recolonization of the *Populus* trunks on the right bank of the river Arve (Quai Ernest-Ansermet) by still small colonies of *Xanthoria parietina* bearing scarce apothecia on 2–3 cm diameter thalli (Turian, unpubl. observations, 1984).

As a conclusion for the 1983–84 status of the air pollution in the urban zone of Geneva evaluated by lichen indicators, we can still circumscribe its lichen desert to ~ 1 km radial transects from the center (Place Bel-Air) but with local desert spots in a 3 km diameter restricted to a few highly polluted places of heavy traffic (Place Cirque, Rond-Point des Eaux-Vives, de Plainpalais, etc.). According to our scale of increasing sensitivity, such desert zones bear as only epiphytes the green *Pleurococcus vulgaris* more or less blackened by its fungal partner, *Coniosporium aeroalgicolum*. Epilithic crustaceous lichens can however be observed on walls of basic stones, with blackish, vertical streaks of Cyanobacteria (Conservatory of Music, etc.)[3]. First epiphytic lichens such as *Physconia grisea* can appear in the peripheral parks (Bastions, less than 1 km from the center), closely followed by *Parmelia sulcata* and *scortea* and preceding the yellow *Xanthoria parietina* now noted at less than 2 km from the center.

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ABSTRACTS

1. Oral Presentations
Medical Microbiology and Virology

Constitutive and ironchelator-inducible hemolysin production of *Escherichia coli*

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Investigating *E. coli* wild strains of our region 14% of 762 strains from fecal flora and 33% of 405 strains causing infections of the urinary tract were hemolytic. 5 strains from the fecal flora could transfer their hemolytic character by conjugation. Their Hly-plasmids are derepressed and determine the production of F-type-pili. 4 of them belong to the FVI, one to the FIV incompatibility group. For the quantification of the hemolysin production we developed a standard method in liquid broth. The hemolysin excretion of all strains can be reduced by $FeCl_3$. This points to a possible correlation between hemolysis and iron metabolism. On the other hand a 10- to 100-fold increase in hemolysin production is observed only by our 5 strains with Hly-plasmids when grown in the presence of iron chelators. The other hemolysin producing strains do not show this effect.

R-plasmid-harboring *Escherichia coli* can secrete beta-lactamases

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We investigated log-phase cultures of ampicillin-resistant *E. coli* wild strains for the ability to secrete R-plasmid mediated beta-lactamases. Most of these strains released beta-lactamases into the liquid media. The R-factors of these strains were transferred into *E. coli* K_{12}921 and enabled the new host to synthesize and secrete beta-lactamases. Mucus producing ampicillin-resistant wild strains did not secrete beta-lactamases. But corresponding transconjugant clones of *E. coli* K_{12}921 released the enzyme into the liquid media. The activity of secreted enzymes varied in a great range between the different strains and did not correlate with the MIC. Secreted and periplasmatic stored beta-lactamase-activity was quantified and the different types of beta-lactamases were determined by isoelectro-focusing.

Plasmid-mediated resistance to phages and colicins

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From a patient with urogenital infections we isolated a colicin producing strain of *E. coli* which is resistant to all tested colicins and phages. After conjugation to a recipient strain of *E. coli* K_{12}921 $r^-m^-lac^-$ we found colicin producing transconjugants which received in 10% lac-property. This lac-positive transconjugant as well as the wild-donator are resistant to colicins (A-, B-, D-, E1-, E2-, E3-, G-, H-, Ia-, Ib-, K-, M- and V-colicin) and also to T2-, T3-, T4-, T5-, T6-, T7-phages and λ-phages, whereas the transconjugant without lac-property is sensitive to these colicins and phages. The wild strain and the lac-positive transconjugant multiply MS_2 phages. The lac-property-giving plasmid is therefore conjugative and of F-pili-type. In the agarose-gel-electrophoresis the colicin producing lac-negative transconjugant shows only one plasmid of about 50 kbp whereas the wild strain and the lac-positive transconjugant have two plasmids of about the same size. The consequence of this plasmid for hospital hygiene is discussed.

The production of siderophores by host strains containing various plasmids

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Beside the chromosomal-determined Enterochelin (Enterobactin), the plasmid-encoded production of siderophores is an important irontransport-mechanism in Enterobacteriaceae, as an example in *E. coli*. With special mutants as indicators we examined the activity of R-factors, colicinogenic and Hly-factors of wild strains, isolated from patients of our region, to produce siderophores. We found some strains harboring plasmids with determinants for siderophores. We conclude that the ability of producing siderophores is not only encoded by Col V as commonly suggested. The importance of these results for the pathogenesis of infections by *E. coli* is discussed.

Staphylocin-typing of nonlysotypable *Staphylococcus aureus* strains

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About 50% of *S. aureus* strains from hospital hygienic investigations are not lysotypable. The main reason for this resistance to phages is the existence of several restriction systems encoded by plasmids and phages or by phages. It is possible to lysotype 80% of these strains by appropriate modification of some phages of the international phageset. The remaining nonlysotypable strains can be typed by means of their sensitivity to staphylocins produced by *S. aureus* and *S. epidermidis* strains, respectively. This staphylocin-typing method can also be used for the typing of *S. epidermidis* strains. It is a methodically simple test and therefore useful for the routine testing.

Vaccinia virus: Mapping of genes coding for structural polypeptides

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Vaccinia, a complex DNA virus, expresses its genetic information in a temporally well regulated fashion. Early genes are expressed shortly after infection. After DNA replication, late genes encoding predominantly structural polypeptides are transcribed. The molecular basis for the switch from early to late gene expression is not understood. This is partly due to the fact that very little is known about the fine structure of late genes and their flanking regions. We have therefore mapped on the vaccinia virus DNA the genes coding for the two major polypeptides of the virus core. In the first step, monospecific antibodies directed against the two polypeptides were prepared in rabbits. Secondly, RNA specific for defined regions of the vaccinia virus genome was selected by hybridization to cloned DNA restriction fragments. The RNA was translated in vitro and the core polypeptides were identified among the products by immunoprecipitation. This allowed us to map the genes to the one end of the *Hind* IIIA fragment. Precise map positions of the 5′ ends as well as the direction of transcription of the mRNAs were obtained by S1 nuclease analysis. This showed that both RNAs are transcribed from the same DNA strand and that the 5′ ends of two genes are separated by approx. 7.5 kilobasepairs of DNA.

Involvement of thiol groups in the fusion process of *Aedes albopictus* cells infected with Semliki Forest Virus (SFV)

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Lowering the pH of the medium to 6 for a few seconds in *Aedes albopictus* (C6/36) cultures 16 h after SFV infection triggers cell-cell fusion. A conformational change of the viral spike proteins located in the plasma membrane is assumed responsible for precipitating the fusion process. A simple and convenient means to arrest the fusion potential is to lower the pH at 4 °C and releasing it simply by reverting back to 28 °C at pH 6 or 7. Utilizing this facility, we treated the thiol groups with sodium tetrathionate to elucidate if these groups are involved in the fusion reaction-chain. Suppressing the free thiol groups prior to lowering the pH has no effect on the fusion reaction. However, derivatizing the thiol groups following the pH change completely inhibits fusion. Thus, we can discriminate an initial conformation change leading to the exposition of additional reactive thiol groups which are functionally essential for membrane fusion.

Fate of microtubuli during Semliki Forest Virus induced cell-cell fusion

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Infection of *Aedes albopictus* (clone C6/36), Vero and HeLa cells with Semliki Forest Virus (SFV) leads to cell-cell fusion upon acidification of the medium (pH $\leqslant 6$), at different times post infection for the respective cells. In the fusion event cellular processes are involved. Because microtubuli are important for the maintenance of the cell shape, we examined the role of tubulin in SFV induced cell-cell fusion. Cells infected with SFV were incubated in presence of microtubuli disrupting drugs, e.g. colcemide, podophyllotoxin and vincristine, for 1 h prior to acidification of the medium (2 min, pH 5–6). Syncytium formation was then observed within ½ h in infected control cells and drug treated cells. Similarly, cells were microinjected with a monoclonal tubulin antibody. No effect on fusion was noticed. However, analyzing the distribution of microtubuli in noninfected or SFV-infected cells before and after fusion striking changes in the microtubular pattern of *Aedes* C6/36 cells (which survive fusion) were observed. In fused cells only the microtubuli started to get rearranged 2–4 h post acidification. After 6 h long, more brilliant bundles of tubulin, which spanned over the length of several cells, were observed. The bundles crossed near or among the multiple nuclei. Conclusively, the following statement can be made: microtubuli are not actively involved during fusion, however they will undergo a striking rearrangement after fusion.

Prevalence of delta-agent infections in hepatitis B patients: A retrospective study from 1978 to 1984

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Delta-antigen and antibody tests were performed on 749 sera from 396 HBsAg positive hepatitis cases: acute hepatitis (294 sera), chronic hepatitis (273 sera), liver cirrhosis and/or hepatocellular carcinoma (38 sera) and asymptomatic carriers including hemodialysis patients and renal graft recipients (144 sera). Delta-markers were determined by solid phase competitive RIA for delta-antibody and by sandwich RIA for delta-antigen. 43 sera (7.3% of patients tested) had markers of delta-

infection: antigen alone was detected in 4 patients, antibody alone in 20, whereas a seroconversion from delta-antigen to antibody could be shown in 5 cases. 21 out of the 29 patients with delta-markers in their sera were persons between 18 and 30 years, and most of them had acute fulminant hepatitis or exacerbation of preexisting infection.

Reactivity of enterovirus specific IgM and IgG antibodies to viral structural proteins of ECHO 9, ECHO 11, Coxsackie B3 and Polio 2 viruses

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To understand serological cross-reactions of antibodies from sera of enterovirus-infected individuals we analyzed the reactivity of enterovirus specific IgM and IgG antibodies to the structural proteins of prototype strains of ECHO 9, ECHO 11, Coxsackie B3 and Polio 2 viruses by the immunoblot technique. Sera were obtained from patients recently infected by different enteroviruses. In general, IgG antibodies reacted to VP 1 of all viruses. In contrast, enterovirus specific IgM antibodies reacted to VP 1, VP 2 and/or VP 3 of the viruses tested. We compared the staining pattern on the immunoblots with data from neutralization tests and M-antibody capture radioimmunoassays.

Interferon-mediated natural resistance to influenza virus: Wild mice express phenotype

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The inbred mouse strain A2G carrying the dominant resistance gene *Mx* has so far been unique among laboratory mice in exhibiting a high degree of resistance to infection by influenza viruses. We have now screened wild mice trapped at various locations in Europe and California for natural resistance by infecting them with a lethal dose of influenza virus. Survivors (approximately 85%) were bred to susceptible laboratory mice and the offspring were again challenged with the same virus dose. The ratio of resistant to susceptible animals in the 7th backcross generation (of resistant F_1 males and susceptible parental females) was approximately 1:1 suggesting the presence of a single dominant resistance gene. In the case of A2G mice, resistance is mediated by interferons-α/β (but not -γ) which, in treated cells, activate the gene *Mx* and thereby induce a nuclear 75,000 MW protein and an antiviral state which is particularly effective against influenza viruses. Similarly, in cultured peritoneal macrophages obtained from wild mice or from wild mouse derived strains, interferons-α/β, but not interferon-γ, were able to induce resistance and, concomitantly, a 75,000 MW protein. This protein was detectable by immunoprecipitation with polyclonal and monoclonal antibodies directed against the interferon-induced *Mx*-associated protein of A2G cells. Likewise, this protein was found by immunofluorescence to accumulate in the nucleus of interferon treated cells. We conclude that genetically determined resistance to influenza viruses is common among outbred wild mouse populations and that the resistance alleles present are the same or very similar to the *Mx* allele of strain A2G.

