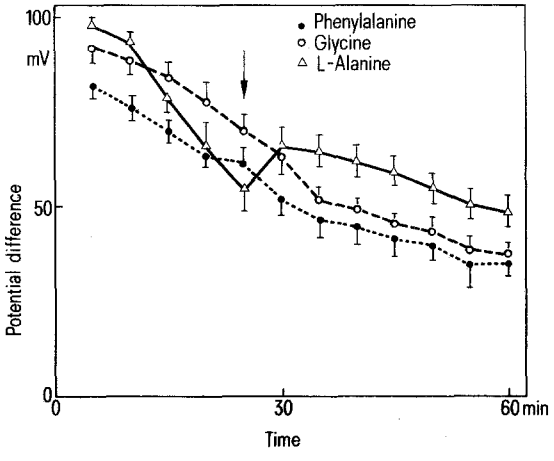
Absorption of glycine, L-alanine and L-phenylalanine in the midgut of the larvae of *Bombyx mori*

V. F. Sacchi and B. Giordana[1]

Istituto di Fisiologia Generale e di Chimica Biologica dell'Università di Milano, Via Mangiagalli 32, Milano (Italy), 12 September 1979

Summary. A net absorption of glycine, L-alanine and L-phenylalanine occurs across the isolated midgut of the larvae of *Bombyx mori*. Although glycine and L-phenylalanine are not metabolized, L-alanine is converted by the midgut and has a metabolic effect on the transepithelial electrical potential difference of the tissue.

The transepithelial transport of metabolites such as glucose and amino acids is coupled to Na absorption in vertebrate intestine: in lepidopteran larvae Na is present in extremely low concentrations (less than 5 mM) in extracellular fluids and intestinal cells. Moreover, the midgut of these insects exhibits an electrical potential difference (PD) of up to 100 mV, with the positive pole in the lumen. This PD is due to an electrogenic haemolymph-to-lumen transport of K ions[2], which has been found to be located in the "goblet cells"[3]. It has been suggested[4] that the absorption of metabolites might take place in the columnar cells. This paper reports some data on the absorption of L-alanine, glycine and L-phenylalanine in the isolated midgut of the larvae of *Bombyx mori*.



Effect of L-alanine (10 mM), glycine (10 mM) and L-phenylalanine (10 mM) on the transepithelial electrical potential difference (PD) in the isolated midgut of *Bombyx mori*. The amino acids were added to the fluids bathing both sides of the tissue when indicated by the arrow. The PD recordings were performed by means of a Keithley microvoltmeter 155 (Keithley Instr.). Each point is the mean of 9 experiments ± SE.

Lumen to haemolymph (influx) and haemolymph to lumen (outflux) fluxes of L-alanine, glycine and L-phenylalanine across the isolated midgut of *Bombyx mori*

	Influx	Outflux	Net flux
L-Alanine	113.2± 6.7 (4)	41.1±7.8 (4)	72.1±10.3
Glycine	101.6± 7.8 (4)	10.4±2.3 (4)	91.2± 8.1
L-Phenylalanine	171.9±22.5 (4)	45.7±7.5 (4)	126.2±23.7

Mean ± SE, number of experiments in parenthesis. The net flux is the difference between the mean influxes and the mean outfluxes. Fluxes are expressed in μmoles/g dry weight·h.

Materials and methods. Midgut sections of larvae of *Bombyx mori* were isolated and mounted as previously described[5]. The saline had a cationic composition similar to that determined in the haemolymph: 1.7 mM NaCl, 21 mM KCl, 25 mM K HCO_3, 44 mM $MgSO_4$, 9 mM $CaCl_2$, 110 mM sucrose. Both luminal and haemolypmphatic solutions were aerated and stirred by bubbling with 95% O_2 and 5% CO_2, pH 7.4. In order to measure the unidirectional fluxes, L-alanine or glycine or L-phenylalanine 10 mM were added to the physiological solutions. 4 μCi/ml of ^{14}C uniformly labeled amino acid (Radiochemical Centre, Amersham) were added to the mucosal solution to measure the influx, and 1 μCi/ml of labeled amino acid was added to the haemolymphatic solution to measure the outflux. Samples were taken from mucosal and haemolymphatic solutions after an equilibration period of 20 min, necessary to obtain steady fluxes, and after 60 min. Radioactivity was measured by means of a liquid scintillation spectrometer (Tri-Carb Packard 3003 series). At the end of the experiments the tissue was removed, gently blotted with filter paper (Whatman No. 1), weighed, put into a tared tube with 1 ml distilled water, frozen, thawed, resuspended and centrifuged for 30 min. The supernatants and flux samples were used to test the integrity of the amino acids by ascending 1-dimensional paper chromatography, the solvent being n-butanol -:- acetic acid -:- water (12:3:5). Sediments were then dried overnight and weighed. The effect of L-alanine, glycine and L-phenylalanine 10 mM on the PD was also tested.

