tered. Moreover the appearance of these resistant organisms has been reflected in an increased failure of the antibiotics in question to treat infections caused by these bacteria. A comparison of the tetracycline resistance plasmid from *H. influenzae* with ampicillin resistance plasmids from the same strain has shown that the 2 types of resistance plasmids are very similar, save that one carries ampicillin transposon (Tn3-like) and the other has one that specifies resistance to tetracycline (Tn10-like)[2]. Thus it seems probable that the emergence of these resistant strains of *H. influenzae* occurred in 2 stages: first the organism acquired a plasmid which specified no resistance traits but which was able to replicate stably in *H. influenzae*. Then this plasmid acquired either an ampicillin-resistance transposon, or a tetracycline resistance transposon as a separate event. Presumably the source of these transposons were members of the Enterobacteriaceae - since the 2 types of transposon involved are very prevalent on plasmids in these species. But exactly when and where the events occurred is quite unclear.

In summary: One can see that bacterial DNA - and this is particularly the case with respect to DNA which specifies antibiotic resistance - is organized at 3 hierarchical levels - that of the whole bacterial chromosome, that of the bacterial plasmid and that of the transposon. In practical terms these hierarchies of DNA are continually interacting. Bacteria infect man: plasmids infect bacteria: transposons infect plasmids. Nor is this a sequence that relates only to antibiotic resistance; but our studies on antibiotic resistance in bacteria do allow us to study evolution in action, and this is becoming increasingly possible at the molecular level.

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DNA rearrangements and their importance in the evolution of gene systems

by Heinz Saedler, Institut für Biologie III, Universität Freiburg, D-7800 Freiburg i.Br. (Federal Republic of Germany)

See the following publications:

1 Transposable Genetic elements as agents of gene instability and chromosomal rearrangements.
P. Nevers and H. Saedler, Nature *268*, 109 (1977).
2 The Role of IS-Elements in *E. coli*.
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3 Tn 951: A new Transposon carrying the Lactose Operon.
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ABSTRACTS

Structure at the ends of bacteriophage Mu DNA

B. Allet
Department of Molecular Biology, University of Geneva, 30, quai Ernest-Ansermet, CH-1211 Geneva 4

Bacteriophage Mu exhibits genetic properties that are analogous in many details to those of established insertion mutants in procaryotic and eucaryotic chromosomes (for a review, see DNA insertion Elements, Plasmids and Episomes [1977], Cold Spring Harbor Monograph Series, edited by A.I. Bukhari, J.A. Shapiro and S.L. Adya). These properties, which include the ability to be transposed, and to delete DNA adjacent to the insertion site, seem to involve invariant DNA sequences in the insertion elements. In Mu these particular sequences must lie at the ends of the viral genome, and these ends are covalently bound to heterogenous bacterial DNA in vegetative, but presumably not in lysogenic Mu DNA.

We have analyzed the nucleotide sequences at the 2 ends of the Mu DNA using the Maxam and Gilbert method. In some cases, these ends were present in a genetic material derived from lysogenic Mu. They were included in plaque-forming *λ-lac*-Mu hybrid particles constructed by recombination between Mu lysogen and *lac λplac*5. In one case the Mu SE (or variable) end was present in a plasmid constructed with a vegetative Mu DNA fragment and pMB 9. Our analyses lead to 3 main conclusions. 1. They show that a short stretch of 5 identical bases is located at each Mu end, oriented as an inverted repeat. 2. They strongly suggest that identical Mu end sequences are present in lysogenic and vegetative DNA. 3. They demonstrate that the heterogenous bacterial DNA bound to vegetative Mu DNA is completely removed during lysogenization, thus implying that the transposition and deletion events are, at least in part, site specific.

Transposable genetic elements in a *Salmonella dublin* wild type strain

F. H. Kayser, M. Devaud and F. Homberger
Institut für Medizinische Mikrobiologie der Universität, Postfach, CH-8028 Zürich

S. dublin was isolated from the blood of a kidney allograft recipient, who was treated with immunosuppressive drugs to overcome rejection of the transplant. Strain 1 (HK247), isolated in August 1976, showed resistance to Sul, Str [AAD (3″)], Cml and Tet. Strain 2 (HK246), isolated in November 1976, was, in addition, resistant to Pen and Cep (TEM), Gen [ANT (2″)] and Kan [APH (3′)-I], but susceptible to Tet. This phenotype was identical with that of R-plasmid containing gram-negative organisms endemic in the university hospital. We assumed, therefore, that strain 2 originated from strain 1 by loss of the Tet-marker and gain of the endemic R-plasmid.

The resistant phenotypic characters of strain 2 could be transferred easily to appropriate receptor strains, while the R-markers of strain 1 could not be transferred at all. Variants of strain 2 selected for loss of resistance to beta-lactams and aminoglycosides retained resistance to Sul, Cml and Str. Further loss of resistance from these variants was rare. No transfer of resistance from the variants was observed. Tet resistance of strain 1 alone or together with other resistance could be eliminated in a frequency of 10^{-4}/cell. Molecular analysis of plasmid DNA revealed that strains 1 and 2 both contained a 52 Mdal cryptic plasmid. Strain 2, in addition, contained a 59 Mdal conjugative R-plasmid. Loss of resistance in strain 1 apparently is the result of excision of genetic elements from the chromosome. Evidence for excision and transposition of DNA was obtained by transferring a 44 Mdal R-plasmid into strain 1 and selecting for transconjugants carrying the R-plasmid as well as the chromosomal R-markers. Analysis of plasmid-DNA of these transconjugants showed a mol.wt of 55 Mdal, indicating the transposition of an 11-Mdal piece of chromosomal DNA into the plasmid.

A lactose-gene in a nonconjugative fi^+-R-factor from *Klebsiella pneumoniae*

G. Lebek and P. Zünd
Institut für Hygiene und Medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3008 Bern

96 strains of *K. pneumoniae* isolated from medical material of a hospitalism outbreak in the children's clinic at the University of Munich belonged to 29 different capsule types. All strains[1] contained an R-factor. When tested 2 years later, all the remaining R-factors showed to be fi^+, repressed and compatible with all the F-groups. Investigated further, these R-factors appeared to have mutual incompatibility. Remarkably these R-factors belonged to *Klebsiella* strains of various capsule types. In these experiments several R-factors with no tetracycline resistance and no conjugating properties appeared. Being mobilized by different R-factors of the fi^+-type a lactose gene showed to be co-transferred. Further examination proved that this gene was localized in the nonconjugating R-factor. It could also jump on other conjugating R-factors and could leave them spontaneously. Additionally, this lac gene appeared in its host cell in a noncurative state. This gives evidence for its insertion into the host chromosome. Based on these phenomenological results we suspect that this lac gene could possibly be a transposon. Should this genetic element be contracted, possibilities of misidentification of Enterobacteriaceae with conventional methods are obvious.

1 We thank Dr W. Marget, Munich, for kindly having given us the strains.

Structure and properties of the R-factor P111 system in different *Escherichia coli* strains

J. Cl. Piffaretti and Y. Froment
Département de Microbiologie, Faculté de Médecine, Université de Genève, 22, quai Ernest-Ansermet, CH-1205 Genève

P111 is a resistance plasmid system isolated from an *E. coli* clinical specimen; it confers resistance to ampicillin (Ap), chloramphenicol (Cm) and streptomycin (Sm).

P111 is characterized by the heterogeneity of its DNA: Several populations of different size can be observed in different *E. coli* K12 strains and for different resistance derivatives. We decided to analyze 2 populations of small molecules: molecules A are 9.2 kbases (kb) long and molecules B are 14.1 kb long. Purification of these molecules by centrifugation through 5–20% sucrose gradient followed by transformation experiments with *E. coli* C600 competent cells demonstrated that these molecules are replicons; A-molecules are related to Sm resistance and B-molecules are related to ApSm resistance. Heteroduplex analysis and transposition assays on λ showed that the Ap determinant is located on a transposon of 4.9 kb flanked by small inverted repeats. This transposon has also been localized on a 80-kb molecule belonging to the P111 system. Evidence is given that in our conditions Ap transposition seems to be much more frequent than usually reported.

The r-determinant of the drug-resistance plasmid R100.1

L. Caro, M. Chandler, L. Silver and D. Lane
Département de Biologie Moléculaire, Université de Genève, 30, quai Ernest-Ansermet, CH-1211 Genève 4

Integrative suppression of a *dnaAts* mutation by the composite drug resistance plasmid R100.1 results frequently in the appearance of small, circular, supercoiled, extrachromosal DNA-molecules. We have demonstrated by cloning various restriction fragments generated from one such molecule, pLC1, that it carries all the known r-determinant associated drug resistance genes of R100.1. Electron microscopic analysis of heteroduplexes formed between *Eco*RI generated fragments of R100.1 and its 2 component parts, the RTF (pAR132) and the r-determinant (pLC1) has shown that excision of both pLC1 and pAR132 from R100.1 involves recombination between the 2 IS1 elements which delimit the r-det- and RTF-regions of the parent plasmid.

We have found no evidence for autonomous replication of pLC1. It could not be introduced and maintained by transformation, under a variety of conditions, whether or not an RTF was present in the recipient cell. No partially replicating molecule was ever observed. Hybrid plasmids formed between pLC1 and a carrier plasmid could be introduced by transformation and express all the drug resistance genes of pLC1. They could not, however, replicate under conditions nonpermissive for the carrier plasmid.

The presence of pLC1 as a 'plasmid' is absolutely dependent on a functional *recA* gene; pLC1 disappears when the $recA^-$ allele is introduced into an Hfr; it does not arise in Hfr's made in a $recA^-$ background; it appears in both types of Hfr's when the $recA^-$ is replaced by a $recA^+$ allele; it is not dependent on the *lexA* gene.

These results suggest that the autonomous copies of r-determinant found in integratively suppressed strains are replicated from the integrated R100 and excised by a precise and efficient mechanism.

IS1-mediated transposition and amplification of drug resistance determinants in *Escherichia coli* and its phage P1

S. Iida, Christine Hänni, J. Meyer, Christine Echarti and W. Arber
Abteilung Mikrobiologie, Biozentrum der Universität, Klingelbergstrasse 70, CH-4056 Basel

The r-determinant of an NR1 derivative carrying genes for resistance to chloramphenicol, fusidic acid, streptomycin, sulfonamide and mercury is an IS1-mediated transposon of 23 kb length. Its transposition is demonstrated from the R-plasmid NR1 to various sites on the genome of bacteriophage P1, from P1 to various sites on the *E. coli* chromosome, and from there to phage P7.
The IS1 elements flanking the r-determinant give rise to occasional deletion formation. In a series of Cm^r deletion derivatives of transposed P1, the extent of the nondeleted material of the r-determinant is shown to be variable. Most of these derivatives still contain the 2 flanking IS1 elements, so that they each bear a new transposon which is shorter than the parental one.
A transposon can duplicate spontaneously. It is then carried in the P1 genome as a tandem dimer. The tandem dimers, but much less readily the monomers, amplify to yield tandem oligomers. These can be selected in the presence of increased concentrations of chloramphenicol, since oligomerization results in increased Cm resistance.
Hybrids between NR1 and P1 can also be formed by IS1-mediated cointegration. The cointegrate genomes segregate deletion derivatives which in some respects resemble the transposition derivatives.

