these values were not significantly different from controls. Carbachol, a chloride secretory stimulus that acts on muscarinic receptors on enterocytes, evoked similar changes in Isc in control (64.5 ± 4.5 $\mu A \cdot cm^{-2}$ or TTX-treated tissues (66.9 ± 4.4 $\mu A \cdot cm^{-2}$), and this lends further support to the idea that TTX did not adversely alter transport characteristics.

The results suggest that forskolin stimulates chloride secretion at low concentrations primarily by activating neurons that influence epithelial function. With increasing concentrations, the proportion of the chloride secretory response that is due to a direct effect of forskolin on the enterocytes increases, so that at the highest concentrations, neural influences become insignificant.

Although the stimulation of chloride secretion by forskolin is consistent with previously reported observations in the rat colon[4], it is not clear from those experiments whether stimulation of enteric neurons was involved in the response. The present experiments cannot distinguish the specific types of neurons that are involved in the secretory response to forskolin; however, direct evidence that forskolin alters activity in one subset of enteric neurons is provided by electrical recording from myenteric ganglion cells.

The results suggest that the use of agents like forskolin in intestinal transport studies should be carried out with the consideration that the agent may have both a direct influence on enterocyte function and an indirect influence mediated by activation of enteric nerves that subsequently alter transport processes through release of neurotransmitters.

1 Acknowledgment. The authors thank E. Martens, S. Bowen for technical assistance and N. Schaad for secretarial assistance. This work was supported by National Institutes of Health Grants, F32-AM07301, K-104-AM1004, RO1-AM29699, RO1-AM26742 and an Eloise Gerry Fellowship.

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0014-4754/85/091156-03$1.50 + 0.20/0

Free amino acid composition of the intestinal contents, intestinal cells and hemolymph of *Philosamia cynthia* larvae[1]

P. Parenti, D. Cidaria, G. M. Hanozet and B. Giordana

Dipartimento di Fisiologia e Biochimica Generali, Università di Milano, via Celoria 26, I-20133 Milano (Italy), 17 September 1984

Summary. Free amino acid composition of the intestinal contents, intestinal cells and hemolymph has been determined in larvae of the moth *Philosamia cynthia*. From the hemolymph/lumen concentration ratio, an active transport could be inferred for neutral and basic amino acids. The values of cell/lumen and hemolymph/cell ratios suggested that the active step in the transport mechanism could be localized at the luminal pole of the enterocyte for neutral amino acids (except aromatic amino acids) and at the basolateral pole of the enterocyte for basic amino acids (except arginine).

Key words. Amino acid analysis; lepidopteran larvae midgut; free amino acid composition; amino acid transport.

In the gut of lepidopteran larvae, amino acids are both actively absorbed and utilized as the main source of metabolic energy[2,3]. The ratio between the concentration of individual amino acids in the hemolymph and in the lumen can indicate the involvement of the intestinal barrier in the active and selective absorption of each amino acid. Therefore, the analysis of the free amino acid composition of the intestinal contents, intestinal cells and hemolymph can give an indication of the physiological activity of the intestinal wall. Hemolymph composition in lepidopteran larvae and the role of aminoacidemia in osmoregulation of the internal environment, as well as its contribution to the biosynthesis of silk protein, have been well established[4,5]. By contrast, no reports are available about the free amino acid composition of the intestinal cells and lumen contents. The composition of this latter compartment is almost constant since lepidopteran larvae are strictly monophagous.

In previous work, we demonstrated the secondary active transport of some neutral amino acids at the luminal membrane of the enterocytes of *Philosamia cynthia* larvae[2,6-8]. In the present paper, the free amino acid concentrations in the intestinal contents, intestinal cells and hemolymph have been measured in the same experimental substrate. The data obtained give further evidence of the selective nature of the active absorption of amino acids in lepidopteran midgut, and they will allow a more physiological choice of amino acids to be studied in transport experiments.

