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].

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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

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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].

Experimental cultures were established under the same conditions as those for control cultures. At the first medium exchange (2 days) the normal nutrient medium was replaced by the particular conditioned medium which was then changed twice a week.

Results and discussion. The evolution of dissociated cells from chick embryo cerebral hemispheres cultured on a collagen substrate and in the basal nutrient medium has been described previously in detail[13,17]. After 3 weeks, dispersed differentiated neurons were observed upon a layer of astroblasts as shown in figure 1, a. The cultures exposed to any fibroblast CM evolved similarly to the control cultures. However, an increased population of large multipolar neurons, with a clear nucleus containing a distinct nucleolus had developed after 3 weeks (figure 1, b). In the presence of any meningeal CM a stimulatory effect was observed as early as the 1st week in culture. The size of the neuronal bodies were larger and the fibres were more abundant than in the control cultures. Some large multipolar neurons appeared after 2 weeks in culture and were still present after 3 weeks (figure 1, c). However, between 2 and 3 weeks more nerve cells were seen to degenerate than in the control cultures. Conditioned medium from the glial cell cultures was observed to enhance all morphological aspects of neuronal differentiation intensively (figure 1, d). The average size of the neurons was greater than those in the control cultures after 48 h and a dense outgrowth of nerve fibres with numerous bifurcations were evident. After 3 weeks most of the neurons had a mature morphology containing a centrally localized clear nucleus, with typically 1 or 2 nucleoli (figure 1, e).

In the control cultures, as well as in the cultures exposed to the various conditioned media, the underlying astroblast layer remained unchanged.

In the presence of the synthetic tripeptide the neurons differentiated in the absence of glial cells, the proliferation of which was greatly suppressed[18] (figure 2, a). However, these neurons which had no contact with glial cells degenerated after 7 days in culture. The fibroblast CM had no stimulatory effect upon the isolated nerve cells and these neurons did not survive longer than 1 week, as observed in the control cultures. In the presence of meningeal CM the average size of some of the isolated neurons was greater than in the control cultures. This CM maintained some neurons without contact with glial cells for an average of 10 days. However, most neurons had degenerated by this time and after 14 days all neurons disappeared from the cultures. In the presence of glial CM some neurons were larger after 5 days (figure 2, b) than the neurons in the control cultures and most neurons survived after 10 days. However, after 2 weeks these neurons also began to degenerate.

The results of this morphological study have shown that conditioned medium obtained from fibroblast cultures had only a light stimulatory effect on the differentiation of brain neurons in culture. Moreover, this effect occurred only when glial cells were present in the culture. Indeed, this CM was unable to enhance the maturation of the isolated neurons or to maintain them for a longer period. In the mixed neuronal-glial cell cultures the meningeal CM influenced the maturation of the neurons, especially during the early stages of the culture. The presence of typical large multipolar neurons after 3 weeks in vitro showed similari-

