of 20 lyophilized substrates are read with a dichromatic spectrophotometer and compared with a data base. Identifications with probabilities (PR) are printed after 4–5 h at 37 °C.
6 E strains (str.) were each tested three times in two different lots of cuvettes with sterile distilled water (SDW, pH 6.3) and SDW ad infundibilia (SDWI, pH 6.0). Different biocodes but identical sp. diagnoses at 97.6% PR were obtained. 594 Str. (479 E, 80 NF, 35 V), identified by CDC methods (E) or API 20 NE (NF, V) were tested for indentification. SDW (262 E+ (NF+V) str.) and SDWI (332 E+ (NF+V) str.) gave no significantly different results. At > 80–99% PR, 77% each of E and NF+V were diagnosed correctly; from < 50% upward, figures were 92.5% and 79%. 24 str. were misidentified, 16 only to species. 62.5% of E were diagnosed at 4 h; the rest and all NF+V at 5 h. Problems occurred with *P. mirabilis, S. liquefaciens, S. paratyphi A,* and *Yersinia* spp. and *E. agglomerans.*

Evaluation of the Cobas-Bact® automated antimicrobial susceptibility testing system

G. Dupuis
Division of Clinical Microbiology and Infectious Diseases, Valais Central Institute, CH-1950 Sion

The Cobas-Bact® is an analyzer for the microbiological laboratory capable of producing 5 h susceptibility tests and kinetic growth curves. The Cobas-Bact® system was evaluated for its efficacy by determining the susceptibilities of 2000 clinical, nonfastidious, falcultative and aerobic isolates compared with standard Kirby-Bauer disk diffusion. For gram-positive isolates, overall full and essential agreements between Cobas-Bact® and reference antibiograms were 90% and 95.5% respectively. For Enterobacteriaceae the overall full and essential agreements were 91% and 95.5% respectively. With *Pseudomonas* spp. the full and essential agreements were 90% and 96% respectively. Reproducibility studies gave essential agreement in 98% of cases. On the basis of this preliminary evaluation, it seems that the Cobas-Bact® can yield rapid, reasonably accurate and reproducible results when testing gram-positive cocci, Enterobacteriaceae and *Pseudomonas aeruginosa.*

A simple rapid technique to measure minimal bactericidal concentrations and combined antimicrobial action

I. Heinzer
Institute of Hygiene and Medical Microbiology, University of Bern, CH-3010 Bern

The methods used most frequently in vitro to measure the interaction of antibiotic combinations are 1) chessboard method (MIC and/or MBC), 2) killing curves and 3) diffusion tests, e.g. the cellophane transfer method of Chabbert. None of these is satisfactory. I have developed a new technique which combines many advantages of the known methods without their major disadvantages – especially the 'carry over' of antibiotics.
Chessboard titrations are done in tissue culture plates (Costar 3424, Mark II, 24 holes). After overnight incubation the plates are centrifuged in an oblique position of 45°. The supernatant is removed and the sediment may be washed several times to remove the antibiotics. After resuspension of the bacteria a double strength agar medium is added. After reincubation for another 24 h 99% or 99.9% bactericidal activity is calculated for each combination of antibiotics.

Evaluation of the Api 20 Strep system for species identification of viridans streptococci from blood cultures

R.G. Hafner
Institut für Hygiene und Medizinische Mikrobiologie, Universität Bern, Friedbühlstrasse 51, CH-3010 Bern

87 (100%) strains of Aerococci and viridans streptococci isolated from blood cultures in the years of 1983 and 1984 were classified according to a modification of Facklman's methods: 3 Aerococci, 10 *S. bovis* I, 3 *S. bovis* II, 15 *S. sanguis* I, 21 *S. sanguis* II, 12 *S. mitis*, 14 *S. milleri*, 4 *S. salivarius*, 2 *S. mutans*, 3 strains unclassified. Api system identifications: 28 (32%) strains after 4 h of incubation by the profile index. 68 (78%) strains after 24 h. 73 (84%) strains by additional testing: Optochin reaction and polysaccharid production. 74 (85%) strains by computer identification. 76 (87%) strains by computer identification and additional testing. 3 strains were misidentified. 8 (9%) strains remained unidentified, including the 3 unclassified strains by the reference method.
The Api system proves to be an accurate method for species identification of viridans streptococci. With additional testing and identification by the profile index the rate of identification was 84%.