The bacteriophage P1 plasmid can serve as a trap for transposing host insertion elements

W. Arber, Helga Jütte, P. Caspers and S. Iida
Abteilung Mikrobiologie, Biozentrum der Universität, Klingelbergstrasse 70, CH-4056 Basel

The genome of bacteriophage P1 has a size of about 90 kb and is carried as a plasmid in P1-lysogenic *Escherichia coli*. When P1-lysogenic strains are stored at room temperature for several months, subclones not producing plaque forming phage any longer are easily detected by replication of single colonies. The vast majority of these derivatives still carry a P1 plasmid. For a number of such derivatives the plasmid was isolated and its structure studied by restriction cleavage analysis. Many of these plasmids carried an insertion of about 1–2 kb length, at different locations. Other plasmids were deleted in the genome region flanking the IS1 element occurring naturally in P1 at map unit 20. No point mutant has yet been detected. Thus insertion and deletion are the most frequent spontaneous causes for loss of the ability of the P1 prophage to give rise to production of plaque-forming phage. Similar insertion elements were also detected by chance in functionally intact subclones of plaque forming phage P1Cm and of the R-plasmid NR1. The data available suggest that independent insertion derivatives carry different insertion elements. Experiments are in progress to explore if the inserted segments originate from the host chromosome. If this assumption is confirmed, the P1 genome could routinely be used to isolate and identify readily transposable elements in particular bacterial strains.

Specificity of the Eco P1 restriction modification endonuclease

B. Bächi, J. Reiser and V. Pirrotta
Department of Microbiology, Biozentrum of the University Basel, Klingelbergstrasse 70, CH-4056 Basel

Eco P1 is a restriction modification enzyme of a type intermediate between class I and class II. It requires ATP for cleavage and AdoMet for methylation of DNA. We have mapped the sites of both cleavage and methylation in SV40 DNA and determined their sequences. The enzyme methylates the sequence AGACC (A: 6-methyl aminopurine; AdoMet: S-adenosyl methionine) and cuts the DNA 25–27 basepairs (bp) from the site of methylation in the 3′-direction, with a 2–4 bp stagger between cuts. Consistent with the fact that the methylation sequence is asymmetric, the enzyme methylates only one strand in vitro. One variant of SV40 has acquired an additional Eco P1 methylation and cleavage site by changing an AGAAC to AGACC.

Drug-resistance survey in Switzerland: an approach

J.-S. Pitton
Commission des Antibiotiques (SSM/SGM), 16, avenue de Frontenex, CH-1207 Genève

This work is designed to determine and to improve the methodology to be used for evaluation of drug-resistance, to set up a collection of reference strains (sensitive or resistant) and to organize collaborative assays for regular controls. 3 assays have been completed so far. They consisted in drug-sensitivity tests for a number of different bacterial strains, including internationally recommended reference strains, as well as wild-type strains isolated from clinical specimens. The participants were requested to use their routine procedure to determine drug-sensitivity to 14 antimicrobial agents. For the 3 collaborative studies, the results show that the overall correlations are 72.7%, 80.7%, 77.4% for 26, 30, 22 laboratories, respectively. The analysis of results for the first collaborative study led the Commission to write down recommendations derived from WHO (Technical Report Series 610, 1977) and NCCLS (Villanova, PA 1975) proposed standards.
Among the most important sources of discrepancies, one can point out the following:
1. improper storage of discs (temperature);
2. content of antibiotic in discs which do not correspond to recommendations;
3. use of inadequate media:
a) media containing specific inhibitors, b) media especially designed to enhance the in vitro sensitivity to certain drugs or their combinations, c) blood-containing media for non-fastidious microorganisms;
4. misunderstanding of heteroresistance to β-lactam antibiotics for staphylococci;
5. lack of regular quality control (performance tests with reference strains);
6. lack of uniformity in guidelines of manufacturers for interpretation of zone diameters.
It is obvious that there is an urgent need for 'harmonization' in the interpretation of sensitivity tests. For some drugs, as well as for some strains, the discrepancies are particularly high. This work should allow one to evaluate and to survey the evolution of drug-resistance for the most important pathogenic strains of human and animal sources and finally to establish a catalogue of the distribution of R-plasmids in Switzerland.

Clinical laboratory evaluation of an automated microbial detection and identification system (AMS™)

F. H. Kayser
Institut für Medizinische Mikrobiologie der Universität, Postfach, CH-8028 Zürich

The AMS is an automated, computerized instrument to detect, quantitate and identify bacteria and yeast in clinical urine specimens. The technique uses freeze-dried, highly selective media placed in disposable cuvettes. An optical system detects growth in the inoculated microculture wells. Results are automatically obtained within 13 h. Susceptibility testing of isolated bacteria to 11 antimicrobials is made possible in a separate cuvette.
The system was evaluated by comparing the results of examination of 399 urine specimens from hospitalized patients and from outpatients with those of a standard method. 189 specimens contained bacteria equal to or more than 7×10^4/ml. Of these, 92% were correctly enumerated by the instrument. 210 specimens showed numbers lower than 7×10^4/ml. Only 1.5% of these samples were wrongly enumerated as too high by the system. Positive identification of significant number of organisms by AMS in 189 urine specimens correlated with 69%. 8% of the urines were misidentified, 23% were only in part identified correctly (i.e. identification of 2 organisms, although only one was present or vice versa). Specific identifications were 21-91%, depending on the organism present. Problems were mainly observed with *Citrobacter freundii* (21%), *Staphylococcus aureus* (27%), *Pseudomonas aeruginosa* (47%) and *Serratia* (50%). A number of *Escherichia coli* strains was examined for resistance to antimicrobials. Agreement with agar diffusion was positive in 91-100% for 11 antimicrobials. A cost analysis revealed that the automicrobic system was twice as expensive as our standard procedure.

Typology and cluster analysis of 1529 *Pseudomonas aeruginosa* strains marked by serological and phage typing

P. Schaffer, D. Grange and J. Lavillaureix
Institut d'Hygiène, Faculté de Médecine, Laboratoire d'Hygiène Hospitalière, F-67085 Strasbourg Cédex, France

Joint correspondence analysis and cluster analysis by ascending hierarchical classification of sero and phage typing of 1529 Hospital germs of *P. aeruginosa* has lead to the following results:
- by studying the phages typology (set of 17 bacteriophages of Lindberg et al.): appearance of phages couples made by phages which lyse germs simultaneously i.e.;
(31, 1214) (16, F8) (M4, 24) (44, 352) (7, 119X);
- by studying interrelations between serogrouping (0 grouping system of Habs I completed by M. Veron) and phage typing:
discrimination in serotypes between those which are good epidemiological typers i.e. serogroups (0:1, 0:2, 0:3, 0:6, 0:9, 0:10, 0:11, 0:13) and those poorly 'informative' (NT, AA, 0:4, 0:5, 0:7, 0:8, 0:12, 0:14);
observation of some phages which simultaneously lyse strains typed by some specific serogroups:
group 1 = (31, 1214, F8, 109) lyses strains frequently typed by serogroup 0:2 and scarcely by 0:11;
group 2 = (7, 24, 119X, M4) lyses strains frequently typed by 0:6 and scarcely by 0:10;
group 3 = (21, 68, 44, 352) lyses strains typed by 0:1, 0:3 or 0:13;
group 4 = (16, 73) lyses strains typed by 0:6 and exceptionally by 0:1, 0:3 or 0:13.
Phage F7 and F10 are not related to any other phage or serotype. M6 lyses strains often typed by 0:6 but never by 0:2.
These associations are plausibly linked to nosocomial factors but more likely they are of structural order. This suggest that the phages overlap partly or fully with those determining serogroups since both are determined by surface structures.
These analyses give a more efficient way of studying the ecology of the *P. aeruginosa* strains. Moreover the quality of the phages set can be asserted and it is possible to remove from our set the phages 1214, 109, M4 which are redundant with phages 31, F8, 24.

Methicillin-resistance does not influence survival of *Staphylococcus aureus* in an in vitro phagocytic assay

P. Vaudaux and F. A. Waldvogel
Infectious Disease Division, Department of Medicine, Hôpital cantonal, CH-1211 Geneva 4

The mechanisms by which *S. aureus* (SA) produces disease are widely unknown. Among various parameters, survival of SA in normal polymorphonuclear leukocytes (PMNL) has been correlated with their pathogenic potential (Rogers and Tompsett, J. exp. Med. *95*, 209, 1952; Mandell, J. clin. Invest. *55*, 561, 1975). Since there is still some debate regarding the degree of pathogenicity and the treatment of methicillin-resistant *S. aureus* (MRSA), their survival was measured over 1, 3, 5 and 24 h in an in vitro phagocytic assay, containing an opsonic source and 5×10^6 PMNL/ml.
Survival of 8, epidemiologically distinct MRSA strains was similar to that of 2 pathogenic methicillin-sensitive strains (MSSA): after an initial decrease in colony counts, growth resumed at 3-6 h despite the presence of viable, phagocyting PMNL. This secondary growth phase of MRSA was not abolished, when 25 µg/ml methicillin (MET) was included in the phagocytic assay.
In order to elucidate whether the demonstrated pathogenicity of MRSA could be dissociated from MET resistance, comparative phagocytosis experiments were performed with one of the MRSA strains and 2 subpopulations derived from it: The 1st one was composed exclusive of cells highly resistant to MET (Sutherland and Rollinson, J. Bacteriol. *87*, 887, 1964), whereas the 2nd one contained only cells sensitive to MET, being the progeny of 'revertant' MET-sensitive colonies, segregating from MRSA upon repeated subculture. The 2 subpopulations and the original population they derived from showed similar survival in the phagocytic assay.
In conclusion, MRSA show the same pathogenic potential in the phagocytic assay as MSSA and this potential can be dissociated from their expression of MET resistance. In contrast to other antibiotics tested (Vaudaux and Waldvogel, 10th Int. Congress Chemother., p. 44, 1977), MET is unable to prevent the secondary growth phase of MRSA, which occurs in presence of actively phagocyting PMNL. MET is more active on MSSA than on MRSA, because it can prevent the secondary growth phase of the former (including the 'revertant' MET-sensitive cells referred above), but not of the latter.