Results and discussion. A net absorption of the 3 amino acids occurred in the midgut of *Bombyx mori* (table) even if the flux measurements were performed in the absence of any concentration gradient. The flux ratios between the influx (lumen to haemolymph) and the outflux (haemolymph to lumen) are 2.7 for L-alanine, 9.7 for glycine and 3.8 for L-phenylalanine. The difference between the influx and the outflux could be ascribed to a solvent-drag effect: however, no net water movement takes place across the midgut, as has been seen both by means of inulin ^{14}C luminal dilution, and by weighing the midgut perfused as a sac at given times (unpublished results). The fluxes have been measured in the presence of the spontaneous PD; however, at pH 7.4 the 3 amino acids tested are virtually uncharged.

The absorption of these amino acids is therefore an active process. An active transport of α-aminoisobutyric acid and of lysine has also been demonstrated in the isolated *Hyalophora cecropia* midgut[6,7].

In order to investigate whether any metabolism of the amino acids occurs, fluxes and tissue extracts have been tested by means of paper chromatography. L-phenylalanine and glycine are not metabolized in the midgut: the former is not metabolized in mammalian intestine either[8], and it has already been seen that the latter is scarcely metabolized by *Bombyx mori*[9] and by the midgut of other insects[10].

On the other hand L-alanine is converted by the midgut, since about 20% of the radioactivity of both tissue extracts and fluxes is found in a spot different from that of L-

alanine; so the unidirectional fluxes of this amino acid are overestimated, and the net flux must be smaller. It is interesting that L-alanine and glycine are not essential amino acids in the larvae of *Bombyx mori:* besides, L-alanine can be obtained in the midgut by transamination of L-glutamic acid[11]. Phenylalanine is one of the 10 essential amino acids[12] and this could explain the high rate of absorption found in vitro (table).

Metabolization of L-alanine was suggested in a previous paper[13] to explain the effect of this amino acid on the PD. The figure shows that only L-alanine causes a relevant rise of the PD when added to the perfusion fluids; the metabolism of this amino acid could supply energy for the activity of the K-pump. From these experiments it is apparent that an active transport mechanism for neutral amino acids is present in the gut wall of the larvae of *Bombyx mori.* The nature of this mechanism can as yet only be hypothesized; the model proposed by Crane[14] for solute uptake in mammalian intestine involves an electrochemical gradient as driving force. In the lumen and midgut tissue, Na concentration is very low and no chemical gradient of this ion is present across the mucosal barrier[15]. Therefore the amino acid would have to be co-transported with a cation different from Na. On the other hand, amino acid absorption could take place via an active transport mechanism located on the lumen side, as demonstrated for *Hyalophora cecropia*[10].

1 Acknowledgments. We are very much indebted to Prof. V. Capraro for helpful advice and criticism. We are also grateful to Prof. G. Reali for his interest and support of this research.