Early spleen cell elicited target cell lysis (ESEL) in cytomegalovirus(MCMV)-infected mice

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In-vitro cell lysis by spleen cells of i.p. MCMV-infected Balb/c mice is attributed to natural killer (NK) cell activity in the first few days followed by specific cytotoxic T (Tc) cells with maximal activity at 8–10 days after infection and finally by killer (K) cell activities when virusspecific antibodies are present. At day 4 after infection ESEL is high against Yac-1 targets (a cell line sensitive for NK cells) and against autologuous non-MCMV-infected embryonic fibroblast targets (MEF after 2 or 3 passages in vitro). ESEL is no longer observed when Tc activity is maximal. As this early cytolytic activity is coincident with maximal MCMV-induced suppression of antibody production against SRBC it is tempting to define the cell populations which may be responsible for the phenomenon. ESEL is not restricted by class I H-2 antigens of the major histocompatibility complex in contrast to Tc. At least 40–50% of the observed lytic activity is sensitive to monoclonal anti Thy-1.2 antibody and lysis against autologuous cells is higher after nylon wool passage. Further analysis must clarify whether the ESEL-phenomenon can be explained entirely by NK cell activities or not.

General and Clinical Microbiology

A new type of membrane-filter system for the demonstration of microorganisms in foodstuffs

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A new type of membrane-filter system (trade name Isogrid™) was introduced. It is designed for the qualitative and quantitative investigation of microorganisms in foodstuffs. On the basis of its microbiological performance, and the considerably simplified procedure, the Isogrid™ system offers a useful alternative to conventional methods for the microbiological examination of foodstuffs. It is suitable for routine work, reliable and economical.

The procedure offers a number of advantages. Both very low and high numbers of organisms (up to 6000 per sample) can be reliably measured by the application of the MPN procedure. There is good agreement between the observed number of organisms and the actual number present. Evaluation of the results is straightforward and can be made early; differentiation can be made between colonies since they are displayed separately. Automated evaluation is possible. The work involved in an assay is reduced because serial dilutions do not have to be made. The separation of particulate material by means of a pre-filter in the filtration apparatus prevents mistakes in interpreting the results (false positives). A simple, standardized method can be used for the investigation of a wide variety of foodstuffs.

Detection of mycoplasma contamination in cell culture by a chemiluminescent (CL) assay

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Mycoplasma contamination of cell cultures represents a serious problem in virology, immunology and biochemistry. Methods to detect mycoplasma are time-consuming or require special expertise. When added to phagocytic cells, free mycoplasma, or cells contaminated with such organisms, were able to evoke on intense burst of CL (Peterhans et al., Eur. J. Immunol., in press; Koeppel et al., J. Immunol., in press).

Based on this observation, we have established a CL assay for mycoplasma. Human or bovine polymorphonuclear leucocytes (PMN) were suspended at 1×10^5/ml and 4×10^5/ml, respectively, in phenol red-free Hanks' balanced salt solution containing 5.6 mM D-Glucose and 20 µl/ml of 4% bovine serum albumin saturated with the chemiluminogenic probe, luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). Cells to be tested for mycoplasma contamination (i.e. target cells) were added to the PMN indicator cells and CL was measured in a liquid scintillation spectrometer operated in the off-coincidence mode. Mycoplasma contamination was readily detected at indicator to target cell ratios of > 1. The sensitivity of this assay was equal or superior to direct DNA staining, cultural and electron microscopic methods used in parallel. However, CL measurement was more versatile than the staining method and results were obtained within 90 min compared to several days with cultural and electron microscopic methods. Since PMN are readily prepared and most liquid scintillation spectrometers are suitable for CL measurement after switching off the coincidence circuit, this method represents a simple means of detecting mycoplasma contamination in cultured cells.

Conventional and radiometric drug susceptibility testing of *Mycobacterium tuberculosis*

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A recently developed method of drug susceptibility testing of *Mycobacterium tuberculosis* which measures the evolution of labeled CO_2 from [1-^{14}C] palmitic acid (Bactec 460 System) was compared to the conventional proportion method (7H10 agar). Drug susceptibility to isoniazid (INH), streptomycin (SM), rifampicin (RMP) and ethambutol (EMB) of 59 isolates was determined by the indirect method. In 92% of the cases, results obtained by the radiometric method were available within 1 week, as opposed to 3–6 weeks needed with conventional methodology. Drug concentrations (in mg/l) used were (Bactec/proportion method): SM (4.0/2.0), INH (0.2/1.0), EMB (10.0/10.0), RMP (2.0/1.0). Individual agreements were 78% for SM, 50% for INH, 98% for RMP and 92% for EMB. Individual disagreements were (B = Bactec, C = conventional proportion method, s = susceptible, r = resistant): 22% (Cr-Bs) and 0% (Cs-Br) for SM; 3% (Cr-Bs) and 7% (Cs-Br) for INH; 2% (Cr-Bs) and 0% (Cr-Bs) for RMP; 8% (Cs-Br) and 0% (Cs-Br) for EMB.

Mycobacterium malmoense, the causative agent of a pulmonary infection in Lausanne

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A 45-year-old patient was treated with a tritherapy (INH-RMP-EMB) for 10 months for a noncaseous tuberculous lesion of the right apex. The diagnosis rested on clinical ground and X-ray but cultures were always negative. Due to chronic ethylism, the patient failed to take his medicine regularly and was hospitalized for a recidivant manifestation. This patient was suffering from a chronic ethylism, heavy smoking and consecutive progressive obstruction syndrome.
Bacteriological findings: The first sputum samples are almost all positive when smeared and stained with auramin or Ziehl-Neelsen. Each sample was cultivated on Löwenstein-Jensen and on Stonebrink medium at 37°C. Part of the first positive sputum was digested with acetyl-cystein and seeded on L-J medium with nalidixic acid. After decontamination with SDS of one sputum, a direct antibiotic sensitivity test by the Canetti method was effected. Within this test, we observed the first growth after 3 weeks on L-J medium pH 5, with and without pyrazinamid. The direct culture of nondecontaminated samples – made possible by an intensive antibiotic treatment – was only positive on L-J and Stonebrink after 4 weeks. Decontaminated samples were positive only in 1 out of 6 trials on L-J and Stonebrink media pH 7, and once on Stonebrink alone after 2 months. However, the transfer of rinses of the surface of L-J media after 4 weeks onto Middlebrook 7H9 ADC Tween, 7H10 agar and L-J. pH 5 were all positive. The identification was based on poor colony formation on L-J medium, growth at 25°C and 37°C, but not at 45°C, microaerophily, resistance to antituberculous drugs, to PNB and to TCH, absence of pigments, niacin production and nitrate reduction, but strong Tween 80 hydrolysis in 24 h. The strain antibiotic susceptibility was tested on 7H10 agar. The most favorable media for the study of this strain were the Tween containing Middlebrook media and the L-J pH 5. Thus, the growth on L-J pH 7 and on Stonebrink media was irregular, delayed and often negative even after repeated passages. It seems important to note that this case is well documented with 6 successive isolations of *M. malmoense*. This contrasts with the rare occurrence of single isolations from man, which might not be significant.

Mycobacterial lymphadenitis in pigs – a risk for human health?

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Tuberculous lesions of lymph nodes associated with the intestinal tract (usually submaxillary lymph nodes) in swine are a common finding worldwide. This subject has been the objective of intensive research in the past two decades mainly in order to elucidate the possibility of transmitting the causative agents to human beings. In these studies acid fast bacilli of the *M. avium-intracellulare* complex could be isolated as etiological agents in over 90% of all lymph nodes examined. We investigated 59 lymph nodes from swines slaughtered in the local abattoir and referred from different farms. In 38 (64%) *M. avium-intracellulare* strains could be isolated from submaxillary lymph nodes. Serotyping of the strains obtained revealed the predominance of serotype 9 (17 of 38, 45%), only one strain (3%) belonged to serogroup 1 or 2 (the avian mycobacterium which typically is pathogenic for chickens). From a total of 5500 mycobacterial isolates of human origin only 16 belonged to the *M. avium-intracellulare* complex. Until now 11 such strains have been serotyped showing the following pattern: group 6, 7 and 14 one strain each (9%), group 8 two strains (18%), the remaining 6 strains (54%) showed spontaneous agglutination and thus could not further be characterized by this method. The pig population in the area from which we receive most material for bacteriological work-up is very high (120 pigs/km^2) and the pork meat consumption is enormous (43 kg/person per year). Although *M. avium-intracellulare* infection in pigs in this area is not uncommon, the most frequently isolated serotype *M. avium-intracellulare* has not been found in humans. Despite the small number analyzed in this survey, these data support the assumption that *M. avium-intracellulare* infection of pigs is not likely to be transmitted to humans.

Opportunist causal agents in AIDS patients in Switzerland

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The Acquired Immuno Deficiency Syndrome (AIDS) has also been diagnosed repeatedly in Switzerland during the last three years. On the basis of the registered cases various epidemiological aspects were discussed, in particular the infections found in these patients. The frequency of occurrence of opportunistic agents (such as *Pneumocystis carinii*, Cryptosporidia, atypical Mycobacteria, among others) was comparable with that found in other countries.

Pasteurella multocida septicemia in compromised hosts

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Pasteurella multocida (P.m.) septicemia in man is a rare event. Most of the cases reported in the literature hitherto are due to close contact with animals and/or to reduced resistance of the macroorganism caused by underlying diseases. Two cases of *P.m.* septicemia are described and clinical data and laboratory findings are discussed. Both patients were elder women, both had underlying diseases (one had diabetes, the other had an ulcerating carcinoma of mamma). In both patients preceding wound infections were suspected. In one case, *P.m.* could be cultured from wound swab specimen together with *Streptococcus viridans, Proteus mirabilis* and *Eikenella corrodens*. Both patients were hospitalized with symptoms of septicemia: fever up to 40.5 °C, leucocytosis with a shift to the left. *P.m.* was cultured from blood during the first day of hospitalization. In one patient one bottle of three inoculated bottle sets was positive, in the other, all bottles of six inoculated bottle sets were positive. The identification of *P.m.* was done by means of biochemical tests of the Institute for Medical Microbiology of the University of Zürich. Both patients were treated successfully with penicillin and were discharged without any signs of systemic infections. The origins of the organisms were probably cats which were held by both patients at home.

Double infection with *Yersinia enterocolitica:* serotype 3 and 9: Implications for laboratory diagnosis

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Enteritis caused by *Yersinia enterocolitica* is not very common, and only few cases of double infection with two different strains of this organism have been reported until now. We present here an additional case showing unusual features in the laboratory diagnosis. Direct plating of stool specimen on CIN medium revealed *Yersinia enterocolitica* serotype 3. After enrichment for 24 h at 29 °C in phosphate buffered saline (PBS) and Rappaport broth (RAP), followed by subculture on CIN medium, serotypes 3 (PBS) and 9 (RAP), respectively, were isolated. After incubation at 4 °C for 14 days, no *Yersinia* was isolated from RAP, but serotype 9 was detected in PBS. In-vitro studies with the two isolates did not explain the fact that after incubation at 29 °C for 24 h serotype 9 was found exclusively in RAP and serotype 3 in PBS, respectively. However, growth in the two media at 4 °C was considerably different: serotype 9 grew much faster in PBS than serotype 3. Both serotypes did not survive in RAP. These results are in good agreement with the initial finding that while serotype 3 was isolated from PBS after 24 h at 29 °C serotype 9 was found after cold enrichment. The value of enrichment procedures is controversial. A review of the results in our routine laboratory indicated that both liquid media are of some value for the isolation of *Yersinia enterocolitica*. Of 30 positive stool cultures, 20 were detected after direct plating on CIN medium. An additional 5 strains were detected after incubation at 29 °C for 24 h (2 in RAP, 1 in PBS and 2 in both), and another 5 strains after cold enrichment for 14 days (1 in RAP, 4 in PBS and none in both).

