

Acta histochemica

**Zeitschrift für
histologische Topochemie
Supplementband XXXVIII**

**Herausgegeben von
Joachim-Hermann Scharf, Halle,
Gerhard E. Voigt, Lund,
Werner Linß, Jena**

Verhandlungen der Gesellschaft für Histochemie
auf dem XXX. Symposium in Gargellen, Montafon (Österreich)
vom 21. bis 24. September 1988

Cytochemical Markers of Neural and Endocrine Cells

Herausgegeben von Jan Drukker, Maastricht

Mit 129 Abbildungen und 34 Tabellen



Gustav Fischer Verlag Jena · 1990

Table of contents

Robert Feulgen Lecture

Larsson, L.-I.: Tracing of neuroendocrine peptides and their corresponding mRNA's: unravelling the neuroendocrine network	9
---	---

Symposium: Cytochemical markers of neural and endocrine cells—cell biology and pathology

Pilgrim, Ch.: Introductory remarks	15
Bock, E., and Linnemann, D.: Cell adhesion molecules in brain	19
O'Connor, D. T., Takiyyuddin, M. A., Cervenka, J. H., Parmer, R. J., Barbosa, J. A., Chang, Y. M., and Hsiao, R. J.: Circulating chromogranin A as a diagnostic tool in clinical chemistry	27
Heitz, Ph. U.: Neuroendocrine markers in pathology	35

Workshop I: Cell surface markers

Organizer and moderator: O. K. Langley, Strasbourg	
Aletsee-Ufrecht, M. C., Langley, O. K., and Gratzl, M.: NCAM expression in endocrine cells	45
Rougon, G., Nédélec, J., Malapert, P., Goridis, C., and Chesselet, M. F.: Post-translation modifications of neural cell surface molecules	51
Rathjen, F. G., Wolff, J. M., Chang, S., and Raper, J.: Membrane glycoproteins involved in neurite-neurite interactions	59
Gennarini, G., Rougon, G., and Goridis, C.: F3: a new developmentally regulated member of the HNK-1 family	65

Workshop II: Intracellular markers

Organizer and moderator: M. Gratzl, Ulm	
Grant, N. J., Aunis, D., and Langley, O. K.: Neurofilament expression in bovine chromaffin cells	71
Schilling, K., and Pilgrim, Ch.: Expression of neurofilament proteins in developing neurons	77
Grube, D., Bargsten, G., Cetin, Y., Jörns, A., und Yoshie, S.: Chromogranine in den endokrinen Zellen des Verdauungsapparats	81
Ehrhart, M., and Gratzl, M.: Chromogranin A in the endocrine pancreas: Extracellular or intracellular function?	87

Workshop III: Neural and endocrine markers as diagnostic tools

Organizer and moderator: H. Höfler, Graz	
Klöppel, G., and In't Veld, P.: Neural and endocrine markers as diagnostic tools in pancreatic and gastrointestinal endocrine tumors	93
de Bruïne, A. P., and Bosman, F. T.: Neuroendocrine tumours in the respiratory tract	99
Becker, H., Wirnsberger, G., Ziervogel, K., and Höfler, H.: Immunohistochemical markers in (ganglio)neuroblastomas	107

Larsson, L. T., and Sundler, F.: Neuronal markers in Hirschsprung's disease with special reference to neuropeptides	115
Falkmer, S., Askensten, U., Grimelius, L., and Abrahamsson, P.-A.: Cytochemical markers and DNA content of neuroendocrine cells in carcinoma of the prostate gland during tumour progression	127
Schmid, K. W., und Weiler, R.: Unterschiedliches Vorkommen von Chromogranin A und B in normalen menschlichen endokrinen und nervalen Geweben und Tumoren neuroendokrinen Ursprungs	133