About effective immunization against tuberculosis

J. Illés
Kempfhofweg 9, CH-8049 Zürich

It is not possible at the moment to get accurate information on the level of protection and on its duration after BCG-vaccination in humans (WHO, Technical Report Series, p. 552, 1974). Robert Koch was able to obtain a 'fast and perfect immunity' on horned cattle if they were immunized

by i.v. injection (route of administration), with suitable vaccine (quality), in sufficient quantity (quantity). He was not able to immunize guinea-pigs effectively by the same method (Gesammelte Werke von Robert Koch, Band 1, p. 591, 1912).
We have immunized guinea-pigs with BCG-vaccine i.c., i.p. and i.v. in parallel tests. These vaccinated animals showed only an enhanced resistance. Effective immunity against tuberculosis could be never achieved in guinea-pig (Bull. Wld Hlth Org. *53,* 3456, 1976), and therefore the following conclusions seem to be justified.
a) Guinea-pigs are not suitable animals for the potency test of BCG-vaccine. b) The importance of the route of administration, the quality and quantity of the vaccine should be tested in suitable animals (e.g. calf or goat). c) The results obtained should be examined for their practical significance in human beings.

Staphylococcal protein A – a tool in diagnostic microbiology and serology

M. Gasser, D. Mohr, S. Strasser and U. Wyrsch
Institut für Mikrobiologie und Hygiene, Petersplatz 10, CH-4003 Basel

Staphylococcal protein A is a single polypeptide chain of mol. wt 42,000 which is synthesized and incorporated into the cell wall by some strains of the genus *Staphylococcus.* The characteristic biological property of protein A is its ability to combine with the Fc part of IgG molecules of the subclasses 1, 2 and 4 of virtually all members of the class Mammalia (Kronvall et al., J. Immun. *103,* 828, 1969; *104,* 140, (1970). In vivo, this property may prevent the staphylococci from being captured by phagocytic cells (Dossett et al., J. Immun. *103,* 1405, 1969). Of the various in vitro applications of protein A for diagnostic purposes some are discussed: a) Characterization of staphylococcal strains by their ability to produce protein A; b) removal of IgG from sera prior to testing for specific IgM (Ankerst et al., J. infect. Dis. *130,* 268, 1974); c) coagglutination, a convenient serologic method in identifying bacteria (e.g. the grouping of streptococci by the Phadebact *Streptococcus* Test), bacterial products and viruses (Rosner, J. clin. Microbiol. *6,* 23, 1977; Eldridge et al., Med. Lab. Sci. *35,* 63, 1978; Zalan et al., Arch. Virol. *56,* 177, 1978); d) detection of cell antigens by use of specific antibodies and protein A, either coated to sheep red cells or FITC-labelled (Ghetie et al., Eur. J. Immun. *4,* 500, 1974; Biberfeld et al., J. Immun. Meth. *6,* 249, 1975); e) adsorption of IgG or immune complexes for solid-phase immunoassays such as radioimmunoassays for viral antigens and antibodies (Premkumar-Reddy et al., J. nat. Cancer Inst. *58,* 1859, 1977; Brunner et al., Med. Microbiol. Immun. *163,* 25, 1977). 2 methods being developed are presented, a sensitive solid-phase neutralization assay which depends on adsorption of antisera on to formalin and heat-killed protein A positive staphylococci prior to the proper neutralization testing, and a slide coagglutination assay for virus antibody.

Oral immunization of foxes against rabies

F. Steck, U. Häfliger, Ch. Stocker and A. Wandeler
Veterinär-bakteriologisches Institut der Universität Bern, Schweizerische Tollwutzentrale, Länggaßstrasse 122, CH-3012 Bern

Oral immunization of wild-living foxes against rabies can be considered as a complement or alternative to other methods of reduction of the susceptible fox population. For many problems of baiting however a satisfactory solution has to be found. Bait uptake has to be in time and in a sufficient proportion by foxes, the vaccine virus has to be liberated in the buccal cavity and induce immunity resistant to challenge, the vaccine virus has to be stabilized against thermal inactivation and should be innocuous for other nontarget animals.
The results of our field and laboratory experiments are summarized as follows:
1. In the field chickenheads and meatballs are taken up to 70% within 12–36 h, a minimum of 28% by foxes.
2. Optimal stabilization against thermal inactivation of the attenuated SAD-strain of rabies virus grown in BHK_{21}-cells in liquid form was obtained by the addition of 5–10% egg yolk. The loss of infectious titer was less than 50% at 4 °C and 22 °C and not more than 90% at 37 °C in 72 h.
3. The 50% protective dose by oral application of SAD-BHK_{21} is about $10^{5.5}$ IU (tissue culture infective units). Foxes with a positive seroconversion are protected against an i.m. challenge with 3×10^3 MLD_{50} of fox salivary gland rabies virus.
Protection was obtained with 2 ml liquid vaccine in small plastic containers in chicken head baits. With 10^7 IU a strong, with 10^6 IU a reduced antibody response was observed, both groups resisted challenge. With HEP-virus grown on BHK_{21} cells the results were unsatisfactory.
4. The oral application of SAD-BHK_{21}-virus to wild mice *(Microtus arvalis, Apodemus silvaticus* and *Mus musculus)* induces rabies in less than 4%. No tendency of virus spread among mice was observed so far in laboratory and field studies.

Bovine herpes mammillitis in Switzerland: a seroepizootologic survey

M. Engels and A. Metzler
Institut für Virologie der Universität Zürich, Winterthurer Strasse 266a, CH-8057 Zürich

Infection by bovine herpes mammillitis virus (BHM-V s. BHV-2) is an ulzerative skin condition of the teats and sometimes of the udder of lactating and recently 'dried off' cows. The disease has been observed in the United Kingdom, Italy, Australia, Bulgaria and the USA. We conducted a seroepizootological survey to find out whether BHM occurs also in Switzerland.
Of a total of 1821 sera examined 135 (7.4%) showed antibodies against BHM-V. The seroepizootological situation varied in different regions: in the eastern parts of Switzerland 86 (6.7%) out of 1267 animals examined showed antibodies to BHM-V. In the western parts the percentage was 1.25% (319 animal sera tested) and in the southern parts of the country 10.1% (235 sera tested).
To our knowledge no clinical outbreaks of BHM have been observed in Switzerland although from the serological point of view the disease should exist. The following explanations for this phenomenon are discussed: outbreaks escaped diagnosis, subclinical course of the disease and crossreactions with unrelated viruses.

The replacement of labelled anti-species gamma-globulines with labelled protein A for the detection of viral antibody titers

W. Bommeli, U. Kihm and H. Fey
Veterinär-bakteriologisches Institut, Universität Bern, Länggaßstrasse 122, CH-3012 Bern

The replacement of anti-species gamma-globulin with protein A, which is known to interact with the Fc fragment of

IgG molecules from several species, results in a lower background reaction and higher sensitivity and moreover it renders the tedious preparation of various anti-species gamma-globulines unnecessary. The isolation of protein A was performed according to Sjöquist et al. (1972). The *Staphylococcus aureus* strain Cowan I was digested with lysostaphin and the protein A precipitated with ammonium sulfate at 80% saturation.

At present we successfully use the protein A labelled with FITC for the detection of pseudorabies, hog cholera and swine vesicular disease antibodies on a semi-quantitative base. Thus the antibody titers are higher in the indirect fluorescent antibody technique than the seroneutralization titers and the reading of the test is very easy.

In preliminary experiments we also checked the protein A for its validity in the ELISA for the detection of pseudorabies antibodies. The pseudorabies virus was propagated in serum-free medium on PK15 cells. The coating of polystyrol tubes was done over night at 4 °C with a 1:25 dilution of the harvested virus in PBS. The tubes were washed 3 times with a 0.85% NaCl, 0.5% Tween 80 solution. 1:20 serum dilutions were placed into the tubes and incubated 1 h at 37 °C. After 3 washes the protein A labelled with peroxidase, using the method of Nakane and Kawaoi (1974), was incubated for 15 min at 37 °C. Again after 3 washes ABTS was used as indicator for the peroxidase reaction. The spectrophotometric measurements are very promising in terms of quantification of antibody levels.

Influence of poliovirus infection on S-phase and mitosis of the host cell

A.-L. Hugentobler, K. Bienz and H. Löffler
Institut für Mikrobiologie, Petersplatz 10, CH-4003 Basel

The influence of a poliovirus infection on different stages of the host cell cycle was studied. Hep-2 cells were synchronized by a double thymidine block and infected with poliovirus type I (Mahoney) in hourly intervals after release (a.r.) from the 2nd thymidine block. A 2nd culture, synchronized identically but not infected, served as control.

The S-phase is not blocked by a polio infection, but when infected 0–3 h a.r., an increase of its duration is found with correspondingly slower DNA-synthesis per cell. When the cells are infected 4 h and more a.r., no difference in the progress of DNA-synthesis through S-phase of infected and control cultures can be demonstrated.

In infected cells, a slightly higher peak of mitotic cells compared to control cultures is found. The mitotic indices are about the same for all cultures infected at different times a.r. The mitotic peak, however, is found at a more or less fixed time (2.5–3,5 h) after infection rather than at a constant time a.r.

In conclusion, poliovirus infection reduces the rate of synthesis of host cell DNA, but does not affect the normal completion of the S-phase. Subsequently, infected cells enter mitosis, but the mitotic peak is no longer controlled by the cell cycle, but depends on the time of infection after release of the cells from the thymidine block.

Physicochemical and morphological properties of virus particles associated with hepatitis A

G. Siegl and G. G. Frösner
Institut für Hygiene und Medizinische Mikrobiologie der Universität Bern, Friedbühlstrasse 51, CH-3010 Bern

Virus particles were purified from stools of patients in an epidemic of hepatitis A. Using reference MS-1 chimpanzee preinoculation and convalescent sera, the close serologic relationship of the purified particles to well-known isolates of hepatitis A could be established both by means of immune electron microscopy and in a specific radioimmunoassay. The majority of the hepatitis A associated particles banded at 1.34 g/ml in CsCl and, like poliovirus, sedimented at about 160 S. In addition, a distinct hepatitis A antigen was observed which banded at 1.305 g/ml and sedimented between 50 and 90 S. A further component accumulated in the density range of between 1.38 and 1.44 g/ml; however, it seemed to be rather labile. Upon sedimentation in sucrose it resolved into a 160 S, a 90–100 S and a 50 S form.

The capsid of the 160 S particles measured 27–29 nm in diameter and, therefore, could be readily distinguished from that of a parvovirus (22–24 nm). Detailed analysis of electron micrographs also provided evidence for the capsid being composed of 32 capsomeres arranged according to the symmetrical requirements of a rhombic triacontahedron. In the presence of 4 M urea the 160 S particles released linear single-stranded RNA molecules which showed a bimodal size distribution with peaks at 1.2 and 1.7 µm. On the basis of the weight per length ratio determined in parallel experiments for poliovirus RNA, this size would be consistent with a mol. wt of 1.3×10^6 and 1.9×10^6, respectively. It was finally concluded that the virus of human hepatitis A has to be classified with the picornaviruses.

