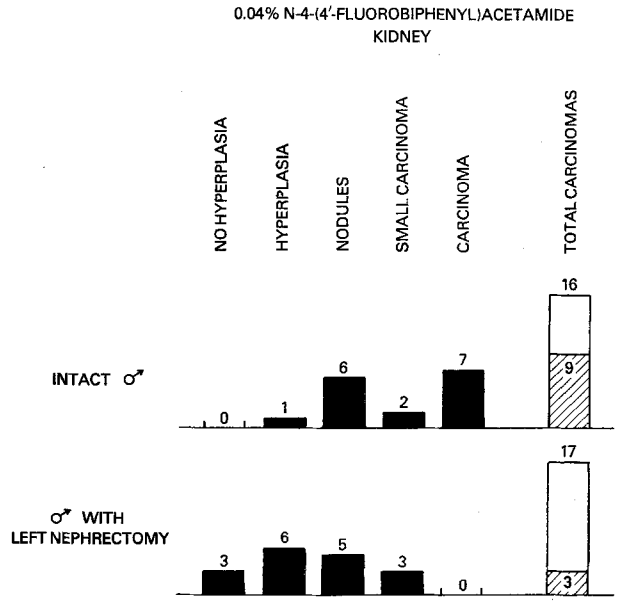
and 5 rats with a uninephrectomy that did not have carcinomas of the kidney.

Seven of 16 intact rats also developed carcinomas of the liver; however, the rats with renal carcinomas either did not have hepatic carcinomas or the carcinomas were less than 5 mm in size. The incidence of carcinomas of the liver was greater in the rats with a uninephrectomy (13 of 17) and the carcinomas were large[12].



The most advanced lesion in a given animal is indicated. Number above each black bar is the total number of animals developing such lesions. 'Total carcinomas' indicates total number of animals in the group, with the shaded area representing the number with carcinomas.

Discussion. Buffalo strain inbred male rats are more susceptible to hyperplasia and carcinomas of the kidney induced by 4'-F-4BAA[8,9]. Other studies concerning the influence of endogenous or exogenous factors on these renal lesions have not been done.

Renal tumors that occur in the intact male golden hamster given s.c. stilbestrol are inhibited by androgen[13]. Stilbestrol-treated hamsters with a uninephrectomy develop renal tumors much earlier than the treated intact animals[14]. It was postulated that stilbestrol may not be metabolized by the liver of the hamster, thus accounting for the fact that the hamster, and not other species, have stilbestrol-induced renal tumors.

4'-F-4BAA is metabolized by the rat liver and the metabolites are excreted by the kidneys. Animals with one kidney apparently were not able to secrete the metabolites of 4'-F-4BAA as readily as rats with both kidneys intact. The metabolites were returned to and acted upon the liver and increased the incidence of hepatic carcinomas[15].

It is not known whether4'-F- 4BAA or some metabolite causes carcinoma of the kidney or why there was a decreased incidence of renal carcinomas in rats with a unilateral nephrectomy.

[12] M. D. Reuber, unpublished data (1975).
[13] H. Kirkman, Progr. exp. Tumor Res. *24*, 1569 (1972).
[14] E. S. Horning, Br. J. Cancer *8*, 627 (1954).
[15] M. D. Reuber, Path. Microbiol. *42*, 119 (1975).

Uptake of L-Glutamate and L-Aspartate in Neurones and Glial Cells of Cultured Human and Rat Spinal Cord

Elisabeth Hösli and L. Hösli

Institute of Physiology, University of Basel, Vesalgasse 1, CH-4051 Basel (Switzerland), 3 December 1975.