Contributed papers

Viebahn, Ch., Lane, E. B., and Ramaekers, F. C. S.: Vimentin and keratin are expressed in the neurogenic tissue of the rabbit embryo during primary neurulation	139
Layer, P. G.: Cholinesterases reveal early patterns of neurogenesis in the chick	145
Becker, I., Paulus, W., Roggendorf, W., and Peiffer, J.: Immunohistologic analysis of neuron-specific enolase and neuropeptides in stromal cells of cerebellar hemangioblastomas	151
Graeber, M. B., Streit, W. J., and Kreutzberg, G. W.: The third glial cell type, the microglia: cellular markers of activation in situ	157
Brückner, G., Delpech, B., Delpech, A., and Girard, N.: Concentration of hyaluronectin and anionic glycoconjugates in perineuronal glial cell processes at GABAergic synapses of rat cerebellum	161
Nitsch, C., and Riesenberger, R.: Ultrastructure of the dynorphin-immunoreactivity in rat brain hippocampal mossy fiber system	167
Christmann, M. Ph., Nokihara, K., Feller, S., and Forssmann, W. G.: Demonstration of the storage form of cardiac hormones by the use of segment-specific antibodies against human Cardiodilatin/human Atrial Natriuretic Peptide (CDD/hANP)	173
Vyberg, M., Horn, T., Francis, D., and Askaa, J.: Immunohistochemical identification of neuron-specific enolase, synaptophysin, chromogranin and endocrine granule constituent in neuroendocrine tumours	179
Schwenk, J., and Makovitzky, J.: The occurrence of carbohydrate antigens (CA 19-9, CA-50) in chronic pancreatitis and pancreatic carcinoma	183
Makovitzky, J.: The sterical order of the sialic acid in the microvillous layer of the human duodenal mucosa	189
Budde, R., Gerok, K., von Deimling, O., and Schaefer, H. E.: The occurrence of β -glucuronidase in erythrocyte inclusions	195
Haider, S. G., Passia, D., and Rommert, F. F. G.: Histochemical demonstration of 11β -hydroxysteroid dehydrogenase as a marker for Leydig cell maturation in rat	203
Aumüller, G., Hüntemann, S., Larsch, K. P., and Seitz, J.: Transglutaminase immunoreactivity in the male genital tract of the rat	209
Staneva, L.: Histochemical and ultracytochemical studies on complex carbohydrates in the cyclic rat endometrium	213
Graf, R., Goßrau, R., and Frank, H.-G.: Enhancement of immunoreactivity of von Willebrand factor in vascular endothelial cells of rat organs after glucocorticoid administration	219
Ribitsch, D., Dohr, G., Hartmann, M., Pilz, G., Salmhofer, H., and Desoye, G.: GZ 100, 101, 106, 107, 111, 112, 116, 121: A series of monoclonal antibodies against human trophoblast antigens	227
Manfredi Romanini, M. G., Frascini, A., Fuhrmann Conti, A. M., Gasperi, G., Giuliani, A., and Pellicciari, C.: Changes of gene expression during long term adaptation of human EUE cells to a hypertonic medium: electrophoretic protein patterns and DNase I digestion in situ	233
Fritz, P., Hoeses, J., Multhaupt, H., Schenk, J., Mischlinski, A., Klein, C., Tuczek, H. V., and Wolf, M.: Application of the chromogenic reaction to conventional silver staining, the Ag-NOR staining and the silver-intensified immunogold technique	239

Papers read by title

Nöhammer, G., Bajardi, F., Benedetto, C., Kresbach, H., Rojanapo, W., Schauenstein, E., and Slater, T. F.: Histophotometry of protein thiols and disulphides in tissue samples from the human uterine cervix and the skin reveals a "field effect" as well as an "extended field effect" of malignant tumours 247

Schulte, E.: The influence of embedding on the stoichiometry of the pararosaniline-Feulgen stain in histological material 255

Lecture by the Robert-Feulgen-Laureate

Thanos, S.: Bidirectional fluorescent labelling techniques for the developing and regenerating visual system 259

NCAM expression in endocrine cells

By M. C. Aletsee-Ufrecht, O. K. Langley¹ and M. Gratzl

(Abteilung Anatomie und Zellbiologie der Universität Ulm, FRG,

¹ Centre de Neurochimie, INSERM U44, Strasbourg, France)