Listeriosis prevention

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Since the last listeriosis epidemic in the Canton of Vaud (Grandguillaume, 1961), few rare cases have appeared (from 2 to 5 per year) not constituting an epidemiological risk. From November 1983 an increase of this disease has been noticed. The distribution of cases is in conformity with the disease epidemiology: patients presenting a pathological deficit of cell-mediated immunity (such as elderly people, cancerous, cirrhotics) about 50%, pregnant women and newborns about 50%. The serotypization is proceeding. Only one case of spontaneous abortion has been detected among cattle, whereas *Listeria monocytogenes* has been shown up in one goat and three dead hares. In contrast to various authors (Campbell, Kachel, King et al.) we have not noticed nosocomial transmission. The *primary prevention* can only be performed on the foetus or the newborn detecting gravidic listeriosis during a fever attack by blood cultures and applying an extended appropriate antibiotic treatment. We have contacted several obstetricians in order to know their standpoints on the importance of this diagnosis method. More than 50% of the questionnaires were sent back, so we can deduce that only 55% of these colleagues practise blood cultures (most of them are hospital doctors), whereas the majority search for listeria by serology, cervix or placental swabs or fecal cultures. Regarding the continuous antibiotic treatment, 6% of the physicians would directly confide it to a colleague and 87% would practise monotherapy (amoxycillin or ampicillin). This inquiry shows the importance of team work between the public health departments and the practicians. Ideally, the *secondary prevention*, consisting in searching for the bacillus in the alimentary sources, should also be worked up.

Construction of cloning vectors that autoamplify in stationary phase of growth

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A set of four new cloning vectors (pKT234, pKT235, pKT252 and pKT254) is described. These vectors are based on the replicon of the plasmid ColD-CA23, a naturally occurring gram-negative colicin plasmid. They contain either two or three antibiotic resistance genes, and several unique sites for gene cloning. Since some of these restriction endonuclease sites are located within the antibiotic resistance genes, hybrid molecules can be identified by 'insertional inactivation' of antibiotic resistance. We have shown by DNA:DNA hybridization, that the copy number of these vectors dramatically increases when cultures of the host bacteria *(Escherichia coli)* enter the stationary phase. Coordinate with this increase of the copy number, a significant elevation in the expression of cloned genes is observed. The autoamplification of these vectors permits the augmentation of expression of cloned genes and does not involve any experimental intervention. Since this spontaneous process occurs towards the end of a fermentation it minimizes physiological disturbance of the culture by expression of the cloned genes.

Inactivation of delta-endotoxin of *Bacillus thuringiensis* by plant extracts

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The delta-endotoxin, produced by *Bacillus thuringiensis* during the sporulation process, is insecticidal against larval stages of several economically important Lepidoptera, e.g. *Heliothis virescens, Trichoplusia ni* or *Choristoneura fumiferana*. The toxin, deposited within the sporulating cell in a crystalline form, is ingested by insect larvae along with the food, dissolved and proteolytically activated by the gut juice, followed by destruction of the gut epithelium. Despite susceptibility of the target insects, *B. thuringiensis* treatments give in some crops unsatisfactory or inconsistent results. *H. virescens* is one of the insects which is easily controlled on soja or tobacco but insufficiently on cotton. A possible direct influence of plant compounds on the delta-endotoxin was investigated. Leaves from field grown cotton (variety Stoneville), from greenhouse soja plants and from red spruce needles were freeze-dried. 100 mg of this material were homogenized in 10 ml of phosphate buffer (0.06 M, pH 6.4). Remaining solid particles were removed by centrifugation and the supernatants were used for incubation with intact crystals, protoxin (dithiothreitol solubilized crystals) and trypsin activated delta-endotoxin, respectively. Following incubation at 23 °C for 30 min, the extracts were bioassayed using early fifth instar larvae of *Pieris brassicae*. When whole crystals were incubated, no measurable change in biological activity occurred. This was however different with protoxin or activated delta-endotoxin. Lethal toxin concentrations (50 ng/larva) had lost their effect following incubation in cotton leaf extracts. Spruce needle extracts had a lower inactivating capacity whereas extracts from soja did not influence the activity of protoxin or activated delta-endotoxin. Tests were also conducted with a commercial tannin preparation. Inactivation comparable to cotton extracts was obtained with a 10^{-2} M tannin solution. No differences could be detected when the toxins of eight *B. thuringiensis* were tested. The inactivating factor is not dialysable and shows considerable heat resistance (10 min, 80 °C).

Environmental nitrate and the nitrate-reducing bacterial flora of the healthy human stomach

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In any evaluation of the environmental pollutant nitrate, and especially of its reduction product, nitrite, the role of the functioning, healthy stomach must be considered. Because of its acid production, the stomach offers a particularly favorable environment for the synthesis of carcinogenic N-nitroso compounds. Nevertheless, the stomach itself has not hitherto been regarded as a source of nitrite produced by bacteria, because the hydrochloric acid present inhibits colonization. It was assumed that the salivary glands selectively absorb nitrate from the blood and secrete it with the saliva into the mouth, where the bacterial flora reduces it to nitrite. When the saliva is swallowed and reaches the acid stomach this nitrite is involved in further reactions.

Studies on fasting gastric juice from 75 young, healthy subjects showed that during the night, when the acidity of the stomach is physiologically reduced, it is colonized by bacteria, often in very large numbers (order of magnitude 10^7 KBE/ml); an analysis of the species present showed that the main source is the mouth and the intestine is a subsidiary source. Analogous to the pH-dependent increase in the total number of organisms and in the number of colonies of nitrite-producing cells is an increase in the level of nitrite from 0.1 ppm at acid pH (1–4) to an average of 1.4 ppm in a neutral environment. Although this connection is quite clear, the observation that the concentration of nitrate increases from 6 ppm at acid pH to nearly 12 ppm at neutral pH is more difficult to explain; an increase in nitrite concentration would be expected to produce a decrease in nitrate. Indications that there are sources of nitrate other than the salivary glands, to be found in the stomach itself, require further investigation. Nevertheless, it can already be stated that in a completely healthy, acid stomach there is bacterial production of nitrite, which is then available for the synthesis of carcinogenic N-nitroso compounds.

2. Posters
A) Medical Microbiology

Investigation of formaldehyde-resistance in clinical isolates

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Using a recently-published micromethod, under the conditions of the qualitative suspension test, a screening programme was carried out to test the resistance of bacteria to formaldehyde. So far, about 700 strains have been tested, in particular isolates of the genera *Pseudomonas, Escherichia, Klebsiella, Enterobacter, Salmonella* and *Proteus*. The results showed that there was no increased resistance to formaldehyde in the so-called 'mucoid variants' of *Pseudomonas aeruginosa, Escherichia coli* and *Enterobacter* sp. in comparison with those strains of the same species which grow without slime-production. Furthermore, in all but a few of the strains tested the level of resistance only reached that found in ATCC reference strains tested at the same time.

Studies on the identification of non-fermenting gram-negative bacteria

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The results of a comparative study on the identification of non-fermenting gram-negative bacteria were reported. 250 strains of more than 30 different species, which had already been identified by a conventional macro-method, were investigated using a commercially-available micro-system (API 20 NE, api Biomérieux), which includes 8 biochemical and 12 assimilatory tests. After optimization of the analytical index it was found that the identification was correct for 95% of the strains. Because of its simplicity in use, the unambiguous results for the various reactions, and the high rate of correct identification, in our opinion this system is valuable for routine microbiological laboratory work.

Patterns of resistance to newer β-lactam antibiotics as aids in the diagnosis of nonfermentative gram-negative rods, *Aeromonas* and *Plesiomonas*

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Patterns of antimicrobial disk susceptibility have previously been used as aids in the diagnosis of nonfermenters (NFGNR) (von Graevenitz and Redys, Health Lab. Sci. 5 (1968) 107). Since NFGNR may develop resistance to antimicrobials, only consistent ('intrinsic') resistance should be used as a marker.

We tested 533 strains of NFGNR representing 40 species of *Pseudomonas, Alcaligenes, Achromobacter, Agrobacterium, Acinetobacter, Bordetella, Moraxella, Flavobacterium,* 51 *Aeromonas* and 15 *Plesiomonas* strains with the Kirby-Bauer method against cephalothin (CF), cefamandole (MA), cefoxitin (FOX), cefuroxime (CXM), cefotaxime (CTX), cefotiam (CFT), ceftriaxone (CTR), cefoperazone (CFP), cefsulodin (CFS), ceftazidime (CAZ), moxalactam (MOX), azthreonam (AZM), mezlocillin (MZ), azlocillin (AZ) and piperacillin (PIP). Using breakpoints provided by NCCLS or by the manufacturers (for CFT, CTR, AZM) we arrived at multiple resistance patterns (which may not necessarily reflect MIC values). As examples, *F. meningosepticum* was regularly resistant to CF, MA, CXM, CTX, CFT, CFS, CAZ, CTR, CFP, MOX and ACM. *P. maltophilia* against CF, MA, FOX, CXM, CTR, CFT, and *P. stutzeri* only against CF. There were specific patterns within one species as well which will be outlined in tabular form.

Rapid latex agglutination test for the direct detection of group A streptococci from throat swabs

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A rapid antigen detection test using latex agglutination (Directigen®, Becton Dickinson) was compared with conventional culture for the detection of group A beta-hemolytic streptococci (GAS) from throat swabs. One throat specimen was collected from each of 208 patients, using rayon tipped swab (Culturette®, Marion Scientific). One sheep blood agar plate was first streaked and incubated in 5% CO_2 for as long as 48 h. Beta-hemolytic streptococci were grouped by a coagglutination procedure (Phadebact®, Pharmacia). After standard inoculation swabs were immediately tested for GAS antigen when possible or kept at 4 °C no longer than 72 h. All swabs were tested for GAS antigen within 72 h. The test consisted of incubating the swabs for 1 h at 37 °C in the Directigen® extraction enzyme. The extraction solution was then mixed with anti-GAS latex and observed for macroscopic agglutination.

	Conventional Positive	Culture Negative
Latex positive	39	4
Latex negative	4	161
Total	43	165

20.7% (43/208) of the specimens were GAS positive by culture. Direct latex agglutination agreed with culture in 200/208 specimens (accuracy 96%). When compared to culture the sensitivity of the direct latex agglutination was 91% (39/43), the specificity 98%, the positive predictive value 91% and the negative predictive value 98%. Three out four of the false negative latex agglutination tests were associated with low numbers (< 100) of GAS colonies in culture. Half of the false positive latex agglutination tests (2/4) were read as weakly positive reactions. The rapid latex agglutination test for the direct detection of GAS from throat swabs compared favorably to culture and has the advantage of providing same day results.

Enzyme immunoassay for detection of *Neisseria gonorrhoeae*

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The Enzyme Immunoassay (EIA) for detection of *N. gonorrhoeae* (gonozyme Abbott) was compared with culture as reference method. The tests were performed with genital tract specimens and under laboratory conditions.

1. Urethral and cervical swab specimens of 198 patients were examined. *N. gonorrhoeae* was detected by both culture and EIA in 50 patients, by culture only in 6 patients, and by EIA only in 5 patients.
2. The sensitivity of gonozyme was tested in laboratory conditions in vitro and compared with the culture. Dilutions of *N. gonorrhoeae* beginning from McFarland 0.5 standard were inoculated to gonozyme, the same inocula were tested by culture quantitatively. Whereas gonozyme was positive from 2×10^3 organisms/ml, the culture was positive already at 10 organisms/ml. Thus, the culture is 200 times more sensitive for detection of small inocula of gonococci than the gonozyme test.

