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Studies on the Na\(^+\), K\(^+\)-ATPase Activity, Membrane Potential and Amino Acid Uptake of Poliovirus Infected HeLa Cells

SCHAEFER, A., ZIBIRRE, R., KABUS, P., KÜHNE, JUTTA, and KOCH, G.

Na\(^+\), K\(^+\)-ATPase activity was studied in a plasma membrane rich fraction isolated from control and poliovirus infected HeLa cells and compared to membrane potential and amino acid uptake in parallel cultures of intact cells. Na\(^+\), K\(^+\)-ATPase activity in membranes from infected HeLa cells increased relative to control with a maximum at 90 minutes post infection (+30\%\) and decreased again (180 min.: -30\%). Similar but slighter changes were observed in the membrane potential dependent tetraphenylphosphonium (TPP\(^+\)) uptake, indicating a correlation between membrane potential in intact cells and our measurement of plasma membrane Na\(^+\), K\(^+\)-ATPase. At approximately 1 h post infection we observed a decrease in the uptake of amino acid (methionine, leucine) in infected cells relative to controls. These results suggest that the decline in amino acid uptake is not mediated by virus-induced changes in the Na\(^+\), K\(^+\)-ATPase activity or membrane potential.

Sequence Homology in Different Strains of FMDV and other Picorna Viruses

MARQUARDT, O.

Restriction enzyme generated subgenomic fragments of cloned cDNA prepared from RNA of the strain 01K of FMDV were compared quantitatively for sequence complementarity with radioactive RNA from strains CObb and A2S or with RNA from mengo or polio virus in hybridization experiments by use of the Southern technique. Nucleic acid sequences neighbouring or including the 3' end of viral genomes are demonstrated to be 80\% homologous in FMDV. In contrast, sequences coding for the capsid proteins VP1 and VP3 were remarkably heterologous (less than 20\%) in FMDV. Sequences coding for non-structural proteins showed 35-50\% homology. Thus no highly conserved coding sequence was detectable in FMDV by this technique. No hybridization could be detected between O1K specific DNA and polio RNA, while weak hybridization was observed with mengo RNA at areas including the 3' end and with a part of the gene for precursor protein P52.
Modification of Poliovirus Capsid Proteins

SCHÄRLI, CLAUDIA and KOCH, G.

Poliovirus type 1, strain Mohoney contains a protein kinase activity. A highly purified (two cycles of CsCl gradient centrifugation) poliovirus preparation is able to transfer the γ-phosphoryl-group of [32P]_ATP to acid precipitable material. When the reaction product is analyzed by SDS PAGE, all structural proteins of polio are phosphorylated. Most of the label is incorporated in the minor capsid protein VPO and to a lesser extent in VP1, VP2 and VP3. – The incorporation of [32P]- into viral proteins is linear up to about 90 min. at 37 °C. The reactions requires Mg⁺ and the PH optimum is around 8. The in vitro phosphorylated proteins are recognized by antiserum directed against viral capsid proteins. – The properties of the kinase as well as possible biological functions and consequences of the phosphorylation are still unknown. Further investigations are in progress to clarify these questions.

Max von Pettenkofer-Inst., D-8000 München 2

Growth of Hepatitis A Virus in Human Diploid Fibroblasts

GAUSS-MÜLLER, V., DEINHARDT, F., SCHEID, R., and FRÖSNER, G.

Hepatitis A virus (HAV) was isolated directly from human stool in diploid human fibroblasts. Viral antigen was expressed only after 210 days of incubation of the infected cultures. In contrast, HAV first adapted to growth in human hepatocellular carcinoma cells caused antigen expression in fibroblast cultures already 90 days after infection. During further passage the time of first appearance of antigen in infected fibroblasts decreased to about 14 days in both passage series (i.e. cultures inoculated with virus recovered directly from human stool or after adaption to human hepatoma cells). Antigen was predominantly cell-associated and was shown by immunofluorescence to be located exclusively in the cytoplasm. – Biophysical properties of HAV particles extracted from infected cells were comparable to those described for HAV extracted from human stool. Those findings are of importance for preparation of large amounts of HAV for vaccine production.

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Cloning of Hepatitis A Virus Genomes

HELM, K. VON DER, WINNACKER, E. L., and DEINHARDT, F.

Subgenomic fragments of the genomic RNA of hepatitis A virus (HAV) were cloned via cDNA into Pst 1 site of pBR 322. By restriction enzyme pattern analysis and hybridization of the cloned HAV DNA with selected fragments of the HAV RNA it was determined that the major part of the cloned HAV DNA fragments are located at the 3’ end of the genome but a few clones are distributed over the entire genome.
Further Studies on the Inactivation of Hepatitis A Virus (HAV)

SCHEID, R., DEINHARDT, F., FRÖSNER, G., and GAUSS-MÜLLER, V.

Some additional data on the temperature stability of HAV at 60 °C and 56 °C were obtained. Virus infectivity was not detected after 6 and 12 h respectively in first passage material; however, after blind passage some residual infectivity was still present, even after treatment for 12 h at 60 °C. At 4 °C virus infectivity was stable for at least 16 weeks - 70% of alcohol reduced the titre by 4.75 log 10 after 12 h, and formalin 1:350 at room temperature reduced the titre by 3 log 10 after 60 min. After 15 min. inactivation, Na-hypochlorite at a concentration of 10 mg/l, iodine at 3 mg/l, and potassium permanganate at 30 mg/l were suitable as water disinfectants. Chloramine T did not inactivate HAV at a concentration of 1 g/l, nor did peracetic acid (15%) at 300 mg/l. - At pH3 (room temperature, 3 h) virus infectivity remained detectable.

Development and First Application of a Hepatitis B-Vaccine

THOMSSEN, R., GERLICH, W., BÖTTCHER, U., ANSORG, R., STIBBE, W. LEGLER, K., BANDLOW, G. (Göttingen), WEINMANN, E. (Marburg), KLINING, O. (Kassel), and PFEIFER, U. (Würzburg)

We developed a hepatitis B-vaccine. Source of HBsAg: Only anti-HBe pos. HBsAg carriers. Purification: 2 X fractionated precipitation with PEG, zonal rotor flotation with KBr, gel-chromatography (Biogel®). Inactivation: 1:425 diluted formalin for 4 days at 37 °C. Safety-tests for sterility, pyrogenicity and for residual infectivity according to recommendations of the WHO. Examination of 6 postvaccin. liver biopsies in each of 4 chimpanzees within an observation period of 6 months p. v. excluded infection not only with hepatitis B but also with hepatitis Non-A, non-B. The potency of the vaccine (1 ml vaccine contains 11 300 Natl. Units of HBsAG activity and 1,0 mg aluminium-hydroxide) was compared with a reference vaccine of the NIH, Bethesda, Md, USA, in guinea pigs. 44 from 47 (94%) human volunteers developed anti-HBs already after the 2. dosis given 4–6 weeks after the 1. dosis in contrast to 30% after the 1. dosis. After the 3. dosis given to three volunteers 5 months after the 2. dosis there was a booster reaction about 10 fold above the previous titer (500–700 m-WHO units/ml). Serious side effects to the vaccine could not be observed.

Differences in the Structure of Cloned DNA among Different HBV Subtypes

BÖTTCHER, U., PAULY, P., HAHN, U., KÜNTZEL, H., and THOMSSEN, R.
we have now identified subtype specific sites in HBV-restriction maps. The maps were aligned to the Eco RI site. Only subtype ay revealed one subtype specific site of each XbaI (2000 BP), HinclI (2200 BP) and Bam HI (2900 BP). They are situated outside the s-gene. Employing this system the subtypes of three HBV-DNA sequences that have been published so far, could be determined. Thereupon the first 54 triplets of a HBV (ad)-DNA sequence of the s-gene could be compared with published data. Nucleotide exchanges in triplets 12 and 13 did not cause an amino-acid (aa)-exchange, but changes in the triplets 45 and 46 do cause such exchange. Thus, in subtype ay, aa 45 is thr, and aa 46 is thr, while in subtype ad, aa 45 is ser and aa 46 is pro. These exchanges may produce different conformation of the peptides.

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Characterization of the Hepatitis B Virus (HBV) Associated Proteinkinase

GERLICH, W. H.

Purified HBV-core preparations were shown to contain a proteinkinase which phosphorylates the major core protein (Albin and Robinson, 1980). In this study it was found that this enzyme copurifies with the light (d = 1.31) but not with the heavy subfraction (d = 1.35) of the core-particles. The enzyme has a high affinity for ATP (K\text{M} = 2.6 \times 10^{-6}) and it transfers approx. one phosphate per particle within minutes. The $^{32}$P-phosphate introduced \textit{in vitro} cannot be removed from core-particles by digestion with alkaline phosphatase. After lysis of the cores with SDS the $^{32}$P can be cleaved from the protein. This suggests that the enzyme and its phosphate acceptor site are located within the particle. After acid hydrolysis the incorporated $^{32}$P co-migrates with phosphoserin but not with phosphothreonin or phosphotryosin.

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A Phosphokinase Activity Associated With the Hepatitis B Virus (HBV) Core

HELM, K. VON DER, ROGGENDORF, M., and SIEGERT, W.

Preparations of core antigen positive (HBcAg) fractions obtained from HBV positive human liver tissue are associated with a phosphokinase which copurifies with the HBcAg in CsCl density- and sucrose velocity gradient centrifugations. The acceptor protein for this kinase is the 19 000 d HBcAg protein; casein as an exogenous acceptor is also phosphorylated to a minor extend. The phosphorylated amino acid is serine or threonine and not tyrosine, as it is frequently the case with tumor virus specific protein kinases.

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Effect of Glycosidases on the Proteins of Hepatitis B Surface Antigen (HBsAg)

STIBBE, W. and GERLICH, W. H.

The protein composition of purified HBsAg was studied by SDS-gel-electrophoresis and staining with silver. In addition to P25 and GP28 of HBsAg two further proteins,
GP33 and GP36, were found consistently in preparations from 9 different blood donors. The glycoprotein nature of GP 33 and GP 36 was shown by the increase in electrophoretic mobility after treatment with endoglycosidase H or neuraminidase. Immune precipitation with anti-HBs in RIPA-buffer confirmed the specificity of the glycoproteins. The change in apparent mol. weight after treatment with neuraminidase was larger for GP36 (900) than for GP33 (600) or GP28 (300). The data suggest that GP 33 and GP36 are multiply glycosylated proteins with N-glycosidic linked carbohydrate side chains of the mixed type.

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Frauenklinik d. Univ. Köln

HBs Antigen Screening in Pregnancy in Order to Prevent a Postnatal Infection of the Child

ZIPPEL, C., EGGERS, H. J., ZIPPEL, H. H., and FUHRMANN, U.

HBs antigen screening was done in the blood samples of 1,790 pregnant women. 33 blood samples were found to be HBs antigen positive. 32 women were asymptomatic carriers, 1 woman suffered from chronic hepatitis. 31 children have been born until now. The examination of cord blood showed HBs antigen in 6 samples; the venous blood samples taken thereupon demonstrated HBs antigen only in two samples. The HBs antigen negative children were treated with HBIG. The follow-up examination of the treated children showed no signs of infection up to now. – One child born to an HBs antigen positive mother was already infected at the time of birth. Another child born to an anti HBs positive mother became HBs antigen positive at the age of 4 months. Unfortunately, this child had not been treated with HBIG at the time of birth.

Staatl. Medizinaluntersuchungsamt, D-3000 Hannover

Perinatal Transmission of Hepatitis B Virus

WILLERS, H., PONNATH, H., SIPOS, S., and MÜLLER, R.

