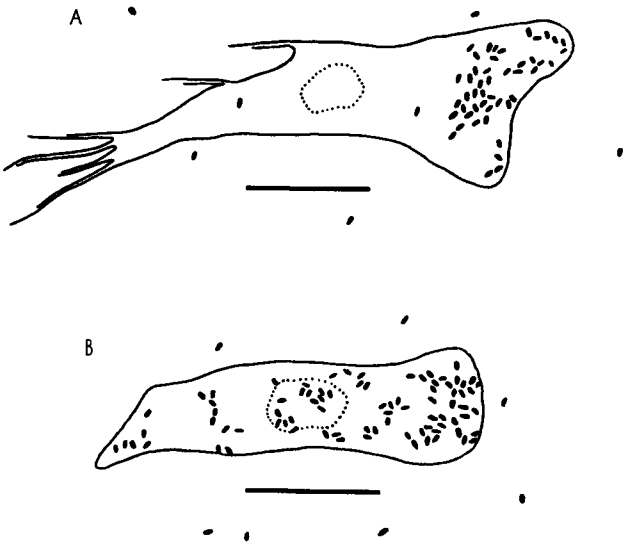
Bacteria as Cell Surface Markers on Normal and Malignant Mammalian Cells[1]

A number of attempts have been made to compare normal and malignant cells by observing differences in their biochemistry, their biophysics, and their surface properties[2-4]. All these approaches yield important information concerning the distinction between the two types of cell. Considerable interest therefore attaches to any additional distinctions that can be made. The results presented in this paper demonstrate just such a distinction in the microtopography of the cell surface using bacteria as markers. In an analogous study, MARCUS[5] used red blood corpuscles, but commented on the difficulty of obtaining red blood corpuscles small enough to give a meaningful resolution. Bacteria, being of the order of 2 to 3 µm in length, circumvent this difficulty. The present experiments should be regarded as complementary to those of SHAFFER[6] in which cationic and anionic exchange resins were used to study the cell surface of slime mould amoebae.

The experiments were conducted as follows. HeLa (calf) and WI 38 human embryonic lung cell lines were obtained from Flow Laboratories, Irvin, Scotland, as early monolayer cultures on coverslips in Leighton tubes. They were maintained at 37.5 °C for 2 to 3 days before an experiment. The medium was changed daily, and consisted of Flow Laboratories MEM + 10% foetal bovine serum. Log phase cultures of *Aeromonas liquifaciens* N.C.I.B. 9233, *Escherichia coli* N.C.T.C. 8196, and *Pseudomonas fluorescens* N.C.I.B. 9046, were resuspended in Hanks solution containing buffered bicarbonate (Oxoid No. BR 19a, and BR 26), and 5 ml of each placed in sterile plastic petri dishes (5 cm ⌀). Coverslips with WI38 or HeLa cells were taken through three dips of Hanks solution, and then placed cell side uppermost into the petri dishes which were shaken at 120 shakes/min for 1 h (vertical arc of 1.5 cm). The coverslips were then removed, rinsed gently in three 5 ml volumes of Hanks solution, and photographed under phase. All experiments were conducted at 37 °C. Counts and drawings were made from projected negatives.

The results of a typical experiment are given in the Table. More bacteria attached to the cells than to the glass between the cells, and the cells/glass ratio was more marked for the HeLa than for the WI 38 cells. Samples taken during the progress of the hour showed that these differences were distinguishable within 15 to 20 min. There were also obvious differences in the patterns of distribution of bacteria on the glass and on the cells, and between the 2 types of cell. Bacteria on the cells were aggregated, while those on the glass were randomly distributed, and there were more aggregates on the HeLa than on the WI 38 cells. The pattern of aggregates on the cells was very characteristic, and was most apparent on cells that were not in contact with other cells. On both HeLa and WI 38 cells, aggregates were commonly found at or near the leading edge of the cell or at both ends of a bipolar shaped cell. On the HeLa cells they were often found on areas of the cell surface close to the nucleus. These points are illustrated by tracings from high magnification phase contrast photographs of 2 cells, in Figure A) and B). These characteristic patterns of adhesion and their differences between HeLa and WI 38 cells are consistent, and have been obtained in many experiments.

The differences in the numbers of bacteria adhering (Table) and in the patterns of adhesion (Figure A and B) must reflect differences in the local characteristics of the cell surface between normal (WI 38) and malignant (HeLa) cells. There is little information on localized specificities of the mammalian cell membrane. Patches of fuzzy coat



A) *Pseudomonas fluorescens* on a WI 38 human embryonic lung cell. B) *Pseudomonas fluorescens* on a HeLA cell. Tracings from a series of ×1000 phase contrast photomicrographs. The area of the nucleus is shown by the dotted line in each cell. The black bar beneath each diagram = 20 µm.

Bacteria attaching to cells

		Aeromonas liquifaciens	*Escherichia coli*	*Pseudomonas fluorescens*
bacteria attached to WI 38 cells	on cells	4.803	2.321	3.563
	on glass	3.234	0.7436	1.780
	cells/glass ratio	1.49	3.12	2.0
bacteria attached to HeLa cells	on cells	6.101	3.480	4.289
	on glass	3.500	0.6262	1.404
	cells/glass ratio	1.74	5.56	3.05

Numbers are given as numbers of bacteria × 10^6/cm² of surface. Statistical analysis of the results (χ^2) showed that there were significantly more bacteria on the cells than on the glass surfaces ($P < 0.001$), and that the cells/glass ratio was more marked for HeLa cells than for WI 38 cells ($P < 0.001$).

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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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