With 4 figures

Introduction

Cell adhesion is one of the primary processes of morphogenesis involved in several stages of development to determine embryonic form. A number of cell adhesion molecules (CAMs) have been described and are named according their presence in different tissues. The best-known is NCAM, the neutral cell adhesion molecule (Thiery et al., 1977). This membrane associated glycoprotein has been shown to exist in brain as three closely related polypeptides (NCAM-180, NCAM-140 and NCAM-120) i.e. of Mrs 180, 140 and 120 kD. They are characterized by an identical or similar extracellular domain which contains the amino-terminal end, the homophilic binding site and the region with large amounts of sialic acid. NCAM-120 is anchored in the membrane via a phospholipid. NCAM-140 and NCAM-180, which are characterized by a transmembraneous segment, differ in the length of their cytoplasmic domains. While NCAM is widely distributed in the embryo (Crossin et al., 1985; Daniloff et al., 1986), in the adult it has been reported to be essentially restricted to cell of the central and peripheral nervous system, with the exception of skeletal muscle (Covault and Sanes, 1986) and chick lung epithelium (Crossin et al., 1985). The present study demonstrates that NCAM is much more widely distributed in the adult than had been originally supposed. We have extended our investigations on endocrine cells of neural and non-neural origin and on endocrine cell lines. The cellular distribution of NCAM was studied immunocytochemically, and the various NCAM determinants expressed were characterized by immunoblots.

Materials and methods

Cell culture: The rat insulinoma (RIN A2) and the pheochromocytoma cell lines (PC 12) were cultured as previously described (Lind et al., 1987).

Immunocytochemistry: Adult rats were perfused with 4% formaldehyde in phosphate buffer (0,12 M), and the excised tissues postfixed in the same solution. 50 µm thick vibratome sections and cells were incubated in rabbit polyclonal anti-NCAM-antiserum (diluted 1:1,000; kindly supplied by C. Goridis) and then in peroxidase-conjugated sheep anti-rabbit IgG (dilution 1:100). Bound immunoglobulin was detected with diaminobenzidine and hydrogen peroxide. Cells and slices destined for electron microscopy were subsequently osmicated and embedded in Spurr resin.

Immunocytochemistry: Adult rat tissues and cells were homogenized in sample buffer (62,5 mM Tris/2% SDS) and the proteins separated electrophoretically under reducing conditions (Laemmli, 1979). After transfer to nitrocellulose (Towbin et al., 1979) the strips were incubated

with polyclonal rabbit anti-NCAM antiserum (dilution 1:1,000), and the bound antibody detected with iodinated protein A. For the quantification of the NCAM polypeptides the optical densities of the immunostained bands were determined by computer-assisted analysis (I. P. S., Kontron, FRG).

Results and discussion

The cellular distribution of NCAM was studied in seven different rat tissues and cell lines at both the light microscopical and ultrastructural levels. The analysis included: posterior lobe, intermediate lobe and anterior lobe of the hypophysis, adrenal medulla, and pancreas, and pheochromocytoma (PC 12) and insulinoma (RIN) cell lines. Light microscopy of vibratome sections of the examined tissues and cells immunostained with anti-NCAM all showed prominent cell surface labelling (Langley et al., 1987, 1988). With the exception of the intermediate lobe, the staining was always very intense: neurohypophysis was the most intensely stained tissue studied. In the adenohypophysis all different types of secretory cells were intensely stained; immunocytochemistry of the adrenal medulla showed that both the adrenergic and noradrenergic cells were positive for NCAM as reported earlier (Langley and Aunis, 1984). PC 12 cells, a tumour cell line derived from a rat pheochromocytoma, were strongly surface labelled, in accordance with data provided by other groups (Jørgensen and Richter-Landsberg, 1983; Prentice et al., 1987).

The most remarkable result was obtained by staining pancreatic tissue (Fig. 1), an endocrine organ considered not to be derived from the neural crest (La Douarin, 1988).

