jugation, with a preferential transfer of the A-determinant. Following these observations, the physical structure of P111-ACS and of its P111-A segregant has been determined. The plasmids were isolated by CsCl gradient centrifugation in presence of ethidium bromide. After removal of the dye, the contour length of the plasmids was measured by electron microscopy, using SV40 DNA molecules as internal standard. In *E. coli* K12 F⁻, P111-ACS shows both the structures of: 1. a plasmid aggregate formed by 19.2 μm molecules (inferred to be the transfer factor) and 3.2 μm molecules (inferred to be the R-determinants, A, C or S); 2. a plasmid cointegrate of 29.6 μm; occasionally a plasmid cointegrate of 22.8 μm was also observed. In the same host-cell P111-A shows only one structure: a molecule of 22.8 μm in length inferred to be the plasmid cointegrate TF-A. These results are in agreement with a cointegrate state of the R-determinants and the TF during the transfer the A-determinant probably having a preferential attachment to the TF compared to the other replicons.

A New Kanamycin/Neomycin Phosphotransferase Found in Staphylococci

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Aminoglycoside-phosphotransferases I, II and III have been reported to be involved in aminoglycoside resistance of gramnegative bacteria. In two strains of *Staph. aureus* and one strain of *Staph. epidermidis* a phosphorylating enzyme was observed, differing from these enzymes in the substrate profile and in pH optimum. Kanamycin/neomycin phosphotransferase IV rapidly phosphorylated and inactivated kanamycin A, B and C, neomycin B and C, paromomycin, gentamycin A and B, butirosin, lividomycin and ribostamycin. After two hours of incubation amikacin was completely inactivated, but phosphorylation was only slow. This certainly is the reason for the susceptibility of the strains against amikacin. Over the range 25–45 °C, there was significant phosphorylation with optimal activity at 37 °C. A temperature of 55 °C for 15 min inactivated the enzyme completely. Enzymatic activity generally was found over the pH range 5 to 9. For the kanamycins and the ribostamycin group, the optimal pH was 5.5 to 6.0 in citrate phosphate buffer, for the neomycin group 8.0 to 8.5 in *Tris*-maleate buffer. In two strains, resistance to aminoglycosides was found to be plasmid-mediated. The characterization of the resistance plasmids by sucrose gradient centrifugation and electron microscopy revealed molecular sizes of 36.5 (*Staph. aureus* E 142) and 21.5 (*Staph. epidermidis* 147) megadaltons respectively. Preliminary experiments indicate that resistance in *Staph. aureus* 170 might be governed by chromosomally located genes.

Mutual Influence Between λ-Phages and R-Factors

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A few years ago we stated that MS_2-phage-infection of cells carrying R-factors does diminish or even prevent the R-transfer (Path. Microbiol. *40*, 153, 1974, and *41*, 194, 1974). Now we examined the influence of fi⁺ R-factors on λ-lysogeny and vice-versa and obtained the following result: If *E. coli* K12 carrying R192 grown in Columbia broth were infected with phage λ at a multiplicity of infection of 10 PfU and incubated for 24 h at 37 °C, we could not detect afterwards any lysogenic clones out of one thousand. If *E. coli* K12, which are not carrying an R-factor, were infected and incubated under the same conditions, we found about 70% out of all cells lysogenic after 24 h. After further incubation for 24 h the R-free progeny was 100% lysogenic, the cells carrying an R-factor however only for 30%. If λ-lysogenic and λ-sensitive cells of the same strain were R-infected under equal conditions, both transferred the R-factor in the same frequency. If, however, R-carrying cells were λ-lysogenic, we detected a serious reduction of the frequency of R-transfer. With derepressed fi⁺ R-factors in doing so the ability for building sex-pili was lost. Therefore the interactions between fi⁺ R-factors and λ-phages seems to depend on which of the two genomes is in the cell first. As these investigations were also done with wild-R-factors and wild-strains of *E. coli*, they allow allusions to the influence of the epidemiology of R-factors. The molecular biological explanation of these phenomenona is at work.

Proteus mirabilis Wild-Strains as Donors and Recipients of Wild R-Factors

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At the microbiological examination of urine isolates coli bacteria are isolated beside cells of *Proteus mirabilis*. Thereby most of all the coli bacteria are containing infectious R-factors while the *Proteus* strains are R-negative and do only possess the well-known chromosomal resistance against polymyxin B and tetracycline. This observation shows that – considering the reception of R-factors – *Proteus* does behave differently from the rest of the *Enterobacteriaceae*. Therefore we investigated the chloramphenicol-resistance-transfer of multiple-resistant *Proteus* into antibiotic-susceptible *Proteus* cells, the R-infection of *E. coli* into *Proteus mirabilis* and vice-versa. We obtained the following results: 2 out of 8 multiple-resistant *Proteus* wild-strains did not transfer their resistances on the 39 recipient-strains. The other 6 donor-strains transferred their resistance on the following number of the recipient-strains: 2, 6, 8, 10, 11 and 31. Six of the 39 recipient-strains did not act as recipients with any of the 8 donor strains, 10 strains conjugated with only 1, 10 with only 2 and the rest with 3 to 5 of the donor strains. The frequency of transfer came to 10^{-6} and 10^{-7}. Besides the resistance for chloramphenicol mostly also the other resistances of the donor strains were transferred. From the 39 *Proteus* recipient strains only 12 accepted – with a low frequency – R192 from *E. coli* K12, and out of 8 *Proteus* donor strains only 1 was able to transfer the resistance into restriction-free cells of *E. coli* K12 with the low frequency of 5×10^{-7}. From these results we conclude that *Proteus mirabilis* is a bad donor and recipient for R-factors.

