chromosome aberration findings many cells had to be rejected, which explains why a total cell number of 500 could not always be analysed.

Conclusions. An exact quantification of radiation-induced chromosome aberrations in human lymphocytes requires the analysis of M1 cells. In blood cultures with a high proportion of M2 cells established after storage of blood for 2 or 3 days a correct identification of M1 and M2 cells is impossible when only 19.4 μM BrdU is used for the FPG staining method. As a consequence the actual chromosome damage in M1 is significantly underestimated. When culture initiation is delayed for more than 24 h and 38.7 μM BrdU is added to the cultures, M1 and M2 cells can be reliably identified and the chromosome damage can be correctly analysed. This should be taken into account for cytogenetic radiation dosimetry purposes when mailing of blood samples, causing a delay of several days, occurs.

Acknowledgments. We wish to thank Mrs R. Giesen for skilful technical assistance, Mrs G. Rocznik for her help in preparation of the manuscript and Prof. Dr. E. Schmid for helpful discussion.

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0014-4754/85/121589-02$1.50 + 0.20/0

A mechanism for macromolecular transfer from glia to neuron cell body in crayfish

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Summary. Small extrusions of glial cytoplasm are endocytosed by neuron cell bodies of the crayfish *Procambarus*. Vesicles are double walled with the external membrane issuing from the neuron and the internal one from the glia. This could be a system for the transfer of glial cytoplasmic free proteins to neurons.

Key words. Crayfish; double-walled vesicles; glia; modified endocytosis; motoneurons.

Glial cells can transfer proteins to the axons of invertebrates[1-4]. Several structures, such as tubular lattices[5,6] and trans-glial channels[7,8] could be concerned with glia-to-axon transfer. Glial exocytosis coupled to axonal endocytosis could also account for the transfer of proteins from glial vesicles through the intercellular space[4]. Nevertheless, no mechanism has yet been described for the glia-to-axon transfer of cytoplasmic free proteins such as actin[4]. Junctional structures for the glia-to-neuron cell body transfer of free cytoplasmic proteins or vesicular macromolecules have not been reported. Moreover, neuron somata of both vertebrates and invertebrates are separated from glia by a space of 10–20 nm which is considered as the microenvironment of the nervous cells, i.e. the site for ionic and macromolecular glia-neuron exchange[1,9,10]. In crayfish, however, gap-like junctions have been reported[11]. Modified endocytosis of small glial cytoplasmic extrusions by abdominal neuron cell bodies of crayfish is described here. It could be involved in glia-to-neuron cell body transfer.

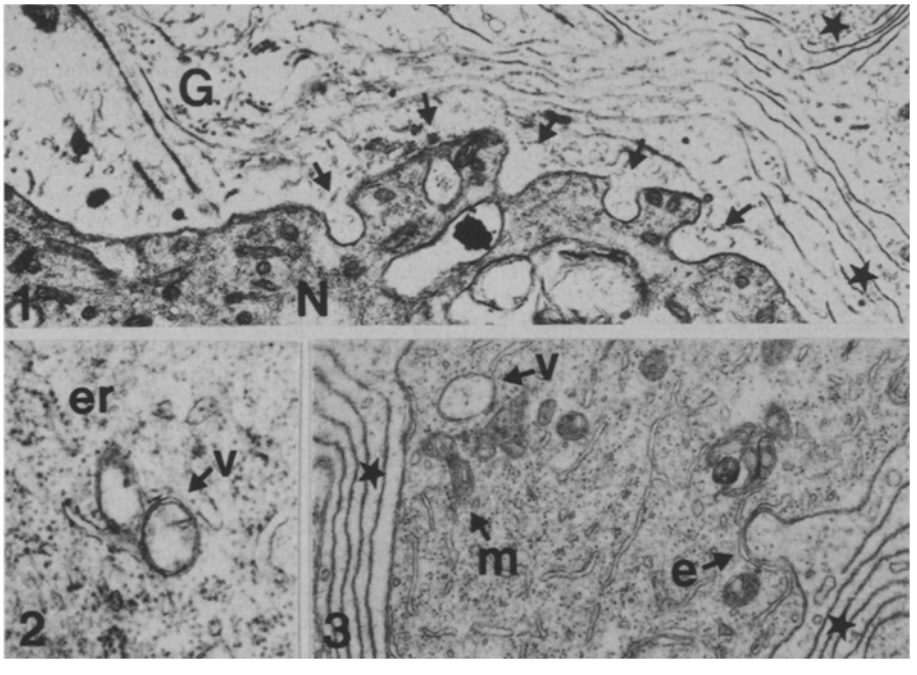

Figure 1. Modified neuronal endocytosis of glial cytoplasmic extrusions. Vesicles (arrows) are shown at different stages of the process. Note the difference in electron density between glial (G) and neuronal (N) cytoplasms. Asterisks: membraneous systems of the glial cells (× 11,540).

Figure 2. A double-walled vesicle (v) with light content within a neuron cell body. er, rough endoplasmic reticulum (× 28,900).