Sera of 5,847 pregnant women living in the Hannover area were investigated for the presence of HBsAg. 56 healthy HBsAg carriers were found: 17 (0.34 %) among 4,962 women of German origin and 39 (4.28 %) among 912 foreign-borne women from Southern European and non-European countries. 10 out of the 56 HBsAg carriers (1/4,962 German women and 9/912 foreign-borne women) were recognized to be HBeAg-positive. – The risk of perinatal transmission of hepatitis B virus in infants of HBeAg-positive mothers is suggested to be 90 % and of HBeAg-negative mothers 12 %. – Thus in the Hannover area 6 out of 10,000 infants born to German mothers and 129 out of 10,000 infants born to foreign mothers become HBsAg carriers due to perinatal hepatitis B virus-infection.

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Specificity and Significance of Anti-HBc IgM Determination

FRÖSNER, G. G.

A radioimmunoassay for anti-HBc IgM using higher concentration for the dilution of serum (5 mg/ml) and HBcAg (10 mg/ml) was compared with an ELISA test \( J. \) clin.
Microbiol. 13 (1981), 618. Only 10 of 157 (6%) HBsAg positive blood donors were positive (40 in ELISA) and no correlation with the total anti-HBc titer could be found. After fractionation of a serum with discrepant results a positive result was found in the ELISA only in the IgG fractions. In a collaborative study with Altorfer et al. (presented also at Lisbon, EASL 1981). RIA results had the following significance: 1) In chronic hepatitis anti-HBc IgM correlates with the degree of inflammatory activity. 2) Anti-HBc IgM is not a good marker for virus replication. 3) The titer of anti-HBc IgM can discriminate between acute (> 1:1000) and chronic hepatitis B.

1. Biotest-Serum-Institut GmbH, Flughafenstr. 4, D-6000 Frankfurt 73;
2. The Lindsley F. Kimball Research Inst. of The New York Blood Center, 310 East 67 Str., New York, N. Y. 10021

β-Propiolactone/Ultraviolet Treatment: Quantitative Studies on Effectiveness for Inactivation of Hepatitis B Virus

STEPHAN, W.¹ and PRINCE, A. M.²

A combined β-propiolactone/ultraviolet irradiation (β-PL/UV) procedure was first proposed by LoGrippo et al. (1956) as an effective means of sterilization of pooled human plasma. Our studies revealed that a standardized procedure could be carried out on a large scale without major denaturing effects on plasma proteins. Prince et al. demonstrated (1981) that β-PL/UV can reduce hepatitis B virus titer about 10⁷ fold. This was confirmed by Stephan et al. (1981), who showed a 10⁶ fold reduction in hepatitis B titer by means of β-PL/UV treatment. The effectiveness of the cold sterilization procedure as regards reducing virus infectivity is considerably greater than that of pasteurization (10 h 60 °C) for which Shikata et al. (1977) showed a 10⁴ fold reduction of hepatitis B virus infectivity. It has also been found that factor IX concentrate and stabilized serum (Biseko®) derived from pooled, cold-sterilization human plasma transmitted neither hepatitis B nor hepatitis non-A, non-B in chimpanzees.

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Comparison of Different Evaluation Systems for Determination of Viral Antibodies of the IgG and IgM Class in the Enzymeimmunoassay

ROGGENDORF, M., ZACHOVAL, R., ZOULEK, G., and DEINHARDT, F.

The enzymeimmunoassays used to demonstrate antibodies of the IgG and IgM class against viral antigens reveal a high sensitivity. At onset of acute viral infections IgM antibodies can be demonstrated up to serum dilutions of 10⁻³. Due to this high sensitivity, the determination of antibody titters is not accurate and reproducible, because titer end points are determined as that dilution of sera giving an O.D. sample/O.D. negative control equal or greater than 2.1. - For the determination of antibody concentrations an evaluation method is proposed which correlates, the measured O.D. of sera at one dilution step to the O.D. of a reference serum which is defined arbitrarily to contain 100 antibody units. Using an ELISA for detection of antibodies to adenovirus, a significant increase in antibody units of acute phase sera over that of convalescent phase sera is observed. Low day to day variations are seen in tests carried out on different days. In an ELISA which is designed to detect antibodies to tick-borne encephalitis virus of the IgM class, the day to day variation using antibody units was significantly lower than using P/N ratios.
Structure and Function of the Core Protein of Alphaviruses

BOEGE, ULRIKE and WITTMANN-LIEBOLD, BRIGITTE

The complete primary structures of the core proteins of Sindbis and Semliki Forest virus have been established by proteinchemical methods. Both proteins contain clusters of basic amino acids and proline in the N-terminal part, suggesting that its function is to bind to the nucleic acid. The C-terminal parts have extensive identical sequence regions, probably providing the recognition sites for protein-protein interactions. Both proteins contain within these highly conserved portions the sequence Gly-Asp-Ser-Gly which is typical for serine proteases and most likely is related to the catalytic function of the core protein as a protease when cleaving its own peptide chain from the nascent protein precursor. Experimental studies using peptides which contain certain sequence regions will help to elucidate the relationships of structure and functions.

Rabies Virus Decreases Agonist Binding to Opiate Receptors of Mouse Neuroblastoma-Rat Glioma Hybrid Cells 108-CC-15

MÜNZEL, P. and KOSCHEL, K.

An acute and a persistent infection with Rabies virus (HEP Flury) was established in the CNS-derived cell line 108-CC-15 (NG 108-15). These cells possess specific membrane receptors to many hormones and neurotransmitters including opiate receptors. In both cases we found increases in the dissociation constant \(K_D\) for the agonist \(^3H\)-etorphine as estimated by Scatchard plot analysis. However, in both cases there was no change in the number of opiate receptors per cell compared to uninfected cells. These studies complete our previous published observations of the impairment of receptor functions in rabies virus infected cells (1).

Reference
(1) Koschel, K. and M. Halbach: J. Gen. Virol. 42 (1979), 627–632.

Effect of Measles (SSPE) Antiserum on Viral Surface Proteins and Hormone Receptor Activity in C6/SSPE Persistently Infected Cells

BARRETT, P. N. and KOSCHEL, K.

It has been reported (1) that measles antiserum can modulate the expression of certain virus proteins in acutely measles infected cells. We have examined the effect of measles (SSPE) antiserum on the expression of viral surface proteins in C6/SSPE infected cells. We have shown an over 50% reduction in the amount of viral antigen present on the membranes of these cells following incubation with SSPE antiserum. This was demon-
strated both by immunofluorescence and radioimmunoassay. However this loss of antigen had no effect on membrane receptor linked cAMP synthesis. This is discussed with respect to the effect of virus antigen insertion in cell membranes on specialised membrane bound functions.

Reference
(1) Fujinami, R. S. and M. B. A. Oldstone: Alterations in Expression of Measles Virus Polypeptides by Antibody. J. Immunol. 125 (1980), 78–85

BAUMANN, E. A. and HAND, R.

We have analyzed the effect of phosphorylation and dephosphorylation on the structure and DNA binding of D2-T antigen. On non-denaturing pore-gradient gels the purified protein migrated with an apparent size of 135,000 daltons. In vitro phosphorylation by the protein kinase associated with the purified protein resulted in a shift of most of the protein to a size of 740,000 daltons, and it was this form that contained most of the phosphate incorporated. This aggregation was completely reversible by treatment of phosphorylated D2-T antigen with alkaline phosphatase. Partial tryptic digestion indicated that phosphorylation of sites in the N-terminal part of the protein is responsible for the observed aggregation. - As shown by protein blotting onto nitrocellulose filters predominantly the 740,000 dalton form bound to SV40 DNA. However, only a fraction of the in vitro phosphorylated protein did bind to DNA, suggesting that aggregation alone is not sufficient for DNA binding.

Fak. f. Biologie, Univ., D-7750 Konstanz

BRAUER, D., WESTPHAL, K.-H., BURGER, C., and FANNING, E.

Subclasses of SV40 large T antigen were separated by zone velocity sedimentation. Three major forms, which sedimented at about 5–6S, 14–16S and 23–25S, have been shown to differ biologically and biochemically (1). Each subclass was tested for specific binding to restriction fragments of SV40 DNA using an immunoprecipitation assay. - All three forms of T antigen bound specifically to a restriction fragment containing the SV40 origin of replication. However, the 5–6S form bound more origin fragment per unit T antigen than the other forms. The 5–6S form bound equally well to origin DNA in the presence and absence of excess cellular DNA, whereas the binding of the 14–16S form was reduced in the presence of cellular DNA. All forms of T antigen from SV40-transformed SV80 cells bound much less origin DNA than that from infected cells.

Reference
(1) Fanning, Nowak, Burger: J. Virol 37 (1981) 92–102
Demonstration of Various Human Papillomavirus Types in Benign and Malignant Tumors of an Epidermodysplasia Verruciformis Patient

PFISTER, H., NÜRNBERGER, F., HETTICH, I., and HAUSEN, H. ZUR

Wart scrapings from several small skin regions of an Epidermodysplasia verruciformis patient were tested for papilomavirus-specific sequences by means of a 32P-labelled HPV8 DNA. Uncleaved wart DNA contained uniforme full length HPV genomes. Cleavage with Bam HI revealed six different patterns and a surprising heterogeneity even within small biopsies. At least two virus types showed only limited cross-hybridization with HPV 8a. One of these closely resembles HPV 5b (Bam HI fragments 2,9 and 2,1; Eco RI fragments 3,6 and 1,1). Hind II fragments A and C of his virus perfectly hybridize with HPV 8a; B, D and 6 anneal only partially and F, G show no detectable hybridization. The DNAs of four subtypes were partially characterized and mapped. Only DNA of the HPV 5b-like virus was detected in a probe from the center of a carcinoma at the patient’s forehead. This DNA persists extrachromosomally with more than 100 genome equivalents per cell.

Gene Expression of Papillomaviruses in Hamster Tumors

FREESE, U. K., SCHULTE, P., and PFISTER, H.

BPV 1 includes fibrosarcomas in hamsters. The tumors contain large amounts of complete virus genomes which persist extrachromosomally but there is no evidence for capsid protein synthesis or virus particle production. We used this system to study early viral gene expression. RNA from the tumors contained a single virus-specific RNA with about 1300 nucleotides which was shown to be polyadenylated by affinity chromatography on poly-U-Sepharose. The transcribed DNA region of BPV 1 was mapped within the two Hpa II fragments, which are next to the Bam HI cleavage site within the 1.4 X 10^6 d Bam HI/Eco RI fragment. Cross-hybridization studies under low stringency revealed some sequence relationship of HPV 1 and HPV 4 DNA to the transcribed region of BPV 1.

The Adenovirus Type 12-Mouse Cell System: Permissivity and Analysis of Viral DNA in Tumor Cells

STARZINSKI-POWITZ, A., SCHULZ, M., and DOERFLER, W.

Interactions between viruses and eucaryotic cells can be studied in a genetically well defined system like the mouse system. We have investigated whether Ad 12 DNA is able to replicate in primary mouse kidney cells or in the mouse cell line L929. It was shown by Southern blot experiments that Ad12 DNA was not able to replicate in L929 cells,
whereas in primary mouse kidney cells of (BALB/c × CS7/B16) F1 origin, viral DNA replicated. Moreover, we subcutaneously injected Ad12 into mice of various genetic origins. In about 100 mice injected, one tumor emerged in a BALB/c mouse almost 8 months after injection. Restriction pattern analysis of the tumor or DNA indicated that about 2–3 copies of Ad12 DNA were integrated and covalently bound to cellular DNA. With the techniques available no deletion or rearrangement of viral DNA could be found. The sites of junction between viral and cellular DNA will now be cloned and sequenced.