Materials and methods. Larvae of *P. cynthia* (Lepidoptera, Saturnidae) in the fifth instar, fed on *Ailanthus glandulosa* leaves, were used. The midgut was dissected, deprived of malpighian tubules and intestinal contents, rinsed with cold 100 mM mannitol, 10 mM HEPES-Tris pH 7.4 and rapidly homogenized in cold 0.6 M perchloric acid (4 ml/g fresh weight) with a glass teflon Thomas homogenizer, 9 strokes at 3000 rev/min. The homogenate was kept in an ice bath for 10 min and then centrifuged at 3000 × g for 15 min at 4 °C. The pH of the supernatant was adjusted to 7.0 by the addition of 2.5 M K_2CO_3. After 15 min at 0 °C, the sample was centrifuged as before and the supernatant was collected. Intestinal contents, free from leaf fragments, and hemolymph were treated with perchloric acid and processed as above described. Aliquots of the supernatants were used for glutamine assay according to Lund[9]. The remaining supernatants were diluted 1:3 with 0.1% trifluoroacetic acid:methanol 70:30 and passed through SEP-PAK C_{18} car-

Free amino acid concentration in the hemolymph, intestinal cells and lumen contents of *P. cynthia* larvae. Mean ± SE of three determinations

Amino acid	Concentration (mM) Hemolymph	Cells	Lumen	Ratio Hemolymph/Lumen	Cell/Lumen	Hemolymph/Cell
Aspartic acid	< 0.01	0.43 ± 0.11	0.77 ± 0.09	< 0.1	0.6	< 0.1
Serine	30.33 ± 4.51	15.48 ± 2.20	4.98 ± 0.39	6.1	3.1	2.0
Glutamic acid	0.14 ± 0.03	6.10 ± 0.70	1.92 ± 0.38	0.1	3.2	< 0.1
Glutamine	15.24 ± 3.36	8.57 ± 1.69	1.31 ± 0.31	11.6	6.5	1.8
Glycine	10.99 ± 0.68	5.23 ± 0.29	2.39 ± 0.23	4.6	2.2	2.1
Alanine	2.73 ± 0.46	9.41 ± 1.77	0.35 ± 0.02	7.9	26.9	0.3
Valine	1.77 ± 0.19	1.17 ± 0.35	0.27 ± 0.06	6.6	4.3	1.5
Metionine	0.21 ± 0.03	0.23 ± 0.07	0.11 ± 0.02	1.9	2.1	0.9
Isoleucine	0.87 ± 0.10	0.76 ± 0.12	0.16 ± 0.02	5.4	4.8	1.1
Leucine	0.58 ± 0.13	0.60 ± 0.16	0.24 ± 0.02	2.4	2.5	1.0
Tyrosine	2.44 ± 0.08	< 0.01	0.37 ± 0.04	6.6	< 0.1	> 100
Phenylalanine	0.70 ± 0.09	0.57 ± 0.30	0.58 ± 0.10	1.2	1.0	1.2
Lysine	4.48 ± 0.87	< 0.01	0.60 ± 0.01	7.5	< 0.1	> 100
Histidine	9.17 ± 1.53	2.42 ± 0.89	1.91 ± 0.22	4.8	1.3	3.8
Arginine	1.22 ± 0.15	1.91 ± 0.17	0.20 ± 0.02	6.1	9.6	1.2
Ornithine	9.15 ± 2.49	< 0.01	1.17 ± 0.13	6.4	< 0.1	> 100

tridges (Waters Associated, Millford, MA). The amino acid analysis on the eluate was performed by HPLC, using the apparatus supplied by Waters, and according to the procedure described in the Waters Technical Bulletin No. 3357a. The method employed a strong cation exchange column (25 × 0.46 cm) and a gradient elution system formed by Na citrate-HNO_3, pH 3.1 (buffer A) and borate-$NaNO_3$-NaOH, pH 9.6 (buffer B). The gradient elution was run through a Waters Automated System Controller mod. 720. Detection of amino acids was carried out fluorimetrically by the post-derivatization method with o-phtalaldehyde as reagent. Integration of peak area was performed by the Waters Data Module mod. 730.

Results and discussion. The table shows free amino acid concentrations in the lumen contents, intestinal cells and hemolymph of *P. cynthia* larvae. In order to calculate intracellular concentrations, tissue values (obtained as mmoles/l tissue water) were corrected for the amino acid concentrations in the extracellular luminal and hemolymph space volumes (ECS_l and ECS_h respectively). The midgut extracellular space volumes have been previously reported[10]: ECS_l and ECS_h are 10.9 ± 3.0 and 29.4 ± 2.1% tissue water (means ± SE, four experiments) respectively. The amino acid pattern of *P. cynthia* hemolymph is in many respects similar to that found in other lepidopteran larvae. Glycine and serine are the most concentrated amino acids, together with the basic amino acids histidine and lysine. These features are shared with the larvae of *Bombyx mori*[3,4,11], of *Spodoptera littoralis*[12], of *Erinnys ello*[13] and of other Lepidoptera[5]. The two neutral amino acids, with threonine, proline, aspartate, glutamate and their amides are taken up by the silk glands directly from the hemolymph for the biosynthesis of the silk proteins, whereas the basic amino acids are involved in the regulation of the hemolymph osmotic pressure[5]. It is worthnoting the high ornithine concentration, as also observed in *S. littoralis*[12] and *B.mori*[14].