Figure 3. Detail of a neuron cell body covered by membraneous processes of perineuronal glial cells (asterisks). e, double-walled endocytotoc figure, m, mitochondrion. v, double-walled vesicle with light content (× 16,250).

Adult specimens of *Procambarus* were anesthetized by cooling. Abdomens were perfused with 4% paraformaldehyde-4% glutaraldehyde in phosphate buffer (pH = 7.3, 4°C). Abdominal ganglia were removed, immersed in fixative (overnight), post-fixed in OsO_4 (2%, 2 h), dehydrated through an ethanol series, stained 'en bloc' with uranyl acetate and finally embedded in araldite. Thin sections of ventral areas of ganglia were stained on grids with lead citrate.

Transmission electron microscopy of neuron perikarya reveals the presence of modified double-walled endocytotic vesicles (300–700 nm). They are formed by apposed neuronal and glial membranes which enclose small extrusions of the glial cytoplasm (fig. 1). The external membrane is of neuronal origin, whilst the internal one is glial. These two membranes are separated by a narrow cleft of 10 nm as in a normal neuron-glia interface[11,12]. These membranes do not show in general gap-like junctions, but can bear neuronal subsurface cisternae. In some cases, a thin lamina of dense neuronal cytoplasm underlies endocytotic figures. Free double-walled vesicles are frequently observed in neuron cell bodies (figs 2 and 3). Their content shows the same density as glial cytoplasm and is lighter than neuron mitochondrial matrix or neuronal cytoplasm. These vesicles are interpreted as modified endocytotic vesicles moving to internal cytoplasm. Both endocytotic vesicles and internalized double-walled vesicles are found in most of the neuron cell bodies, irrespective of their size or situation in the ganglia. They are not found in glia/axon pairs or in neuropile.

The presence of gap-like junctions[11], and the observed modified endocytosis suggest that the neuronal microenvironment concept is no longer applicable to the intercellular space between perikarya of abdominal neurons of crayfish and associated peri-neuronal glia cells. Gap-like junctions could allow the glia-to-neuron transfer of small proteins up to 1000–1500 dalton[13]. Larger free cytoplasmic macromolecules could be transferred through endocytosis of glial cytoplasmic extrusions. In this case, some coupled mechanism to free the content of the double walled vesicles and to transport it to destination is probably needed[14].

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0014-4754/85/121590-02$1.50 + 0.20/0

SV40 transformed fibroblasts recognize the same 140 kD fibronectin chemotactic fragment as non-transformed cells

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Summary. SV40 virus-transformed human embryonal fibroblasts show an enhanced chemotactic response to the glycoprotein fibronectin. However, they recognize the same chemotactic active region as non-transformed fibroblasts. The result suggests that an enhancement of chemotaxis by fibroblasts which have been transformed with Simian Virus 40 is due not to the utilization of further chemotactic domains in the molecule, but to an increased sensitivity of the cells to the chemoattractant.
Key words. Fibronectin; domains; chemotaxis; SV40; fibroblasts.

The high molecular weight glycoprotein fibronectin (FN) is composed of apparently independent domains, which exert different biological activities, among them binding to different biological substances, such as bacterial walls, heparin, gelatin, fibrin, actin and proteoglycans[2]. FN promotes cell attachment to collagen as well as cell spreading on tissue culture substratum. Large FN fragments with cell binding activity have been demonstrated to be chemotactic for fibroblasts[3,4]. Further work has shown that FN peptides which do not contain the cell binding site are also unable to attract fibroblasts[5].

Virus-transformed and tumor-derived cells have been shown to have an enhanced chemotaxis to FN if compared with normal fibroblasts[6,7]. These cells have also an increased random migration in the presence of FN, if compared with control fibroblasts. However the chemokinetic component accounts for only 10% of the migration. An increased chemotaxis by tumor cells is thought to be involved in tumor invasion and metastasis[8,9]. Enhancement of chemotaxis by tumoral cells has been reported in response to other chemoattractants, like collagen[10] or complement derived factors[11]. The major source of chemotactic activity for fibroblasts, leukocytic cells and tumor cells, elicited by the complement, derives from the C5 component[12,13]. However, different fragments might be responsible for the attraction of the different cell types.

In the present study we demonstrate that two different lines of SV40 virus-transformed human embryonal fibroblasts recognize the same FN fragment as non-transformed fibroblasts, although their chemotactic response to the protein is enhanced.

Material and methods. Human embryonal fibroblasts (HEF), derived from a 16-week-old fetus after legal abortion, SV40 virus-transformed human embryonic fibroblasts SV40/WI 38 (American Type Culture Collection, strain ATCC CCL 75.1) and SV40/WI26 (strain ATCC CCL 95.1) were a present from Dr P.K. Müller, Max-Planck-Institut für Biochemie (FRG).

Cells were grown in Dulbecco's MEM supplemented with glutamine, antibiotics and ascorbic acid as previously described[14]. Purified plasma FN[15] was used for the chemotactic assays and for the production of specific peptides. Briefly, the large cathepsin D fragments Ca70, Ca140 and Ca75-S-S-65 were prepared by mild digestion of human FN[16]. Fragments Ca95 and Ca23 were