Innate resistance against influenza is expressed in cultured liver cells

H. Arnheiter, O. Haller and J. Lindenmann
Institut für Medizinische Mikrobiologie, Experimentelle Mikrobiologie, Postfach, CH-8028 Zürich

Adult mice homozygous (A2G) or heterozygous (e.g. AxA2G F_1) for the dominant gene *Mx* survive infection with pneumotropic, neurotropic and hepatotropic influenza viruses up to 10^4 LD_{50} (titrated in susceptible mice). Resistance develops early after birth and is independent of surveillance by T-cells and normal antibody production (Haller and Lindenmann, Nature *250*, 679, 1974).

In vivo resistance is correlated with in vitro resistance of peritoneal macrophages obtained from adult animals (Lindenmann et al., J. exp. Med. *147*, 531 (1978). Cultured fibroblasts or kidney cells from *Mx*-bearing embryos are fully susceptible. Parenchymal cells from adult animals should represent the in vivo situation more realistically. Therefore, primary monolayer cultures of adult hepatocytes known to be nondividing were prepared free of macrophages and challenged with a hepatotropic variant of influenza A/TUR/Engl/63 (Havl, Nav3).

Infection at a multiplicity of 100 virus particles per cell resulted in similar virus growth in cultures prepared from susceptible and resistant animals. Low virus doses (10^{-2} virus particles per cell) led to distinct restriction of virus reproduction in cultures from resistant mice as compared to control monolayers. No differences of viral replication steps were found by immunofluorescence and electron microscopy.

Thus, liver monolayers from resistant animals proved to be susceptible, when the majority of cells was infected simultaneously. However, when few cells were involved in first replication cycles following low challenge dose, liver cells appeared to express resistance in vitro without the help of any particular nonparenchymal cell. These findings suggest resistance mechanisms to be based on some interference phenomenon, be it of cellular (interferon?) or of viral (interfering particle?) origin.

Genetic heterogeneity among different isolates of parapoxviruses

R. Wittek, P.A. Bachmann, A. Mayr and R. Wyler
Institut für Virologie der Universität Zürich, Winterthurer Strasse 266a, CH-8057 Zürich, and Institut für Mikrobiologie und Infektionskrankheiten der Tiere der Ludwig-Maximilians-Universität München, D-8 München 22, Federal Republic of Germany

Parapoxviruses form a group of morphologically clearly distinct viruses within the family poxviridae. Very little is known about the genetic relatedness of viruses belonging to the parapoxvirus group. By serological methods, orf virus and stomatitis papulosa virus have been found to be very closely related.

The genetic relatedness of parapoxviruses was examined by comparison of restriction endonuclease digests of the genomes of 5 stomatitis papulosa virus isolates from infected calves and of an orf virus isolate from an infected sheep. The DNAs of 3 stomatitis papulosa virus isolates displayed almost identical restriction patterns with the enzymes *Hind*III, *Hind*II, *Eco*RI and *Kpn*I. The DNAs of 2 further isolates of stomatitis papulosa virus yielded identical restriction patterns to each other but having no similarities to the former 3.

The DNA of the orf virus strain examined yielded restriction patterns which were entirely different from those obtained from the genomes of the stomatitis papulosa virus strains. We conclude from our analysis, that subclassification of parapoxviruses ought to take into account the existence of different species even among the serologically closely related stomatitis papulosa viruses showing similar clinical manifestations.

Aedes albopictus (mosquito) cells showing strong response to Semliki Forest Virus (SFV) infection

P.J. Späth and H. Koblet
Institut für Hygiene und Medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3008 Bern

A cell line of *A. albopictus*, fortuitously obtained by subcultivating loosly attached cells from a well-developed culture of the Singh-line, shows an unusually strong response to SFV infection never observed in the original line: destruction of cells, formation of syncytia-like cell complexes and formation of granular masses.

Cells were grown and infected in Mitsuhashi Maramorosch medium (MM-M) at 28 °C. The cellular reaction after infection with different numbers of PFU was followed under the light microscope. After 1 day the normal cellular appearance was lost and a granular mass developed. Gradually holes appeared in the granular mass in which single cells were observed. Finally the granular mass disappeared and within 3 weeks a new normal looking culture had grown. These cells could be subcultivated and were virus-resistant.

Fresh medium immediately before or within 24 h after infection prevented the sequence of events described. Small changes of the medium composition may therefore play a role.

If the cells were frozen in 10% dimethylsulfoxide (DMSO), thawed, and left for 2 h in 1% DMSO and were then grown in MM-M, they did not show susceptibility until a first subcultivation with trypsin was made. All further subcultures demonstrated virus sensitivity. However, 1% DMSO before infection (20 h) and after infection did not prevent susceptibility.

Subviral particles from Semliki Forest Virus (SFV) infected chick embryo fibroblasts (CEF)

F. Reigel and H. Koblet
Institut für Hygiene und Medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3008 Bern

To find intermediary products and free messenger ribonucleoprotein complexes during the replication cycle of SFV, we infected CEF. 6 h after infection the cells were disrupted in hypotonic buffer containing non-ionic detergents. The lysates were centrifuged in such a way in sucrose gradients containing high salt that all polysomes were in the sediment. We found in the 25 S to 120 S region several particles with RNA of viral origin. They sediment with about 27 S, 43 S, 57 S, 84 S and 110 S as compared to the ribosomal subunits. The particles in the lower S-range contain the 26 S viral RNA; the others contain the 42 S RNA. They can be degraded with RNAase (25 µg/ml, 30 min, 20 °C). The incorporation of radioactivity into the RNA of some particles varies in different experiments for unknown reasons. Proteins are labeled in the region of 43 S, 57 S, 84 S and 110 S. The particles at 84 S and 110 S contain a protein which could correspond to viral coreprotein as judged from polyacrylamide gel-electrophoresis; prelabeling of cells before infection gave no analogous peak. When the cells are disrupted without detergents, all particles except the 27 S appear, suggesting that this peak could correspond to the detached, free 26 S viral RNA.

Rubella immunofluorescence as an alternative to hemagglutination-inhibition

U. Schilt
Institut für Hygiene und Medizinische Mikrobiologie der Universität Bern, Friedbühlstrasse 51, CH-3008 Bern

To assess rubella immunity the hemagglutination-inhibition test (HI) is considered most convenient. Unfortunately the test is insufficient in some situations. e.g. the significance of low positive titers is questionable. In urgent cases performance of the test takes too much time. Because complement fixation and neutralization cannot be considered as routine procedures to establish rubella immunity the author determined immunity in 1621 sera in both HI and IF. Measurement of HI-antibodies was done according to a standard procedure. IF-antibodies were measured by the indirect method using rubella virus infected Vero cells and the serum diluted 1:5. The test was read with blue-light epiillumination. Agreement between the 2 tests was 98.4-100% with respect to the age group tested. From our experience IF may be used as an efficient alternative test in rubella virus serology.

The influenza virus transcriptase and its transcription products

M.A. Horisberger and R. Brambilla
Pharma-Forschung, Ciba-Geigy AG, CH-4002 Basel

Influenza A viruses are negative strand RNA viruses with a segmented genome consisting of 8 distinct RNA pieces coding for 8 polypeptides. An RNA-dependent RNA transcriptase is associated with complexes containing the genome RNA pieces, a nucleoprotein and 3 large mol.wt polypeptides. These complexes are found not only in purified virions but also in infected cells.

We have shown that these virion- and cell-associated transcriptase complexes are able to synthesize transcripts in vitro which can be separated on acrylamide gels and which are functional since the synthesis of 5 viral polypeptides

were unequivocally shown in a coupled system for transcription and translation (Content, DeWit and Horisberger, J. Virol., in press).
We have asked the question whether initiation of transcripts and not only their elongation could take place in vitro on the transcriptase complexes isolated from infected cells. Analysis of the nucleotides at the 5'-end of the transcripts gave a direct proof that initiation occurred in our transcriptase assay. The RNA was transcribed in vitro in the presence of 3H-labelled nucleoside triphosphates. After purification the radioactive transcripts were completely digested with T_2-ribonuclease and 5'-ends with charge - 4 were isolated corresponding to pCp and pAp.
Our results show that the transcriptase complexes isolated from infected cells are able to initiate and fully transcribe, in vitro, complementary RNA. These transcriptase complexes may represent the precursors of the ribonucleoprotein complexes with transcriptase activity from influenza virions.

Studies on vaccinia mRNA biosynthesis using in vitro translation systems

W. Bossart, D. Nuss and E. Paoletti
Institut für Mikrobiologie, Petersplatz 10, CH-4003 Basel, and Division of Labs and Research, NYS Health Department, Albany, New York 12201, USA

The biogenesis of vaccinia mRNA is supposed to proceed via synthesis of a large RNA transcript and subsequent processing to translationally active mRNA. This hypothesis was tested using an in vitro system where RNA synthesis by purified vaccinia viruses was coupled to the translation of the resulting RNA products in a cell-free protein synthesizing system derived from wheat germ. The synthesis of virus-specific proteins in the coupled system was differentially inhibited by exposure of the virus particles to UV-light: The sensitivity of single proteins to UV-light was shown to be proportional to their molecular weights. Each translationally functional mRNA species produced by vaccinia virus is therefore synthesized from individual promotor sites.
Translation of purified virion-associated high-molecular-weight (HMW) RNA resulted in the same polypeptides that were expressed in the coupled system. The relative translational efficiency of capped and uncapped HMW transcripts was identical to that of virion-released 8–12S RNA. A cap-mediated step in the polypeptide chain initiation is required for efficient translation of both transcript types as was shown in experiments using m^7GMP. Pulse-chase experiments after inhibition of polypeptide chain initiation with ATA suggest that only sequences at or near the 5'-end of the large RNA transcript are translated. The question whether vaccinia virion-associated HMW transcripts result from faulty termination or really represent precursor transcripts to mature vaccinia mRNA can not be answered until we can decide whether sequences further downstream are only translated after processing of the large transcripts.