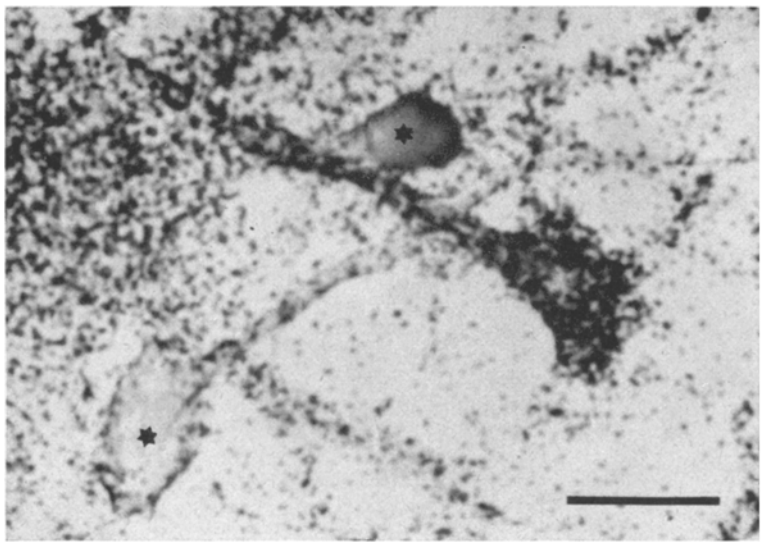
Summary. Autoradiographic investigations on the uptake of L-glutamate and L-aspartate have shown that the amino acids were taken up by neurones as well as by glial cells of cultured human and rat spinal cord. The activity of glutamate and aspartate varied considerably between individual neurones, whereas glial cells showed a more even distribution of the labelled amino acids. Our results suggest that both neurones and glial cells are involved in the uptake of amino acid transmitters.

Investigations on the regional distribution of L-glutamate and L-aspartate in the cat spinal cord have shown that the glutamate concentrations were highest in the dorsal grey and in the proximal part of the dorsal roots whereas aspartate levels were highest in the ventral grey[1-3]. It has been observed by electrophysiological studies that glutamate and aspartate depolarize spinal neurones (for ref. see[4]) and that this depolarization is associated with an increase in sodium permeability[5-7]. From these results it has been suggested that both amino acids may function as excitatory transmitters in the spinal cord, glutamate being released by primary afferent fibres[1,2,8] and aspartate being associated with interneurones[2].

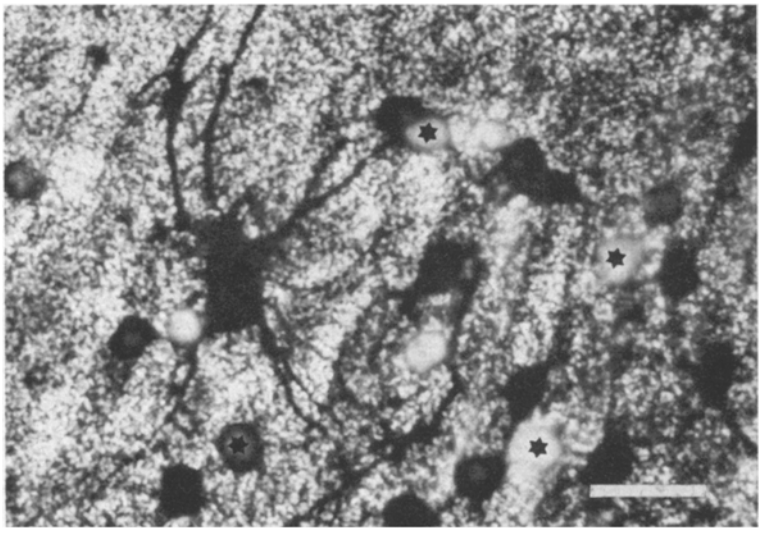
It has been proposed that uptake may be an important mechanism for terminating the action of transmitter

[1] A. W. Duggan and G. A. R. Johnston, J. Neurochem. *17*, 1205 (1970).
[2] L. T. Graham jr., R. P. Shank, R. Werman and M. H. Aprison, J. Neurochem. *14*, 465 (1967).
[3] J. L. Johnson and M. H. Aprison, Brain Res. *24*, 285 (1970).
[4] D. R. Curtis and G. A. R. Johnston, Rev. Physiol., Berl. *69*, 97 (1974).
[5] L. Hösli, E. Hösli and P. F. Andrès, Brain Res. *62*, 597 (1973).
[6] L. Hösli, P. F. Andrès and E. Hösli, Experientia *29*, 1244 (1973).
[7] L. Hösli, P. F. Andrès and E. Hösli, Experientia *31*, 710 (1975).
[8] J. L. Johnson, Brain Res. *37*, 1 (1972).

A



B



C

Fig. 1. Autoradiographs of the uptake of amino acid transmitters in spinal cord cultures. A) Human spinal neurones (fetus 9 weeks in utero, 14 days in vitro) after incubation with ^{3}H-glycine (10^{-6} *M* for 30 sec). Unlabelled cells are marked with asterix (*). Bar: 20 μm.