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**Virus-Cell DNA Recombinants in Human Cells Lysically Infected by Ad2**

NEUMANN, R., WEYER, U., and DOERFLER, W.

There is ample evidence for the notion that virus-cell DNA recombinants are formed in human cells productively infected with adenovirus type 2 (Ad2). These high molecular weight forms were detected at 1–2 h postinfection and were generated at high frequency. A limited set of rather specific recombinants was produced (Neumann and Doerfler, J. Virol. (1981) 887 suggesting that recombination exhibited a certain specificity. We now succeeded in molecularly cloning DNA fragments excised from the high molecular weight DNA of Ad2-infected human cells by the restriction endonuclease EcoRI in λgtWES – λB or in λCharon 4B. Some of these cloned fragments had sequence homology to both viral and cellular DNA. This result provided proof for the occurrence of virus-cell DNA recombinants.

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**Unmethylated DNA Sequences in the Promoter Regions of Integrated Adenovirus Genes Correlate with Gene Expression**

KRUCZEK, I. and DOERFLER, W.

An inverse correlation was established between the levels of DNA methylation at 5'-CCGG-3' sites in specific segments of integrated adenovirus DNA and the extent to which these segments were expressed (Sutter and Doerfler, PNAS 77 (1980) 253. Similar correlations were reported in other viral and non-viral systems. More recently, the results of in vitro experiments provided direct evidence for the notion that DNA methylation at specific sites led to gene inactivation (Vardimon et al., PNAS, in press). – In the present study a detailed methylation map at 5'-CCGG-3'(HpaII/MspI) sites in the expressed early and the silent late genes of Ad12 DNA was determined in three Ad12-transformed hamster cell lines. The early regions of integrated Ad12 DNA were unmethylated; in particular their promoter regions were unmethylated at the HpaII sites in all three lines investigated. The late regions including the promoter sites were completely methylated.
Abstracts of the 38th Meeting of the DGHM 423

Inst. of Genetics, Univ., D-5000 Cologne

Patch Homologies and the Insertion of Viral DNA into the Host Chromosome

GAHLMANN, R., DEURING, R., STABEL, S., WINTERHOFF, U., VARDIMON, L., and DOERFLER, W.

The sites of junction between viral and cellular DNA were sequenced to investigate two problems: 1. Are the sites of insertion of viral DNA specific? 2. What type of recombinatorial events occur in viral DNA integration? We have studied junction sites from the Ad2-transformed hamster line HE5, and from the Ad12-induced hamster tumor lines CLAC1 and CLAC3. The virus-cell DNA junctions were cloned in the DNA of bacteriophage λgt·λB. Appropriate restriction fragments were sequenced by the Maxam-Gilbert technique. There was no direct sequence identity apparent at the viral and cellular junction sequences. Deletions at the termini of the viral genome were seen involving 5(HE5), 45(CLAC3) or 174(CLAC1) nucleotides. Peculiar patch type homologies between the cellular and viral sequence adjacent to the site of junction and also in remote areas were observed. These patches may have an important function in integrational recombination.

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Primary Structure of the Coding Region of the Adenovirus Type 2 DNA Binding Protein

BÜTTNER, W., VERES-MOLNÁR, S., and BLOCK, J.

As a first step to understand the relationship between structure and function of the adenovirus type 2 DNA binding protein (Ad2 DBP) we have begun to determine the primary structure of the DBP gene. - The DNA sequence of 1956 nucleotides including the translated region of the Ad2 DBP gene was elucidated. The coding region of the DBP gene comprises 1587 nucleotides, information for 529 amino acids. - The only open reading frame in the mRNA homologous r-strand of the DBP gene begins with the initiation codon ATG at nucleotide position 2263 and ends before a stop codon TAA at nucleotide position 676. (Arbitrarily nucleotide 0 was positioned at the BglII cleavage site 60.2 of the Ad2 genome).

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Isolation and Preliminary Characterization of Temperature-Sensitive Mutants of Adenovirus

DARAI, G., HEINZELMANN, D., and FLÜGEL, R. M.

The isolation and characterization of Tupaia adenovirus (TAV) has been reported. In order to construct a genetic map of the TAV genome the use of temperature-sensitive mutants (ts) of TAV was necessary. - A variety of ts mutants of TAV, which were
generated by treatment of TAV virions (1 × 10^{10} pfu) with hydroxylamine, were isolated and preliminary characterized. Six of these mutants (ts: 1, 4, 11, 12, 13, and 54) did not replicate in Tupaia embryonic kidney cells at 39.5 °C, but did replicate well at 32 °C. The characterization of these mutants was carried out using complementation tests, host range studies and DNA analysis using different restriction enzymes. According to complementation analysis four groups were determined: group I = ts 1, group II = ts 12, group III = ts 13, and group IV = ts 4, 11, and 54. The host range study revealed that ts 1 and 54 also had properties of host range mutants. These mutants did not replicate in Tupaia baby fibroblasts in contrast to wild-type virus. Genome analysis of these mutants revealed that the mutated region is located between 69.9 to 74.5 map units. In addition, in ts 1 and 54 mutants a deletion from 0.77 Kb to 1.39 Kb (map unit 76.5 to 100) was detected.

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Interaction of Viral Substructures with Serum and Serum Components resp.

DENNIN, R. H.

During the final stage in the course of many viral infections complete virus particles as well as viral substructures enter the intercellular space, e.g. HBsAG, HBcAG in hepatitis B virus infections. The present study deals on the interaction of the potentially infectious adenovirus cores with DNA-specific antibodies and immunoglobulin solutions which had been absorbed by DNA-antigens. Cores were prepared by sarcosyl treatment of purified adenovirus typ 5. By electron microscopy immunocomplexes could be demonstrated which are composed of several individual core units. By buoyant density centrifugation in metrizamide gradients a drastic rise in the density of cores could be shown too. With immunoglobulin solutions, absorbed with native as well as with denatured DNA, so far no complexes could be assessed.

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Detection of a Retrovirus in Bovine Skin Tumour Cells

KAADEN, O.-R., LANGE, S., MARSCHALL, H.-J., and MOENNIG, V.

A cell line designated SBL-H12578 and several cell clones were established from skin tumours of the sporadic leukosis form. The cells were proved to be free of bovine leukemia (BLV), and some other bovine viruses. By indirect immunofluorescence macroscopic maps of a Theileria species were seen in the cytoplasm. The cells originated from a BLV-antibody negative animal and carried a female karyotype with some morphological aberrations. Evidence for a possible T cell origin of SBL-H12578 cells was obtained. After inoculation of 8 × 10^6 cells into 'nude mice' transplantable SBL-H12578 tumours developed. By incorporation of 3H-uridine and electron microscopy, the production of retrovirus particles by the cultured cells was detected. In the simultaneous detection test a high-molecular weight RNA co-migrating with FeLV 70 S RNA was demonstrated. No antigenic or genetic relationship between the skin tumour virus isolate and BLV or other major mammalian retroviruses has been found. – (Supported by Stiftung Volkswagenwerk).
Insectpathogenic Baculoviruses: Studies of the Activation of Endogenous C-type Retroviruses in Mammalian Cell Cultures

SCHMIDT, J. and ERFLE, V.

Activation of endogenous C-type retroviruses by Baculoviruses was studied in “in vitro” cell culture systems of four mammalian species: mouse, rat, monkey and man. Cells were treated with Baculoviruses (from larvae and insect cell cultures), Baculovirus-DNA, C-type retrovirus-activating chemicals and chemical insecticides alone and in combination. The activation of retroviral genomes was tested by the determination of reverse transcriptase activity in concentrated cell culture supernatants and by the demonstration of the intracellular localisation of retrovirus structural protein p30 applying the indirect immunoperoxidase technique. - C-type retroviruses were activated in mouse cells only by the halogenated pyrimidine analogue iododeoxyuridine. In Baculovirus-treated cell cultures no C-type retrovirus activation was detectable. In simultaneous treatments of the cells with Baculoviruses and chemicals no potentiating effects could be detected. Virions of Baculoviruses in mammalian cell cultures showed unaltered morphology and upon reisolation their infectivity in homologous insect cell cultures was lowered by approximately one log. No influence upon growth or morphology of the cells could be observed.

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Cleavage of Oncornaviral Fusion Proteins by the Viral Protease p15

HELM, K. VON DER, KONZE-THOMAS, B., KRAUSSLICH, H. G., and BORN, M.

The gene products of replication-defective oncornaviruses are high molecular weight proteins which comprise a gag related and onc gene product. They are probably not cleaved because the p15 protease gene is lacking. In vitro, however, the gag-specific peptide sequences are cleaved off upon addition of the purified viral p15 protease; in the case of the replication-defective, transforming avian sarcoma virus PRC II the cleaved non-gag part has a tyrosine-phosphorylating kinase activity similar to that described for the RSV src-gene product pp60src.

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Processing of Pr92gp, the Precursor to the Viral Glycoproteins of Rous Sarcoma Virus

BOSCH, V., SCHWARZ, R. T., ZIEMIECKI, A., and FRIIS, R. R.

The viral glycoproteins of Rous sarcoma virus gp85 and gp35 are synthesized via a precursor polyprotein Pr92gp. This precursor is already glycosylated and contains the polypeptides of both gp85 and gp35. We have studied the nature and site of processing of Pr92gp to mature disulfide-linked gp85 and gp35 (VGP). We could show that in addition to proteolytic cleavage, processing involves conversion of the high-mannose oligosac-
charides found in Pr92 to the complex, sialidated oligosaccharides found in VGP. Experiments pertaining to the site of processing indicate that processing does not occur extracellularly as has been proposed by others (Klemenz and Diggelmann, J. Virol. 29 (1979) 285–292. We have determined that the small amount of mature VGP found in infected cell lysates is localized chiefly within the cell, not at the cell surface. We favour the view that further glycosylation and proteolytic cleavage occur concomitantly on smooth membranes within the cell and that subsequent to this, export in virus is rapid.

Evidence that the Physiological 38,000 d Proteinsubstrate of the pp60src Kinase is the Subunit of Malic Dehydrogenase

RÜBSAMEN, H., FRIIS, R. R., and EIGENBRODT, E.

A protein of a molecular weight of about 38,000 d has been found to be phosphorylated 1 h after the onset of cell transformation by Rous sarcoma virus (RSV). It is assumed to be a physiological target of the pp60src kinase, since, apart from being phosphorylated in the transformed cell, it can be phosphorylated in vitro by the pp60src kinase in tyrosine (1, 2). - Using 6 different chromatographic procedures (chromatography on DEAE-Sepharose, poly(A)-Sepharose, Blue Sepharose, and hydroxylapatite, isoelectric focusing and gel filtration) the 38,000 d protein could not be separated from cytosolic malic dehydrogenase activity (c-MDH). Antiserum against the 38,000 d protein inhibited c-MDH. - In vivo, phosphorylation of the 38,000 d protein occurred in serine and tyrosine, resulting in altered kinetic properties of c-MDH. Possible implications of the modified kinetic properties of c-MDH after transformation will be discussed. - Supported by grant Ru 242 from the Deutsche Forschungsgemeinschaft.

