Broth) was filtered on Millipore (0.45 μm), concentrated 50-fold by ultrafiltration (Amicon PM 10) and filtered on Sephadex G-100 (elution buffer: phosphate 0.1 *M*, pH 7.2); for the 11 strains, 1 hemolytic peak was found (apparent molecular weight 46,000 ± 3000). The hemolytic fractions were pooled, dialyzed against glycine 1%, pH 7 and electrofocused in a pH 5–8 gradient of Ampholine. 2 major and 1 minor hemolytic peaks were found, corresponding respectively to pI 6.54; 6.13 and 5.85. A second isoelectricfocusing of each major peak confirmed these values. Moreover, by Sephadex gel filtration, the same apparent molecular weight (46,000) was found for each pooled fraction. Samples were applied to polyacrylamide gels for electrophoresis and the hemolytic bands were detected by placing the gels on blood agar plates. For all the strains tested, the hemolysin migration appeared to be identical. From these results it seems that the 11 (toxigenic and non-toxigenic) strains of *Cl. tetani* have a hemolytic system which cannot be differentiated by their molecular weights, their isoelectric points or by polyacrylamide gel electrophoresis. The hemolytic system of each serotype tested can be resolved into three fractions by isoelectricfocusing.

Interaction of Tetanus Toxin with Cultured Neuroblastoma Cells: Analysis by Antitoxin Immunofluorescence

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The membrane binding of tetanus toxin and toxoid was studied with intact cells. 24 h monolayer cultures of mouse Neuroblastoma C1300 cell line clone NB2A (gift of Dr. D. MONARD, Friedrich-Miescher-Institut, Basel) were incubated for increasing time periods with 20 Binding Units of either toxin or toxoid. Each Binding Unit (BU) as defined by KRYZHANOVSKI (Bull. exp. Med. Micro., 1975, in Russian) is approximately equal to 1 L_f (RAMON, Rev. Immun., Paris 1940). Cells were grown in petri dishes containing glass cover slips and maintained at 37 °C in an 8% CO_2 atmosphere. Samples were fixed in acetone, cells stained by the Giemsa method and bound antigens visualized by indirect immunofluorescence with FITC- conjugated antiserum. Preparations were observed by phase contrast for cell morphology and with fluorescent microscopy for binding evidence. The toxin bound increased over a 24-h period, however no evidence of toxoid binding was demonstrated by immunofluorescence. Exposure to the toxin in growth (presence of serum) and 'differentiation' (absence of serum) cultures showed a marked difference in binding distribution. Observations of toxin treated cells pointed out a net difference in cell morphology in absence of serum, but no change was visible in growth cultures, either in cell morphology or population numbers. Chemical modulators of membranes were assayed for effect on toxin binding both by preincubation and coincubation with the agents: poly-D-glutamate, NH_4Cl, neuraminidase (*V. cholerae*) and β-galactosidase (*E. coli*). In these preliminary studies the technique has not only proven useful as a method to visualize and an immunofluorescent probe to evaluate the distribution of cell surface receptors and their character, but also as a qualitative back-up to [^{125}I]-toxin binding experiments (in preparation).

Production of Cholera Toxin by *Vibrio cholerae* Strain 569 B and B 1307

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Strains 569 B and B 1307 of *Vibrio cholerae* synthesize in vitro an exo-enterotoxin. The rate of this synthesis was determined by immunochemical and biological methods. Measurements in the supernatants from cultures of strain 569 B revealed, that this toxin remained stable over a long period of time, whereas in supernatants from cultures of strain B 1307, a decrease in the specific biological activity was found although the immunochemical values remained constant. Strain B 1307 was also elaborating a proteolytic enzyme. The assumption, that this enzyme causes the loss of biological activity could not be confirmed. During cultivation of strain B 1307 a toxoid was spontaneously formed, which could not be differentiated immunochemically from the toxin. The decrease in biological toxin activity can partially be explained through the formation of this toxoid.

Analysis of PBSX⁻ and Cell Wall Turnover Reduced Mutants of *B. subtilis*

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MNNG mutagenized populations of *B. subtilis* 168, *thy*⁻, *trp*⁻, were successively treated with mitomycine C and UV and the survivors screened for mutants defective in production of active PBSX particles. 11 such mutants, designated Ni1–8, Ni10, Ni11 and Ni15 were isolated and back-crossed into a multiple auxotroph by co-transformation with *met* C. Derivatives of strain Ni1 were of 2 types designated 1 and 16 (see below). Strains Ni1–8, 11 and 15 did not lyse upon treatment with various phage inducing agents. Strains Ni10 and 16 showed a normal lysis curve upon induction and their lysates, as determined by electron microscopy, contained respectively phage heads (*xtl* mutation) and phage tails (*xhd* mutation). Using PBS1 mediated transduction all PBSX mutations were mapped by 3 point crosses between *arg C* and *met C*. The apparently reduced autolysis of stationary phase cultures of Ni strains prompted us to examine the turnover of their cell wall. It was found that all original strains have a reduced wall turnover. Genetic analysis has shown that this property of different PBSX⁻ non-inducible strains is due to different mutations all mapping in the PBSX region. Reduced turnover of the original strain Ni10 is due to an unlinked *gta* C mutation. PBSX inducible back-crossed strains 10 and 16 exhibit a wild-type wall turnover. It would appear that proteins involved in cell metabolism are under prophage PBSX control.

Cell Wall Turnover, Autolysin Levels, and Surface Expansion in *B. subtilis* and a Mutant, Ni 15

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Ni15, a mutant of *B. subtilis* (see previous communication), growing on a casein hydrolysate supplemented medium at 45 °C (generation time = 20 min) has a markedly reduced cell wall turnover (POOLEY, J. Bact. *125*,