All the amino acids found in the hemolymph are also present in the midgut luminal fluid, but the two profiles are quite different. As it is apparent from the hemolymph/lumen ratio reported in the table, all but acidic amino acids are more concentrated in the hemolymph than in the lumen. Therefore, transepithelial active mechanisms for amino acid transport must exist. In addition, the midgut of *P. cynthia* as well as of other lepidopteran larvae shows a transepithelial electrical potential difference of about 100 mV, lumen positive, and separates two environments with a ΔpH of about three units (luminal pH = 10, hemolymph pH = 6.8; unpublished results). In these conditions neutral amino acids bear a negative charge in the lumen and should be actively absorbed by the midgut, especially glutamine, alanine, serine, tyrosine, valine and isoleucine, which exhibit the highest hemolymph/lumen concentration ratio. Basic amino acids carry almost no charge at the luminal pH, and their accumulation in the hemolymph cannot be accounted for by the transepithelial electrical potential difference, as already observed in the *Cecropia* midgut[15].

The active step suggested by a hemolymph/lumen ratio higher than 1 could occur in principle either on the luminal or on the hemolymph pole of the enterocyte, or on both poles. Some evidence about this is given by the cell/lumen and hemolymph/cell ratios reported in the table. The ratio of cell/lumen concentrations of amino acids shows that an uphill movement of neutral amino acids, except phenylalanine and tyrosine, should occur at the luminal pole of the enterocyte. As a matter of fact, a secondary active K-dependent transport mechanism for some neutral amino acids has been characterized in brush border membranes from *P. cynthia*[2,6-8]. The potassium excretion into the lumen is the primary active transport mechanism responsible for the transepithelial electrical potential difference which provides the driving force for K-dependent amino acid absorption[2]. On the other hand, a very high hemolymph/cell ratio is present for tyrosine and basic amino acids, except arginine. At the intracellular pH, basic amino acids are present as cations and their transport across the basolateral membrane is hampered by the polarity of the transmembrane potential (−32 mV, cell negative[16]). Therefore, an active transport mechanism should be present at the basolateral membrane for these amino acids and for tyrosine. Alternatively, other tissues could contribute to their high concentration in the hemolymph. In every case, the basolateral membrane might play a role in maintaining the gradient. Up to now, only the transport of the neutral amino acids alanine, glycine, phenylalanine and α-aminoisobutyric acid has been studied with membrane preparations from lepidopteran midgut and a secondary active transport mechanism demonstrated[2,3,6-8]. The data reported in this paper suggest that active transport mechanisms possibly exist also on the basolateral membrane of the enterocyte. Therefore, a definite insight into the transport mechanisms involved in the absorption of amino acids in lepidopteran midgut is open to investigation.

1 This work was supported by grants from Italian Consiglio Nazionale delle Ricerche and from Ministero della Pubblica Istruzione, Rome. The authors are indebted to Prof. V. Capraro for helpful discussion.

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0014-4754/85/091158-03$1.50 + 0.20/0

Role of inorganic phosphate in total biliary phosphorus determination[1]

M. J. T. Jones and N. Tavoloni

Departments of Medicine (Polly Annenberg Levee Hematology Center) and Physiology, Mount Sinai School of Medicine, New York (New York 10029, USA), 15 October 1984

Summary. Total phosphorus, inorganic phosphate, and phospholipids were measured in bile of rats and guinea pigs during choleresis and cholestasis produced by taurocholate and taurolithocholate, respectively. Under either experimental condition, total biliary phosphorus concentrations increased significantly in both species, due primarily to an increase in inorganic phosphate. These studies indicate that, if total phosphorus is taken as an estimate of biliary phospholipid concentration, correction for inorganic phosphate is essential under conditions associated with changes in bile secretory function.
Key words. Biliary phospholipids, bile flow; taurocholate; glycochenodeoxycholate; taurolithocholate.