Plasmids and Transposons

Replication of the tetracycline resistance plasmid pSC101

G. Churchward, Xia Guixan, T. Goebel, D. Manen, E. Othon-Izaurraulde and L. Caro
Université de Genève, Département de Biologie Moléculaire, 30, quai Ernest Ansermet, CH-1211 Genève 4

We have a series of mutations in the replication genes of the *E. coli* plasmid pSC101 caused either by insertion in vivo of the transposon Tn*1000*, or in vitro of a segment of DNA carrying an antibiotic resistance marker. These mutations have enabled us to map the origin of replication, a gene, *repA*, whose product is essential for replication and regions implicated in the regulation of replication. The RepA protein represses transcription of its own gene. We are currently isolating and analyzing mutations which affect replication control and using gene fusion techniques to map transcriptional units within the replication origin. We want to elucidate the molecular mechanisms which determine plasmid copy number.

Ω mutagenesis in gram-negative bacteria: a selectable DNA fragment which terminates transcription in a wide range of bacterial species

J. Frey, J. Deshusses and H.M. Krisch
Départements de Biochimie et Biologie Moléculaire, Faculté des Sciences, Université de Genève, CH-1211 Genève 4

A novel method for in vitro insertional mutagenesis of genes cloned in *E. coli* has been previously described (Prentki and Krisch, Gene *29* (1984) 303). It employed the Ω fragment, a 2.0 kb DNA segment consisting of the antibiotic resistance gene *aadA*$^+$ (Sm^r/Spc^r) flanked by small inverted repeats carrying transcription and translation termination signals and synthetic polylinkers. In *E. coli* the Ω fragment has been shown to terminate RNA and protein synthesis prematurately at the site of insertion. In this communication we demonstrate that Ω mutagenesis is equally effective in a wide range of gram-negative bacteria other than *E. coli.* To facilitate this analysis, we constructed in a broad host range vector, a hybrid plasmid which contains the entire *meta*-cleavage pathway operon of the *Pseudomonas putida* TOL plasmid pWWO. Since one of the downstream genes in the polycistronic mRNA encodes an easily assayable enzyme, the Ω fragment was inserted between this gene and the promoter. Comparison of the enzyme levels produced by the plasmid with and without the Ω insertion indicates that in all the strains examined Ω reduced transcription beyond the point of insertion at least 50-fold. We conclude that Ω mutagenesis is equally applicable as a method to study gene structure and function on these organisms.

Integration of an RP1 derivative into the *Pseudomonas aeruginosa* chromosome results in the formation of stable HFR strains

C. Reimmann and D. Haas
Mikrobiologisches Institut ETH, CH-8092 Zürich

We used an RP1 derivative which is temperature-sensitive for replication and has an inactivated resolvase gene in its transposon Tn*1*. When selecting for plasmid markers at nonpermissive temperature in a Rec$^-$ *P. aeruginosa* strain we obtained plasmid integration due to Tn*1* transposition. The strains thus constructed were stable and showed good Hfr donor properties. The origins for chromosomal transfer lay at different sites on the chromosome, giving a useful system for genetic mapping. We observed rearrangements in the integrated plasmid that seem to be necessary or at least advantageous for RP1 maintenance in the chromosome. In many strains we found a intramolecular transposition of IS*21* into the *trfA* region, which is required for autonomous RP1 replication. When such plasmids were excised from the chromosome they could replicate autonomously only if the *trfA*$^+$ replication function was provided in *trans*.

IS-like repeated sequences clustered around the NIF region of the *Rhizobium japonicum* genome

K. Kaluza, M. Hahn and H. Hennecke
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Two different repeated sequences (RS) were discovered in the *Rhizobium japonicum* genome: RSRjα is 1126 bp long, and is reiterated 12-fold; RSRjβ is approximately 950 bp long, and is repeated at least 6 times. Their arrangement in root nodule bacteroid DNA is the same as in DNA from bacteria grown in culture. Deletion analysis has shown that many copies of α and β are clustered around the nitrogenase genes *nifDK* and *nifH*, or in general, they are found within a genomic region harboring genes which are nonessential for growth. One copy each of α and β are located upstream of *nifDK*, and adjacent to each other. Neither of them, however, is involved in the expression of *nifDK*. Nucleotide sequence analysis of three copies of RSα has revealed many characteristics of prokaryotic IS elements: potential inverted repeats at their ends, potential target site duplication, and large open reading frames. Despite this, their genomic positions appear to be stable, as we could not demonstrate any transposition event. One possible function of RS is in deletion formation, probably *via* recombination between them rather than induced by an IS-like mechanism.