Stimulation of 2-deoxy-D-glucose uptake in HeLa cells after rabbit poxvirus infection

D. Schümperli, E. Loepfe, E. Peterhans and R. Wyler
Institut für Virologie der Universität Zürich, Winterthurer Strasse 266a, CH-8057 Zürich

The uptake of the glucose analogue, 2-deoxy-D-[1-^{3}H]glucose (2-DG), by HeLa cell monolayer cultures was measured after infection with purified rabbit poxvirus (strain Utrecht) at a multiplicity of 5 to 10 plaque forming units per cell.
The addition of fresh medium greatly stimulated 2-DG uptake both in infected and control cultures and a plateau was eventually reached about 2 h later. However, as early as 0.5 h post-infection (p.i.), 2-DG uptake was significantly higher in infected cultures than in controls and the difference persisted at least until 12 h p.i. In comparison, the penetration of infected cells by [^{14}C]-sucrose for which the plasma membrane is normally relatively impermeable increased only around 10 h p.i.
The time course of the uptake reaction was analyzed at 3 h p.i. The reaction rate was constant between 10 and 60 min after the addition of 2-DG and curves of infected and control cultures were similar in shape, but higher values for the former were obtained throughout the reaction.
The enhancement was observed over a wide range of 2-DG concentrations (5×10^{-2} M to 2.5×10^{-7} M). The K_M of 2-DG uptake was estimated from double-reciprocal plots to be 2.3×10^{-3} M in control and 2.1×10^{-3} M in infected cultures. However, values for v_{max} were also altered.
When cells were infected with UV-irradiated virus or with native virus in the presence of 300 µg/ml cycloheximide, a slight and temporary stimulation of 2-DG uptake was observed. These results suggest that the observed enhancement of 2-DG uptake may in part be due to some structural component(s) of the input virus, but that some early translation product(s) are required for a complete development of the effect.

Method for the study of irritation and superficial aggressivity of antiseptics applied topically to the skin

F. Lautier, D. Razafitsalama and J. Lavillaureix
Institut d'Hygiène et de Médecine Préventive, Faculté de Médecine, F-67085 Strasbourg-Cédex, France

Irritation and superficial aggressivity of antiseptics applied topically to the skin can be studied on guinea-pigs. Our method derives from the standard one for cosmetics, given in the 'J.O. de la R.F.', 21 April 1971. The tests for irritancy consist in a closed patch test in 3 days giving a primary irritation index, and in prolonged applications over periods of 90 days giving a superficial aggressivity index. We changed the standard method in order to apply it to antiseptics; specially we established new intervals of index values going from 0 to 5. The antiseptics with values from 0 to 1.5 are available; those values between 1.5 and 5 cannot be used in medical and surgical applications. For the past 2 years we tested about 20 antiseptics: different dilutions of chlorhexidine, products with chlorine, iodine, soap with hexachlorophene, etc., with brushing or not, with rinsing or not. The primary irritation test is available for perfecting a formula; the superficial aggressivity one to show up all possible kinds of cutaneous reactions. This last test gives a better guarantee.

Bacteriology of mineral waters: Influence of PVC on the growth of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*

A. Masson, R. Michel and E. Richoz
Laboratoire cantonal, Les Croisettes, CH-1066 Epalinges

W.H. Stahl and H. Pessen have studied in 1953 the degradation of plastic films by 2 microorganisms: *Aspergillus versicolor* and *P. aeruginosa*. We have investigated the action of PVC on the growth of *P. aeruginosa* and *P. fluorescens*.

3 experiments have been carried out. The 1st one shows a 25-time rate increase in the development of *P. aeruginosa* in the presence of PVC. The 2nd one shows a 44-time rate increase for *P. fluorescens* under the same conditions. The 3rd experiment shows an important development of *P. aeruginosa* and *P. fluorescens* after 1 month, in either glass or plastic bottles. This development is faster for *P. fluorescens* than for *P. aeruginosa*. However, the *P. aeruginosa* develops faster in plastic than in glass bottles. The reason of these phenomena may be attributed to the appearance of mutants.

Combination of intercalation and antibiotic treatment with R-host cells

G. Lebek and L. Petri
Institut für Hygiene und Medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3008 Bern

After the isolation of the first R-factors (Zentbl. Bakt. I Abt. Orig. *188*, 494, 1963) we investigated the spontaneous and drug-induced R-loss of bacteria from medical material. We found that after a longer period of antibiotic abstinence a spontaneous R-loss appears rapidly with *Salmonella* and *Pseudomonas* and slowly with *Escherichia coli* strains (Zentbl. Bakt. I Abt. Orig. *191*, 387, 1963). While treated with antibiotics, however, there is to be expected neither a spontaneous nor a drug-induced R-loss. A recently published paper (F.E. Hahn, Naturwissenschaften *64*, 632, 1977) contradicted our findings but presented optimistic data which necessitated our reexamination of our former conclusions.
Using the authors method we tested the combination of ethidiumbromide with the following antibiotics: tetracycline, ampicillin and kanamycin. The tested R-factors originated from *E. coli* bacteria of medical material. They contained resistant determinants against the 3 antibiotics mentioned above and were repressed, derepressed fi^+- and fi^--R-factors. As host cells we used *E. coli* K_{12} C600 and *S. typhimurium* LT_2.
We concluded that in no case an R-loss could result from the combination of ethidiumbromide and antibiotic and that a minimal decrease of cells is possibly due only to the activity of ethidiumbromide. In no case did the additional use of antibiotic reinforce the decreasing effect of ethidiumbromide.

The effect of an *Escherichia coli* $dnaA_{ts}$ mutation on the replication of the plasmids col E1 and RTF-TC

J. Frey and L. Caro
Département de biologie moléculaire, 30, quai Ernest-Ansermet, Sciences II, CH-1211 Genève 4

The replication of the plasmids col E1 and RTF-TC, a derivative of the drug resistance factor R 100.1 has been investigated in a $dnaA_{ts}$ strain and has been compared to the replication of the host chromosome at permissive and nonpermissive temperatures. We have developed a DNA-DNA hybridization method that allows us to follow simultaneously in one strain the replication of various DNA species. The host strains for the plasmids carry in addition to the $dnaA_{ts}$ mutation prophage Mu inserted close to the chromosome origin and λ integrated at its normal site distant from the origin.
After a shift to the nonpermissive temperature of 42 °C, chromosome initiation of the $dnaA_{ts46}$ strain stops rapidly but not immediately as judged by the replication of the prophage Mu. Replication of prophage λ continues for a further 20 min after temperature shift and stops then as expected from a lack of initiation. The RTF-TC plasmid seems not to be affected directly by the temperature shift. It continues replication for some time after chromosome replication has stopped. The replication of col E1 on the other hand decreases as rapidly as chromosome initiation even if col E1 is coharboured with RTF-TC in a $dnaA_{ts}$ strain. This result however is in contrast to our recent finding that col E1 is able to replicate in a integratively suppressed $dnaA_{ts46}$ strain at 42 °C.

Relationship of cleavage with structural transformation in giant T4 capsids

J. L. Carrascosa and A. C. Steven
Abteilung Mikrobiologie, Biozentrum der Universität, Klingelbergstrasse 70, CH-4056 Basel

We have analyzed the surface lattices of giant pro-capsids derived from canavanine-treated cultures infected with T4.21$^-$ and T4.17$^-$ mutants. These particles exemplify respectively the 'uncleaved, unexpanded' and 'cleaved, unexpanded' states of the pro-capsid shell. The results correlate cleavage of gp23, prior to the following surface lattice expansion, with a subtle change in unit cell morphology. The most significant aspect is a change in the capsomer orientation angle from 9.5° to 3.5°. This change can be accounted for by the removal of 6 stain-excluding portions from the uncleaved hexamer. These portions may possibly represent the pieces of the gp23-molecules excised by the T4 prohead protease. Study of the rarely observed 'intermediate' giants, different zones of whose surface lattices are in different structural states, give clues as to the transformation dynamics. Both the cleavage pattern and the expansion process appear to be polar, initiating in 1 cap and propagating along the particles in sharp transition zones.

Study of the ε-particle, a new intermediate in bacteriophage T4 head maturation

C. Schärli, R. Gyalog and E. Kellenberger
Abteilung Mikrobiologie, Biozentrum der Universität, Klingelbergstrasse 70, CH-4056 Basel

Last year we presented evidence for a new intermediate precursor particle in the pathway of the maturation of the head of phage T4. The shell of this particle (ε-particle) is defined as being made of gp23* (product of gene 23 after in situ proteolytic cleavage) and unexpanded, that is still of the size of the prehead which is made of gp23. The content of this particle is now under investigation, after it had been demonstrated, that in vitro the expansion can very easily be triggered (J.L. Carrascosa and E. Kellenberger, J. Virol. *25*, 831, 1978).
The following results illustrate the difficulties encountered when studying highly fragile particles, which can undergo alterations or breakdowns induced by fixation or lysis of the cell. We have found that on thin sections this ε-particle can appear either as full or as empty, according to fixation procedures used. The content is not yet determined to be (processed?) core proteins alone or with DNA. When full it is not distinctly identifiable and thus invisible in the surroundings of ribosomes. When 'cytoplasmic leakage' happens then more or less empty small particles appear, in numbers which agree with the amount of gp23*. Experiments suggest that under some conditions of fixation expansion might be triggered and thus the observed empty large particles might be artificial products. The same might be true for heads which are partially filled with DNA. The decision of whether DNA filling is always initiated by the (small) ε-particle or can occur also on an expanded (empty)

particle is primordial for the understanding of DNA packaging.

Possible mechanism of inhibition of DNA polymerases sensitive to β-lapachone

A. R. Schürch and F. Bieri-Bonniot
Pharmaceuticals Division, Ciba-Geigy Ltd, CH-4002 Basel

β-Lapachone has been shown to inhibit oncornavirus reverse transcriptase and eukaryotic DNA polymerase α after a time lag of ~15 min in standard assays (1 h, 37 °C), not however polymerase β. Dithiothreitol (DTT) is essential for the development of the inhibitory effect of the drug, and no other SH-reagent tested could substitute for DTT (Schürch and Wehrli, Eur. J. Biochem. *84*, 197, 1978). DTT also enhances the activity of reverse transcriptase and DNA polymerases α and γ, but not of polymerase β. Enzyme activities without DTT and β-lapachone are reduced only little, excluding simple removal of DTT by the drug.
A systematic study on the effects of β-lapachone on the activity of DNA polymerases γ in addition to α and β, each partially purified from calf thymus, HeLa- and P 815-cells, and on reverse transcriptase was performed. DNA polymerase γ is the only enzyme examined which shows extents of inhibition in standard assays varying with its source.
Preincubations (30 min, 37 °C) with β-lapachone followed by initiation of the reaction with the missing essential component reveal that:
a) absence of substrate does not change the pattern of inhibition (in comparison to the not preincubated controls),
b) absence of enzyme leads to a reduced inhibitory effect,
c) absence of template/primer increases drastically the extent of inhibition and/or reduces the lag period.
Polymerases β remain unaffected by β-lapachone, whatever conditions used.
From these results a possible model can be drawn, wherein the template/primer protects to some extent the SH-groups involved in structure and/or activity of sensitive enzymes from inhibition by β-lapachone or by an active inhibitor generated by reaction of DTT with the drug.