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The Transformation-specific Protein “v-myc” of the Acute Avian Leukemia Virus MC29

DONNER, P., GREISER-WILKE, IRENE, and MOELLING, KARIN

Avian acute leukemia viruses transform cells through a virus-coded oncogene. In the case of the myelocytomatosis virus, MC29, which transforms fibroblasts as well as bone marrow cells in vitro, this oncogene is fused to a viral structural component, p19. The fusion protein, v-myc, was characterized by using monoclonal antibodies against p19. MC29 transformed quail fibroblasts which do not produce any virus, MC29–Q8, were analyzed by immunofluorescence. A nuclear fluorescence was observed which was not detected in normal cells or virus-producing cells. The monoclonal antibodies were used to purify the v-myc protein by immuno-affinity chromatography. The purification achieved by this single-step purification was 3,500 fold. The eluted protein was precipitable by anti-sera and will be further characterized for its biological properties.
Biochemical Characterization of Antigens in Human Leukemic Sera that Crossreact with SiSV p30 and BAEV p30

LEIB, C., SCHETTERS, H., ERFLE, V., and HEHLMANN, R.

Antigens crossreacting with the core proteins p30 of baboon endogenous virus (BaEV) and/or of simian sarcoma associated virus (SiSV) have been isolated from human leukemic sera by immunoaffinity chromatography. The antigens have an apparent molecular weight of 70,000 in SDS-polyacrylamide gel electrophoresis. Peptide maps performed with the antigens from two different leukemic sera show that the two antigens are identical. Peptide analyses of SiSV p30 and of BaEV p30 and simultaneous mapping of p30 proteins mixed with human antigens show that 11 out of 21 major peptides of SiSV p30 and 10 out of 20 major peptides of BaEV p30 have identical mobility with peptides of the human antigens. Human serum albumin, transferrin, fibrinogen, IgG and IgM share clearly less peptides of identical mobility with the human antigens.

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GP69 and GP71 of Friend (F) Murine Leukemia Virus (MULV) (Purification and Partial Characterization)

LINDER, D., HUNSMANN, G., OROSZLAN, S., SCHLÜTER, M., SCHNEIDER, J., SMYTHERS, G., and STIRM, S.

The isoglycoproteins gp69 and gp71 were purified from F-MuLV particles (propagated in Eveline cells) by solubilization (freezing and thawing), ion exchange chromatography (phosphocellulose) and preparative SDS-PAGE. Prior to amino acid and NH$_2$-terminal amino acid sequence analysis, the purified glycoproteins were subjected to high performance liquid chromatography (HPLC) to remove contaminants. The NH$_2$-terminal amino acid sequences (23 residues) of gp71 and gp69 were found to be different (in 10 positions) but highly related. F-MuLV gp69 shows 41% homology to gp71 but lacks the potential glycosylation site (sequon) at position 12 in both F-MuLV gp71 and R-MuLV gp70.

Antigens Crossreacting with MMTV gp52 in Malignant and Benign Human Breast Lesions

HEHLMANN, R., BAUMGARTEN, A., SCHREIBER, M. A., SCHETTERS, H., LUZ, A., MESA-TEJEDA, R., LOHE, K. J., and PRECHTEL, K.

Recently, antigens have been found in human breast cancers that crossreact with the envelope glycoprotein gp 52 of the mouse mammary tumor virus (MMTV) (Spiegelman
et al., 1980). In our laboratory 99 breast cancers, 60 normal breast tissues, 44 benign breast lesions and 10 other carcinomas from south german patients were tested for crossreactivity with MMTV-gp52. The tests were done by indirect immunoperoxidase staining on paraffin-sections using antiserum against gp52 provided by Dr. Spiegelman, N. Y. Specificity of positive reactions was controlled by preabsorption with purified MMTV-gp52. 86 breast cancers (87 %) and 13 benign breast lesions (30 %) gave positive reactions, whereas normal breast tissue and other carcinomas were negative. – The test might be an additional useful diagnostic tool for the early detection of micrometastases, for the assignment of metastases from unknown primary tumors and in doubtful cases of breast cancer. It can possibly serve as an additional criterion for the classification of mastopathies.

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Biological Consequences of Altered MHS Antigen Expression on Murine Leukaemia Cells

SCHMIDT, W. and FESTENSTEIN, HILLIARD

Major histocompatibility system (MHS) encoded cell membrane antigens play a key role in immune cell interactions. Modulation of the expression of MHS alloantigens may influence cellular interactions including those between the immune system and tumor cells. We present here a study of the MHS profile of several spontaneous leukaemias from AKR mice; in a majority of these tumors the H-2K antigen is either completely absent or greatly reduced. These results were correlated with the ability of mice carrying the tumors to reject them and to generate cytotoxic T effector cells against a panel of leukaemias. The ability of effector cells to recognize leukaemia virus induced target antigens on the AKR leukaemias was correlated with the expression of H-2K MHC antigens. These findings have important implications for immunosurveillance of tumors.

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Aetiology of Virus-Meningitides in Lower Saxony 1967–1980

KNOCKE, K.-W., WILLERS, HILDEGARD, and SIPOS, SUSANNE

From 1967 until 1980 specimens from 8,609 patients with meningitis were analysed. Viruses were found in 3,691 patients. Nearly all illnesses were caused by mumps- and entero-viruses. Other viruses were found in less than 5 % of all cases. The mumps-meningitides were ascertained constantly over all these years. In 1967, 1974 and 1980 an increased incidence of meningitides caused by Echo-virus type 30 was seen. – There was no seasonal dependence on the occurrence of mumps virus meningitides. Meningitides, caused by entero-viruses was found more often in the autumn. Male patients fell ill twice as often as female patients. Mumps-meningitides were rarely found in the first year of life. In contrast, enterovirus meningitides could be detected during the first year and caused meningitides to age of 14. Virus meningitides among adults were rarely found.
Virological Findings in Cases of So-Called Idiopathic Peripheral Facial Pareses

MERTENS, TH., THOMAS, J. P., ZIPPEL, C., and EGGERS, H. J.

We suspected that a number of peripheral facial pareses (P.F.P.) considered "idiopathic" might, in fact, have a viral aetiology. 71 patients of the Cologne University E.N.T. Clinic with so-called idiopathic P.F.P. were examined under the aspect of a viral aetiology. In 32 cases conditions for virological investigations were optimal (paired sera, first serum within the first week after onset of disease). In 10 of these 32 cases a viral infection could be proven (varicella zoster virus: 7, herpes simplex virus: 2, coxsackie B4:1). In 5 of 7 VZV cases minimal zoster lesions were observed, 4 facial and 1 thoracic. Among the 39 other patients (with late sera) no viral infection could be proven. In conclusion, by means of alert clinical and virological examinations, a considerable fraction of idiopathic P.F.P. could be associated with a virus infection.

Rotavirus Infections of Neonates

STEINMANN, J. and KIOSZ, D.

152 neonates were screened from the delivery to the time of discharge for rotavirus infections. Daily faecal specimens were examined by an enzyme-linked immunosorbent assay (ELISA) and a subgroup of positive specimens were also tested by a negative staining electronmicroscopic method. - 22 babies (14.5%) were found to excrete rotavirus. 20 of them were asymptomatic infected and 2 showed mild gastrointestinal symptoms. With one exception viruses were not detected in babies less than 24 h old, but 10.9% of them excreted virus during the second day of life and after 10 days 30% of the neonates were positive for rotavirus. - Excretion persisted for 1 to 5 days. According to our study most babies (40%) excreted rotavirus for 3 days. - A great number of the stools (29%) from the first day which were tested by ELISA were found to have nonspecific activity in the absence of rotaviral antigen. Therefore such stool specimens should only be examined by electron microscopy.

Effect of Coupling Reagents in the Single Radial Hemolysis Test

STEINMANN, J. and MARZOCK, H.-J.

The single radial hemolysis test (SRH-test = hemolysis-in-gel test) is a suitable technique for detection of rubella and influenza antibodies in a large number of sera. In our study we looked for the effect of attachment of the viral antigens to the erythrocytes by different coupling reagents. Chromic chloride, cyanogen chloride, glutaraldehyde and tetrazotized – o – dianisidine (TOD) were used for the sensitization of the erythrocytes. – In the rubella SRH-test no improvement on regard to sensitivity of the test was seen. Moreover it is not possible to detect IgM specific antibodies after different kinds of attachment.
in the SRH-test. - In the influenza SRH-test with allantoic fluid of eggs infected with H₃N₂ virus it was possible to increase the sensitivity with TOD, chromic chloride and potassium periodate. If tween-ether treated hemagglutinin was used only after sensitization with TOD, chromic chloride and periodate hemolytic zones were detectable.

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**RNA-Electropherotyping of Human Rotaviruses 1978–1981**

FORSTER, J. and PASTOR, S.

Rotaviruses contain a double-stranded ribonucleic acid genome consisting of 11 segments. This can easily be extracted from crude stool suspension (method by Rodger et al., J. Clin. Microbiol. 1981). 55 out of 210 Rota antigen positive samples contained sufficient RNA to produce a satisfying pattern in the SDS-acrylamide-electrophoresis. We demonstrate six electropherotypes with differences in the relative mobility of segments 2, 3, 4, 5, 7, 8, 9, 11. Of these diarrhea producing strains at least two different electropherotypes were found during every outbreak of Rotavirus gastroenteritis. Our findings are in good agreement with the results reported by Rodger et al. (1981), Espejo et al. (1980, 1979), and Kalica et al. (1978, 1976).

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**Attempts to Select and Isolate a Strain of EMC Virus Capable of Inducing B Cell Dependent Diabetes in Mice**

KRUPPENBACHER, J., MERTENS, TH., MÜNTEFERING, H., and EGGERS, H. J.

The so-called M-type of EMC virus is capable of inducing diabetes mellitus in mice by a selective B cell damage. The M-EMC strain used in our laboratory has partially lost this capacity. We attempted to reisolate a diabetogenic variant and to elucidate the causes of the change. Seven serial heart passages in mice did not enrich such a variant. Cloning of the virus stock yielded one clone diabetogenic in two of ten animals (5 clones tested so far). - In a different substrain of M-ECM virus (the highly diabetogenic D-variant, obtained from Dr. Petersen) we found both diabetogenic and non-diabetogenic clones. Incidence and severity of diabetes has been shown to be age-related.

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**Comparative Investigations of Virus Detection from Patients with Acute Respiratory Diseases by Radioimmunoassay and Tissue Culture Isolation**

EHRLICHER, L., HOFFMANN, H., and HABERMEHL, K.-O.

In winter 1981 146 patients with an acute respiratory infection were examined. Nasopharyngeal specimens were obtained by intranasal catheter. Comparative investigations
were performed by cultivation in tissue culture (primary monkey kidney cultures, diploid fibroblasts and FL-cells) or by a four-layer radioimmunoassay (P. E. Halonen).

Relationship between RIA and Culture Results

| Virus             | Culture + RIA | Culture + RIA | Culture + RIA |
|-------------------|---------------|---------------|---------------|
| Adenovirus        | 10            | 3             | 0             |
| RSV               | 2             | 0             | 5             |
| Influenza A       | 21            | 6             | 4             |
| Influenza A (H1N1)| 12            | 3             | 1             |
| Influenza A (H3N2)| 9             | 3             |               |
| Influenza B       | 2             | 1             | 12            |
| Parainfluenza Type 3| 1             | 0             | 0             |

In some cases (adenovirus, influenza A virus) tissue culture seems to be more sensitive, in other cases (respiratory syncytial virus, influenza B virus) antigen detection by RIA is more efficient.

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Selective and Controlled Mass Rubella Vaccination. Experiences with an Economical Method

ZIPPEL, C., FEDERMANN, G., and EGGERS, H. J.