Phospholipids (lecithins, lysolecithins, sphingomyelins, and cephalins) are, together with cholesterol, the major lipids present in bile of laboratory animals and man. Determination of phospholipid content of bile is of practical importance in studies of cholesterol gallstone formation and dissolution, and of bile secretory physiology and pathophysiology. For several years, biliary phospholipids have been measured by the Fiske and Subbarow reaction[2], following acid digestion of their molecules to inorganic phosphorus[3]. Recently, an enzymatic assay has been developed[4,5], but it measures only choline-containing phospholipids. Thus, determination of total biliary phosphorus is still commonly used for quantitating total biliary phospholipids. Although it has been demonstrated that inorganic phosphate is present in bile[6], it is assumed that the contribution of the latter to the total phosphorus content is minimal compared to that made by phospholipids. Hence, extraction of phospholipids from bile is not commonly carried out, and the total phosphorus concentration is often taken as an estimate of biliary phospholipid content. In research settings, however, biliary phospholipids are frequently measured under experimental conditions associated with changes in bile secretory function, yet no studies have ever examined the inorganic phosphate content of bile during changes in bile secretion rate. In the present report, our objective was to quantitate biliary inorganic phosphate in laboratory animals during choleresis and cholestasis, and to determine its contribution to total biliary phosphorus concentration.

Methods. Male Sprague-Dawley rats (270–320 g) were anesthetized with pentobarbital (50 mg/kg, i.p.), whereas albino male guinea pigs (400–500 g) with urethan (500 mg/kg, i.p.) and pentobarbital (20 mg/kg, i.p.). Animals (fasted for 24 h) were surgically prepared for bile collection by cannulating one jugular vein, one carotid artery, and the common bile duct. In the guinea pigs, the cystic duct was ligated, after the bile in the gallbladder was aspirated. Choleresis was produced by infusing 180 μmoles/kg/30 min of sodium taurocholate (rats, n = 5) or 120 μmoles/kg/30 min of sodium glycochenodeoxycholate (guinea pigs, n = 6) through the jugular vein cannula. Taurocholate and glycochenodeoxycholate are the physiological bile salts for these respective species. Cholestasis was induced by an i.v. injection of sodium taurolithocholate at 15 μmoles/kg to both rats (n = 4)

Bile flow and biliary concentrations of inorganic phosphate, total phosphorus, and phospholipids in rats and guinea pigs

Bile	Animal species	Spontaneous[a] secretion	Choleresis During[b]	Choleresis After[c]	Cholestasis During[b]	Cholestasis After[c]
Flow (μl/min/kg)	Rat	72.5 ± 4.9	121.3 ± 9.7*	66.4 ± 5.7	26.2 ± 3.7*	64.4 ± 5.3
	Guinea pig	193.6 ± 11.7	252.4 ± 13.5*	190.8 ± 9.9	72.4 ± 6.9*	184.5 ± 10,8
Inorganic phosphate	Rat	0.88 ± 0.17	0.79 ± 0.16*	2.14 ± 0.31*	1.49 ± 0.23*	1.27 ± 0.24*
(mmoles/l)	Guinea pig	0.79 ± 0.24	0.72 ± 0.21	3.39 ± 0.85*	2.75 ± 0.52*	2.94 ± 0.76*
Total phosphorus	Rat	6.75 ± 1.33	6.52 ± 1.15	8.26 ± 1.38*	8.39 ± 1.64*	7.19 ± 1.57
(mmoles/l)	Guinea pig	1.33 ± 0.41	1.27 ± 0.43	4.23 ± 0.77*	3.61 ± 0.72*	3.54 ± 0.56*
Phospholipids (mmoles/l)	Rat	5.15 ± 0.76	4.85 ± 0.59	5.47 ± 0.83	6.95 ± 1.13*	5.96 ± 1.22
	Guinea pig	0.27 ± 0.05	0.24 ± 0.05	0.34 ± 0.07*	0.56 ± 0.08*	0.43 ± 0.12*

Values are means ± SD and were obtained from 4–6 experiments for each group (see text). [a] Values were obtained 30 min after the common bile duct was cannulated, when a steady state bile flow rate was observed. [b] During maximal increase or decrease in bile flow (see text). [c] 60 min after the bile acid infusion (choleresis) or injection (cholestasis) was given. * Significantly different (paired t-test) when compared to the value observed during spontaneous secretion ($p < 0.05$–0.001).