The genomes of the morphologically different bacteriophages LP52 and theta are related

J. Meyer, M. Stålhammar-Carlemalm, H. Štorchová and J. Doskočil
Abt. Mikrobiologie, Biozentrum der Universität, CH-4056 Basel, and Institute of Molecular Genetics, Czechoslovak Academy of Sciences, ČSSR-166'37 Praha 6

Bacteriophages LP52 and theta replicate in several strains of *Bacillus licheniformis*. In spite of different virion morphology these phages form viable recombinants, and two long regions of homology within the genomic DNA have been determined by restriction fragment analysis and blot hybridization (Forstová et al., Molec. gen. Genet. *187* (1982) 138). The denaturation maps also reflect this DNA relationship. Mapping of sequence homology between LP52 and theta DNA by electron microscopic heteroduplex analysis revealed about 50% homologous segments interspaced by 14 nonhomologous regions. This illustrates the importance of DNA rearrangements in these phages' evolution. Several recombinant phages of LP52 virion architecture were analyzed and shown to contain DNA consisting of a large interior segment of theta DNA flanked on either side by the respective LP52 DNA. All hybrid genomes contained within the theta DNA a 2 kb DNA segment which was nonhomologous to either parental DNA at this position, indicating that they resulted from a complex recombination process.

The spreading of an unknown plasmid in a children's hospital

C. Bourquin and G. Lebek
Institute for Hygiene and Medical Microbiology, University of Bern, CH-3010 Bern

While typifying *E. coli* strains isolated in a newborn unit of a children's hospital we observed periodically strains with resistance against all colicines and phages. We therefore searched for a plasmid responsible for these properties. Conjugation experiments between the wild strains and laboratory strains lacking colicine- and phage-resistance proved the existance of the postulated plasmid. The plasmid formed (40 kbp) belongs to the F 4-type. Beside the properties mentioned above it also contains genes encoding (an) enzyme (s) for lactose fermentation and for flagella formation (mobility). At present we do not know the origin of this plasmid. Currently further studies on the epidemiology as well as biochemical and genetic studies are under investigation.

An epidemiological study of two *S. typhimurium* outbreaks by means of plasmid fingerprints

A. Baumgartner and C. Breer
Institute of Clinical Microbiology and Immunology, CH-9000 St. Gallen

An outbreak of *S. typhimurium* infection occurred during November 1984 in an old age home. Three persons became ill, and 6 clinically inapparent carriers were found from 25 fecal samples examined. At the same time 16 of 21 cows, belonging to the old age home, were observed to have enteritis. In the feces from all the sick animals *S. typhimurium* could be isolated. Within 4 weeks a second outbreak occurred with 2 cases of *S. typhimurium* enteritis in the butchery of the same village.
By means of plasmid fingerprinting we could demonstrate that all the Salmonella strains isolated carried the identical plasmids. The identity of the strains was further confirmed by phage lysotyping and antibiotic susceptibility testing. These findings suggest that both outbreaks might have a common cource.

Insertions of ampicillin transposons into the plasmid DNA of *N. gonorrhoeae*

A. Arini and J. C. Piffaretti
Istituto Cantonale Batteriologico, CH-6904 Lugano

Penicillinase producing *Neisseria gonorrhoeae* (PPNG) strains isolated up to now throughout the world harbor a 7.3 kb resistance plasmid or its 5.1 kb deletion derivative: both genetic elements contain 40% of the transposon Tn*A*.
In order to better understand the emergence of such plasmids and to establish whether these can originate from multiple events of Tn*A* insertions into a specific site of a core plasmid, we constructed *bla*$^-$ derivatives of the PPNG resistance elements. Genetic experiments using phage λ::Tn*A* showed indeed that these recombinant plasmids have a hot-spot insertion site for the transposon Tn*A*.
Experiments are in progress to ascertain whether, when introduced in *Neisseria gonorrhoeae*, these Tn*A*-inserted plasmids will show a specific deletion process resulting in the stable presence of the 40% moiety of the Tn*A* transposon, similar to that found in the naturally occurring resistance genetic elements isolated from PPNG.