B) and C). Rat spinal cord cultures, (18 days in vitro) after incubation with L-^{3}H-glutamic acid (B) and L-^{3}H-aspartic acid (C) (10^{-6} *M* for 5 min). Some neurones show a heavy accumulation of silver grains over the cell bodies and processes; other neurones (*) are almost free of label. Bar: 30 μm.

substances such as monoamines and amino acids[9,10]. Investigations on uptake kinetics have revealed that in the spinal cord, glutamate and aspartate are taken up by a high affinity transport system[3,11,12]. Since there is only little information on the cellular localization of the uptake of glutamate and aspartate in the mammalian spinal cord, we have used the technique of tissue culture[13–16] to study the uptake pattern of these amino acids in cultured human and rat spinal cord by autoradiography.

The cultures were prepared from lumbar and sacral segments of spinal cord of human fetuses (8–11 weeks in

[9] L. L. Iversen, *The Uptake and Storage of Noradrenaline in Sympathetic Nerves* (Cambridge University Press, Cambridge 1967), p. 253.
[10] L. L. Iversen and M. J. Neal, J. Neurochem. *15*, 1141 (1968).
[11] V. J. Balcar and G. A. R. Johnston, J. Neurochem. *20*, 529 (1973).
[12] W. J. Logan and S. H. Snyder, Brain Res. *42*, 413 (1972).
[13] E. Hösli, Å. Ljungdahl, T. Hökfelt and L. Hösli, Experientia *28*, 1342 (1972).
[14] E. Hösli, U. M. Bucher and L. Hösli, Experientia *31*, 354 (1975).
[15] L. Hösli and E. Hösli, Brain Res. *45*, 612 (1972).
[16] L. Hösli, E. Hösli, P. F. Andrès and J. R. Wolff, in *Golgi Centennial Symposium: Perspectives in Neurobiology* (Ed. M. Santini; Raven Press, New York 1975), p. 473.