Alcohol pretreatment facilitates isolation of *Clostridium difficile* from feces

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The aim of this study was to assess the influence of alcohol pretreatment of feces on routine isolation of *C. difficile*. Fecal samples from children less than 1 year old were used since colonization with *C. difficile* in this age group is not unusual (P.L. Stark and A. Lee, J. Pediatr. *100* (1982) 362). Fecal samples were cultured on CCFA selective agar (Oxoid CM 60) with and without pretreatment with 50% ethanol for at least half an hour to select sporulating bacteria (S.P. Borriello and P. Honour, J. clin. Path. *34* (1981) 1124). For assessing selectivity after 48 h incubation each colony type was identified according to macroscopic and microscopic morphology, biochemical reaction patterns, gas liquid chromatography and cytotoxin assay. Of 111 fecal samples analyzed, 17 strains of *C. difficile* were isolated, one of these strains was detected only after alcohol pretreatment. 7 of the 17 isolates were cytotoxin producers. While 13 (77%) of the *C. difficile* isolates grew in pure culture after alcohol pretreatment, after direct plating there were only 2 (12%) isolates in pure culture. Furthermore after alcohol pretreatment no growth was observed in 65 (69%) of the samples without *C. difficile*, but only 11 (12%) of the same samples yielded no growth after direct plating. Bacteria other than *C. difficile* include the following: enterococci and lactobacilli as well as bacteroides sp., different enterobacteriaceae, pseudomonadales, staphylococci, other clostridia and anaerobic non-spore-forming bacteria and fungi. The results can be summarized as follows:

From 111 fecal samples were isolated	Pretreatment none	Alcohol
C. difficile total	16	17
C. difficile in pure culture	2	13
Strains other than *C. difficile*	187	50

Our study is in agreement with data reported by S.H. Willey and J.G. Bartlett, (J. clin. Microbiol. *10* (1979) 880), and shows clearly that a combination of alcohol pretreatment and culture on selective media facilitates isolation of *C. difficile* from feces.

Value of macroscopic filamentation (MF) on routine solid media for the rapid differentiation between *Candida albicans* and *Candida* others than *albicans*

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Routine identification of *Candida albicans* (CA) is usually based on the production of chlamydospores on special media,

the production of filaments in serum (germ-tube) being considered as presomptive identification. Macroscopically visible filamentation (MF) on primary solid culture media are often seen after overnight incubation around yeast colonies. We assessed the value of early MF as a means to identify CA on 400 consecutive fresh clnical specimens that were culture positive for yeasts. All specimens were inoculated onto blood agar and chocolate blood agar plates, incubed for 48 h at 35 °C and read at 24 h and 48 h. Yeast colonies on both media were observed for the presence of MF. Definitive identification was done as follow: 1. germ-tube tests were done on all yeast isolates: 2. germ-tube positive isolates were plated onto rice Tween 80 agar medium to observe the production of chlamydospores; 3. germ-tube negative isolates were further identified with API 20 C yeast identification system and microscopic morphology.

Definitive identification	Macroscopic filamentation (MF) Positive			Negative
	at 24 h	at 48 h	Total	Total
C. albicans	257	36*	293	24
C. others than *albicans*	0	0	0	83

* 34/36 exhibited no growth at 24 h; 2/36 exhibited growth but not MF at 24 h.

The sensitivity of the MF determination was 92.4%, the specificity 100%, the positive predictive value 100% and the negative predictive value 77.6%. MF permitted the definitive identification of 293/317 (92.4%) CA isolates, which represented the 3/4 of all *Candida* isolated in the study.
We propose to use the MF as a first step procedure in identifying CA in yeast isolates and to reserve further tests (germ-tube, assimilation and fermentation tests) for the MF negative isolates.

Evaluation of methods enabling a rapid etiological diagnosis of calf-diarrhea

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Calf-diarrhea, occurring mainly during the winter season, causes considerable losses in our country. Because of the diversity of the causative organisms, the therapeutic approach can only be symptomatic. This stresses the importance of an efficient etiological diagnosis. We analyzed fecal specimens of calves suffering from diarrhea. The samples were obtained from veterinarians collaborating with our laboratory. Special attention was paid to the SDS-PAGE analysis of stool specimens for the presence of rotavirus dsRNA, one of the most frequent agents associated with calf-diarrhea. Different RNA patterns observed were compared with the RNA patterns found in rotavirus associated diarrhea in childhood. Furthermore, we searched for the following agents known to cause calf-diarrhea: enterotoxigenic *E. coli* (K 99 antigen), *Campylobacter* sp., *Salmonella* and *Cryptosporidia*. An approximately equal amount of feces samples from clinically normal calves served as control group.

Characterization of microaerophilic cocci isolated from various pathological material in cattle and sheep

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84 strains of peculiar microaerophilic cocci were isolated from various lesions in cattle and sheep affected with pneumonia (39), endometritis (19), orchitis (1), arthritis (12), septicemia (1) and sundry abscesses (12). 26 of these strains were taken from pure cultures. The organisms stain weak and irregular gram-positive. They appear as small coccoid bacteria occurring mostly in pairs but also in short chains and clusters. In smears of lesions, they are surrounded by leucocytes, whereby phagocytosis is often observed. Primary isolates on blood and chocolate agar are growing only in a narrow zone, close to 'nursing' colonies of some other kind of bacteria. This dependence is lost in subcultures on chocolate agar enriched with serum and yeast extract. Maximal growth is registered after an incubation period of 2–3 days at 37 °C in a 10% CO_2-atmosphere. Under aerobic and anaerobic conditions, growth is slow and scanty. Colonies are small, translucent, greyish, smooth and they do not adhere strongly to the agar. When incubated for long periods they produce slightly greenish zones of hemolysis on blood agar. Serum and yeast extract are required for good growth in liquid media. When 46 strains were examined, acid was produced from lactose, glucose, trehalose, maltose and occasionally from salicin. No acid was produced from raffinose, arabinose, xylose, saccharose, mannitol, sorbitol and inulin. Other negative biochemical reactions were: indole, H_2S, urea, esculin, nitrate, Voges-Proskauer, methyl red, catalase and oxydase. The strains were non-motile. Susceptibility testing was done on 23 strains: 100% were sensitive for penicillin G, ampicillin, oxacillin, chloramphenicol and bacitracin; 96% for spiramycin and erythromycin; 91% for furadantin; 39% for tetracycline; 22% for streptomycin and 9% for gentamicin. All strains were resistent to neomycin, polymyxin B, colistin, sulfadiazine and trimethoprim-sulfamethoxazole. These organisms seem to be similar to those isolated from cases of dry-cow mastitis. Although their taxonomic position remains unclear, they might be related to streptococci or peptococci.

In vitro antibiotic resistance patterns in *Bacteroides fragilis* group isolates. Evaluation of the implications in antibiotic therapy

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The potential use of recent broad spectrum antibiotics as monotherapy in abdominal or gynecological severe infections is of interest. There is a need to establish at each institution the susceptibility patterns of anaerobic gram-negative bacilli. We determined the 'in vitro' susceptibility of 75 *B. fragilis* group isolates (53 *B. fragilis*, 7 *B. vulgatus*, 5 *B. ovatus*, 3 *B. thetaiotaomicron* and 7 other species or non speciated) identified from clinical specimens in 1983. All these strains were sensitive to metronidazole and chloramphenicol (disk-elution method). The antimicrobial agents tested were: clindamycin, moxalactam, cefoxitin, ceftriaxone, ticarcillin and the association ticarcillin-clavulanic acid. The NCCLS proposed agar dilution method was used. The results were (μg/ml):

	Range	MIC_{50}	MIC_{90}
Clindamycin	< 1– > 16	1	4
Ceftriaxone	< 4– > 64	8	64
Ticarcillin	< 32– > 512	32	128
Moxalactam	< 4– > 64	4	64
Cefoxitin	< 2– > 32	4	16
Ticarcillin	< 8– 32+	8+	8*

+ Clavulanic ac. (* Ticarcillin conc.)

B. fragilis on the other hand was more sensitive to the antibiotics, than the other members of the group. The clinical importance of resistance in *B. fragilis* group isolates was assessed and will be reported.

New cephalosporins: 'In vitro' comparative studies

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This study concerns the in-vitro antimicrobial activity of some recent cephalosporins, in comparison with well-known other β-lactam antibiotics. One of the aim of this study was to determine, for the clinical microbiologist, which cephalosporin can be used in routine for antibiotic sensitivity tests, without being compulsed to test systematically five or more cephalosporins, now available in Switzerland. All the strains tested were isolated in an hospital area in Geneva. The identification was done with the API-20 system and/or by slide agglutination. The sensitivity tests were executed according to the method of Chabbert (Pasteur Institute, Paris). The interpretation for sensitive, intermediate and resistant patterns was performed by determination of the MIC's, using a special home-made program established for an HP-41C. For the most of the strains tested, it appears that moxalactam and ceftriaxon are generally more active than the other β-lactamins tested. It seems that there is a small advantage for moxalactam against strains of *P. aeruginosa*. It must also be pointed out that the activity of the 'new' cephalosporins is much less spectacular on gram-positive cocci than it is on gram-negative rods. In conclusion, it seems that, actually, cefadroxil is a good marker for the determination of cephalosporin's activity 'in vitro', because we can assume that if a strain is sensitive to this derivative, it will be also sensitive to other members of the 'family'.

The minimal bactericidal concentration (MBC) divides artificially the viridans streptococci into sensitive and tolerant strains

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The bactericidal effect of antibiotics predicts the therapeutic effectiveness in bacterial endocarditis (BE) both in animals and humans. Furthermore, it affects the efficacy of endocarditis prophylaxis. It is usually assessed by the MBC, defined as the lowest antibiotic concentration killing $> 99.9\%$ of the inoculum (0.1% of survivors) over 24 h of incubation. When the MIC/MBC ratio is $\geqslant 32$, the strain is defined as tolerant. We determined the MBC of amoxicillin and vancomycin (amoxi, vanco, advocated for prophylaxis and treatment of BE) in 24 strains of viridans streptococci isolated from patients with BE. When testing amoxi, all strains had a MIC $\leqslant 0.25$ µg/ml. The MBC was found to be either low ($\leqslant 0.5$ µg/ml) in 6 strains (sensitive strains), or high (> 128 µg/ml) in 18 strains (tolerant strains). In contrast, when evaluating the bactericidal effect of amoxi by counting the actual number of survivors (% surv.) after 24 h of incubation, the maximal effect was seen at or near the MIC, and remained constant with increasing amoxi concentrations up to 128 µg/ml. 6 strains showed an increasing % of surv. above the MIC with increasing concentrations of amoxi (Eagle effect). As expected, the 6 strains with a MBC of $\leqslant 0.5$ µg/ml had $< 0.1\%$ of surv. ($> 99.9\%$ kill). Of the 18 strains with a MBC of > 128 µg/ml (tolerant), 10 had from 0.1 to 1% surv. ($> 99\%$ kill), 4 from 1 to 10% surv. ($> 90\%$ kill), and 4 $> 10\%$ surv. ($< 90\%$ kill). Thus, for these 18 strains a uniformly high MBC corresponded to widely differing bactericidal effects of amoxi. Similar observations were made when testing vanco. The MIC for the 24 strains were $\leqslant 1$ µg/ml, the MBC $\leqslant 2$ µg/ml for 3 strains and > 128 µg/ml for 21 strains. The % surv. was $< 0.1\%$ for the 3 strains with MBC $\leqslant 2$ µg/ml. Of those with a MBC > 128 µg/ml, 4 had from 0.1 to 1% surv., 8 from 1 to 10% surv., and $10 > 10\%$ surv. The maximal bactericidal effect was seen at or near the MIC. These results suggest that the % of surv. represents more accurately the bactericidal effect of cell wall active antibiotics than the MBC, which artificially divides the strains into either sensitive or tolerant organisms.