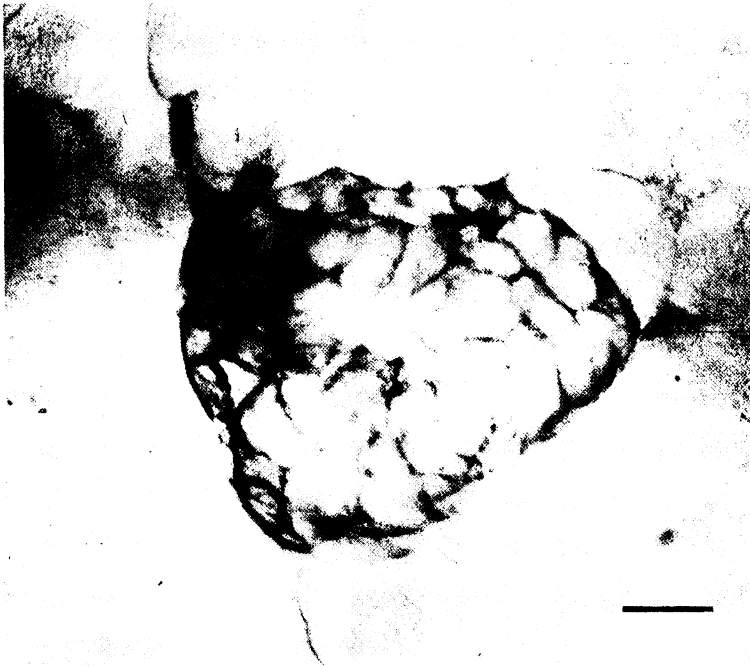


Fig. 1. Adult rat pancreas immunoperoxidase labelled for NCAM. It is evident that the reaction product is limited to the islets of Langerhans. Scale bar, 20 μ m.

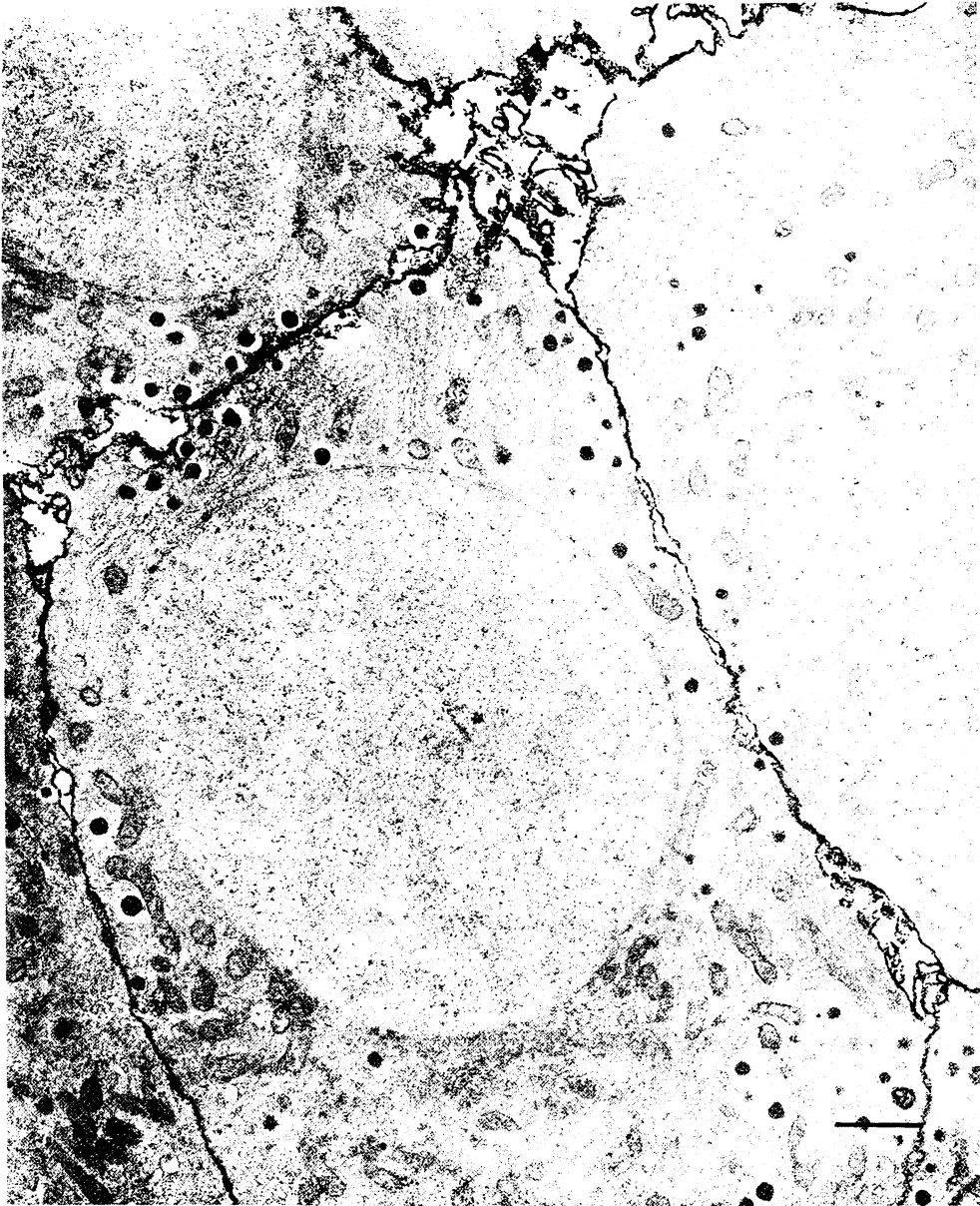


Fig. 2. Electron micrograph of a pancreatic islet labelled with anti-NCAM-antibody. Only the surface membrane is immunoreactive. No intracellular staining is detectable (section not counterstained). Scale bar, 1 μ m.

