and of short filaments have been described[7], while microvilli are recorded on cell surfaces but are not known to be localized at any particular site[4]. Bacteria may be adhering to areas of the cell surface such as these, since MARCUS[5] records red blood corpuscles adhering to NAD+ giant HeLa cells by microvilli. It is also possible that areas of the cell surface to which bacteria adhere have a different surface charge, are related to virus binding sites, or are places where new plasma membrane is being encorporated into the cell surface. In particular, the patterns of adhesion may be relevant to cell locomotion[6] (c.f. adhesion at the front end of the cells in the area of the ruffled membrane) and to surface differences between malignant and ordinary cells[8] (c.f. the differences in patterns of adhesion on the HeLa and WI 38 cells).

Zusammenfassung. Bakterien haften an der Oberfläche kultivierter Säugetierzellen in charakteristischer Weise. An Krebszellen haften sie nicht nur am Vorderteil, sondern auch an der Zelloberfläche und in Kernnähe.

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The Effect of a Single Dose of N-Ethyl-N-Nitrosourea on the Fine Structure of the Brain of the Rat

Resorptive carcinogens were introduced in experimental neuro-oncology by DRUCKREY et al.[1,2]. One of these compounds, an acyl-alkyl-nitrosamide derivative, N-ethyl-N-nitrosourea (ENU), when given intravenously in a single dose to pregnant rats in the latter part of gestation, induced tumours and malformations selectively in the nervous system of the offspring (DRUCKREY, IVANKOVIC and PREUSSMANN[3]). The onco- and teratogenic effects of ENU have been studied in recent years[4,5]. It is known that a single dose of ENU[6] or of N-methyl-N-nitrosourea (MNU)[7], another nitrosourea derivative, blocks DNA synthesis almost completely within 6 h, causing a concurrent cytotoxic effect on proliferating cell populations, and that MNU, when infused into the carotid artery, brings about an immediate suppression of the electrocortical activity of the brain[8]. However, the possible toxic effects of these compounds on the ultrastructure of the brain have not been investigated. In this study we have examined the acute toxic effects of a single high dose of ENU on the fine structure of the brains of adult rats.

Materials and methods. Male Wistar rats weighing about 200 g were given 240 mg/kg (LD_{50}) of ENU[9] by i.p. injection. The ENU was dissolved in citrate buffer at pH 6.0 and used immediately. Control rats were injected with the buffer. The animals were anaesthetized with pentobarbitone sodium (30 mg/kg) and perfused 6, 12 and 24 h after the injection with half strength Karnovsky fixative[10]. Blocks from the CA 1 region of the hippocampus and those from the anterior part of the wall of the lateral ventricle were post-fixed in 1% osmium tetroxide in 0.1 *M* phosphate buffer after washing overnight in 0.067 *M* cacodylate buffer containing 0.25 *M* sucrose, dehydrated in ethanol and embedded in Epon 812.

Results and discussion. Changes in the fine structure of the examined regions were observed after 6 h. The most prominent feature was swelling of astrocytic processes around the capillaries and throughout the neuropil. The degree of oedema varied, but the plasma membranes were usually intact. Relatively well preserved organelles were dispersed in the watery, sometimes floccular cytoplasm. The mitochondria were of variable size and shape with an increased matrix density and irregular cristae. The cisternae of the rough endoplasmic reticulum were dilated: several distended cisternae contained floccular material.

Astrocytes are especially susceptible to chemical and mechanical damage. FRIEDE, HU and CECHNER[11], investigating the fine structure and chemistry of glial footplates in the bowfin, concluded that the plasma membrane of these glial cells is characterised by an exceptionally active transport of sodium ions which renders astrocytes particularly sensitive to swelling. A highly toxic compound like ENU may represent the chemical challenge, to which the non-specific response of the astrocytes is oedema. Astrocytic swelling as a fixation artefact or post-mortem deterioration was excluded by comparison of the brains of ENU injected animals with those of controls.

Pericytes showed irregular contours and their extensive cytoplasm was occupied by lipid droplets, vacuoles of different sizes and by many dense bodies (Figure 1).

After 12 h many glial cells showed advanced degeneration and necrosis (Figure 2). Coarsely clumped chromatin had accumulated beneath the nuclear membrane, the nucleus had become pyknotic with breakdown and disintegration of the nuclear membrane and the cytoplasm contained much debris. Cell organelles could hardly be distinguished; occasionally mitochondria with broken membranes could be discerned together with ballooned cisternae of the endoplasmic reticulum. These necrotic cells were frequently adjacent to neurons and myelinated fibres. Their position, together with their size and shape, suggests that they are probably oligodendrocytes.

