meters of isolated mitochondria and also of intact infected cells decayed beginning from 10th h p.i. At this time infected cells exhibited only slight morphological differences to controls in light microscopy. Since mitochondria play important roles both in energy supply and also in regulation of cellular ionic environment progressive functional impairment may contribute to cytopathic damage due to viruses.

Studies on Semliki-Forest-RNA Containing Polysomes

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The genomic RNA of Semliki-Forest-Virus (SFV) sediments at 42S. In the infected cell several viral RNAs can be found: a 42S-, a 26S- and a double stranded 20S RNA among others. Polysomes were extracted from SFV infected chick embryo fibroblast cultures with a hypotonic medium containing Triton-X-100 and cycloheximide to prevent ribosomal run-off; they were then isolated by centrifuging through layers with high sucrose concentrations. Such polysomes are highly active in cell-free systems with sap from uninfected chicken embryos. The different parameters of the cell-free system have been studied. If the viral RNA has been labelled in vivo in the presence of actinomycin to inhibit labelling of host RNA, all individual polysomal peaks contain label, though the main RNA is always the 26S-RNA. Therefore, as opposed to polysomes from uninfected cells, different spacings between ribosomes on this single type of RNA have to be assumed. The inhibition of over-all protein synthesis in infected cells in vivo cannot be explained by a polysomal defect: equal concentrations of polysomes in uninfected and in infected cells (irrespective of time after infection up to 14 h) yield in vitro the same amount of incorporation. A better explanation for inhibition of protein synthesis in vivo is given by the observation of an increasing amount of single ribosomes and a decreasing amount of polysomes in extracts dependent on duration of infection. Under condition of EDTA-desintegration of polysomes to ribosomal subunits in vitro, the 26S polysomal RNA sediments with the 40S ribosomal subunit.

Some Ultrastructural Aspects of the Replication of Coxsackievirus A9 in CV-1 Monkey Kidney Cells

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A lot of biochemical data concerning the replication of Enteroviruses are available but only little is known about the morphological structures involved in the synthesis of the virus progeny. The presented study deals with the ultrastructural changes in monkey kidney cells (CV-1) infected with Coxsackievirus A9. Giant polysomes containing about 35 ribosomes appear at 1 h p.i. in infected cells. According to biochemical data (SUMMERS et al., Virology *31*, 427–435, 1967; JACOBSON et al., Proc. natn. Acad. Sci. USA *61*, 77–84, 1968) they probably contain the transcript of the whole genetic information of the virus. After 4 h p.i. considerable alterations in the ultrastructure of the nucleus become obvious. At the same time areas of membrane bound vesicles arize in the cytoplasm in place of the Golgi zones. In parallel with the appearance of the vesicles the polysomes disintegrate. Different functions were attributed to the vesicles, e.g. they were thought to be the site of virus RNA synthesis (CALIGUIRI et al., Virology *42*, 100–111, 1970). The fact however that they appear after the extensive formation of the giant polysomes suggests that they are at least not the only site of viral RNA synthesis. Titration experiments show a sharp rise in infectious virus after 5 h and 7 h p.i. for intracellular and for extracellular virus respectively. Various granular and fibrillar components in the cytoplasm are though to consist of virus material but no virus progeny is detectable in their neighbourhood. All of the newly synthesized viruses are found in cell protrusions. Not until late in the replication cycle groups of virus particles are located in the inner part of the cell too. Most of the viruses are arranged in parallel linear arrays which are separated from each other by thin fibrils. Virus cristals were never observed.

Anucleate Cells: their Preparation and their Reaction to Poliovirus Infection

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Virus-host cell interactions were studied in poliovirus-infected, enucleated HEp-2 cells. Cytochalasin B causes spontaneous enucleation of mammalian cells at low percentages, which can be considerably enhanced by ultracentrifugation of whole monolayers. Some cell lines (like HEp-2 cells), however, do not adhere firmly enough to a carrier to be centrifuged. Therefore, a new method for mass enucleation was developed: S-shaped density gradients, containing colloidal silica, were adjusted to have their shallow part between the density of the cytoplasm and that of the nuclei. More than 70% of HEp-2 cells could be enucleated by ultracentrifugation in such gradients, containing cytochalasin B. Purification up to 97% was carried out by centrifugation at low speed through a second, preformed gradient. Purity of the anucleates was tested in Giemsa-stained smears as well as by ^{3}H-thymidine and ^{3}H-uridine uptake. The viability of the enucleated cells, tested by ^{3}H-leucine incorporation was comparable to that of normal cells. For further details see BOSSART et al., Expl. Cell Res. *96*, 360–366 (1975). In enucleated cells, the amount and kinetics of virus-induced RNA synthesis was measured by ^{3}H-uridine incorporation, the number of RNA synthesizing cells being determined by autoradiography. In contrast to ^{3}H-uridine uptake, the ^{3}H-leucine incorporation showed no virus-induced peak. Accordingly, virus yield was low and no virus-induced redistribution of lysosomal enzymes occurred. The available data indicate, that protein synthesis is the limiting factor of virus growth in the polio-HEp-2 system.

Comparative Study of the Hemolysin Produced by Different Serotypes of *Clostridium tetani*

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The hemolysins produced by 9 toxigenic (Tulloch Types I to IX) and 2 non-toxigenic (types III and V) strains of *Clostridium tetani* have been partially characterized. The supernatant of a 24 h culture (Brain Heart Infusion

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*,