The immunoreactivity was restricted to the islets of Langerhans, the acinar cells of the exocrine pancreas remaining unstained. It can thus be concluded that NCAM is expressed by endocrine cells of both neural and non-neural origins. Immunocytochemical studies of the closely related RIN cells, a tumour cell line derived from a rat insulinoma, demonstrated again the presence of NCAM antigenic determinants at the cell membrane.

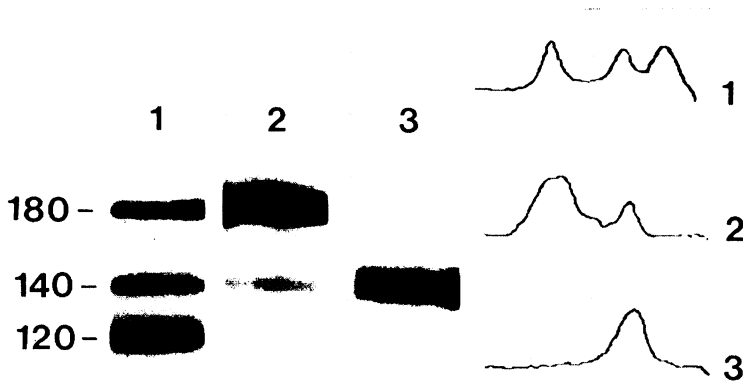


Fig. 3. Immunological identification of NCAM in cerebellum (lane 1), neuro- (lane 2) and adenohippophysis (lane 3). The optical densitis of the immunostained bands were quanticated. Control immunoblots with normal rabbit serum revealed none of the specific immunoreactive bands at 180, 140 and 120 kD. Molecular masses (kD) of NCAM are indicated.



Fig. 4. Immunological identification of NCAM in adrenal medulla (lane 1), PC 12 cells (lane 2), pancreatic islets (lane 3) and RIN cells (lane 4). The optical densitis of the immunostained bands were quanticated. Molecular masses (kD) of NCAM are indicated.

Electron microscopy of the immunostained sections confirmed the results obtained by light microscopy: N-CAM was found to be expressed by all the endocrine cells studied, distributed over the entire surface. Significant intracellular labelling was not noted. Controls employing normal rabbit serum produced none of these specific staining features. Fig. 2 demonstrates the staining of the pancreatic islet cells by antigenic structures.

Comparison of the immunoblots of the endocrine cells and tissues showed marked differences in the pattern of the three different NCAM glycoproteins. Cerebellum (Fig. 3, lane 1) was used as a reference. The presence of the three antigenic determinants NCAM-180, NCAM-140 and NCAM-120 is well documented (Langley et al., 1982;

Edelman, 1984). The pattern of the neurohypophysis (Fig. 3, lane 2) when compared to cerebellum, clearly differed in the amount of the 3 glycoproteins. This endocrine tissue expressed NCAM-140, high contents of NCAM-180 and only trace levels of NCAM-120 (Langley et al., 1988). In extracts of the adenohypophysis (Fig. 3, lane 3) and the adrenal medulla (Fig. 4, lane 1) only the 140 kD variant was present. By contrast, immunoblots of the pheochromocytoma cell line (PC 12, Fig. 4, lane 2) were characterized by an additional immunoreactive band of molecular weight 180 kD, which is in agreement with data reported by Friedlander et al. (1986). The relative amounts of the two NCAM determinants varied in the different cell extracts. Isolated pancreatic islets (Fig. 4, lane 3) contained only the 140 kD NCAM-immunoreactive band whereas the pattern of the insulinoma cell line (Fig. 4, lane 4) resembled that observed in PC 12 cells: The RIN cell extracts showed mainly a band of 140 kD with a less prominent band of 180 kD. Compared to the corresponding tissues it is evident that both tumour cell lines express an additional NCAM-180 polypeptide, suggesting that the malignant transformation is associated with a change in the regulation of NCAM mRNA processing.