JÄNISCH et al.[12] have shown recently that oligodendroglial cells may react more readily to the carcinogenic stimulus of MNU than astrocytes. Moreover, brain tumours induced by i.v. administration of ENU to pregnant rats

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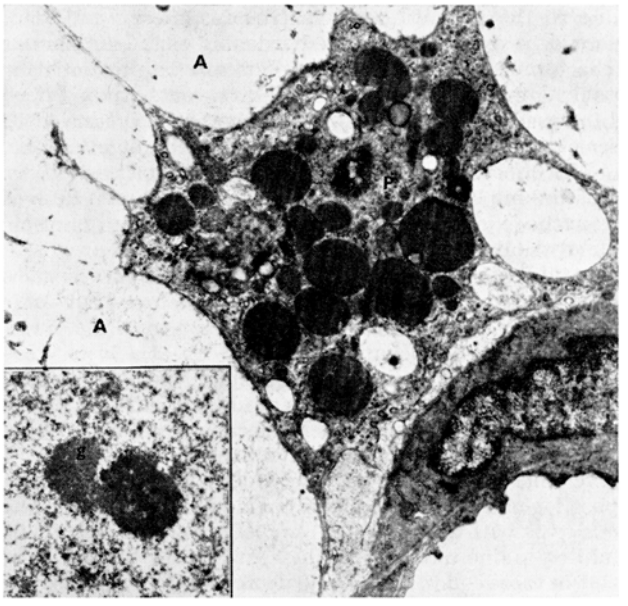

Fig. 1. Pericyte (P) surrounded by oedematous astrocytic processes (A). ×7 870. Inset: Separation of the fibrillar (f) and granular (g) components of the nucleus of a neuron. ×8 000.

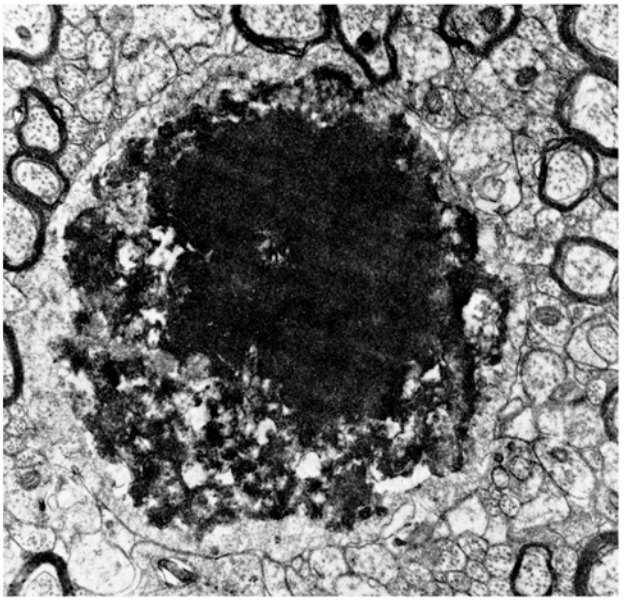

Fig. 2. Cell necrosis 12 h after the injection. The dead cell with pyknotic nucleus (n) is surrounded by unaffected cell processes. ×14 000.

have frequently been classified as oligodendrogliomas[13]. It cannot be excluded, however, that ENU may preferentially cause necrosis of proliferating cells known to persist postnatally in the subependymal layer[14].

All types of glial cells displayed an increased population of dense bodies after 24 h, including the formation of autophagic vacuoles. The degenerate cell organelles found in astrocytic processes were sometimes surrounded by concentric layers of thin astrocytic lamellae. Microglial cells had numerous dense bodies of variable size and complexity; these often occupied a large proportion of the cell, displaying granular and/or lamellar structure.

The neurons of the regions examined showed no consistent changes throughout the experiment with the exception of irregularity of the nuclear profiles and of nucleolar segregation (Figure 1, Inset). Such separation of the nucleolar fibrillar and granular components is regularly seen in carcinogen-treated rat liver[15].

To summarize, ENU causes extensive degenerative changes in all types of glial cells, while neurons react less obviously to the toxic stimulus.

Résumé. Une forte dose de composé carcinogène N-éthyle-N-nitrosourée (ENU) a été injectée à des rats Wistar mâles adultes: des changements ultrastructuraux des cerveaux furent observés. La dégénérescence et la nécrose des oligodendrocytes, l'oedème des astrocytes et l'accroissement du nombre des corps denses dans les cellules gliales ont été les réactions du cerveau à ce stimulus chimique.

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Effects of Various Inhibitors of Protein Cross-linking on the Formation of Fertilization Membrane in Sea Urchin Egg

At fertilization, sea urchin eggs produced a membrane. A few min after its formation, the fertilization membrane exhibited an increase of its mechanical resistance[1] and of its stability towards various chemicals. Runnström[2] distinguished 2 stages in the differenciation of the fertilization membrane: the assembly stage and the solidification or hardening stage. It is suggested that the 2 stages correspond to the formation of various types of binding necessary for the cohesion of the membrane. The increase of the stability of the fertilization membrane should involve new types of links. Two ways of studying this problem appear experimentally possible: in the first place, the study of effects of various chemical reagents on the membrane at different stages of its formation. The knowledge of the properties of reagents can help to investigate the nature of the links involved in the structure of the membrane. In the second place the use of agents able to pre-

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