Immunity to rubellavirus was examined in 5,870 school girls. 22.7% of the girls required immunisation. Successful vaccination could be demonstrated in 99.6% of the vaccinated girls. The hemolysis in gel test (HIG) (3,552 samples) and an ELISA (1,134 samples) were compared with a sensitive hemagglutination inhibition test (HI). HIG and HI gave identical results, whereas with the ELISA diverging results were obtained in 14% of the samples. 13% have been proven to be false positive. For the examination of larger population groups the HIG seems to be a well-suited technique. We have shown in comparative tests involving 3,224 girls that the HIG can be performed with capillary blood as reliably as with venous blood. This greatly facilitates the collection of samples in large population groups.

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Safety-Studies of Granulosis Virus: Replication in Vertebrates and Non-Immunological Interaction with Mammalian Sera

DÖLLER, GABRIELE

This safety study was to demonstrate whether or not granulosis virus (GV) of Laspeyresia pomonella can replicate in vertebrates. After feeding GV to NMRI-mice, no virus
induced antibodies could be detected within eighty days by radioimmunoassay (RIA) and no vertical virus transmission was observed. GV did not replicate in mice. Human sera, as well as sera from horses, cattle, sheep and swine reacted with GV in the RIA. By characterization the positive reacting human immunoglobulin class(es), IgG showed the strongest positive reaction, less positive reactions were shown by IgE and IgM and no reaction by IgA and IgD. By concentrating the immunoglobulins of the negative reacting sera to 250 μg IgG/ml, all sera could be recognized as positive. Thus a non-immunological reaction has been suggested.

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Cytomegalovirus-Host Cell Interaction: Synthesis and Distribution of Actin in Infected Cells

LÖSSE, D., LAUER, R., WEDER, D., and RADSACK, K.

Infection of human fibroblasts with cytomegalovirus induces typical cytopathic alterations. Cell rounding within 5–6 h postinfection (p.i.) is followed by an increase in cell size, appearance of cytoplasmic and nuclear inclusions and a morphological change to an epitheloid cell shape by 48 h p.i. In order to elucidate the participation of the cytoskeleton in this alteration of cell morphology experiments were initiated in serum-starved human fibroblasts to visualize changes in actin arrangement by indirect immunofluorescence. As early as 3 h p.i. cytoplasmic microfilaments had shortened and were rearranged to a more irregular pattern. At 12 h p.i. actin fibers were absent from rounded cells. The same was observed in epitheloid cells at 48 h p.i. Cultivation of infected cells with phosphonoacetic acid resulted in a partial preservation of the normal actin fiber distribution. In contrast, infected cells did not exhibit major changes in actin synthesis as estimated from the specific radioactivity of cytoplasmic actin isolated from 3H-leucine pulse labelled infected cells by DNAase I-Sepharose affinity chromatography and SDS-polyacrylamide electrophoresis with fluorography.

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Serologic Diagnosis of Cytomegalovirus Infection with a Modified Passive-Haemagglutination Test (PHAT)

DOERR, H. W., HUSCHKA, U., and STENGEL, H. H.

Chicken erythrocytes were coated with glycine-extracted CMV antigen and negative control antigen by treatment with formaldehyde and CrCl₃ and were used for detection of CMV specific serum antibodies in PHA tests. Their sensitivity was proven to be in good agreement and their specificity superior to results seen in CFT. IgM-specific CMV antibody detection was performed either after a simple and rapid DEAE-cellulose exchange chromatography of serum samples or by IgG immunoprecipitation combined with ME-reduction controls. The main advantage of the modified CMV-PHAT was seen in the stability of sensitized erythrocytes, which can be lyophilized.
Comparison of Herpes Simplex Virus Typing Methods, and Results of Isolation Attempts from the Genitourinary Tract of Asymptomatic Males

LANVERS, A., MERTENS, TH., and EGGERS, H. J.

There are reports that herpes simplex virus (HSV) could be isolated from the genitourinary tract of up to 15% of males without manifest herpes. In order to confirm these results we first wanted to establish a method for typing possible HSV isolates. We used 3 published methods: a plaque test in chick embryo cells, a neutralisation test, and the analysis of the early HSV-proteins (SDS-PAGE). All 3 methods yielded identical results. We then tried to isolate HSV from 192 materials of 181 asymptomatic males (89 urethral swabs, 55 seminal fluids, 48 fresh tissue probes). All materials were immediately inoculated into tube cultures of two cell lines shown to be highly HSV-susceptible. Additionally, the tissue probes were co-cultivated with permissive cells. We also induced cell fusion (PEG) in such cocultures. Despite all efforts we did not isolate any virus from all these materials.

Peripheral Infection of Mice with HSV: Lethal and Non-Lethal Genetic Virus Variants

KÜMEL, G., KIRCHNER, H., SCHRÖDER, C. H., and KAERNER, H. C.

For an experimental approach to answer the question as to which viral genes may control pathogenesis after peripheral infection of inbred mice with HSV, we have isolated a variant strain (ANG-path) that proved highly neuropathogenic both upon i.p. – or intravaginal infection from the apathogenic strain HSV-1 ANG. Two alterations of the ANG genome have been detected by restriction enzyme analysis: the loss of the amplifiable 500 b.p. nucleotide sequence typical for ANG and a 400 b.p. deletion approximately at the position mapped for the structural viral glyco-protein D. Despite the induction of interferon and NK-cells both variants multiply to a similar extent, probably in the same target cells of the peritoneum. The spread from the peritoneum, spleen, liver, thymus and local lymph nodes to the CNS seems, however, to be controlled by the action of gene products coded for in only one of the variants.

Influence of Genetic Factors and Bestatin on Antibody Formation Following HSV-1 Infection of Mice

KNOBLICH, A., FRIEDRICH, D., GOERTZ, J., and FALKE, D.

Herpesviruses are known to cause infections in men and animals. Strong differences in resistance to Herpes simplex virus (HSV-1) are noted among mice of various strains. Anti-HSV-activity of T-lymphocytes, macrophages and NK cells has been demonstrated in the last years. Our interest was focused on neutralizing antibodies in primary HSV-1
infections of mice. Antibodies become detectable by day 5 after infection and reach a plateau level at day 21, the day we chose to test the sera. Comparison of antibody titers in 18 strains of mice revealed titers always to be higher in female mice, whereas no clear influence of either H-2-haplotype or background genome could be detected. Sexual steroids produced in ovaries and testes were identified to exert influence on antibody formation by castration experiments. Treatment of mice with silica once between day 1 before and day 12 after infection resulted in a strong increase of antibody titers both in females and males at the same time abolishing the difference in antibody titers between the sexes. Silica could enhance antibody levels also after immunisation of mice with a formol-inactivated HSV-vaccine. Bestatin is a small peptide known-selectively to stimulate DNA metabolism in T-lymphocytes and to enhance HSV-antibody titers maximally when given at day 5 after infection. After pretreatment of mice with silica the antibody augmenting effect is already achieved at day 1 after infection. In secondary HSV-1 infection Bestatin acts best 1 day after infection, too.

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Induction and Characterization of Herpes Simplex Virus Reisolates, Isolated after Intertypic Superinfection of Latent Infected Tupaias

DARAI, G. and SCHOLZ, J.

The susceptibility of juvenile and adult Tupaias to Herpes simplex virus type 1 and 2 had been reported. The intertypic recombination of herpes simplex virus using temperature-sensitive mutants of HSV-1 and 2 and superinfection with wild-type HSV-1 and 2 was studied. It was found that animals survived an infection of $1 \times 10^7$ to $1 \times 10^8$ pfu of ts mutants of HSV-1 and/or 2 which were inoculated intravenously ($LD_{50}$ for HSV-1 = $10^{-3.75}$ and for HSV-2 = $10^{-2.49}$). The inoculated animals were protected against a superinfection of HSV-1 or 2 ($5.0 \times 10^6$ pfu/animals). The state of viral latency in surviving animals was investigated. It was found that infectious virus was recovered from ganglia of those animals which had initially been infected with wild-type HSV-1 or 2 and/or superinfected with wild-type viruses. In contrast, the infectious viruses were recovered only from spleens of those animals which had initially been infected with ts mutants of HSV-1 or 2 and superinfected with wild-type HSV-2 and/or 1. It was found that recovered viruses from the spleen of the animals lost their pathogenicity and their natural tissue tropism. Significant changes in the genome of the recovered viruses from the spleen were detected. Recombination between ts mutants of HSV-1 and 2 and challenged wild-type viruses was observed. Thus, the pathogenicity and genomic properties of recovered viruses were altered. This observation is the first description of generation of intertypic recombinants of HSV-1 and 2 in vivo.

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The Functions of the HSV-Coded dPyK-Complex Enzyme

LABENZ, J., BRAUER, D., MÜLLER, W. E. G., and FALKE, D.

Analysing the phosphorylating capacity of the HSV-coded dPyK of HSV-1 and 2 by glycerol gradient centrifugation we detected each three peaks differing in molecular weights using ATP, ADP or AMP as phosphate donors. Also by PAGE peaks with differ-
ing Rj-values could be seen. Indeed, $^{32}$P-AMP and $^{32}$P-ADP were used for phosphorylation of dThd. The AMP-dependent activity could be purified 1300-fold. Two antisera against HSV-coded PdyK neutralized all three activities. The TK- mutant MDK (B2006) did not induce in TK- cells the respective activities, only the 3 cellular TK's were detected and identified by their Rj-values. Further experiments have shown that only the HSV-1-dPyK has thymidylate kinase activity, but not that of HSV-2. The HSV-1 thymidylate kinase activity could be neutralized by a TK-antiserum. The pH-optimum, sensitivity to Mg$^{++}$, Fe$^{++}$, Zn$^{++}$, Co$^{++}$ and Mn$^{++}$ ions differed. The susceptibility to thiol reagents was different, the AMP-dependent activity proved to be susceptible to phenanthroline. Also the $K_m$ and $V_{max}$-values were detected. A diagram summarizes the biochemical function of the HSV-coded dPyK-complex. Finally there is some indication that the enzyme phosphorylates Ado by using dTMP or ATP as a phosphate donor. The importance of the enzyme complex in HSV-infected cells is discussed.

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Deletion of Nucleotide-Sequences in Cloned HSV-1 Fragments during Propagation in the rec A E. coli, HB 101

GRAY, C. P., JELLINGHAUS, U., and KAERNER, H. C.

Passage of HSV at high MOI results in the generation of defective genomes which are of full length, consisting of a relatively short region of the wild type genome that is repeated. They are packaged into mature virus particles and thus leave the cell in a state that is capable of entering a new host. Such defective molecules are of interest as they represent a simpler model in which to study replication, recombination and packaging of HSV. Such a defective molecule arising from the serial passage of HSV-type 1-ANG has been mapped, and restriction fragments have been cloned into pBR 322 using the rec A E.coli, HB 101, as host. All the resulting clones were found to be unstable and to contain deletions, one of which has been characterised in more detail. Fine mapping of the original genome and of the clones was facilitated by selective labeling of the variable nucleotide in the Hinf I recognition site.

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Structure and Function of Defective Herpes Simplex Virus Genomes: The Defective HSV ANG DNA of Class II

KNOPF, K. W., STRAUSS, G., OTT-HARTMANN, A., SCHATTEN, R., and KAERNER, H. C.