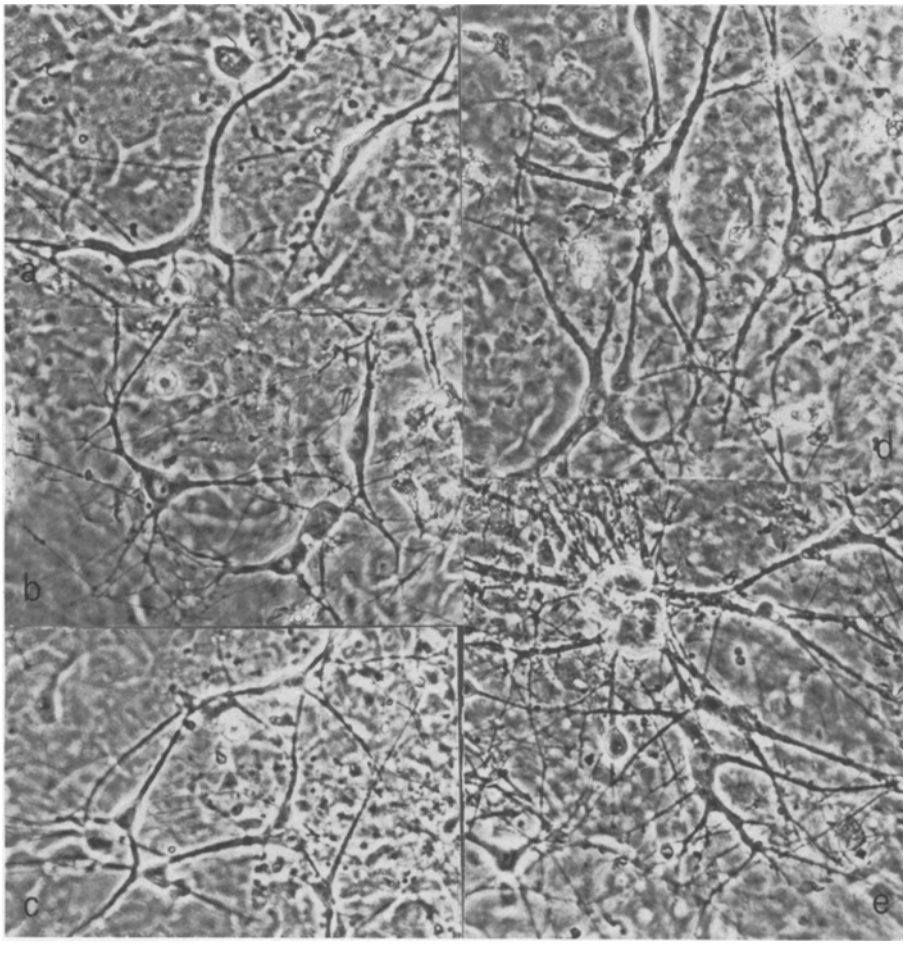

Fig. 1. Dissociated nerve and glial cells from 7-day-old chick embryo brains in culture. Phase contrast micrographs. *a* 3-week-old culture in the basal nutrient medium; *b* 3-week-old-culture exposed to fibroblast CM; *c* 3-week-old culture exposed to meningeal CM; *d* 2-week-old culture exposed to glial CM; *e* 3-week-old culture exposed to glial CM. × 190.

ties to the effect of the meningeal cell layer on nerve cell differentiation which were described previously[15]. It was also observed that some of the isolated neurons can survive in this condition for a longer period of time in culture. Therefore, the meningeal CM seemed to contain active factors which influenced the maintenance of some neurons. However, the most pronounced stimulatory effect was observed with the glial CM both on the neurons in the mixed population and on those which were well isolated. Suggestions that there are exchanges between neurons and glial cells have been made for a long time. Our results show that substances released by glial cells had a favorable effect upon neuronal maturation and survival. A similar fact has been described by Monard et al.[10], who observed that CM obtained from tumor glial cells stimulated the differentiation of neuroblastoma cells in culture. Luduena[12] showed that spinal ganglionic neuron differentiation is stimulated equally by heart fibroblast and by glial cell conditioned media. Our observations indicate that neurons from the central nervous system are more dependent on the specific presence of glial cell conditioned medium in the culture.

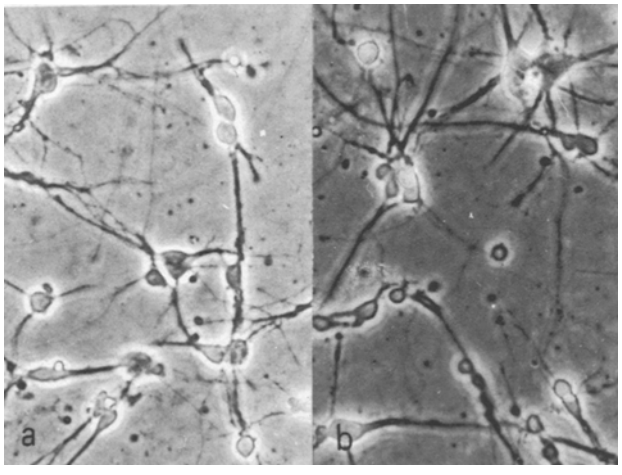

Fig. 2. Isolated neurons from 7-day-old chick embryo brains in culture. Phase contrast micrographs. *a* Control culture after 5 days; *b* 5-day-old culture exposed to glial CM. × 190.