Rifampicin-Resistance in *E. coli*: Comparison of Microbiological and Enzymatic Properties

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The antibiotic rifampicin inhibits the growth of *Escherichia coli* by forming a tight complex with the bacterial RNA polymerase and thus inhibiting the enzyme. Cells

with various degrees of resistance towards rifampicin arise by spontaneous mutation. One aim of the present study was to see whether there exists a correlation between the sensitivity of the bacteria and of the enzyme towards rifampicin. A large number of rifampicin resistant *E. coli* were obtained by incubating cells with increasing concentrations of the drug. From these, 6 mutants were selected which showed an increasing MIC (minimal inhibitory concentration), ranging from 10-fold that of the wild type to complete drug resistance. It could be shown that an increasing MIC was closely paralleled by a corresponding decrease in sensitivity of the RNA polymerase. None of the mutants showed an RNA polymerase with a rifampicin sensitivity comparable to that of the wild type, ruling out, for these mutants, alterations in permeability as a major cause of rifampicin resistance. In order to examine the reasons for the increased enzyme resistance, the association and dissociation kinetics of rifampicin with RNA polymerase isolated from the wild type and two moderately resistant mutants were measured. It could be demonstrated that the stability of the drug-enzyme complex drastically decreases in resistant mutants as compared to the wild type, whereas the rate of complex formation seems to be little affected.

Evidence for Intestinal Duovirus (Rotavirus) Infections in Switzerland

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The presence of duovirus (rotavirus) in Switzerland was investigated using different techniques. Nebraska calf diarrhea virus infected cells were demonstrated by fluorescent antibody technique in feces and in gut mucosa smears of scouring calves from different locations in the canton of Berne. The conjugate was prepared from a calf hyperimmunized with commercial anti reolike scours vaccine (Norden Labs). Duovirus particles were observed in negative contrast stained fecal preparations of the same animals. Attempts to isolate the agent in tissue culture resulted in only very few infected cells demonstrated by FA technique. The low yield was usually lost while passaging. One isolate was successfully adapted to Vero cells. Precipitating antibodies have been found in immunodiffusion tests in sera of all species (human, cattle, roe-deer, horse, swine, guinea-pig) tested so far. The soluble antigen used was prepared from tissue cultures infected with Swiss and foreign isolates. The same antigens proved to be highly anticomplementary and not suitable for CF tests. Neutralizing antibodies are common in cattle sera. Performing neutralization tests it has to be considered that virus adsorption is very slow, temperature dependent, and impeded by high protein contents of the media.

Detection of Specific IgM Neutralizing Antibodies in Naturally Acquired Sporadic Human Enterovirus Infections

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In order to improve enterovirus diagnostic work the author tried to demonstrate a so called primary immune response in a group of patient sera. 5 sera from patients (2 to 20 years old) were analyzed. From all these patients an enterovirus was isolated from a stool specimen. These enteroviruses have been isolated in a 2-year-period from different parts of the country. The following types have been isolated: Echo type 6, 11, 22 and Coxsackie B2 and B5. A serum sample of each patient from the time of virus isolation was fractionated by gelfiltration. Fractions for neutralization tests were chosen in which only one class of screened immunoglobulin (IgG, IgA, IgM) could be detected by the Mancini-Carbonara method. In all sera the whole neutralizing activity detected against the homologous isolated enterovirus was found in the IgM fraction. No IgM neutralizing activity could be recovered against the panel of heterologous enterovirus types. On the other hand most sera showed neutralizing activity against one or more enterovirus in the IgG fraction and in unfractionated sera as would be expected. From the viewpoint of specificity and reproducibility the findings are encouraging enough for us to analyze a set of sera from patients where no enterovirus can be recovered and where an enterovirus etiology is presumed.

Genetic Relationship Between Two Poxviruses Determined by Restriction Analysis of their DNAs

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The giant genomes (185,000 basepairs approx.) of two biologically closely related poxviruses (Vaccinia strain Elstree and Cowpox strain Brighton, red mutant) were cleaved with the restriction enzymes *Eco*RI and *Hind*III. A great variety of specific DNA fragments, ranging in size from 46,000 to 48,000 basepairs and less could be separated from each other by specially adapted agarose gel electrophoresis techniques. Addition of the sizes of the separated *Hind*III restriction fragments confirmed the genome size determined by electron-microscopy (GESHELIN and BERNS, J. molec. Biol. *88*, 785, 1974). *Hind*III digestions of Vaccinia and Cowpox DNA yielded 13 and 17 easily distinguishable fragments, respectively, of which 5 were identical in size in both DNAs. *Eco*RI cleavage resulted in at least 30 different fragments, at least 9 of which were common to both DNAs, again indicating relatedness between the two DNA sequences. The actual base sequence homology was estimated to be about 90%.

Mitochondrial Functions in Semliki Forest Virus Infected Cultures of Chick Embryo Fibroblasts

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Monolayer cultures of chick embryo fibroblasts were infected at a multiplicity of 20 plaque forming units/cell. 5, 10, 15 and 20 h post infection cells were harvested. ADP/O quotients, acceptor control ratios and oxygen consumption/mg protein of isolated mitochondria were determined (substrates: malate-pyruvate, α-ketoglutarate, β-hydroxybutyrate and succinate). Ca/O quotients, acceptor control ratios and oxygen consumption/mg protein of intact cells were also measured with the Clark electrode (substrate: succinate). Both functional para-