Defective virus particles arising during serial undiluted propagation of herpes simplex viruses (HSV) and displaying only a subset of the genomic information of the standard virus, represent a powerful tool to understand a number of virus-directed processes such as gene expression, and mechanisms of replication and propagation of the more complex standard HSV genome. We have examined in more detail the structure of one class of defective HSV ANG DNA (dDNA1), both by establishing physical maps as well as by molecular cloning. A model is presented explaining the defective DNA concatemers differing in size by insertion of a 550 bp nucleotide sequence originating from the IRS sequences of the HSV ANG genome. The concatemers are composed of tandem repeat
units of 8900 bp size comprised of sequences of 6500 bp from the $U_L$ – and of 2400 bp from the terminal redundancies. By various molecular biological techniques it was demonstrated that the $U_L$ – sequences code for the major DNA binding protein of about 130K molecular weight, and for parts of the HSV DNA polymerase gene, both proteins playing a dominant role in viral replication.

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**Generation of Recombinants Between Tupaia Herpesviruses and their Characterization**

KOCH, H.-G., DELIUS, H., MATZ, B., FLÜGEL, R. M., and DARAI, G.

The isolation and characterization of Tupaia Herpesviruses (THV 1 to 4) has been reported. The analysis of DNA of these viruses showed the absence of submolar DNA fragments, when the DNA was cleaved with different restriction enzymes, as well as of a stem loop structure, when analysed by electron microscopy. With respect to this genomic structure it was of interest to study the recombination events between THV 1 to 4. Recombinants were generated between these viruses using a co-infection technique in vitro on Tupaia fibroblasts. Recombinants were selected after the stocks of new progeny virus were treated with specific antiserum against each parental virus. Different recombinants were isolated, plaque-purified and characterized. Results for one recombinant THV-R-26 between THV-2 and 3 were as follows: (i) The in vitro host range and the in vivo pathogenicity in juvenile Tupaia was altered compared to parental viruses. (ii) Physical properties of recombinant DNA (namely the Tm value (G+C content) and the buoyant density in caesium chloride) were significantly different from the values of parental viruses. The physical map of the recombinant virus genome was constructed using restriction enzyme Hind III. (iii) Changes in the polypeptide pattern of the recombinant virions were detected.

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**Virion-Associated Protein Kinase in Tupaia Herpesvirus**

FLÜGEL, R. M.

Phosphorylation of proteins is a posttranslational modification which is regulatory for the activity of several enzymes. Most animal viruses code for phosphoproteins and the degree of their phosphorylation is thought to be a controlling factor during viral macromolecular synthesis. Recent studies on protein kinases from a number of tumor viruses have raised the possibility that the phosphorylation of cell proteins is involved in the processes leading to cell transformation. – Incubation of purified tree shrew (Tupaia) herpesvirus (THV) particles with $\gamma$-32P ATP resulted in the incorporation of 32P labelled phosphate into proteins. A nonionic detergent such as NP-40 was necessary for the detection of protein kinase activity. The incorporation of 32P phosphate was proportional to the quantity of TH viral proteins, indicating that the viral proteins can serve as substrates for the viral enzyme. $\alpha$-32P dATP or $\alpha$-32P ATP did not function as phosphate donors. A divalent cation such as Mg$^{2+}$ or Mn$^{2+}$ is essential for the enzymatic activity. A product analysis revealed that six viral polypeptides are phosphorylated. The predominant sites of phosphorylation are the $\beta$-OH groups of serine and threonine.
Properties of the Goat Herpesvirus (BHV-6)

ENGELS, MONIKA, GELDERBLOM, H., DARAI, G., and LUDWIG, H.

In 1972 a herpesvirus was isolated from young goats with a severe generalized infection in California. This isolate has been characterized in some detail and named caprine herpesvirus 1. Recently a herpesvirus was isolated in Switzerland from goats with a similar infection. We report a further characterization of both isolates and propose their classification as Bovid Herpesvirus 6 (BHV-6). - BHV-6 multiplies rapidly and shows a broad host cell range. Crossneutralization only could be observed with BHV-1 (IBR/IPV-Virus), in a one way reaction. In gel immunoelectrophoresis the serologic relationship involved the major antigenic components of BHV-1. The analysis of BHV-6 DNA resulted in a contour length of approximately 44 μm, a buoyant density of 1.7285 g/cm³, a melting point of 86°C and a G+C content of 71-72%. Finally the goat herpesviruses could clearly be distinguished from other bovid herpesviruses by means of restriction enzyme analysis.

The Appearance of Antibodies against EBV-specified Proteins During Infectious Mononucleosis

STOLBERG, M., BAYLISS, G. J., HENLE, W., and WOLF, H.

We have evaluated two methods for the analysis of antibodies directed against EBV-specified proteins: 1. Indirect immunoprecipitation (IP) and 2. Radioimmunoassay of electroblots of SDS PAGE separated EBV-specified proteins (RIAB). Using IP we have identified 20 proteins against which antibodies are made during infection, some of these proteins have also been found using the latter technique. Many of the proteins are only reactive with EA'VCA+ sera and not reactive with EA'VCA+ sera and may therefore be candidates for the EA specifying proteins. It seems likely that the failure to identify all proteins using the RIAB technique is probably due to the strong denaturating conditions used during the SDS PAGE step. Only those antibodies directed towards the primary sequence of the protein will react with the blotted proteins, whereas with IP analysis the native proteins are available for interaction with the sera.

Regulated Expression of Epstein-Barr Virus (EBV): On the Control of the Synthesis of Secondary Proteins

BAYLISS, G. J. and WOLF, H.

Previously we demonstrated that the synthesis of EBV-induced proteins in superinfected Raji cells (Raji SI) could be divided into 3 phases: Primary, secondary and tertiary (Bay-
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Liss and Wolf, J. Gen. Virol., 1981, in press). Recent experiments show that incubation of Raji SI in the presence of canavanine and the absence of arginine allows only a limited expression of the viral genome. If the cells are released from a canavanine block (applied from 0 to 8 h post infection) then between 2 and 4 h later several new proteins are synthesized, however, if m-RNA synthesis is inhibited (with actinomycin D) after removal of the canavanine then these new proteins fail to appear. Amongst these proteins are members of the EBV-specified early antigen complex (EA). These results indicate that an arginine-containing protein is synthesized soon after infection and that this protein is required in an active form in order to synthesize m-RNA for the secondary group of proteins. Amongst these proteins are the major components of the early antigen (EA) complex.

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Methods for the Detection of EBV-Genomes in Tumor Cells

SEIBL, R., RICHTER, W., ZENG, Y., GU, S.-Y., and WOLF, H.

Antibody titers of IgA antivirus capsid antigen (VCA) can be used for screening early tumor cases, confirming histological diagnoses and longtime surveillance of therapy. However, in high risk areas (S. China, incidence of NPC: 20/10^5) 2% of the population have IgA anti VCA antibody indicating a need for methods which allow monitoring of additional parameters for deciding on the need for therapy. The same need exists in case of long term (1-2 years) survivors of NPC with constant IgA anti VCA titers. – We have developed a system to collect cell specimens by application of buffer to the tumor site and collection of cells directly on to nitrocellulose filters. These cells can be examined cytologically or for EBV DNA in nucleic acid hybridization. For the latter tests a modification of the Grunstein Hogness colony hybridization test and cloned EBV DNA have been used. In reconstruction experiments 25 virus-producing cells could be detected.

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Functional Mapping of the Herpesvirus saimiri Genome with Recombinants

KEIL, G. and FLECKENSTEIN, B.

After infection of permissive cell cultures with overlapping restriction-fragments of viral DNA derived from different strains of Herpesvirus saimiri (H. saimiri) a number of recombinants could be isolated. The analysis of the viral proteins of the wt-strains 11, OMI and S295C led to the identification of four proteins that differed within these strains with respect to molecular weight. We are able to localize these proteins in the internal region of the M-DNA by comparing the protein patterns of recombinant and parent strains. The exact localization was so far not possible because the recombination events occurred mainly in regions near the ends of the L-DNA. – Furthermore recombinants were constructed to identify the genomic region responsible for oncogenicity. The wt-strains of H. saimiri are oncogenic while attenuated strain 11-att, that was obtained from strain 11 has lost this property. This may be due to a deletion of 1,1 md at the left end of the L-DNA. Recombinants between this attenuated strain and wt-strains were constructed. The test for oncogenicity in vivo is in progress.
Molecular Structure of Episomal Herpesvirus saimiri and Herpesvirus ateles DNA in Lymphoid Tumor Cells and in vitro Transformed Cell Lines

KASCHKA-DIERICH, CH.*, WERNER, F.-J., SCHIRM, S., BAUER, I., and FLECKENSTEIN, B.

The structure of circular H. saimiri and H. ateles genomes isolated from five different cell lines was analysed by partial denaturation mapping. Three different types of circular viral DNA structure were found: 1. Circular viral genomes consisting of one L-DNA and one H-DNA region of the same length as in virion DNA. 2. Circular viral genomes consisting of one L-DNA region with a deletion of various length which is compensated by a prolongation of the H-DNA region. 3. Circular viral genomes showing rearrangements of the viral sequences with duplications of the left end of unique L-DNA and a deletion within the L-DNA. This approach helps to identify regions of viral DNA which are not necessary for the maintenance of the transformed state (deletions) and those regions which might play an important role in the process of oncogenic transformation (duplications).

Herpesvirus saimiri-Specific Transcription in Lysically Infected and Transformed Cells

KNUST, E. and BODEMER, W.

The highly oncogenic primate herpesviruses, H. saimiri and H. ateles, infect owl monkey kidney cells lytically, whereas T-lymphoid tumor cell-lines are persistently infected. The T-lymphoid cells can contain up to 300 genome copies per cell as episomes. We have begun to study translation and transcription in transformed cells in comparison to lytically infected OMK-cells. About 25 new proteins can be detected in lytically infected cells having apparent molecular weights between 12-200 kd. At early and late stages of infection the right part of the genome is preferentially transcribed and each DNA-fragment encodes a series of specific RNAs. In contrast, we do not find any virus-specific proteins in the transformed cells after labeling with 35S-methionine and subsequent immunoprecipitation. Preliminary results may suggest that the only virus-specific RNA found in the transformed cells are small RNAs with molecular weights of about 0.13 kb. A more detailed analysis has to be performed in order to confirm these hybridization data.

Characterization of Herpesvirus saimiri Glycoproteins

MODROW, S. and WOLF, H.

For the identification of Herpesvirus saimiri glycoproteins we separated H. saimiri 11 induced cell proteins on SDS-polyacrylamide gels and transferred the polypeptides by elec-
trophoretic blotting (2 h, 3.7 mA/cm²) to nitrocellulose paper using carbon electrodes and buffer soaked sponge to cover the gel/filter layer. Lectins, which are known to bind very specifically to certain sugar residues (Concanavalin A to D-glucose and D-mannose derivates, Soy bean agglutinin to N-acetyl-D-galactosamine and D-galactose, Dolichos biflorus agglutinin to N-acetyl-D-galactosamine, Ulex europaeus agglutinin to L-fucose) were iodinated using the NEN-lactoperoxidase system. The glycoproteins bound to the nitrocellulose sheets were detected by incubation with the ¹²⁵I-labelled lectins. By this, eight viral glycoproteins could be identified, two of them were synthesized in the presence of canavanine, and characterized according to the type of glycosylation.

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Anatomy of Lymphocystis Disease Virus of Pleuronectes

DARAI, G., ANDERS, K., KOCH, H.-G., DELIUS, H.¹, GELDERBLOM, H., and FLÜGEL, R. M.