RNA synthesis in *Escherichia coli* cells made permeable to nucleoside triphosphates by various methods

H. R. Felix and W. Wehrli
Departement Forschung, Division Pharma, Ciba-Geigy AG, Postfach, CH-4002 Basel

In recent years, methods have been developed which overcome the permeability barrier in bacterial cells to certain molecules such as nucleoside triphosphates. Thus it has been possible to assay certain enzyme reactions directly under conditions which resemble the in vivo situation. In the present work the measurement of RNA synthesis in *E. coli* cells made permeable by various methods is described.
The characterization and optimalization of the transcription reaction in *E. coli* cells, treated with ether according to the method of Vosberg and Hoffmann-Berling, shows that the optimal reaction conditions are identical to those found with an isolated and highly purified RNA polymerase. The reaction rate is constant for 30 min and the subsequent decrease in rate can be explained by an inactivation of the RNA polymerase in permeabilized cells.
The treatment of *E. coli* cells with ether seems to be optimal for the analysis of the RNA polymerase. Permeabilization methods using other organic solvents, antibiotics such as polymyxin, or physical treatments such as temperature shock are less favorable.
During optimalization of the permeabilization procedure with ether, it was found that the suspension of *E. coli* cells in Tris-buffer containing magnesium ions, and treatment for 1 min with ether gave optimal results. The solvent-treated cells had to be frozen quickly and stored at −20 °C in order to maintain high activity.

Mutants of *Pseudomonas aeruginosa* with increased antibiotic sensitivity: properties and possible applications

W. Zimmermann
Departement Forschung, Division Pharma, Ciba-Geigy AG, Postfach, CH-4002 Basel

Most strains of *P. aeruginosa* are resistant to many antibiotics that have excellent activity against gram-positive bacteria. It is generally assumed that resistance is at least partially a result of the outer membrane functioning as an efficient permeability barrier. Additional factors such as antibiotic-inactivating mechanisms or intrinsic resistance of the target enzymes are also involved.
In order to obtain a better understanding of the relative importance of such factors we isolated mutants of *P. aeruginosa* K 799 (PAe K 799) with increased sensitivity towards a large spectrum of antibiotics with different modes of action. Mutants were isolated by replica plating single colonies from mutagenized cultures onto plates containing novobiocin (5 μg/ml) or cephalosporin C (0.5 μg/ml) and picking colonies growing on the master plates, but not on the replicas.
2 mutants, PAe K 799/1 and PAe K 799/61, were studied. Both grew normally at 37 °C. They were much more sensitive than the wild type to antibiotics like novobiocin, tetracycline, rifampicin and erythromycin. There was, however, only a small increase in sensitivity to aminoglycosides, indicating good penetration of this group of antibiotics also in wild type *Pseudomonas*. In a preliminary analysis only minor differences in the cell surface proteins between the mutants and the wild type could be detected, and the mechanism for the better access of the first group of antibiotics to their targets is still unknown.
The mutant PAe K 799/61 not only had a reduced diffusion barrier, but was also no longer inducible for the β-lactamase normally present in wild type cells of *Pseudomonas*. It was extremely sensitive to most β-lactam antibiotics tested. Its pattern of penicillin-binding proteins was indistinguishable from that of the wild type. The mutant PAe K 799/61 should therefore be useful in determinations of intrinsic activities of β-lactam antibiotics against *P. aeruginosa*.

The inhibition of glucan synthesis in yeast protoplasts by papulacandin, a glucose-containing antibiotic

B. C. Baguley
Biological Research Laboratories, Ciba-Geigy Ltd, CH-4002 Basel

Papulacandin B is a new antifungal antibiotic containing 2 long chain unsaturated fatty acids, glucose and galactose (P. Traxler, H. Fritz and W. J. Richter, Helv. chim. Acta *60*, 578, 1977). In this study, yeast cell protoplasts have been used to investigate its mode of action. Protoplasts of logarithmic phase *Saccharomyces cerevisiae* or *Candida albicans* were prepared by digestion of the cell walls with snail gut enzyme and were incubated in an osmotically stabilized medium with ^{14}C-labelled glucose. The polysaccharides formed were fractionated into alkali-insoluble glucan, alkali-soluble glucan and mannan fractions. Papulacandin B, at concentrations of 0.2 and 2 μg/ml, inhibited

the incorporation of glucose into alkali-insoluble glucan by 60% and 90%, respectively, while causing little or no inhibition of incorporation into the other 2 fractions. In a papulacandin-resistant strain of *C. albicans*, the concentration of papulacandin required for 50% inhibition of incorporation of glucose into alkali-insoluble glucan was 2.5 µg/ml, compared with 0.25 µg/ml in a sensitive strain. In membrane preparations derived from lysed protoplast preparations, papulacandin inhibited the transfer of glucose from uridine diphosphoglucose to dolichol phosphate over a concentration range (2–20 µg/ml) which had no effect on the transfer of mannose from guanosine diphosphomannose to dolichol phosphate. It is proposed that yeast glucan synthesis proceeds via glucolipid intermediates, and that papulacandin B inhibits the formation of one or more of these intermediates.

Allosteric behaviour of O-acetyl-L-serine sulfhydrylase of *Cephalosporium acremonium*

H. Döbeli and J. Nüesch
Ciba-Geigy AG, Werk Klybeck, CH-4002 Basel

O-Acetyl-L-serine sulfhydrylase (OASS) is the enzyme catalyzing the final step in the anabolic biosynthesis of cysteine from H_2S and O-acetyl-L-serine (OAS). We separated this enzyme from the closely related O-acetyl-L-homoserine sulfhydrylase (OAHSS) which represents an alternative pathway for cysteine biosynthesis. OAHSS is not specific for the reaction leading from O-acetyl-L-homoserine (OAHS) and H_2S to homocysteine. It also catalyzes the conversion of OAHS and CH_3SH to methionine and the formation of cysteine from OAS and H_2S. Although the rate of cysteine synthesis by OASS is about 30 times higher than it is by OAHSS a physiological role for the latter may be possible since our results obtained earlier are compatible with the presence of 2 proteins with OASS activity (Treichler et al., Central Role of Sulfur Metabolism in Biosynthesis of Cephalosporin C and Penicillin, GIM symposium, Madison, Wisc. 1978, in press).

It could be demonstrated that OASS is an oligomeric enzyme. Whether dissociation into monomers or association into the oligomer occurs, depends on the buffer system used. The OASS activity was eluted as a single peak with a mol.wt of 50,000 from a sephadex G-200 column equilibrated with a 0.1 M pH 7.2 tris buffer. On the other hand activity separated into 2 peaks when the column was equilibrated with potassium phosphate pH 7.2 buffer containing 20% glycerol. The proteins contained in these 2 peaks showed mol.wts of approximately 200,000 for the 1st and 70,000 for the 2nd peak. It was concluded that the enzyme must be a tetramer with subunits each having a mol.wt of 50,000.

Kinetic behaviour depends on the buffer system used. In 0.1 M pH 7.2 tris-buffer a plot of enzyme activity versus OAS concentration yields a hyperbolic curve. The apparent K_m in respect to OAS variation is 1.8×10^{-3} M. Using 0.01 M pH 7.2 phosphate buffer the enzyme displays a sigmoidal plot of the reaction velocity versus OAS concentration. OAHS, a substrate analogue of OAS, accelerates the reaction in low concentrations but inhibits it in higher concentrations. This cooperative behaviour suggests that the enzyme is in the oligomeric state when assayed in this buffer system.

Whether the activation of OASS by OAHS plays a physiological role or if this phenomenon is only due to the structural similarity of OAHS could not yet be decided. With regard to the 1st step in methionine biosynthesis, which begins with the condensation of cysteine and OAHS to cystathionine, an activation of OASS by OAHS might be important.

Glycoproteins in mycoplasmas. Contamination with serum aggregates from growth media

Ph. Paroz and J. Nicolet
Institute of Veterinary Bacteriology, University of Bern, CH-3012 Bern

In a study of a random choice of various SDS-solubilized mycoplasmas and acholeplasmas from human and animal origin, we observed by flat polyacrylamide gel-electrophoresis in all examinated strains a major periodic acid Schiff positive band (PAS+) with an apparent mol.wt of 60,000, suggesting a cell glycoprotein. Another minor anodic PAS+ band was also seen in *M. suipneumoniae* and *M. flocculare*. These PAS+ bands were present even after preparation of membranes by osmotic or digitonin lysis. Treatment of whole cells with pronase eliminates these bands, while trypsin and nuclease have no effect. Fresh uninoculated growth medium, fresh horse or swine serum show PAS+ bands with quite different migration patterns. However a few days incubation at 37 °C of uninoculated with serum enriched growth media, or whole serum or even native PPLO serum fraction (Difco) provokes the formation of aggregates containing proteins, carbohydrates and lipid constituents. Electrophoretically these aggregates migrate as a single PAS+ fraction corresponding to the major band observed in our mycoplasma and acholeplasma preparations. After aqueous phenol extraction of whole horse serum we were able to demonstrate a PAS+ fraction similar to the minor PAS+ band. This suggests that the serum in the medium after incubation forms aggregates, mainly of glycoproteins, which contaminate mycoplasma culture preparations. These growth medium glycoproteins show a migration pattern (major band) similar to the erythrocyte glycoproteins and to those described in *M. suipneumoniae* (Kahane and Brunner, Infect. Immun. *18*, 273, 1977). We draw the attention to the possible misinterpretation of such contamination and to the undesirable effect of such aggregates in serological works with mycoplasmas. Attempts to eliminate the major PAS+ band from serum with chromatographical methods were unsuccessful, only a pre-incubation of whole serum at 37 °C during 5 days and high speed centrifugation reduce considerably the formation of aggregates of the growth medium. The minor PAS+ band however can be easily withdrawn after passage on a Sepharose Con A column. We did not find evidence that these aggregates are essential for the growth of mycoplasmas in broth cultures.

Genetic characterization of albino mutants of *Streptomyces glaucescens*

T. Kieser, R. Crameri, M. Suter and R. Hütter
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Albino mutants of *S. glaucescens* are unable to form melanin due to lack of tyrosinase activity. They arise with high frequency upon prolonged storage at 10 °C or after ethidium bromide treatment, under circumstances where no auxotrophic mutants have been observed. Genetically the albino mutants fall into at least 3 classes. Class I and class II mutants map chromosomally on alternative sides of the his-2 marker on the circular linkage map of *S. glaucescens*. Class III mutants differ from class I and class II mutants by their being inhibited by 0.1 mg/l streptomycin compared to 15 mg/l for the wild type strain. In crosses of class III mutants with suitably marked melanin positive strains an exceptionally high fertility approximating 1 was observed, which is about 100- to 1000-fold better than in ordinary crosses. Besides the 2 parental types predominantly melanin positive, streptomycin resistant recombinants were

consistently recovered irrespective of the selection imposed and therefore no chromosomal map position is indicated. This could mean that in class III mutants a plasmid loss is involved. However, experiments to isolate plasmid DNA failed.