Epidemiological survey of *Klebsiella* cross-infection by biotyping. Its relations with resistance to antibiotics

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From 1978 to 1983, 629 *Klebsiella* strains have been analyzed for an epidemiological study purpose of these germs based on biotypes and antibiotypes. Two levels are reached:
1. The biotyping, which allows the diagnosis of the species, is a first epidemiological aspect.
2. The serotyping is performed when a cross-infection is suspected, because of iedentical biotypes.
The following tests are labeled in three groups: indole, Voges-Proskauer, citrate, lactose, sucrose, malonate, gluconate, dulcitol, lysine, ornithine, urea. Out of different biochemical reaction combination of each group, results a code number. For each strain we express the biotype by juxtaposing the numerical code of each of the three groups (Rennie and Duncan scheme). Certain biotype groups correspond to given species (Ewing and Edwards). Although the 629 studied strains belong to two species only (402 *K. pneumoniae* and 227 *K. oxytoca*), they also belong to 33 different biotypes, which is epidemiologically useful. The most frequently isolated biotype (30.7% of strains) is 1.1.2, *K. pneumoniae*, originated for the 43.5% from urines. Next come the biotypes: 5.1.1, *K. oxytoca* (18.8%) and 1.1.1, *K. pneumoniae* (17.8%). Although *K. oxytoca* is being gnerally more sensitive to tetracyclines and co-trimoxazole than *K. pneumoniae*, and at the same time less frequently multi-resistant, the study of the resistance to antibiotics do not enable the specification and cannot be epidemiologically used. On the other hand, the biotyping brings a necessary element to a cross-infection diagnosis evaluation and constitutes a reliable marker. For the inquiries performed in 6 years on 629 strains, most of the suspicions of cross-infections have been lifted by biotyping, in spite of the presence of several identical *Klebsiella* strains of the same species, at the same time, in the same service.

Plasmids of a novel type from penicillinase producing *Haemophilus influenzae*

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H. influenzae, as a causative agent of meningitis, septicemia, and epiglottitis in infants, is an important pathogen. Since 1975, penicillinase producing strains have been reported throughout the world; these strains are characterized by two types of plasmids: those belonging to the first type are larger than 45 kb and share a common core; those belonging to the second type are 7.4 kb large and are similar to the resistant genetic elements found among *N. gonorrhoeae*, both types of plasmids carry transposons of the Tn*3* family or deletion derivatives of them.
During an epidemiological survey of the resistant *H. influenzae* in Switzerland, two penicillinase producing clinical isolates characterized by a novel type of resistant plasmid were found, one in Bern and the other in Geneva. These genetic elements, pPJ301 and pPJ302, are 10.0 kb large and carry Tn*3*-like transposons as demonstrated by transposition assays on λ DNA, heteroduplex analysis, and Southern experiments using TN*2301* (a Tn*3*-like transposon) as a probe. A restriction map with different endonucleases has been constructed. Transformation experiments showed that pPJ301 and pPJ302 can replicate in *E. coli;* however, when introduced into E. coli C600, these plasmids

frequently originate larger derivatives characterized by DNA insertions up to 1.0 kb. Work is in progress to analyze the nature of these insertions so as the origin of these plasmids of a novel type in *H. influenzae*.

Investigation of the final steps in cephalosporin C biosynthesis in cell-free extracts of *C. acremonium*

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During the industrial production of cephalosporin C, the commercially uninteresting precursors penicillin N, deacetoxycephalosporin C (DAOC) and deactylcephalosporin C (DAC) are released into the culture medium. This represents a loss of potential cephalosporin C production. The accumulation of these cephalosporin C precursors is thought to be due to inhibition or repression of the enzymes DAOC-synthetase and -hydroxylase and DAC-acetyltransferase which catalyze the final steps in cephalosporin synthesis. DAOC-synthetase and -hydroxylase from an industrial strain of *C. acremonium* were partially purified using Sephadex G-75, DEAE-Trisacryl M with subsequent isoelectric focusing and SDS-PAGE. Strong evidence was obtained that ring expansion of penicillin N to DAOC and the following oxygenation to DAC are catalyzed by the same enzyme with a molecular weight of 33,000±2000, a pI of 4.6±0.1 and with strict dependence on O_2, Fe, α-ketoglutarate and ascorbate. To show the activity of the next enzyme in the biosynthetic pathway of cephalosporin C, DAC-acetyltransferase, a new test system was established. The reaction mixture contained crude desalted $(NH_4)_2SO_4$ precipitated enzyme extract and the substrates DAC and acetyl-CoA. Formation of cephalosporin C was followed by HPLC. The activities of DAOC-synthetase/hydroxylase and DAC-acetyltransferase during the course of a fermentation showed great similarities. The maximum level was reached shortly after glucose was exhausted (~ 80 h) and the enzyme activities decreased to a low level during the second part of the fermentation, although cephalosporin C production continued. This would serve to indicate that there is no strict correlation between the specific activities of these two enzymes and cephalosporin C production.

Cyclosporin A suppresses immunity to virus and bacteria

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Cyclosporin A (CS-A) is a lipophilic, cyclically arranged undecapeptide which effectively suppresses immunity to virus, alloantigen and bacteria by specifically interfering with Interleukin-2 (IL-2) production. In vivo and in vitro experiments in mice, using lymphocytic choriomeningitis virus (LCMV), vaccinia and vesicular stomatitis virus and *Listeria monocytogenes* (*L.m.*) as a facultative intracellular bacteria, demonstrated the following effects by CS-A: 1. Daily injections of 60 mg/kg · d CS-A i.p. are able to suppress primary and secondary antiviral response against all three viruses used. 2. CS-A added to secondary in vitro cultures inhibits antiviral T response and alloreactivity in a dose-dependent manner, 180 ng/ml leading to complete abrogation of any response. 3. CS-A has to be added early in secondary in vitro restimulation to abrogate T-cell response. 4. IL-2 containing medium, e.g. 10% ConA supernatant and El-4 supernatant added to cultures are able to counteract CS-A in vitro. 5. CS-A treated mice injected with LCMV carry this virus for prolonged periods but may be able to eliminate it at a later timepoint. 6. LCMV, injected intracerebrally into mice, induces a lethal autoimmune disease. CS-A protects most mice from death, long-term survivors being free of virus. 7. Daily injections of 50 mg/kg · d CS-A during a primary or secondary immunoresponse against *L.m.* in vivo lead to a 10^1–10^4-fold increase in bacterial titers measured in liver or spleen. Therefore CS-A inhibits cell-mediated immunity to the viruses and bacteria tested, in both primary and secondary ('memory') responses. Acknowledgments: We thank Dr. Jean Borel for the gift of CS-A and discussion. Supported by SNF 3.323-0.82 and NIH AI-17285-04.

Bacteriostatic and bactericidal effects of synthetic iron chelates ethylene-bis-hydroxyphenyl-glycine (EHPG) and ethylene-diamine-diacetic acid (EDDA) in the presence of iron

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The iron chelates EHPG and EDDA were used to reduce free iron in culture media. The effects were inconsistent with the sole bacterial needs for iron. The following experiments were made to study:

1. Effect on plating efficiency. Serial 1:10 dilutions of overnight cultures of *K. pneumoniae* in Davis-Mingioli (DM) medium were spread over DM_9 agar or DM containing one of the chelates. Colony counts gave the number of cells in the cultures at 10^4 ml^{-1}. The count reduction was 100-fold on EHPG at the concentration of 500 μg ml^{-1} and on EDDA at 1 mg ml^{-1}. Twice these concentrations suppressed the formation of colonies.
2. Effect on morphology. Agar blocks cut between colonies in partially inhibited agar cultures were observed with the phase contrast microscope at 400 and 1000 magnification. On EHPG-agar, the cells were the usual short rods. On EDDA-agar, they were seen as long filaments (30–50 μm). Few were able to grow in this filamentous form to become microcolonies seen in the microscope.
3. MIC/MBC measurements. Serial 1:2 dilutions of EHPG and EDDA in appropriate media were seeded with en equal volume of *K. pneumoniae* dilution in the same media and containing 10^5 cells ml^{-1}. The results are shown in the table.

	MIC		MBC	
	EHPG	EDDA	EHPG	EDDA
In Davis-Mingioli	0.025	5	1	40
In Mueller-Hinton	0.125	20	1	40
In M-H-+20 μM Fe^{III}	0.125	20	1	40

These results are expressed in mg ml^{-1}.

The iron chelates EHPG and EDDA are thus bacteriostatic and bactericidal at milligram concentrations. The bactericidal effect is insensitive to the environmental conditions such as iron concentration or complexity of the medium. The bacteriostatic effect is slightly susceptible to some nutritional change due to medium composition but is also insensitive to the concentration of iron.

Properties of desferroxyamine B in relation to the synthetic iron chelates ethylene-bis-hydroxyphenyl-glycine (EHPG) and ethylene-diamine-diacetic acid (EDDA)

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Some properties of desferrioxamine B (DFB), an iron chelate from *Streptomyces pilosus*, were studied by diffusion in Davis-Mingioli agar (DM) containing either inhibitory or subinhibitory concentrations of synthetic iron chelates EHPG or

EDDA, or in control DM with its normal iron content or a reduced iron content after treatment on Chelex 100 resin. Controls were obtained by diffusing the synthetic chelates in DM containing DFB, or by diffusing either one of the synthetic chelates against the other in DM. As noted by Miles and Khimji (1975), DFB acts as an antagonist to EHPG. Their conclusion was that DFB displaces iron from its complexes with EHPG and binds it. *Klebsiella* were able to use this newly bound iron. We found that DFB inhibited the growth of *Klebsiella pneumoniae* by diffusing into DM with a diminished iron content. However, DFB antagonized EHPG in the same medium. These results do not support the hypothesis of the possible use of iron displaced by DFB. We present the different relationships between the 3 chelates in the next table.

Diffusing chelate	Incorporated chelate DFB	EHPG	EDDA
EHPG	Antagonism	Synergy	–
EDDA	Synergy	–	Synergy
DFB	–	Synergy	–

Adding oligoelements did not alter these behaviors. Allowing FE^{III} to diffuse into EHPG or EDDA inhibited cultures immediately after seeding or after 24 h incubation partially antagonized the chelates but only about 20% of the cfu's were expressed due to permanent damage to the majority of the cells. We conclude that iron chelates find binding sites on the cell envelopes resulting in severe damage to some cell functions. The antagonistic effect of iron cannot be due to the neutralization of the chelate and thus is probably due to the binding of iron to cell structures near or at the chelate binding site of EHPG and thus permitting a partial restauration of the damaged cell function. A similar mechanism would explain the reversal of the EHPG inhibition by DFB. The lack of such reversal for EDDA might expressed the binding of the latter to a different site on the cell envelopes.

Electron transmission microscope study of *Limax amoebae* cultured in the presence of mycobacteria

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In order to study possible relations between free-living amoebae of the 'Limax' group and mycobacteria, we have placed them into mixed cultures. The culture were made in the C.G.V. liquid medium at 28°C and sub-cultured once a week. For the purpose of this study we chose two species of free-living amoebae of the *Acanthamoeba* genus, i.e. *A. culbertsoni* (Lilly strain) and *A. castellanii* (Neff strain), which were initially axenic. As mycobacteria we chose *Mycobacterium marinum (balnei)* and *Mycobacterium chelonei.* (The strains were supplied by Prof. J.B. Jadin, Institute of Tropical Medicine in Antwerp.) According to this author, mycobacteria, including *Mycobacterium leprae,* multiply in free-living amoebae and the latter can play the role of vectors, whereby this role is important in the epidemiology of leprosy and tuberculosis. Samples of one month mixed cultures were prepared for electronic transmission microscopy, according to the classical method. Mycobacteria from the two strains were observed in a partly digested from inside the amoebae, in their lysosomes. According to the pictures observed, most of the amoebae merely contain these mycobacteria which appear to be about to be digested, in their food vacuoles. If this were so, the hypothesis which says that mycobacteria are capable of multiplying inside amoebae and keep their pathogenic power would be invalidaded. The same would apply to the vector role played by the host amoebae.