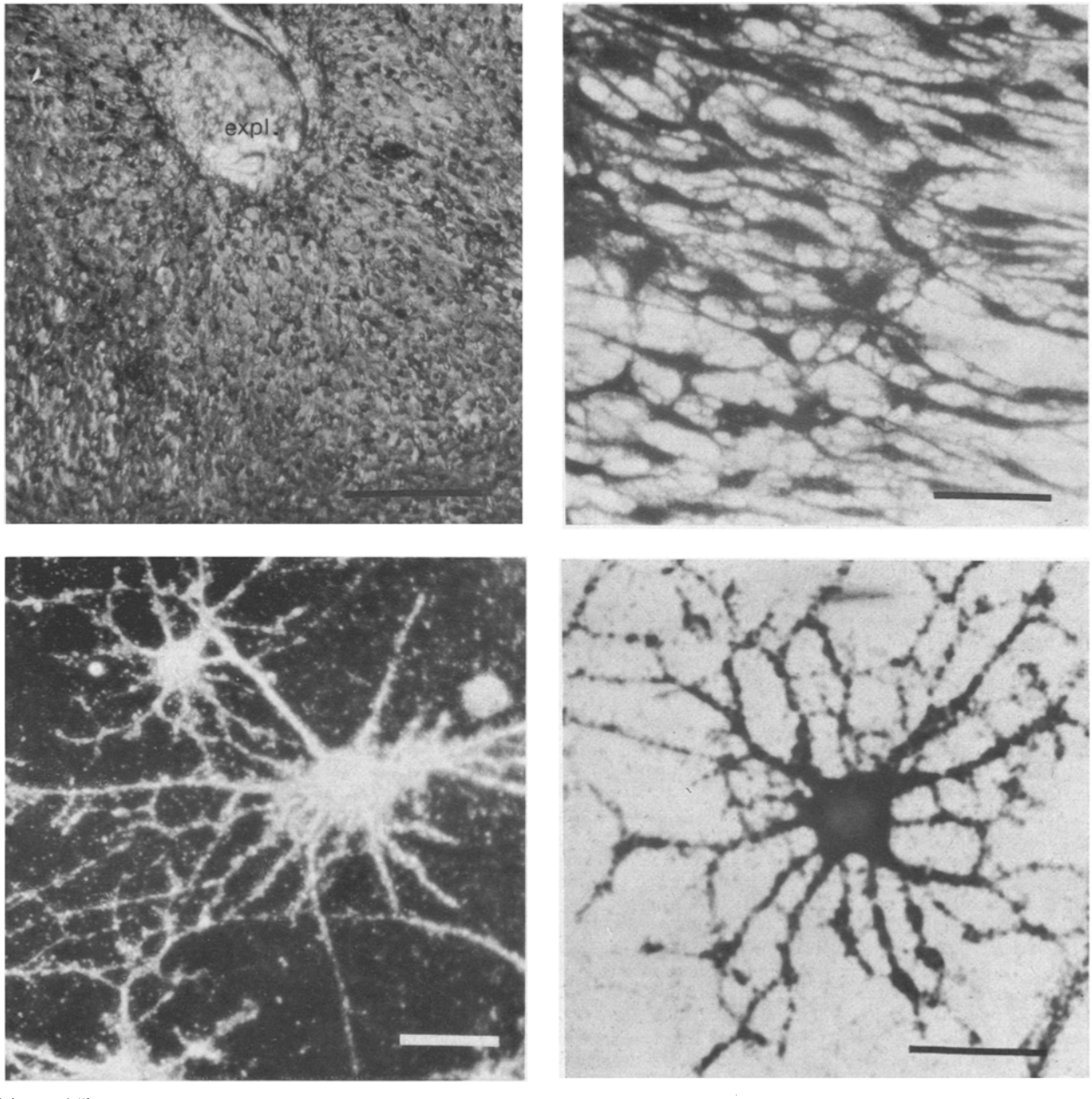


Fig. 2. A) Human spinal cord culture (fetus 9 weeks in utero, 18 days in vitro) after incubation with L-^{3}H-glutamic acid (10^{-6} *M* for 5 min). Labelled neurones and glial cells are found in the dense zones of the culture as well as in the outgrowth zones. expl. = explant. Bar: 200 µm.

B) Labelled glial cells forming a network in the outgrowth zone of a human spinal cord culture (fetus 9 weeks in utero, 18 days in vitro) after incubation with L-^{3}H-glutamic acid (10^{-6} *M* for 10 min). Bar: 50 µm.

C) Dark field illumination of intensely labelled astrocytes of a rat spinal cord culture (28 days in vitro) after incubation with L-^{3}H-glutamic acid (10^{-6} *M* for 5 min). Bar: 30 µm.

D) Astrocyte showing a strong accumulation of L-^{3}H-aspartic acid (10^{-6} *M* for 10 min). Rat spinal cord culture, 28 days in vitro. Bar: 20 µm.

utero), of rat fetuses (18 days in utero) and of newborn rats. Explants were grown on collagen-coated coverslips in the Maximov assemblies (for details see[16]). For the autoradiographic studies the cultures were rinsed in Tyrode solution and incubated in Hank's solution containing either L-^{3}H-glutamic acid (New England Nuclear Corp. (NEN), specific activity, 20.4 Ci/mM) or L-^{3}H-aspartic acid (NEN), specific activity, 23.66 Ci/mM). The concentration of the amino acids varied between 10^{-7} to 10^{-6} M. The incubation time ranged from 30 sec to 10 min. After the incubation, the cultures were rinsed in Tyrode solution, fixed with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), dehydrated, air-dried and mounted on object slides. The dried cultures were covered with Ilford L4 emulsion (Ilford Ltd., Essex/England) by means of the loop technique described by JENKINS[17] and stored in light-tight boxes for 2 weeks at 4 °C. The autoradiograms were developed with Kodak D 19b and fixed in 30% sodium thiosulphate. Observations of the autoradiograms were made with a Wild-epi-illumination microscope.

The outgrowth pattern and the morphological appearance of the various cell types of human and rat spinal cord cultures have been described previously[16]. After incubation of the cultures with L-glutamate or L-aspartate, a great number of neurones and glial cells revealed a strong accumulation of the amino acids. Neurones showing an intense autoradiographic reaction were mainly located in the dense zones of the cultures although a few labelled neurones were also found in the outgrowth zones (Figure 2A). The activity of labelled amino acids varied considerably between individual neurones; some neurones were completely free of an autoradiographic reaction. Figure 1 illustrates examples of autoradiographs of the uptake of glutamate (B) and aspartate (C) into neurones of rat spinal cord cultures. The cell bodies and processes of some neurones show a heavy accumulation of silver grains whereas other neurones (*) seem to be free of label. Similar results have also been obtained after incubation with glycine (Figure 1A) and GABA (not illustrated) using light and electron microscopic autoradiography[13,16].

