicro and Micro Methods* (Analytical Biochemistry Laboratories, Inc., Columbia, Mo. *1968*).

Relative mole percent of amino acids in hemolymph of *O. fasciatus*

Peak	Relative mole percent							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Alanine								
Fed	29 ± 2[a]	32 ± 9	21 ± 1	25 ± 1	29 ± 3	31 ± 4	29 ± 3	23 ± 2
Starved		18 ± 8	23 ± 4	24	22 ± 4	22 ± 2	21	29 ± 1
Threonine and glycine								
Fed	12 ± 1	13 ± 5	12	16 ± 5	15 ± 3	14 ± 3	12 ± 2	11 ± 2
Starved		6 ± 2	8 ± 2	10 ± 3	13 ± 3	10 ± 2	11 ± 2	7 ± 2
Serine and valine								
Fed	8	8 ± 2	8 ± 2	7	9 ± 3	7 ± 4	11	11
Starved		8 ± 4	9 ± 2	10 ± 2	7 ± 1	9 ± 3	8 ± 3	11 ± 2
Isoleucine and leucine								
Fed	8 ± 1	5 ± 2	7 ± 2	5 ± 3	5 ± 1	5 ± 1	3 ± 1	4 ± 2
Starved		4 ± 1	4 ± 1	4	5	5	4 ± 1	4 ± 1
Hydroxyproline, proline								
Fed	4	6 ± 1	7 ± 2	5 ± 2	5 ± 1	4 ± 2	5 ± 1	5
Starved		6 ± 1	9 ± 2	7	7 ± 1	8 ± 1	9 ± 1	6 ± 1
Methionine								
Fed	7	4	4 ± 1	5	3	2 ± 1	1	
Starved		5	6 ± 2	5 ± 3	5 ± 10	6 ± 6		5 ± 3
Phenylalanine, aspartic acid and histidine								
Fed	3 ± 1	5 ± 2	4	4	4 ± 1	3	5	4
Starved		8 ± 1	6	6	5 ± 2	5 ± 1	7 ± 1	4 ± 1
Tyrosine								
Fed	5 ± 2	2	3	3 ± 1	3	3	4	4 ± 1
Starved		3	3	3	4 ± 1	7	7 ± 1	7 ± 1
Glutamic acid								
Fed	18 ± 2	20 ± 7	16 ± 7	13 ± 3	11 ± 4	13 ± 2	14	14 ± 1
Starved		27 ± 12	19 ± 4	18 ± 2	17 ± 4	12 ± 4	15 ± 1	17
Lysine								
Fed	2	2 ± 1	3	3	3 ± 1	5 ± 2	4 ± 2	4 ± 2
Starved		4	2	3	3	3	3 ± 1	2 ± 1
Arginine								
Fed	0	0	0	0	1	1		11
Starved		3	3 ± 1	2	2 ± 2	2	3	2
Tryptophan								
Fed	4 ± 1	3 ± 1	5 ± 1	6 ± 2	5	7	7 ± 2	6
Starved		10 ± 2	6 ± 4	8	9 ± 2	9	11 ± 1	9
Cystine								
Fed	6 ± 3	11	13 ± 6	9 ± 5	9 ± 2	11 ± 1	14	15 ± 3
Starved		0	2 ± 1	0	0	0	5	6

[a] Mean $\pm$ 1 standard deviation.

tions were considerably lower for the starved bugs than for the fed but the highest concentration for the starved bugs was also on the last day of the instar.

Discussion. In addition to demonstrating the application of gas liquid chromatography to the study of blood metabolites in insects, we have shown that the state of nutrition of the insect affects the concentration of some of the free amino acids. These changes do not necessarily correspond to the relative proportions of amino acids in the diet (unpublished data). Since the values for the fed and starved conditions were the same for most of the amino acids, the milkweed bug apparently regulates the concentration of most of the amino acids in its hemolymph.

Alanine and glutamic acid were present in the highest quantity. Glutamic acid is also the amino acid in highest concentration in milkweed seeds (unpublished data), the laboratory diet of these insects. AUCLAIR[5] found that dietary glutamic acid produced an increase in blood glutamine and alanine in the German cockroach.

The use of the GLC for amino acid studies is complex and it seems to have some inherent problems; however, it is the only method that allows the analysis of non-pooled samples from small animals.

Zusammenfassung. Aminosäuren in Proben von 2 µl Haemolymphe von gefütterten und hungernden *Oncopeltus fasciatus* wurden mittels Gaschromatographie analysiert und gezeigt, dass dieses Insekt die Konzentration mehrer Aminosäuren aus Haemolymphe regulieren kann.

ELIZABETH McCLAIN[6] and DOROTHY FEIR

Department of Biology, St. Louis University, St. Louis (Missouri 63103, USA), 20 April 1972.

[5] J. AUCLAIR, J. Insect Physiol. *3*, 127 (1959).

[6] Present address: Division of Entomology, University of California, Berkeley, Calif. 94720, USA.