Neutralizing activity of bovine milk antibodies against enterotoxigenic *E. coli*

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We have previously reported the utilization of specific bovine milk immunoglobulin concentrates (MIC) with an activity against enteropathogenic *E. coli* (EPEC), as an orally applied therapy in infantile gastroenteritis caused by EPEC (Mietens et al., Eur. J. Pediat. *132* (1979) 239–252). Here we describe the in vivo and in vitro activity of MIC against enterotoxigenic *E. coli* (ETEC), heat-labile enterotoxin (LT) from *E. coli* and cholera toxin (CT). MIC were prepared from cows which were hyperimmunized against 6 different ETEC serotypes or cholera toxin. Antibody response was measured by passive hemagglutination, and 5% protein solution gave titers of 1:80 to 1:640 against heat-stable antigen extracts, and titers of 1:32,000 and 1:320 against CT and LT, respectively. In a mouse protection test 50 µg protein/0.1 ml protected against lethal infection with *E. coli* 0 78.H 12 LT^+/ST^+ by the intraperitoneal route. Specific antibodies to surface structures were visualized by marking with colloidal gold labeled with protein-A. The enterotoxins CT and LT show immunological cross-reactivity and MIC prepared against CT neutralized the activity of both toxins in Y1 adrenal cell cultures, and prevented fluid accumulation induced in the mouse ligated intestinal loop. Clinical trails are to be carried out to determine whether specific MIC directed against common virulence factors such as enterotoxins and adhesins can be used in the prophylaxis of infantile gastroenteritis caused by ETEC, or whether serotype specificity remains an important factor.

Evaluation of serum IgG antibody patterns in patients with leptospirosis

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SDS extracts from homogenous cultures of different *Leptospira interrogans* serogroups were electrophoresed on 8 to 20% discontinuous SDS-PAGE. The proteins were subsequently transferred to nitrocellulose according to Towbin et al. (PNAS *76* (1979) 4350), with slight modifications. Thin strips cut from the nitrocellulose sheets were then incubated with sera obtained from patients suffering from acute leptospiral infection. Serum IgG antibodies bound to leptospiral antigens were indentified by further incubating the strips with a peroxidase conjugated anti-human IgG and subsequent substrate addition. The preliminary data shown reveal the presence of IgG antibodies in the human sera reacting with selected leptospiral antigens. The specifity of these antigens has still to be examined, however, the first results indicate that it may be feasible to evaluate the serogroup of the infecting leptospiral strain in the patient's serum based on its IgG antibody pattern. Since, in addition, the method is simple, quick and more sensitive than the conventional macroscopic agglutination test as described by Abdussalam et al. (Bull. WHO *47* (1972) 113), it could be further developed as a rapid technique for the diagnosis of acute or past human leptospirosis. Acknowledgment: The *Leptospira* strains have been kindly provided by Dr. W.J. Terpstra, WHO/FAO Collaborating Centre for Reference and Research on Leptospirosis.

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Identification of *C. trachomatis* specific antibodies of different isotypes in human serum and secretions using Western blot analysis

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C. trachomatis type L2 was cultivated on cycloheximide-treated McCoy cells. Elementary bodies were isolated and purified by density gradient centrifugation. The elementary bodies obtained were solubilized, the proteins were separated by SDS-PAGE electrophoresis on a 8–20% discontinuous slab gel, and subsequently transferred to nitrocellulose sheets by Western blot technology. *C. trachomatis* specific proteins were identified by molecular weight analysis as well as immunologically using antisera obtained from chickens immunized with *C. trachomatis*. Thus at least 5 *C. trachomatis* specific antigens could be determined, in particular the most characteristic and abundant 40 kD major outer membrane protein (MOMP). Nitrocellulose strips thus prepared were then used to detect *C. trachomatis* specific antibodies of different isotypes in human serum and secretions such as breast milk, saliva and tears. Clinical samples were obtained from patients with culturally proven *C. trachomatis* infection. The system has proven to be highly sensitive and allows the visual identification of *C. trachomatis* specific antigen/antibody interaction, therefore, problems related to cross-reactivity often observed using conventional ELISA techniques or complement fixation assays are avoided. In addition, since antibodies binding to various chlamydial antigens can be quantitated and the isotype specific patterns of antibody response to chlamydial infection can be evaluated, this method provides a useful tool to investigate more basic aspects of humoral immunity (such as e.g. systemic versus mucosal immunity) in this infection.

Blastocystis hominis: culture and morphological study

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This protozoan whose biological cycle is still inadequately defined was, for a long time, considered a fungus of the *Blastomyces* genus. In spite of this, it has the physiochemical and biological structures and characteristics of protozoa, as shown in the studies carried out by C. Zierdt et al.. Its considerable degree of cellular differentiation is probably due to the presence of two modes of reproduction. Its incidence in the human digestive tube is so frequent that it has stimulated us to isolate it, cultivate it and study some of its morphological aspects with the help of phase contrast optical microscopy and electronic microscopy (scanning and transmission). It was isolated from faecal substances received in the laboratory and the cultures were made in the Boeck-Drbohlav diphasic medium. *Blastocystis hominis* develops in the latter, together with the associated feacal flora. The microscopic study showed us the three different evolutive stages in which it can be found, i.e. amoeboid, vacuolar and granular. These forms contain the ultrastructural elements which are specific to protozoans, i.e. the cellular membrane, the cytoplasm, marked off by a membrane which is covered by a mucoid layer, the central corpuscule (vacuole), one or more peripheral nuclei containing the nucleolus, numerous mitochondria of varying sizes, Golgi's complex near one of the nuclei, the pinocytotic vesiculae containing debris and other cytoplasmic inclusions, such as ribosomes, glycogenic and lipidic inclusions. This structural and biological set of features, as observed and described, is in line with earlier observations and confirms the view that *Blastocystis hominis* is a protozoan.

On the distribution of intestinal protozoans in single and multiple infestations

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The findings of the parasitology laboratory during 5 years of stool examination for protozoan detection were statistically analyzed. 76% of samples were negative. Positive samples were grouped according to the number of species they carried. The resulting frequency distribution was fitted by a Poisson model with mean m=0.3. The observed frequency of single infestations is smaller than the corresponding frequency given by the model, but everyone of the multiple infestations is more frequent than expected. The relative difference between observed and expected frequency increases with the multiplicity of the infestation. Another statistical analysis shows that samples carrying *Blastocystis hominis* or *Chilomastix mesnili* are more likely to show additional infestations than samples without these species. We conclude that single infestations were found in individuals who, at least a large fraction of them, were able to eliminate the ingested protozoans whereas the multiple infestations revealed a class of individuals which were less aggressive towards their guests and tolerated them for a much longer time. This view is supported by the fact that the more associated protozoans are generally not considered major pathogens (*Blastocystis hominis* and *Chilomastix mesnili* as compared to *Entamoeba histolytica* and *Lamblia giardia intestinalis*).

Culture and morphology of the FSL strain of *Entamoeba histolytica*

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10-year-old strain of *Entamoeba histolytica* isolated from intestinal amebiasis can still cause liver abcesses in the hamster. It was maintained in diphasic medium with its associated bacterial flora. We have adapted it to two liquid media based on Ringer's and Locke's solutions and containing 10% horse serum. The best harvests result from an alternation of these two media. As culture containers we used small Falcon tissue culture bottles to which bottom the amoebae adhered. They were incubated at 37°C and subcultured every other day. For a better understanding of some aspects of the biology of this strain, we carried out a morphological study with the help of scanning and transmission electron microscopes. Thanks to the scanning process we were able to observe the surface morphology of the vegetative forms. They are generally elongated and some of them have a pseudopodium. The less active or precystic cells are spherical in shape. Their surface is smooth, although it looks wrinkled. The uroid is at one of the amoeban poles and, in this area, the membrane has some folds and filiform evaginations which are hidden by the adhering bacteria. Fine sections of trophozoites observed under the transmission microscope make it possible to see all the organites which exist in eukaryots, excepting the mitochondria, the Golgi's system and the endoplasmic reticulum. In some cells, especially in pseudopodia, we can distinguish the ectoplasm which is more transparent to electrons than the endoplasm. The cytoplasm, with its granular appearance, contains an abundant and differentiated vacuolar system: many phagocytic vacuoles, both micro- and macropinocytes, lysosomes which are mostly mixed, containing bacteria and starch grains being digested, and autophagic vacuoles. In some places we can see the fusion between the cytoplasmic membrane and the vacuolar membrane. A kind of membranous reticulum, composed of fine tubules, would be the equivalent of the typical endoplasmic reticulum; the ribo-

somal helices are easy to see, scattered in the cytoplasm or organized in aggregates constituting the chromatoid bodies. The cytoplasm also contains free ribosomes. Cylindrical bodies forming a rosette which are found in most cells, either singly or in pairs, are considered rhabdoviruses, because of structural similarities. These rosettes consist of 10 to 30 cylindrical elements arranged in a circle round a finely granulated cytoplasm. Lastly, the spherical nucleus during the interphase, is limited by a double granular membrane; it contains the central endosoma in the form of a granular mass and the peripheral chromatin arranged under the membrane separated by an electron transparent space. The nuclear chromatin particles resemble cytoplasmic ribosomes.

Toxocara canis larval antigens (E/S-Ag), analysis and evaluation

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Toxocara canis, a common intestinal parasite of dogs with world-wide distribution, quite frequently infects humans, especially children. Second stage larvae migrating to various organs may cause a disease (visceral larva migrans). An etiological diagnosis of *Toxocara* infections in humans in only possible by the serological antibody detection. Excretory/secretory (E/S) antigens produced by second stage larvae of *T. canis* in a protein-free medium under axenic conditions in vitro proved to be highly sensitive and specific for the serological diagnosis of human toxocariasis by ELISA. With the aim to develop standardizable serological procedures, two batches of E/S antigens of *T. canis* were prepared independently in two laboratories. SDS-PAGE and Western-Blotting techniques were used to analyze the composition of these antigens. At least 10 components with different molecular weights ranging between 31 and 120 kdaltons were detected. One of the antigens had a more complex composition of proteins in the range of 30–90 kdaltons. All bands observed in SDS-PAGE were immunogenic as revealed by Western-Blotting. Despite of these differences an 80% accordance of serodiagnosis was obtained when 25 sera of patients with suspected *T. canis* infection were examined by ELISA in the two laboratories. The antigens exhibited a high degree of specificity. The results demonstrate that *T. canis* E/S antigens may well be applicable for the standardization of ELISA for the serological diagnosis of human toxocariasis.

Multiple forms of 3-phosphoglycerate kinase in *Trypanosoma brucei brucei*

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The long-slender (LS) bloodstream form of *Trypanosoma brucei* has the highest known glycolytic rate. In order to increase the efficiency of this pathway, glycolytic enzymes are grouped in a particle called 'glycosome'. One of the ATP producing enzyme, 3-phosphoglycerate kinase (3-PGK) is included in the glycosome in the bloodstream form, but is cytoplasmic in the procyclic form (midgut of the tse-tse fly) in which energy is produced by other ways. Polyacrylamide isoelectrofocusing gel with 3-PGK enzymatic activity development showed the presence of two cytosolic isoenzymes in the procyclic form having pI of 7.0 and 7.6. The glycosome of the LS form presented only one enzyme activity at pI 7.9.