The determination of the final step of the rifamycin B biosynthesis by a chromosomal gene

T. Schupp and R. Roos
Pharma-Forschung, Ciba-Geigy AG, K-125.2.20, CH-4002 Basel

A genetic analysis based on a gene exchange of a conjugation type is known in the rifamycin producing strains of *Nocardia mediterranei* (T. Schupp et al., J. Bact. *121*, 128, 1975). This method of genetic analysis was used to map the final step in the rifamycin B biosynthesis. By means of such experiments it should be possible to obtain basic information on the organization of genes controlling the rifamycin synthesis and whether a plasmid is involved.
It is known that the final step of the rifamycin B biosynthesis is the transformation from rifamycin S into rifamycin B. This reaction consists structurally in an addition of a glycolic acid moiety via an ether linkage. For our studies a blocked mutant was used which had lost the ability to transform rifamycin S into B. Auxotrophic markers were introduced into this rifamycin S producing strain either by mutation or by genetic recombination. The strains prepared in this manner were then crossed with rifamycin B producing strains carrying different auxotrophic markers and a streptomycin resistant marker of known position on the linkage map. The recombinants of these crosses were analyzed for the segregation of the known markers in relation to the segregation of their ability to transform rifamycin S into B. The data from this linkage analysis showed clearly that the gene under investigation which determines the final step of rifamycin B synthesis is located on the chromosome and not on a plasmid. The position of this gene is very close to the str-2 gene on the linkage map of *N. mediterranei.*

Induction of albino mutants in *Streptomyces glaucescens*

M. Suter, R. Hütter and Th. Leisinger
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Generation of melanin negative (albino) mutants from *S. glaucescens* was attempted by methods of plasmid elimination. Although liquid cultures grown in the presence of curing agents showed no increase in albino mutants, 2 procedures were found that generate albino mutants to a high extent: 1. Storage of overnight cultures at 10 °C for 80 days resulted in 13% of the survivors being albino. 2. Growth on agar plates containing ethidium bromide (6 μM) led to 90% of the colonies arising from spores being albino. Apart from the loss of tyrosinase (the enzyme responsible for melanin synthesis), some albino mutants have also lost a β-glucanase, have a different chitinolytic phenotype and exhibit increased sensitivity to UV-irradiation, N-methyl-N′-nitro-N-nitrosoguanidine and also streptomycin. General mutagenesis can be excluded, as no auxotrophic mutants are induced by our treatments. Controls also exclude selection as the responsible event. However, as mutants affected in only some of the above traits were also obtained, complete loss of a single plasmid can not sufficiently explain the genesis and properties of the albino mutants. Other models, based for example on the loss of transposable elements from plasmid(s) or from the chromosome, have to be tested.

Release of cellbound tyrosinase by *Streptomyces glaucescens*

J. Vetterli and L. Ettlinger
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

The tyrosinase (E.C. 1.14.18.1) of *S. glaucescens* in minimal medium is accumulated in the medium and only a hardly measurable amount can be detected in the cells. Tyrosinase is inducible in this organism by the amino acids methionine, leucine and phenylalanine. After induction, the cellbound tyrosinase pool rises to a high level within a short time. Studies on the release of this 'intracellular' tyrosinase were performed.
In shake flask cultures, spontaneous release takes place at a constant rate per cell mass, independent of the size of the intracellular tyrosinase pool. The maximal rate of this release seems to be limited. Artificially the cellbound tyrosinase can be solubilized by 2 methods: treatment with ultrasound or with high salt concentrations. With sonification during 20–30 sec, the cells are destroyed and most protein is released. By very short sonification, up to 5 sec, most of the protein stays cellbound, but a high percentage of tyrosinase is released. This gentle treatment therefore leads to a high specific activity of tyrosinase in the surrounding fluid. Other enzymes, e.g. fumarase, are not released at the same time. Similar results were obtained by incubating mycelia in buffer of high ionic strength during 3–5 h at room temperature. Sodium azide was added to the suspensions to prevent growth and further enzyme synthesis.
The reason for the specific release of tyrosinase by these mild treatments is not yet understood. It is assumed that a great part of the cellbound tyrosinase is located in the cell wall or on the outer surface of the cell membrane.

Glaucescin, a bacteriocin-like substance from *Streptomyces glaucescens*, killing germinating spores

W. Schurter, M. Kissling-Abderhalden and Th. Leisinger
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

S. glaucescens strain ETH 22794 produces a variety of antibiotic substances. Besides several antibiotics with low mol.wt (W. Weber, Tübingen, unpublished results), this strain excretes glaucescin, a high mol.wt substance which was detected on plates by cross-streaking *S. glaucescens* against spores of *Streptomyces canadiensis.* Glaucescin activity, originally masked by the large inhibition zone of the antibiotics, could be revealed by testing against mutants of *S. canadiensis* which are resistant to the antibiotics produced by *S. glaucescens.* Supernatants of liquid cultures of *S. glaucescens* were tested for glaucescin activity after the antibiotics had been removed by desalting on sephadex G-25.
Glaucescin was produced in complete and minimal media in the middle or at the end of the exponential growth phase, respectively. Addition of mitomycin C in concentrations from 0.5 to 8 μg to a growing culture of *S. glaucescens* did not induce glaucescin production. A crude preparation of glaucescin was resistant to DNase, RNase and various proteinases. Its mol.wt was estimated by gel-filtration and by sucrose gradient centrifugation as 196,000 and 186,000, respectively.
In a population of spores of *S. canadiensis* only about 30% were killed by glaucescin. During germination the spores passed through a phase in which 100% were killed. Mycelium was completely resistant to glaucescin. The binding of glaucescin followed a similar pattern: intermediate binding capacity of resting spores, high binding capacity of germinating spores and almost no binding of glaucescin to mycelium of the sensitive *S. canadiensis.*

The activity spectrum of glaucescin was limited to spore forming Actinomycetales: 23 among 29 *Streptomyces* species tested and 4 among 5 other spore forming Actinomycetales but none among 5 non spore-forming Nocardiae were sensitive to an antibiotic-free preparation of glaucescin. The specificity of glaucescin for spores of Actinomycetales was supported by the resistance of *Bacillus* endospores to glaucescin.

Pyruvate orthophosphate dikinase from *Acetobacter aceti*

J.-P. Schwitzguébel and L. Ettlinger
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

A. aceti is able to grow on pyruvate as the only source of carbon and energy. The production of phosphoenolpyruvate (PEP) is required to initiate the gluconeogenetic pathway and to produce oxaloacetate via a PEP-carboxylase (Schwitzguébel, results to be published). This reaction is catalyzed by a pyruvate orthophosphate dikinase (POD), which according to its kinetics appears to be different from the enzyme isolated from *A. xylinum* by Benziman and Palgi (J. Bact. *104*, 211, 1970).

The POD has been purified 64× (hydroxylapatite, ammonium sulphate, DEAE-cellulose, sephadex G-200) and functions in the forward (formation of PEP) or in the reverse direction (production of pyruvate).

In both directions Mg^{++} is required (K_m forward 1.7 mM; K_m reverse 0.83 mM) and no other divalent cation can replace it. The optimum pH is about 8 for the forward and about 7 for the reverse reaction.

The K_m-values for pyruvate, ATP and phosphate are 30 µM, 0.15 mM and 0.8 mM, respectively, in the forward reaction. The K_m-values for PEP, AMP and pyrophosphate are 0.11 mM, 6 µM and 68 µM, respectively, in the reverse reaction. The substrate-product pairs pyruvate-PEP, ATP-AMP and phosphate-pyrophosphate are competitive inhibitors to each other in both directions.

These results suggest that the POD of *A. aceti* should give nonclassical, 3-site Tri(Uni Uni) Ping Pong kinetics, as described by Wood for the same enzyme from *Propionibacterium freudenreichii* subsp. *shermanii* and from *Fusobacterium* (Bacteroides) *symbiosum* (Fed. Proc. *36*, 2197, 1977).

The 'general control of amino acid biosynthesis' in *Saccharomyces cerevisiae* acts also on the carbon metabolism

P. Niederberger and R. Hütter
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

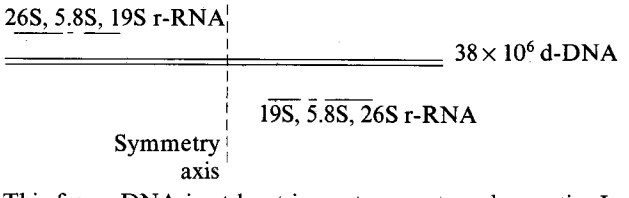
In the yeast *S. cerevisiae*, as in other eukaryotic microorganisms, a regulatory system called the 'general control of amino acid biosynthesis' has been described. This system coregulates several amino acid biosynthetic pathways, including those of the basic, the aromatic and the branched chain amino acids (Schürch et al., J. Bact. *117*, 1131, 1974; Delforge et al., Eur. J. Biochem. *57*, 231, 1975; P. Niederberger, Dissertation ETH Nr. 5882, 1977). Mutants defective in this regulatory system have been isolated: one class is unable to derepress amino acid biosynthetic enzymes under amino acid limitation (non-derepressing = ndr-phenotype), a 2nd class has those enzymes constitutively derepressed (= cdr-phenotype). As a consequence ndr-mutant strains grow, on average, 40% slower than the wild type strain when limited for an amino acid, whereas cdr-mutant strains are less inhibited to about the same percentage.

In this report we present data showing an involvement of the 'general control' not only in amino acid biosynthesis but also in carbon metabolism. To study the influence on the latter, the phenomenon of 'catabolite repression' of glucose was investigated. The wild type strain of *S. cerevisiae* (X 2180-1A) metabolized glucose to ethyl alcohol and CO_2 at a doubling time (t_d) of about 2.4 h (glucose limited minimal medium; growth was monitored on the basis of dry weight measurement). After exhaustion of glucose, a lag period of about 2 h occurred and growth resumed at a t_d of 13 h while ethyl alcohol was respired. The ndr-mutant strain needed 7 h to adapt to growth on ethyl alcohol after the exhaustion of glucose. The cdr-mutant strain, however, switched with a barely detectable lag from one carbon source to the other. Enzymes of the tricarboxylic acid cycle (fumarase E.C. 4.2.1.2), the glyoxylic acid cycle (isocitrate lyase E.C. 4.1.3.1), and the gluconeogenesis (fructose-1,6-diphosphatase E.C. 3.1.3.11) were measured throughout the diauxic growth. All enzymes appeared to derepress more slowly and to a lesser degree in the ndr- and cdr-mutant strains compared to the wild type, when glucose was exhausted from the medium. Experiments are under way to see if differences in respiratory capacity are the reason for the different adaptation pattern of our mutant strains compared to the wild type strain in diauxic growth.