As shown immunocytochemically, all endocrine cells examined express NCAM at their surface. However the pattern of the three NCAM polypeptides observed was different. It may be concluded that the expression of NCAM in the adult is a common feature of endocrine cells, irrespective of their embryonic origin, though the pattern of antigenic determinants is very different from that of central nervous system. In addition, with the exception of neurohypophysis, the NCAM endocrine phenotype is characterised by a predominance of NCAM-140.

Acknowledgements. We thank Dr. G. Ahnert-Hilger for generously providing PC 12 and RIN cells, Dr. K. Schilling, H. Frey and W. Podschuweit for their help with densitometric scans and Mrs. M. Rudolf for expert technical assistance.

This work was supported by Deutsche Forschungsgemeinschaft (Gr681), by Forschungsschwerpunkt no. 24 of the State of Baden-Württemberg, and by a twinning exchange PROCOP fellowship.

References

- Covault, J., and J. R. Sanes: Distribution of N-CAM in synaptic and extrasynaptic portions of developing and adult skeletal muscle. *J. Cell Biol.* **102**, 716–730 (1986).
- Crossin, K. L., C.-M. Chuong and G. M. Edelman: Expression sequences of cell adhesion molecules. *Proc. Natl. Acad. Sci. USA* **82**, 6942–6946 (1985).
- Daniloff, J. K., C.-M. Chuong, G. Levi and G. M. Edelman: Differential distribution of cell adhesion molecules during histogenesis of the chick nervous system. *J. Neurosci.* **6**, 739–758 (1986).
- Edelman, G. M.: Cell-surface modulation and marker multiplicity in neural patterning. *Trends in Neurosci.*, March 78–84 (1984).
- Friedlander, D. R., M. Grumet and G. M. Edelman: Nerve growth factor enhances expression of neuron-glia cell adhesion molecule in PC 12 cells. *J. Cell Biol.* **102**, 413–419 (1986).
- Jørgensen, O. S., and C. Richter-Landsberg: D2-protein in PC 12 pheochromocytoma cells after nerve growth factor stimulation. *Neurosci.* **9**, 665–672 (1983).
- Laemmli, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685 (1970).
- Langley, O. K., M. S. Ghandour, G. Gombos, M. Hirn and C. Goridis: Immunocytochemistry of monoclonal antibodies in the cerebellum. *Neurochem. Res.* **7**, 343–356 (1982).
- Langley, O. K., and D. Aunis: Ultrastructural immunocytochemical demonstration of D2-protein in adrenal medulla. *Cell Tissue Res.* **238**, 497–502 (1984).
- Langley, O. K., M. C. Aletsee and M. Gratzl: Endocrine cells share expression of N-CAM with neurones. *FEBS Lett.* **220**, 108–112 (1987).

- Langley, O. K., M. C. Aletsee-Ufrecht, N. J. Grant and M. Gratzl: Expression of the neural cell adhesion molecule NCAM in endocrine cells (submitted).
- Le Douarin, N. M.: On the origin of pancreatic endocrine cells. *Cell* **53**, 169–171 (1988).
- Lind, I., G. Ahnert-Hilger, G. Fuchs and M. Gratzl: Purification of alpha-toxin from *Staphylococcus aureus* and application to cell permeabilization. *Analyt. Biochem.* **164**, 84–89 (1987).
- Prentice, H. M., S. E. Moore, J. G. Dickson, P. Doherty and F. S. Walsh: Nerve growth factor-induced changes in neural cell adhesion molecule (N-CAM) in PC12 cells. *EMBO J.* **6**, 1859–1863 (1987).
- Thiery, J.-P., R. Brackenbury, U. Rutishauser and G. M. Edelman: Adhesion among neural cells of the chick embryo. II. Purification and characterization of a cell adhesion molecule from neural retina. *J. Cell Biol.* **252**, 6841–6845 (1977).
- Towbin, H., T. Staehelin and J. Gordon: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354 (1979).

First author's address:

Dr. M. Cäcilie Aletsee-Ufrecht, Abteilung Anatomie und Zellbiologie der Universität Ulm, Postfach 4066, D-7900 Ulm.