The cytoskeleton of *Trypanosoma brucei:* a possible target for chemotherapy

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Several species of trypanosomes are the infective agents of important tropical diseases of humans as well as of cattle. African Sleeping Sickness is caused by *Trypanosoma brucei*. A procyclic form of this protozoon can easily by cultivated in vitro. The most abundant protein of *T. brucei* is tubulin. Since it differs somewhat from the homologous protein of the mammalian host, it might serve as a target for chemotherapy. The genes for α- and β-tubulin habe been cloned and characterized. All or most of the 10 to 15 tubulin genes are arranged in a single cluster, in which the α- and β-genes alternate in a regular fashion. There are no introns. DNA sequencing confirms the enormous conservation of the protein in evolution. The tubulin proteins have been identified by taxol-induced polymerization, by immunology and by peptide mapping. The α-chain is posttranslationally modified by tyrosine addition at the C-terminus. The several subforms of tubulin revealed by 2D electrophoresis may derive from different cellular compartments: pellicule, flagellum and mitotic apparatus. Phenothiazines in micromolar concentrations were found to inhibit the growth of *T. brucei* and to disrupt microtubular structures. Members of this group of compounds may be useful chemotherapeutics.

B) General Microbiology

Studies on the bacteriophage P7 DNA inversion system which alters the host range

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Bacteriophage P1 contains two related sets of genes determining the host range, which are alternately expressed due to a DNA inversion system (S. Iida et al., EMBO J. *1* (1982) 1445; S. Iida, Virology, in press (1984)). This DNA inversion system is composed of a gene for the site-specific recombinase, a 3-kb unique DNA sequence flanked by inverted repeats of about 0.6 kb which contain the cross-over sites at the outside end. Phage P7, closely related to P1, carries a largely homologous DNA inversion system except for a 250 bp deletion in one of the flanking inverted repeats. We have mapped a) the deletion to be in the righthand inverted repeat in the C (+) orientation (S. Iida et al.; EMBO J. *1* (1982) 1445; S. Iida, Virology, in press (1984)) and b) the crossover sites to locate at the outside ends of the inverted repeats.

Arrangement and regulatory behavior of genes *TRP1* and *TRP3* in different *Saccharomyces* spp. and *Schizosaccharomyces* spp.

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The commonly used strain of *Saccharomyces cerevisiae* X 2180 shows an arrangement of genes *TRP1* and *TRP3*, which differs from that of other ascomycetes (*N. crassa, A. nidulans, Sch. pombe*). Whereas the latter fungi carry a gene encoding three catalytic functions in the order NH_2-glutamine amidotransfe-

rase–indole glycerole phosphate synthase–phosphoribosyl anthranilate isomerase-COOH, *S. cerevisiae* strain X 2180 *TRP3* gene encodes only the first two N-terminal functions, and PRA-isomerase is encoded by a separate gene *TRP1*. By Southern-hybridization technique we screened *Saccharomyces* spp. from all four continents, including two *Schizosaccharomyces* spp. to compare the arrangement of the two genes with our wild-type strain X 2180. We found strains with a changed *TRP1* and/or *TRP3* hybridization-pattern, but we could not find a *Saccharomyces* spp. showing a fused *TRP3–TRP1* gene. Furthermore we compared the regulatory behavior of the two genes in strain X 2180 with that of other yeast strains. In strain X 2180 only *TRP3* is derepressed unter the general control, whereas *TRP1* responds very little. We found strains with different regulatory behavior, e.g. in a strain isolated from African soil, both genes showed only very weak derepression of InGP-synthase and PRA-isomerase. The coincidence of altered southern-pattern and regulatory behavior is the basis for a comparision of the promotor-structures of these genes.

Low-, intermediate- and high-copy-number cloning vectors derived from the *Pseudomonas* plasmid pVS1

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The *Pseudomonas* plasmid pVS1 was reduced to a minimal replication of circa 3 kb and used to construct gene cloning vehicles. Several nonmobilizable or PR1-mobilizable plasmids were obtained. Each carried two resistance markers for selection and insertional inactivation, respectively. The Mob^- vectors pME290 (Cb Km) and pME291 (Hg Km) could be introduced by transformation and maintained in *P. aeruginosa, P. putida, P. fluorescens, P. stutzeri, P. acidovorans, P. mendocina, P. cepacia* and *P. syringae*. Since the vector copy number appears to influence critically the stability of recombinant plasmids in *P. aeruginosa*, we isolated mutants of pME290 (ca. 7 copies) with different copy numbers: pME292 (1–2 copies) and pME294 (ca. 15 copies). The *argF* gene of *P. aeruginosa* cloned into these vectors was expressed according to their copy numbers and the recombinant plasmids were stable in *P. aeruginosa* during *argF* repression (in arginine medium) and derepression (in glutamate medium). By contrast, an $argF^+$ recombinant plasmid derived from the vector pKT240 ($\geqslant 20$ copies) was entirely unstable in *P. aeruginosa* under repressive conditions.

Nuclear ratios in transformants of a microconidiating strain of *Neurospora crassa*

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A derivative (fluffy 268: aro-9; fl; inos; qa-2) of the microconidiating strain fluffy of *N. crassa* has been constructed and found to be transformable by the chimeric plasmid pDV 1001 (Hughes et al., 1983). Transformants grow and produce uninucleate microconidia on acetate medium and on 0.3% quinic acid. A protocol has been developed to study the somatic segregation from the heterocaryotic transformants. 3 types of transformants were obtained. Type I transformants (fl 268-10) generate no arom segregants and are likely to result from the transformation of uninucleate spheroplasts. Type II transformants are stable heterocaryons producing 80 to 90% segregants. Homocaryotic derivatives can be isolated by platings of microconidia. Surprisingly, type III transformants (fl 268-19 and 268-6) practically do not transmit the transformed phenotype through microconidia (0.01 to 1% of transformed microconidia on acetate medium, respectively). The percentage of transformed microconidia strongly increases after growth on quinic acid. Type III transformants are fully stable on non selective medium. No autonomously replicating plasmids could be detected in the transformants 268-10 and 268-6 either by hybridization or by transformation of *E. coli* HB 101. Southern hybridizations of ^{32}P-labeled pDV 1001 with DNA of these transformants indicate that the plasmid integrated into the *N. crassa* genome at different sites.

Gas-liquid chromatography for differentiation of thermophilic campylobacters and *Campylobacter pyloridis*

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Thermophilic campylobacters include *Campylobacter jejuni, C. coli, C. laridis* and several groups of poorly organisms. The differentiation between *C. jejuni* and *C. coli* is of medical importance because these two species have a different distribution in the animal environment, and the sources of human infections are therefore not the same. *C. jejuni* and *C. coli* are usually differentiated by hippurate hydrolysis (rapid test of Hwang and Ederer, Harvey). This test is not reproducible. We have found some reasons. Gas-liquid chromatography (GC) however is a highly reproducible method for this differentiation. *Campylobacter pyloridis* is a 'new' organism found in enormous numbers in the stomach of patients with gastritis and duodenal ulcers. These isolates are characterized by a typical GC profile.

Properties of purified dichloromethane dehalogenase from *Hyphomicrobium* DM2

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Hyphomicrobium DM2 utilizes dichloromethane as the sole carbon and energy source. The enzyme catalyzing the dehalogenation of this substrate requires glutathione as a cofactor and hydrolyses dichloromethane to formaldehyde and hydrochloric acid. Dichloromethane dehalogenase was strongly inducible by its substrate. It constituted 15 to 20% of the soluble protein of dichloromethane-grown cells. The enzyme was purified to electrophoretic homogeneity by protamine sulfate precipitation, ion exchange chromatography on DEAE-cellulose, hydrophobic chromatography on pentyl-sepharose, chromatography on hydroxylaptite and ammonium-sulfate precipitation. This procedure resulted in 5-fold purification with 60% yield. The specific activity of the pure preparation amounted to 17.4 mkat/kg protein and was retained during several months of storage in 25% (v/v) glycerol at −15°C. Gelfiltration of purified dichloromethane dehalogenase yielded a molecular weight of 195,000±10,000 and a single band corresponding to a molecular weight of 33,000 was observed upon polyacrylamide gel electrophoresis under denaturing conditions. Cross-linking experiments confirmed that the enzyme is a hexamer consisting of six identical subunits. Dichloromethane dehalogenase exhibited Michaelis-Menten kinetics for dichloromethane and glutathione with K_m-values 30 μM and 320 μM, respectively. While the enzyme dehalogenated dihalomethanes other than dichloromethane it was inactive towards a number of chlorinated C1- and C2-compounds tested.

Accumulation of N^{α}-succinylarginine in arginine nonutilizing mutants of *Pseudomonas aeruginosa*

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Certain mutants of *P. aeruginosa* strain PAO are unable to use arginine as the only carbon source aerobically and appear to be unaffected in the enzymes of the arginine deiminase and arginine decarboxylase pathways. The precise lesions in these mutants are unknown. Feeding experiments with 2 mM [U-^{14}C]-L-arginine, [guanidino-^{14}C]-L-arginine or [carboxy-^{14}C]-L-arginine or [carboxy-^{14}C]-L-arginine revealed that some of these mutants accumulated arginine-derived metabolites which, under identical conditions, were not formed by cell suspensions of the wild-type strain PAO1. A major radioactive derivative of ^{14}C-L-arginine accumulated by the mutant strains PAO968 and PAO982 was isolated by preparative high-voltage paper electrophoresis at pH 6.4. It was identified as N^{α}-succinylarginine by cochromatography with synthetic N^{α}-succinylarginine in a reversed phase HPLC system. The two radioactive compounds obtained from the metabolite upon acid hydrolysis comigrated with L-arginine and succinate, respectively, in high-voltage paper electrophoresis and cochromatographed with these two compounds in HPLC. It is proposed that N^{α}-succinylarginine represents the first intermediate of a third, as yet unknown arginine degradative pathway of *P. aeruginosa.*

Deamination of 6-chloro-1,3,5-triazine-2,4-diamine by *Pseudomonas* sp. strain A

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Pseudomonas sp. strain A grew with 6-chloro-1,3,5-triazine-2,4-diamine as sole and growth-limiting source of nitrogen (μ=0.27 h^{-1}). The *s*-triazine substrate was utilized quantitatively and concomitantly with growth, and a growth yield of about 59 g of protein/mol of *s*-triazine was observed. In a control experiment with NH_4^+ as nitrogen source the growth yield was about 50 g of protein/mol of nitrogen. We thus presumed that strain A utilized 1 mol of nitrogen/mol of 6-chloro-1,3,5-triazine-2,4-diamine. A product was formed concomitantly with growth. This product was identified as 2-chloro-4-amino-1,3,5-triazine-6(5H)-one by 3 independent methods: cochromatography on a reversed-phase HPLC system, UV-spectrophotometry and mass-spectrometry. On prolonged incubation with strain A, the concentration of the otherwise stable intermediate decreased and growth (μ=0.07 h^{-1}; 243 g of protein/mol of intermediate) was observed.

In nongrowing cell suspensions, 0.96 mol of 2-chloro-4-amino-1,3,5-triazine-6(5H)-one and 1.0 mol of NH_4^+ were formed per mole of 6-chloro-1,3,5-triazine-2,4-diamine. On prolonged incubation, small amounts of another intermediate were excreted, which was identified tentatively as 6-chloro-1,3,5-triazine-2,4(1H, 3H)-dione. These results indicated a specific degradation rate for the first deamination of 1.2 mkat/kg of protein and 0.08 mkat/mol of protein for the second. Further, dechlorination to cyanuric acid is hypothesized. Crude extract of strain A was separated by colum chromatography on DEAE-cellulose and then on G200-Sephadex. An enzyme which deaminated 6-chloro-1,3,5-triazine-2,4-diamine to 2-chloro-4-amino-1,3,5-triazine-6(5H)-one was observed. It had a molecular weight of about 180,000 and a relatively wide substrate specificity. By analogy to the other deaminations catalyzed by this enzyme, the reaction was hydrolytic.