Lymphocystis disease (LD) is a virus disease of marine fish with an almost world-wide geographical distribution. This disease is characterised by papilloma-like tumour lesions. Lymphocystis disease virus (FDLV) has been tentatively classified as belonging to the family of Iridoviridae. This report describes the anatomy of FDLV: The FDLV virions were isolated from a total of 22 fish with LD lesions, caught near the Doggerbank, including 12 flounders, 6 dabs, and 4 plaice, which were analysed individually. The purity of the virus preparations was examined by a negative-staining technique and the virion diameters were determined: flounder 227.5 ± 12.5 nm, LDV-plaice 198.8 ± 12.9 nm, and LDV-dab 200.5 ± 12 nm. DNAs of these different LDV isolates were cleaved with different restriction endonucleases and the resulting DNA fragments were separated electrophoretically on agarose slab gels. The fragment patterns demonstrate that LDV DNA of flounders and of plaice are indistinguishable, but clearly different from those of dab. The determination of the molecular weights of FDLV DNAs using contour length measurements by electron microscopy resulted in a value ranging from 60 to 150 × 10⁶ daltons. In contrast the molecular weight estimations by restriction enzyme analysis resulted in lower values ranging from 60 to 90 × 10⁶ daltons. This discrepancy is probably due to a restricted, permuted structure of the LDV genome similar to the genome of frog virus 3 as reported by Granoff.

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ATPase Activity in Purified Virions of Lymphocystis Disease Virus of Flatfish

FLÜGEL, R. M. and DARAI, G.¹

Fish lymphocystis disease virus (FLDV) causes papilloma-like lesions in a great number of species of flatfish, particularly in the northern hemisphere. The structure of FLDV constituents and its interaction with host cells remains obscure. In an effort to clarify the viral components and their functions we have studied the proteins and purified FLDV
and searched for virion-associated enzymes. - At least 33 distinct viral polypeptides were detected by polyacrylamide gel electrophoresis under denaturing conditions. The polypeptide patterns are remarkably specific for a given fish species (flounder, plaice, and dab) although some heterogeneity was found when proteins of individual fish of the same species were analysed. – It was found that a nucleoside triphosphate phosphohydrolase activity is closely associated with FLDV particles. This activity hydrolyses ATP with a high preference. The reaction requires a non-ionic detergent and a divalent cation, such as Mg²⁺. Reaction rates and substrate specificities were determined. The products of the reaction are nucleosides diphosphates and inorganic phosphate.

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Characterization of a Poxvirus Isolated from White Rhinoceros (Ceratotherium s. simum)

PILASKI, J., SCHALLER, K., OLBERDING, P., and FINKE, HANNELORE

In September 1977 an outbreak of pox disease occurred in White Rhinoceroses (Ceratotherium s. simum) in the Münster zoo. At the same time a similar outbreak was observed in elephants (Elephas maximus, Loxodonta africana) of the zoo in Frankfurt (air-line distance about 230 km). In both cases Orthopoxvirus strains could be isolated which were similar but not identical in their biological properties (small efflorescences on the CAM with hemorrhagic center, inclusion bodies of type A V+, high pathogenicity for rabbit skin, characteristic skin lesions in adult mice) and their DNA restriction patterns (Xho I, Eco R I, Hin d III). Both virus strains were incorporated into the group of “cowpoxlike viruses” (Baxby and Ghaboosi, 1977). The results indicate that both outbreaks had occurred independently from each other.

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Characterization of a 37K Protein Specifically Associated with Released Extracellular Vaccinia Particles

HILLER G. and AULBACH, H.

Infectious vaccinia virus can be isolated from infected cells after experimentally induced lysis (intracellular virus) or from the growth medium of infected cultures (extracellular virus). We have characterized a 37K protein only present on extracellular particles by its amino acid composition and its behaviour on isoelectric focusing. In addition we have used a 37K-specific antiserum to detect its distribution within infected cells. – 37K protein is a late viral protein appearing 5–6 h p.i. It is predominantly found associated with the cellular Golgi complex but never with structures representing pox virus “factories”. Later in infection 37K protein is incorporated into single viral particles preferentially found in the cell periphery. Upon electron microscopy approximately 30–40% of morphologically mature virions inside the cell are enwrapped by a double-membranate vesicular structure. Thus 37K viral protein is probably a component of this vesicular structure and only vesiculated virions can be released by the cell before lysis occurs.
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Epidemiology of Influenza in Lower Saxony

WILLERS, H. and HÖPKEN, W.

The influenza surveillance in Lower Saxony is mainly based on laboratory investigations especially on the attempts to isolate influenza viruses throughout the year. In the winter 1977-78 and in the winter 1980-81 the two influenza subtypes H3N2 and H1N1 circulated at the same time. The H3N2 subtype affected during the last years persons of all age-groups whereas the H1N1 subtype affected only persons younger than 30 years. In the winter 1978-79 influenza B was found in an epidemiological extent. The disease caused outbreaks mostly in schools and kindergartens but also affected adults. In 1980-81 from mid-January to mid-February scattered outbreaks were caused by the A subtype H1N1, which particularly affected schoolchildren contrarily to 1978 where the H1N1 subtype mostly infected young adults. Influenza of the H3N2 subtype circulated from January to March.

Antibodies against Influenza C Virus in the Population of Germany, Kenya and Australia

PFEIL-PUTZIEN, C. and MEIER-EWERT, H.

Over one hundred human sera from each of the three countries Germany, Kenya and Australia were tested for antibodies against influenza C virus, using the conventional hemagglutination inhibition test (HI). The rate of positive sera, showing a HI titer 8 amounted to 59 % for Germany, 93 % for Kenya and 96 % for Australia. In the age group up to 5 years, 34 % of German and 94 % of Kenian sera had already antibody titers against influenza C virus. The Australian sera were tested for the age group of 16-25 years and showed 95 % seropositivity. The results show that influenza C virus is circulating to a higher extent in the populations of countries with subtropical climate, as compared to the more temperate middle European zones.

The Proteolytic Activation of Influenza Hemagglutinin, Structure of the Cleavage Site and the Mechanism of Cleavage

GARTEN, W. and BOSCH, F. X.

The hemagglutinin precursor HA is posttranslationally cleaved by proteases to the complex H1,2. In vitro cleavage of HA by trypsin and trypsin-like proteases yields infectious virus. Cleavage by thermolysin or chymotrypsin yields non-infectious virus. We have analyzed the cleavage sites of H1, H3 and H10 hemagglutinins. The amino acid sequences at the cleavage site, i.e. C-terminus of HA and the N-terminus of HA are identical when virus is activated in vivo and in vitro. Under both conditions, an arginine residue
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Connecting HA₁ and HA₂ in the precursor is eliminated. The elimination of arginine results in a shift of the isoelectric point of the hemagglutinin as demonstrated by isoelectric focusing. Non-activating enzymes cleave only one peptide bond in the HA₂-N-terminal region of activated HA and thus do not affect the isoelectric point. We have also analyzed the cleavage site of the hemagglutinin of fowl plaque virus (H7). A connecting peptide containing several basic amino acids is eliminated. – The data show that activation of the influenza hemagglutinin involves the action of a cellular protease with trypsin-like specificity followed by the action of an exopeptidase of the carboxy-peptidase B-type. The latter enzyme activity is associated with purified virus and can be analyzed by an assay employing peptides bearing ³⁵H-arginine at the C-terminus.

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Strain Dependent Variations of the Carbohydrates of Influenza Virus

SCHWARZ, R. T. and KLENK, H.-D.

The carbohydrates of the glycoproteins of 21 influenza A strains containing hemagglutinin and neuraminidase of all serotypes known to date have been compared by analysis of glycopeptides labeled with radioactive sugars. Analysis of incompletely glycosylated glycoproteins synthesized in the presence of glycosylation inhibitors allowed the determination of the number of oligosaccharide side chains on HA₂. With all strains, the neuraminidase contains side chains of both the complex type I and the mannose-rich type II. There are distinct quantitative and qualitative differences between the strains in the distribution of type I and type II side chains on the hemagglutinin fragments HA₁ and HA₂. The majority of the hemagglutinin oligosaccharides is located on HA₁. These side chains are usually of type I. Only the hemagglutinins of serotype H3 have, in addition, a substantial amount of type II side chains on HA₁. Most strains have on HA₂ a single side chain which is usually of type I. With serotype H5 this side chain is free of fucose, and with serotype H8 it appears to be missing completely. Serotypes H7 and H10 have, in addition to the type I, a type II side chain on HA₂. These observations strengthen the concept that the primary structure of the polypeptide chain is an important determinant for the carbohydrate moiety of the hemagglutinin.

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Acylation of Viral Glycoproteins

SCHMIDT, M. F. G.

Covalent binding of fatty acids to viral glycoproteins was first detected with Sindbis virus and vesicular stomatitis virus (1,2). Studies on acylation in other enveloped viruses revealed that covalent addition of fatty acid to spike glycoproteins is a more general feature. While in Sindbis and in Semliki Forest virus both species of spike glycoprotein (E₁ and E₂) carry fatty acid chains, acylation with the other viruses studied (corona-, influenza A- and paramyxovirus family) seems restricted to those glycoproteins that are known to carry fusion activity. This new type of modification of viral glycoproteins occurs in a wide variety of host cells including those of human, bovine, hamster, avian and insect origin (3). – With the aid of controlled digestion of ³⁵H-palmitic acid labelled virus particles and by the analysis of cyanogen bromide fragments of fatty acid
labelled glycoprotein the fatty acid binding site in influenza hemagglutinin (HA₃), VSV G-protein and Sindbis virus E1 and E2 could be located to the membrane spanning portion of the respective proteins (3).

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3. Schmidt, M. F. G.: Virology (1982), in press.

Mouse-Neurotropic Recombinants of Influenza Virus

BONIN, J., REINACHER, M., and SCHOLTISSEK, C.

Previous studies have shown, that mouse-neurovirulent recombinants can be obtained from mixtures of avirulent influenza A-viruses provided one of the parents had previously been adapted to that host. This study shows that prior adaptation of parental strains is not necessary and that generation of neurovirulent recombinants is frequent. The gene constellation for neurovirulence was predictable for recombinants derived from a particular pair of parental strains, but varied from pair to pair. After intranasal inoculation virus invasion of the brain occurred via trigeminal and olfactory nerves.

Post translational Modification of the Influenza C Virus Glycoprotein

NAGELE, A. and MEIER-EWERT, H.

In influenza C virus-infected cells a virus-specific protein of a molecular weight of 65,000 Dalton can be detected when glycosylation is inhibited by Tunicamycin. Since this protein can not be found in untreated control cells, it probably constitutes the unglycosylated precursor of the viral glycoprotein gp 88. The difference of the molecular weight between the precursor and the fully glycosylated gp 88 is about 17,000 Dalton, indicating a carbohydrate portion of the glycoprotein of more than 20 percent. Gp 88 and the two glycosylated cleavage products of this precursor protein, gp 65 and gp 30, regularly appear as doublets in SDS-polyacrylamide gels. Since the peptide pattern is identical for the doublet bands, it remains to be established whether this reflects a differential glycosylation or dissimilar proteolytic cleavage sites. The coding capacity of the viral genome RNA-segment No. 4 with a molecular weight of \(0.66 \times 10^6\) Dalton correlates well with the size of the unglycosylated precursor of the viral spike protein.
Replica-Immunocytochemical Data at the Plasma Membrane of HeLa Cells after Infection with Measles Virus

MANNWEILER, K., BOHN, W., RUTTER, G., and HOHENBERG, H.