2 K. Zerahn, in: Transport of ions and water in animals, p.381. Ed. B.L. Gupta, R.B. Moreton, J.L. Oschman and B.J. Wall. Academic Press, London/New York/San Francisco 1977.
3 J.T. Blankemeyer and W.R. Harvey, in: Water relations in membrane transport in plants and animals, p.161. Ed. A.M. Jungreis, T.K. Hodges, A. Kleinzeller and S.G. Schultz. Academic Press, New York/San Francisco/London 1977.
4 T.V. Schultz and A.M. Jungreis, Tissue Cell *9,* 255 (1977).
5 B. Giordana and V.F. Sacchi, Rc. Accad. naz. Lincei *60,* 151 (1976).
6 S. Nedergaard, J. exp. Biol. *56,* 167 (1972).
7 S. Nedergaard, in: Transport mechanism in epithelia, p.372. Ed. H.H. Ussing and N.A. Thorn. Munskaard, Copenhagen 1973.
8 R.P. Spencer and A.H. Samiy, Am. J. Physiol. *200,* 501 (1961).
9 G. Bosquet, J. Insect Physiol. *22,* 541 (1976).
10 S. Nedergaard, in: Transport of ions and water in animals, p.239. Ed. B.L. Gupta, R.B. Moreton, J.L. Oschman and B.J. Wall. Academic Press, London/New York/San Francisco 1977.
11 T. Fukuda, Nature *180,* 245 (1957).
12 T. Inokuchi, Bull. seric. Exp. Stn. *24,* 407 (1970).
13 B. Giordana and V.F. Sacchi, Comp. Biochem. Physiol. *61 A,* 605 (1978).
14 R.K. Crane, Fedn Proc. *24,* 1000 (1965).
15 B. Giordana and V.F. Sacchi, Comp. Biochem. Physiol. *59 A,* 17 (1978).

Effect of conditioned media on nerve cell differentiation

M. Sensenbrenner[1], G.G. Jaros, G. Moonen and B.J. Meyer

Centre de Neurochimie du CNRS, 11 rue Humann, F-67085 Strasbourg Cedex (France); Department of Physiology, University of Pretoria (South Africa); Institut d'Histologie, 20 rue de Pitteurs, B-4020 Liège (Belgium), and Institut de Médecine, Secteur de Neurologie, Université de Liège, B-4020 Liège (Belgium), 27 June 1979

Summary. Glial conditioned medium strongly stimulates the morphological maturation of cultured neuronal cells, while fibroblast and meningeal conditioned media have weaker effects.

Conditioned media contain factors that influence growth[2-4] and differentiation[5-9] of various animal cells in vitro. More specifically concerning the differentiation of nerve cells, it was found that tumoral glial cells[10] and brain primary cultures[11] released factors which induced differentiation of neuroblastoma cells. Conditioned medium from spinal ganglionic cell and heart cell fibroblast cultures was demonstrated to promote neurite outgrowth of ganglionic neurons[12].

In previous reports from our laboratory concerning the study of chick embryo cerebral hemisphere nerve cell differentiation, we reported that brain extracts enhance the maturation of neuronal and glial cells in culture[13,14]. Recently, we reported that a preformed glial cell layer significantly enhanced the differentiation of dissociated neuroblasts settled on this layer, while a preformed meningeal cell layer or a fibroblast layer had a less pronounced effect[15,16].

The aim of the present study was to extend investigations concerning the influence of factors released by other cells on nerve cell differentiation. For this purpose the effects of conditioned media from cultures of fibroblasts, of meningeal cells and of glial cells were studied.

Materials and methods. Cell suspensions were obtained by passing the chick embryo tissues through a nylon sieve (48 and 82 µm pore size)[15]. The resulting dissociated cells were harvested in nutrient medium which consisted of Eagle's basal medium (GIBCO) supplemented with 20% fetal calf serum (GIBCO), 50 units of penicillin/ml and 50 µg of streptomycin/ml. The cells were cultivated in Falcon plastic Petri dishes (60 mm) and incubated at 37 °C in a humidified atmosphere of 95% and 5% CO_2. The nutrient medium was changed twice a week.

Conditioned media were obtained from cultures of fibroblasts, of meningeal cells and of glial cells. Fibroblast cultures were derived from 6-, 8- or 15-day-old chick embryos. Meningeal cell cultures were established from the meningeal membranes of 8- or 15-day-old chick embryo brain. Glial cell cultures were obtained from 15-day-old chick embryo cerebral hemispheres[15]. After 10 days of incubation, when a cell monolayer had been formed, the supernatant conditioned medium (CM) was collected every 24 h during 1 week. This CM was then centrifuged for 10 min at 1000 × g to remove any residual cells and stored at −20 °C.

Control cultures of a mixed neuronal and glial cell population were obtained from cerebral hemispheres of 7-day-old chick embryos and cultivated on a collagen substrate as described previously[17]. Control cultures composed essentially of neuronal cells were prepared from 7-day-old chick embryo cerebral hemispheres and the cells were cultivated on a collagen substrate in Eagle's basal medium supplemented with 1% fetal calf serum and with 200 ng/ml of a chemically synthesized tripeptide Gly-His-Lys[18].