Ribosomal DNA: extrachromosomal genes of *Physarum*

R. Braun and T. Seebeck
Institut für allgemeine Mikrobiologie der Universität Bern, Altenbergrain 21, CH-3013 Bern

The genes coding for ribosomal RNA are extrachromosomal in the myxomycete *Physarum polycephalum*. Each haploid nucleolus contains about 150 free r-DNA molecules, every one of which is a palindrome with 2 coding sequences arranged head-to-head:



This free r-DNA is at least in part present as chromatin. It is possible to isolate the r-DNA in the form of an active transcription complex containing RNA polymerase I. Replication of r-DNA takes place during both the S-phase and the G-phase: At the molecular level it involves eyeloops with an origin close to the axis of symmetry of the palindrome. It is not known whether the free r-DNA is the product of gene amplification: Plasmodia, spherules, amoebae and spores all contain the same relative amount of r-DNA.

Transcription of r-DNA first gives a precursor containing the nucleotide sequences for 5.8S, 19S and 26S r-RNA as well as some additional nonconserved sequences. In the mitotic cycle r-RNA is made continually (except during mitosis); the rate of synthesis, however, increases by a factor of about 5 from early S to late G_2. This implies a specific control of transcription over and above the control exerted by gene dosage.

Cleavage of echinocandin B with polymyxin acylase liberating the fatty acid and reacylation of the peptide moiety

W. Pache, C. Keller and M. Kuhn
Sandoz AG, Pharmazeutisches Departement, Chemische Forschung, CH-4002 Basel

Echinocandin is the major metabolite of the group of antifungal antibiotics produced by *Aspergillus rugulosus*. It

consists of 6 amino acids arranged in a cyclic peptide and a linoleic acid linked to the α-amino group of the 4,5-dihydroxyornithine bound in the peptide ring. In order to investigate the contribution of the fatty acid to the biological activity of the molecule attempts were made to split off the fatty acid side chain using an enzymatic method.
A tetrahydro-echinocandin B derivative substituted at the 4,5-dihydroxy-ornithine with a dimethylaminoethyl-group was used for the cleavage experiments. Splitting off the stearic acid was achieved with polymyxin-acylase, an enzyme from *Pseudomonas* M-6-3 (Kimura and Hirai, Bull. Mukogawa Wom. Univ. *14*, 243, 1966) in 60–70% yield. This yield could be improved to 85–90% by immobilizing the enzyme on octyl-sepharose CL 4B (Pharmacia), and repeated use of the immobilized enzyme was also possible.
The peptide moiety now containing one free primary amino group was purified and reacylated using the hydroxy-succinimide esters of different fatty acids. Whereas the peptide moiety itself is void of antifungal activity, the reacylated compounds show inhibition of *Canida* strains within a narrow range of fatty acid chain length.

Bacterial degradation of dichloromethane

W. Brunner and Th. Leisinger
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

A facultative methylotrophic *Pseudomonas* sp. was grown in continuous culture (D = 0.05, t = 30 °C, working volume = 1000 ml) under nonsterile conditions on a minimal salts medium containing a mixture of methanol (0.04% v/v) and the non-utilizable carbon source dichloromethane (0.2% v/v). After 20 generations the optical density (OD) of the culture increased and free chloride was detected in the effluent. Analysis of the culture showed that the ability to degrade dichloromethane was not due to a mutation of the original *Pseudomonas* strains but to another bacterium which had taken over.
The dichloromethane-degrading isolate was a strictly aerobic, gram-negative, oxidase positive rod (0.8–1.6 $\mu m \times 0.4$–0.6 μm) motile by a single polar flagellum. The organism was facultative methylotrophic. It grew on methanol, formate, mono-, di- and trimethylamine and on dichloromethane. A number of C-2, C-3 and C-4 compounds served as carbon sources whereas glucose and citrate were not utilized.
When a pure culture of the dichloromethane-utilizing strain was grown in a chemostat (D = 0.07) with automatic pH-control on media with a fixed concentration of methanol (0.04% v/v) and varying concentrations of dichloromethane (up to 0.3%) OD and chloride concentration in the effluent increased in proportion to the amount of dichloromethane. Under these conditions the growth yield for dichloromethane was 0.18. The maximal degradation rate of dichloromethane amounted to 0.16 $g \times l^{-1} \times h^{-1}$. The organism was also grown in batch culture on media-containing dichloromethane as the only source of carbon and energy. Again chloride was released at the same rate as growth occurred. The maximum specific growth rate in batch culture on 0.03% (v/v) dichloromethane was $0.11 \times h^{-1}$. Higher substrate concentrations were strongly inhibitory.

Microbial degradation of hydroxy-derivatives of s-triazine herbicides

J. Zeyer, J. Bodmer and R. Hütter
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Triazine and triazine derivatives occurring in the wastewater of production plants are degraded very slowly and can only be eliminated from the wastewater by costly chemical and physical methods. We started a programme to search for microorganisms, which can degrade hydroxytriazines.
Microbial strains were isolated from soil samples, treated with triazine herbicides over many years, from sewage plants from triazine fabrication as well as from other sources. Their metabolic capabilities were investigated with ^{14}C-ring labelled hydroxytriazines, which were present in the growth media at a concentration of 5 ppm. Samples from the incubation mixtures were taken from the supernatant and analyzed by thin-layer chromatography (TLC), followed by autoradiography. In the case of cyanuric acid, the amount of $^{14}CO_2$ formed was measured.
Strains of microorganisms were found, which are capable of performing the following metabolic reactions:

I N-isopropylammeline to N-isopropylammelid (yield: 5% in 3 weeks)
II N-ethylammeline to N-ethylammelid (yield: 30% in 3 weeks)
III Ammeline to cyanuric acid (yield: 96% in 2 days)
IV Cyanuric acid to CO_2 and presumably NH_3 (yield: 100% in a few hours)

In contrast to parent herbicides such as atrazine, dealkylations of hydroxatrazine, N-ethylammeline and N-isopropylammeline could not yet be detected.
The reaction sequences **III** and **IV** have been studied in more detail. The reaction sequence **III** can be accelerated by the addition of cytosine as nitrogen-source. Cyanuric acid decomposition (reaction sequence **IV**) was found to be most efficient with a fungus, which can use this compound as a nitrogen- but not as a carbon-source. The reaction is catalyzed by enzymes, which are at least partially particulate. With the help of this fungus, ^{14}C-cyanuric acid can be determined quantitatively in form of $^{14}CO_2$, even in complex media without previous clean up, quite in contrast to TLC. This microbial assay will therefore be a useful tool to screen for cyanuric acid as an intermediate in the degradation of hydroxytriazine.
In a next step we will try to optimize the degradation of ammeline to CO_2 and NH_3 by using mixed cultures of the strains performing the reaction sequences **III** and **IV** individually.

Bacterial substance similar to parathyroid hormone

H. Klaus-Fuchs, F. Tschopp, R. Hütter and J. Fischer
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich, and Forschungslabor für Calciumstoffwechsel, Orthopädische Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich

A substance in the culture liquid of *Pseudomonas aeruginosa* strain PAO-1 was found to react with antibodies raised to bovine parathyroid hormone (bPTH). The immunoreactive material appeared in the culture liquid mainly during the stationary phase. It was never observed in crude cellular extracts of the organism. The technique of radioimmunoassay was used for the detection and immunological characterization of the PTH-like material. The inhibition of the antigen-antibody reaction by the PTH-like material was not due to proteolytic degradation of either labeled ligand or antibody.
The PTH-like material was recognized with antibodies raised to a trichloroacetic acid extract of bovine parathyroid glands which are specific for a sequence in the middle part of the bPTH-(1-84)molecule, but also with antibodies to synthetic bPTH-(1-34), suggesting that the PTH-like material was related to the biologically active NH_2-terminal part of the native molecule. A 100-fold purification of the PTH-like material was achieved by salt precipitation, gel-

filtration on sephadex G-75 and ion exchange chromatography on carboxymethyl cellulose (recovery 7%). The elution profiles of the PTH-like material and of bPTH were similar on gel-filtration analysis and on ion exchange chromatography.

The action of the δ-endotoxin of *Bacillus thuringiensis:* an electron microscope study

H. R. Ebersold, P. Lüthy, P. Geiser and L. Ettlinger
Mikrobiologisches Institut der ETH, Universitätsstrasse 2, CH-8092 Zürich

The crystalline δ-endotoxin of *B. thuringiensis* acts immediately, following uptake by susceptible insect larvae. The protein crystals are digested by the gut juice proteases, probably yielding an active peptide. This peptide presumably induces the intoxication process at the gut epithelium, which is the primary site of action.

All the experiments were carried out with 5th instar larvae of *Pieris brassicae.* The δ-endotoxin was derived from the variety *thuringiensis* (serotype 1).

Changes in the permeability of the gut epithelium induced by the δ-endotoxin were shown with ruthenium red, an inorganic stain (mol. wt 552) which gives good contrast under the electron microscope. Ruthenium red was not able to penetrate into the epithelium of healthy cells. 5 min after administration of δ-endotoxin, the stain was found within the microvilli, which cover the epithelium as a thick brush border. Somewhat later, ruthenium red was evenly distributed within the cell, indicating a severe disturbance in the permeability balance. With the exception of the nuclear region, all intracellular components, such as endoplasmatic reticulum, mitochondria and the golgi complexes, underwent drastic structural changes in the presence of the δ-endotoxin. The fine 3-dimensional network of the endoplasmatic reticulum, which carries the ribosomes, forms vacuoles which steadily increase in size. The intermembraneous connections seem to be disrupted. The ribosomes fall off their membranes. The mitochondria exhibit various kinds of structural changes. They either become spherical or elongated in shape. The cristae are dissolved. Finally, the remains of the mitochondria consist of empty shells which continue their enlargement.

Concomitant with the changes in the endoplasmatic reticulum and the mitochondria, a considerable increase in the formation of autolytic vacuoles could be observed. The dictiosomes formed by the golgi complexes contain enzymes for the supplementation of the gut juice, whereas the cytosegrosomes perform autolysis of intracellular components.

It can be concluded that the δ-endotoxin causes drastic changes in the membrane systems of the cell organelles. The interconnections of the membranes are loosened and followed by an enlargement of the membrane fragments to vacuole-like structures. It is not yet known if the δ-endotoxin acts exclusively at the surface of the gut epithelium or has to penetrate into the cells, in order to induce the above described destructions.

Editorial remarks

to the publication of F. Wagner, Methanol: a fermentative substrate, in Experientia *33*/1, 110 (1977).

It has escaped from our attention, that the author has taken extensive parts of his text from the dissertation of J. P. van Dijken (Oxidation of methanol by yeasts; VRB Offsetdrukkerij bv, Groningen 1976) as well as from a paper by J. P. van Dijken and W. Harder (Biotech. Bioeng. *23,* 15, 1975). The author has abstained from mentioning the original source of his information. We regret the behaviour of the author and we apologize to J. B. van Dijken and W. Harder.

Committee of Swiss Society of Microbiology

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