Glutamate and aspartate were found to be accumulated by large as well as by small neurones. In our studies it is not possible to determine whether these amino acids are taken up by the same or by a separate neurone population. It has been suggested that amino acid transmitters are mainly taken up by those neurones utilizing or synthesizing these substances[18–22]. The differences in uptake pattern might, however, also depend on the functional state of the individual neurone.

Aspartate and glutamate were also found to be accumulated to a great extent by glial cells. In contrast to the uptake pattern into neurones where a great variability between the individual cells was observed, almost all glial elements have accumulated the amino acids. Figure 2B shows labelled glial cells in the outgrowth zone of a human spinal cord culture after incubation with L-^{3}H-glutamic acid. Strong accumulation of glutamate and aspartate into protoplasmic astrocytes of rat and human spinal cord cultures is illustrated in Figures 2C and D respectively. Similar observations were also made in brain stem and cerebellar cultures (unpublished observations). In contrast to the amino acid transmitters, uptake into glial cells was not observed after incubation with monoamines[14].

Using incubation times between 30 sec and 5 min it appeared that neurones showed a stronger autoradiographic reaction than glial cells whereas after 10 min incubation time, it seemed to be no difference in the intensity of labelling between neurones and glial cells. A similar uptake pattern was observed after incubation with glycine and GABA[13,15,16]. These observations suggest that there might be different transport systems for the amino acids in neurones and in glial cells[20].

The uptake of the amino acids was temperature-dependent being considerably reduced after incubation at 0 °C. In contrast to our studies with glycine and GABA[15,16], removal of sodium ions from the incubation medium seemed to affect the uptake of glutamate and aspartate only moderately. Preliminary experiments using perchloric acid extraction have shown that after incubation with L-^{3}H-glutamic acid (10^{-5} M for 15 min), about 13% of the isotopes were associated with acid insoluble material suggesting that proteinbound glutamate contributes only to a small extent to the autoradiographic reaction[21]. In the present study we have not investigated the rate of metabolism of glutamate and aspartate. It has, however, been described that in slices of the cat spinal cord, more than 90% of the radioactivity remained associated with the original labelled glutamate and aspartate after an incubation time of 5 min at 25 °C[11].

Our results demonstrate that glutamate and aspartate are taken up by neurones as well as by glial cells of cultured human and rat spinal cord. Uptake of glutamate has also been described in Bergman glia of cerebellum[18], in Müller cells of the retina[23], in satellite cells of rat sensory ganglia[24] and of the peripheral neuromuscular junction of insects[25]. Investigations by HAMBERGER[26] on the uptake of glutamate by homogenates of CNS tissue have shown that the amino acid is taken up at a higher rate by the glial fraction than by the neuronal fraction. From these findings it is suggested that glial cells might also be involved in the uptake of amino acid transmitters and that the transport systems might be different for neurones and for glial cells.

[17] E. C. JENKINS, Stain Technol. *47*, 23 (1972).
[18] T. HÖKFELT and Å. LJUNGDHAL, in *Studies of Neurotransmitter at the Synaptic Level, Advances in Biochemical Psychopharmacology* (Eds. E. COSTA, L. L. IVERSEN and R. PAOLETTI; Raven Press, New York 1972), vol. 6, p. 1.
[19] L. L. IVERSEN and F. E. BLOOM, Brain Res. *41*, 131 (1972).
[20] L. L. IVERSEN and J. S. KELLY, Biochem. Pharmac. *24*, 933 (1975).
[21] We should like to thank PD Dr. H. P. VON HAHN, Department of Neurochemistry, Neurological Clinic, University of Basel, for carrying out the perchloric acid extractions.
[22] C. G. HONEGGER, L. M. KREPELKA, V. STEINMANN and H. P. VON HAHN, Experientia *30*, 369 (1974).
[23] B. EHINGER and B. FALCK, Brain Res. *33*, 157 (1971).
[24] F. SCHON and J. S. KELLY, Brain Res. *66*, 275 (1974).
[25] I. R. FAEDER and M. M. SALPETER, J. Cell Biol. *46*, 300 (1970).
[26] A. HAMBERGER, Brain Res. *37*, 169 (1971).