In replica-immunocytochemical (RIC) – and ultrathin section (US) preparations the ultrastructures of the specific alterations and of virus antigens, which appear at the plasma membrane of HeLa cell coverslip cultures after infection with an adapted measles virus strain were investigated. As immunomarker protein A-coated gold particles were used (1). This method is sufficiently sensitive to enable labeling of even small altered areas of the plasma membrane (70–100 nm). Due to the high atomic number contrast in the TEM and the small size of the marker (≤ 10 nm) the ultrastructure of characteristic alterations morphologically still remains visible with ease in a three-dimensional aspect. Data obtained by RIC and US preparations after labeling with antimeasles immune serum or with monoclonal antibodies against HA (2) are demonstrated, compared and discussed.

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Production and Characterization of Monoclonal Antibodies Against Nucleocapsid and Envelope Associated Measles Virus Polypeptides

BOHN, W., RUTTER, G., and MANNWEILER, K.

By use of the mouse hybridoma technique, monoclonal antibodies were obtained with specificity for the HA (79K), P (72K) and M (36K) polypeptides of measles virus. Balb/c mice were immunized with native measles virus and measles virus treated with detergents and heat. Clones obtained after immunization of mice with native measles virus showed specificity for the HA polypeptide only. After immunization with measles virus, treated with 1% sodium sarkosyl sulfate (SSS) at 20 °C a clone was obtained producing antibodies to the M polypeptide. Heating of measles virus in the presence of 1% SDS under reducing conditions elicited a selective immune response to the P and NP polypeptides. Thus, clones producing antibodies to the P polypeptides were isolated.

Replication of a Persistent Sendai-6/94 Virus in Serial Cell Passages Independent of Trypsin Treatment

NEUBERT, W. J.

The persistently infected cell line CL-E-8 originally established by H. Koprowski and V. ter Meulen releases continuously Sendai-6/94 virus. This virus, termed 6/94 cl virus,
contains a 50s RNA, but it does not show any infectivity even after trypsin treatment. An
activation of the 6/94 cl virus can be obtained by i) cocultivation of the CL-E-8 cells with
standard cells (e. g. BHK-21) and ii) serial passaging of the 6/94 cl virus in several cell
lines (e. g. BSC-1). Infectious 6/94 cl virus can be detected in the cell supernatant after a
period of 5–10 days and 25–30 days respectively. This virus cannot be propagated in
chicken eggs, but it can replicate in serial cell cultures without trypsin treatment; more­
over trypsin treatment does not influence the viral replicaiton. In comparison 6/94 virus
released from an in-vitro generated persistent infection (BSC-1 cells infected with egg­
grown 6/94 standard virus) can be propagated in chicken eggs. This virus termed 6/94 pi
virus also can grow on serial cell passages without trypsin treatment, whereas the serial
replication of 6/94 standard virus on cell cultures depends on the trypsin-activation.

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Alteration in Phospholipid Methylation and Impairment of Signal
Transmission in Persistently Paramyxovirus Infected C and Rat
Glioma Cells

MÜNZEL, P. and KOSCHEL, K.

The paramyxoviruses measles (SSPE) virus and canine distemper virus (CDV) cause
an impairment of the catecholamine induced β-adrenergic receptor dependent c-AMP
generation in persistently infected C6 rat glioma cells. In CDV persistently infected C6
cells the number of receptors is greatly reduced. Hirata and Axelrod have shown that the
number of β-adrenergic receptors could be regulated by methylation of phosphatidyl
ethanolamine (PE) resulting in lecithin synthesis (1). We have therefore studied the
methylation of PE in persistently infected cells by the incorporation of (3H) methyl
groups from (3H-methyl)-Methionine into PE. In both infected systems, C6/SSPE and
C6/CDV, we observed a total loss of catecholamine stimulated β-adrenergic receptor
dependent methylation whereas the β-receptor independent methylation of phospholipids
is unchanged.

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Isolation and Preliminary Characterization of a Tree Shrew Paramyxovirus

KURZ, W., GELDERBLOM, H.1, FLÜGEL, R. M.2, and DARAI, G.

A paramyxovirus was isolated from a kidney biopsy of a Tupaia (three shrew) and
termed (TPV). The detailed host range study revealed that only Tupaia embryonic
fibroblasts and Tupaia kidney cells are the cells of choice for the efficient propagation
of TPV. TPV can be plaque-assayed on Tupaia embryonic fibroblasts and this cell line
was used for the continued propagation of TPV. Electron microscopy of purified TPV
revealed the presence of typical paramyxovirus particles. – The hemagglutination test was
performed with erythrocytes with a variety of different species. It was found that guinea
pig erythrocytes were agglutinated with TPV. The buoyant density of purified virions
was determined in sucrose gradient and found to be 1.19 g × mL⁻¹. – The biological
characterization of TPV which was performed by host range study in vivo revealed that TPV is highly pathogenic for new born mice and hamsters. – The characterization of viral RNA and proteins of this new member of paramyxoviridae is now in progress.

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The Biosynthesis of Coronavirus Glycoproteins

NIEMANN, H. and KLENK, H.-D.

Coronaviruses contain two glycoprotein species E2 (180 K) and E1 (23 K) which are both synthesized in the RER of the infected host cell. Glycosylation of E2 is initiated at the cotranslational level and it can be inhibited by 2-deoxyglucose and tunicamycin indicating the presence of N-glycosidic carbohydrate protein linkages. Particles formed in the presence of these inhibitors are noninfectious and lack detectable amounts of E2. – Cell fractionation experiments show that glycosylation of glycoprotein E1 occurs posttranslationally in smooth membranes. The carbohydrate protein linkages in E1 are susceptible to mild alkaline reductive conditions and N-acetylgalactosamine was determined to be the reducing sugar of the released oligosaccharides. This together with the finding that glycosylation of E1 is not sensitive to inhibitors of N-glycosylation suggests that glycoprotein in E1 of coronaviruses is the first structural virus glycoprotein containing O-glycosidic side chains exclusively.

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Target Cells of Infectious Bursal Disease Virus (IBDV) of Chickens

MÜLLER, H. and BECHT, H.

Infectious bursal disease virus (IBDV), the causative agent of a highly contagious disease of young chickens resulting in severe necrotic lesions in the bursa of Fabricius (Gumboro disease), is a non-enveloped icosahedral particle with a diameter of about 60 nm. Its genome consists of 2 segments of double-stranded RNA with molecular weights of 2.2 \( \times 10^6 \) and 2.5 \( \times 10^6 \) daltons. The virion is composed of 5 structural polypeptides with molecular weights of 90 kd, 48 kd, 40 kd, 32 kd and 28 kd. The 40 kd polypeptide, one of the two main structural proteins, is derived from the 48 kd polypeptide, perhaps by proteolytic modification (1). With immunofluorescence and “infectious center assays” we were able to show that 1. after infection of isolated lymphoid cells in vitro only 20 % of bursa cells, 2 % of thymus cells and 5 % of spleen cells produce infective virus (i.e. plaques in infectious center assays) although the donor chickens were in the most susceptible age of 4 to 5 weeks. 2. the number of virus producing cells is not correlated with the appearance of slgM or slgG. 3. virus yields seem to be influenced by the cell cycle: the number of chick embryo fibroblasts producing plaques in “infectious center assays” is increased after synchronisation of the cells before infection. 4. cells that produce infective virus show an increased uptake of \(^{3}H\)-thymidine. 5. isolated lymphoid cells from thymus or spleen as well as blood lymphocytes can be stimulated by mitogens to produce higher virus yields.

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Retino-Cerebral Manifestation of Experimental Borna Disease in Rhesus Monkeys

KREY, H., ROGGENDORF, W., and LUDWIG, H.

Inflammation of the uveal tract, the retina meninges and brain represent uveo-meningo-encephalitic syndromes of unknown origin. One of these, the Vogt-Koyanagi-Harada syndrome is primarily manifested by inflammation of the retinal pigment epithelium, the uveal tract and meninges. Severe visual and neurological impairment can occur. In our experimental studies 14 rhesus monkeys were experimentally infected with Borna disease virus. After a 4–7 week incubation period a progressive retino-cerebral syndrome was observed. Focal inflammatory lesions in the retinal pigment epithelium and the uveal tract were accompanied by encephalitic and meningeal infiltrates. Infectious virus could be demonstrated in the retina and in the brain. Experimental Borna disease can serve as an appropriate model for uveo-meningo-encephalitic syndromes in men.

The EEG of Borna Disease Virus Infected Rabbits

GIEREND, M., ADALOFF, W., and LUDWIG, H.

Borna Disease (BD) virus induced encephalitis in horses, sheep, rodents and primates shows similarities in many aspects with other so-called slow virus diseases. The EEG has shown to be a specific tool in studying encephalitides of different types in man. This is a report on the EEG of the BD virus specific encephalitis. Twenty-eight rabbits inoculated by different routes and with different virus preparations were screened for EEG changes. The basic frequency was measured optically and an analysis of the EEG was performed. The following conclusions were made: 1. A significant slowing down of the basic frequency was observed in the EEGs of BD virus infected rabbits. 2. Spikes and spike-waves were present rather regularly and correlated with epileptic seizures at the end of the disease when they appeared rhythmically in intervals of twenty seconds. Rademaker-complexes appeared from the third week on. Virological and serological data collected from all animals demonstrated a strong correlation of BD virus specific reactions with the EEG alterations. The patterns of EEG changes are reminiscent of those in SSPE. EEG features in this kind of slow virus diseases may have rather similar characteristics which could suggest that they may underlie common pathophysiological mechanism.

Borna Virus Infection in Embryonic Nerve Cells and in Cultures of Non-Neural Origin

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We established the primary cultures of neural retina and pigment epithelium of rabbit and of the brain of chicken embryo to study the sensitivity of each kind of cells to the
infection of Borna virus without any influence of the immunoresponse. The antigens first
appeared in the nucleus of neurons and sometimes fibroblasts, then filled the cytoplasm,
most brilliantly 7 days post infection. Thereafter they disappeared from the cytoplasm, but
remained persistently only in the nucleus in point shape. No morphological changes were
seen in the infected cells during 60 days post infection. We can say that the virus does not
kill the cell. For the destruction of nerve cells in in vivo conditions it can be pointed to
the importance of the immunological events, which might cause the clinical pictures.

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Large Scale Production of Biologically Active VSV in EAT-Cells

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Ehrlich ascites tumor cells maintained in mice were used to prepare milligram quanti­
ties of biologically active VSV. At the 6th day after passage when the cell number reached
approx. 7 × 10^8 cells/mouse, mice were infected by intraperitoneal injection of appro­
priate concentrations of VSV. Ascites fluid was harvested after 20 h. Virus production
was exponential for at least 16 h and continued for at least 20 h p.i. – Approximately
3–4 mg of viral protein/mouse and 2 × 10^{11} PFU/mouse were routinely obtained. The
specific infectivity of VSV isolated from EAT-cells reached nearly 1.5 × 10^8 PFU/μg
protein. The endogenous transcriptase activity of VSV produced in EAT, BHK, and HeLa
cells showed no significant differences. Large amounts of biologically active VSV may be
produced rapidly and much less costly with the described procedure than using tissue
culture cells. It should be possible to adapt the procedure for the production of other
viruses.

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The Quantitative Determination of Bardac-22, Formaldehyde, Glyoxal and
Glutardialdehyde in Disinfectants

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Several institutes of hygiene have been provided with two disinfected formulations, A
and B, with the intention to analyse these mixtures, containing different amounts of
quaternary ammonium compounds, formaldehyde, glyoxal and glutardialdehyde. – The
quantitative analysis of the components of the two mixtures depends on well known
volumetric and spectrophotometric methods. – The results of this analysis have shown
that it is possible to use the described procedure for routine check ups of disinfectants
without using microbiological tests.