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2 N.C. Dulak and H.M. Temin, J. Cell Physiol. *81,* 153 (1973).
3 N.C. Dulak and H.M. Temin, J. Cell Physiol. *81,* 161 (1973).
4 S.W. Melbye and M.A. Karasek, Exp. Cell Res. *79,* 279 (1973).
5 H.P. Gordon and M.C. Brice, Exp. Cell Res. *85,* 303 (1974).
6 V.L.P. Schacter, Exp. Cell Res. *63,* 19 (1970).
7 M. Solursh and S. Meier, Devl Biol. *30,* 279 (1973).
8 K. Watanabe, Devs. Growth Differentiation *13,* 107 (1971).
9 N.K. White and S.D. Hauschka, Exp. Cell Res. *67,* 479 (1971).
10 D. Monard, E. Solomon, M. Rentsch and R. Gysin, Proc. natl Acad. Sci. *70,* 1894 (1973).
11 Y. Schurch-Rathgeb and D. Monard, Nature *273,* 308 (1978).
12 M.A. Luduena, Devl Biol. *33,* 268 (1973).
13 M. Sensenbrenner, N. Springer, J. Booher and P. Mandel, Neurobiology *2,* 49 (1972).
14 P. Athias, M. Sensenbrenner and P. Mandel, Differentiation *2,* 99 (1974).
15 M. Sensenbrenner and P. Mandel, Exp. Cell Res. *87,* 159 (1974).
16 M. Touzet and M. Sensenbrenner, Devl Neurosci. *1,* 159 (1978).
17 J. Booher and M. Sensenbrenner, Neurobiology *2,* 97 (1972).
18 M. Sensenbrenner, G.G. Jaros, G. Moonen and P. Mandel, Neurobiology *5,* 207 (1975).

Germination inhibition activity of a naturally occurring lignan from *Aegilops ovata* L. in green and infrared light

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Summary. The naturally occurring lignan, monoepoxylignanolide (MEL) from *Aegilops ovata* L., inhibits germination of lettuce achenes (seeds) in incandescent light but not in the dark. The 'action spectrum' after preincubation of MEL in darkness shows inhibition in the regions of 399 nm and 712–804 nm, but after pretreatment with incandescent light inhibition is seen at 500–577 nm and 712–804 nm. The infrared inhibition by MEL is not reversible by red light. The pretreatment of MEL with incandescent light gives rise to a photoproduct which, together with MEL itself, inhibits the germination of lettuce achenes in the dark.

We have previously reported[1,2] the occurrence of a naturally occurring lignan from *Aegilops ovata* L. which inhibits the germination of lettuce achenes in incandescent light but not in darkness. So far, the known naturally occurring germination inhibitors[3,4] have been found to be more effective in darkness than in light; this report was the first describing such a compound (a monoepoxylignanolide (MEL)) possessing a reverse activity. This novel phenomenon has now been investigated further. The activity of MEL was tested at various concentration levels and the effect of variations of wavelength of light on MEL during the inhibition of germination was examined. Irradiation of MEL using incandescent light shows that this lignan is photolabile and gives rise to a photoproduct which, when tested together with MEL, inhibits the germination of lettuce achenes in the dark, although neither compound alone shows such an effect.

The solubility of MEL in water was initially determined using UV calibration on standard solutions in ethanol. The maximum solubility was found to be 160 ppm. From this saturated stock solution of MEL in H_2O different dilutions were prepared, and tested by placing a filter paper with 50 lettuce achenes, *Lactuca sativa* cv. 'Great Lakes' in a Petri dish containing 1.5 ml of solution, and germination tests were carried out in darkness and under light. Although not effective in darkness, MEL inhibits germination in light even at concentrations of 20 ppm (figure 1).

In a separate set of experiments using materials and methods already described[1], filter papers were impregnated with 1 mg quantities of pure MEL and put into Petri dishes. Each of these papers was covered with a 2nd untreated filter paper, and distilled water (1.5 ml) was added. The dishes were then left for about 22 h for equilibration. These pretreatment conditions were necessary since MEL, as