Converging catabolic pathways in the bacterial degradation of *s*-triazines

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The biodegradation of alicyclic aromatic compounds is known to be funneled by converging catabolic pathways to a few derivatives of catechol, which are subject to ring-cleavage whereby carbon moieties are led into the central, catabolic pathways to serve as carbon and energy sources for growth (Callely, Prog. Ind. Microbiol. *14* (1978) 205–281). In contrast, many pathways are known for the ring cleavage of heterocycles, all of which supply carbon to the central pathways, but little is known about the channeling of heterocyclic compounds to ring cleavage. The *s*-triazines are now known to yield no ring-carbon for the growth of heterotrophs, because biodegradation releases CO_2 directly (Cook et al., Experientia *39* (1983) 1432). *s*-Triazines thus serve only as nitrogen sources for growth and organisms able to cleave the *s*-triazine ring obtain neither carbon nor energy for growth from this pathway. We have now examined the catabolic pathways for deethylsimazine, vetrazine, melamine and 6-chloro-1,3,5-triazine-2,4-diamine using methods analogous to those of Cook et al. (see above). The catabolic pathways of deethylsimazine (and presumably of simazine), vetrazine, melamine and, hypothetically, 6-chloro-1,3,5-triazine-2,4-diamine all converge at, or are funneled to cyanuric acid, which is hydrolyzed to NH_4^+ and CO_2. Thus the generalization, derived from the degradation of alicyclic aromatics, is seen to be valid for the (heterocyclic) *s*-triazines, whose catabolism is separate from carbon and energy metabolism.

Methyl mercaptan, dimethyl sulfide and their S-oxo derivatives as sulfur sources for bacteria

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Methyl mercaptan and dimethyl sulfide are obnoxious, naturally-occurring toxicants. Dimethylsulfoxide and dimethylsulfone occur naturally and are widely used as solvents. Methylsulfonic acid is highly corrosive. Biodegradation of dimethylsulfoxide and dimethyl sulfide only is known (de Bont et al., Gen. Microbiol. *127* (1981) 315–323). Enrichment cultures were prepared with each of the sulfomethyl derivatives as sole and growth-limiting source of sulfur: the inoculum was prepared from municipal sewage analogous to a published procedure (Cook and Hütter, Appl. envir. Microbiol. *43* (1982) 781–786). Growth was observed with each sulfomethyl derivative, whereas, in the controls, negligible growth occurred in the absence of an added source of sulfur. Two organisms were isolated, strains DZ9 and DZ12, each of which could grow with each of the sulfomethyl derivatives. The organisms were bacteria and were tentatively identified as strains of *Pseudomonas*. Utilization of each sulfur source was quantified as the molar growth yield. Strain DZ12 had a yield of about 5 kg of protein/mol of sulfur with SO_4^{2-} or any one of the named sulfomethyl derivatives. Utilization of the obnoxious compounds was confirmed by loss of the stench, and it was presumed that each substrate was utilized quantitatively. Strain DZ12 could quantitatively utilize ethyl mercaptan but could not degrade propyl mercaptan. Strain DZ9 could quantitatively degrade propyl mercaptan but dit not totally degrade the ethyl homologue. The specific activity of the system is presumably low (Cook and Hütter, Appl. envir. Microbiol. *43* (1982) 781–786). It could possibly be increased by introducing the genes for the degradative enzymes into methylotrophs, thus enabling utilization of the substances as carbon sources which yield 6 g of protein/mol of carbon (cf. 5 kg of protein/mol of sulfur).

C) Virology

Rapid detection and grouping of rotaviruses in stool specimens by SDS-PAGE

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Electrophoretic separation of the 11 double-stranded RNA (dsRNA) genome segments was used to detect rotaviruses in stool specimens. The method in short is as follows: Clarified stool suspensions were phenol extracted and 20–40 µl of the extract were applied directly to a 10% polyacrylamide gel. After the run, the gel was fixed and RNA was visualized by a silver staining method. Using this procedure, results can be obtained within 6 h. The sensivity of the method is comparable to a radioimmunoassay detecting rotaviral antigens. Various groups of rotaviruses can be differentiated on the basis of different dsRNA patterns, thus allowing more refined epidemiological studies and, in particular, tracing nosocomial infection as illustrated in this study. In addition, since this method allows to identify directly physical characteristics of the virus (rotaviral genoms) and does not depend on antigen/antibody detection, which are generally less well controllable and are highly dependent on the antibody binding properties, we use this technique as the method of choice to investigate rotavirus associated diarrhea. The incidence of rotavirus infection was studied by analyzing stool specimens from all children below 2 years of age admitted to the Childrens' Hospital of St. Gallen. The relationship between clinical findings and virus excretion was analyzed. The study period was between the beginning of January 1983 and the end of March 1984. During this period of 15 months two peak seasons of rotavirus associated diarrhea was observed.

C1q-Binding activities (C1q-BA) in sera of patients positive for HBs antigen (HBsAg) and HBs antibody (anti-HBs)

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Occasionally, the simultaneous occurrence of HBsAg and anti-HBs (rarely also of HBeAg together with anti-HBe) is observed in sera of patients with hepatitis B-virus (HBV) infection. This may be due to two consecutive infections with two different HBV subtypes. Alternatively, concomitant antigen and antibody may reflect beginning seroconversion and suggests the presence of circulating immune complexes (CIC). CIC can occur due to persistence of antigen together with its antibody in chronic HBV infection. CIC were observed in various forms of HBV-infections. Furthermore, the extent of damage of hepatocytes was suggested to be related to the presence of CIC. However, the role of CIC in the pathogenesis of hepatitis B remains unclear. C1q-BA was assessed in 160 sera (mostly individual patients) positive for both HBsAg and anti-HBs. 54 (34%) of these sera showed C1q-BA. Among 3 HBsAg pos./anti-HBs pos. sera with concomitant HBeAg and anti-HBe only one showed C1q-BA. These results indicate that presence of antigen (HBsAg and/or HBeAg) together with antibody (anti-HBs and/or anti-HBe) in sera of patients with HBV-infection does not implicate presence of C1q-BA in more than ⅓ of the cases. 75 out of the 160 HBsAg pos./anti-HBs pos. sera were further analyzed by the 'extended C1q-binding test'. This test allows the detection of immune aggregate mediated and nonimmune aggregate mediated C19-BA. In 34 of these sera (45.3%) no indication for either type of C1q-BA was seen. Only in 7 of the sera (9.4%) C1q-BA was mediated exclusively by complexed Ig. In 25 sera (33.3%) a nonimmune aggregate mediated C1q-BA was found but an additional immune aggregate mediated C1q binding could not be excluded definitively. In a further 9 sera (12%) the presence of an exclusively nonimmune aggregate mediated C1q-BA was ascertained. Summary: The majority of sera with concomitant HBsAg and anti-HBs (and/or HBeAg with anti-HBe, respectively) may not contain the corresponding specific CIC. However, lack of C1q-BA does not necessarily imply the absence of such CIC.

Risk of CMV transmission to non-immunocompromised patients from seropositive blood donors

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An iatrogenic means of CMV transmission via blood products has been well documented in recent years. This route of CMV infection can be curtailed by using only blood from CMV seronegative donors for seronegative recipients as has been demonstrated in the case of exchange transfusions in newborns and for CMV seronegative patients receiving organs from seronegative donors. To evaluate the need to extend this practice to other recipient groups, including non-immunocompromised patients, we initiated a study to follow up CMV seronegative patients after transfusion to detect CMV seroconversion. We have used an IgG-EIA to check the CMV antibody status of our blood donors and patients receiving transfusions. 595 CMV seronegative patients were followed up on the average 6 months after transfusion; 7 were seen to seroconvert. Seronegative blood donors studied as controls were found to seroconvert at a rate not significantly different ($p > 0.05$) from the patient group. Of the healthy controls who seroconverted (3 men and 5 women), all were < 40 years old. The patients who seroconverted included 1 man (48 years) and 6 women (3 > 40 years; 3 < 40 years). They received on the average 10 (range 6–19) units of blood products and 6 of the 7 received fresh whole blood (2.6 units average, range 2–4), compared to 4.8 total units on the average for patients who did not seroconvert (11% received whole fresh blood; average 2.9 units). No patient or control suffered from a severe CMV infection or complications. There is no significant risk of transmission of CMV via blood products to normal non-immunocopromised individuals. Of further interest indeed is the search for a possible marker of CMV infectivity in donated blood. Although it has been reported that CMV can be isolated from the urine of about 3% of all blood donors, this method is not practicable in the routine lab. We are therefore checking the blood donations to the seroconverting patients for IgG and other classes of antibodies to CMV. These results will be presented and discussed. – This work was partly supported by the St. Gallen-Appenzell Cancer League.

Neurotropic variants of bovine herpesvirus 1: genome analysis by means of restriction endonuclease digestion and cross-hybridization

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Common strains of bovine herpesvirus 1 (BHV-1) could be separated into two main classes, based on the restriction enzyme patterns of their genomes, on their polypeptide profiles and on their reactivity with a type specific monoclonal antibody. These two classes of common BHV-1 strains, derived from various clinical entities, did not reflect exclusively a distinct organ tropism. However, 3 BHV-1 strains, isolated in

Argentina from calves with meningoencephalitis, could not be assigned to either of the two classes, shown by restriction enzyme analysis. Digestion with various endonucleases resulted in restriction patterns which differentiated the encephalitis-derived isolates unequivocally from common BHV-1 strains. These unique restriction patterns were confirmed by digestion of the DNA of a further neurotropic BHV-1 strain (T.J. Bagust, J. comp. Pathol. *82* (1972) 365–374). On the other hand, cross-hybridization experiments showed an almost complete nucleotide sequence homology between the neurotropic and common BHV-1 DNAs. This is reflected by the close antigenic relationship found in cross-neutralization tests (see Metzler et al., this meeting). Based on our observations we propose the neurotropic variants to represent a third class of BHV-1 strains.

Monoclonal antibodies to bovine herpesvirus 1 (BHV-1) indicating the existence of neurotropic variants

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Bovine herpesvirus 1 (BHV-1) is associated with various clinical entities. Presently no viral marker(s) are known that would allow to group viral strains according to tissue tropism or virulence. Although some strains have been shown to differ antigenically from each other there is no definitive evidence that BHV-1 viruses comprise distinct groups differentiable with respect to antigenic properties. This is reflected by our observation that 37 out of 42 monoclonal antibodies, generated against a reference BHV-1 strain, recognized more than 50 BHV-1 isolates (common strains) tested do date. The remaining 5 monoclonal antibodies failed to react with either 1 or 2 of these isolates. In this communication we show that 3 viral isolates, recovered in Argentina from calves with meningoencephalitis, are members of the BHV-1 virus group as evidenced by reciprocal crossed serum neutralization tests. However, of the 37 monoclonal antibodies recognizing common strains of BHV-1, only 18 reacted with the 3 isolates under study. From the remaining monoclonal antibodies 15 consistently failed to recognize the newly recovered viruses. This was an indication, that these strains lack a substantial number of epitopes being shared by common strains of BHV-1. Comparative analysis by SDS polyacrylamide gel electrophoresis of radiolabeled viral proteins revealed that the encephalitis-derived virus strains possessed a unique polypeptide profile when compared with common strains of BHV-1. These observations were substantiated by the finding that another neurotropic strain of BHV-1 (Hall et al., Aust. vet. J. *42* (1966) 229), was essentially identical to the viruses originating from Argentina. Alltogether the available data (see also Engels et al., this meeting) indicate, that the analyzed viral isolates represent BHV-1 viral variants which have not been defined before. These viral variants can serve as valuable tools for future studies of BHV-1-host relationships.

Synthetic events in MRC-5 cells infected with hepatitis A virus

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MRC-5 cells infected with hepatitis A virus (HAV) at 3 multiplicities of infection were examined for the presence of progeny infectious virions and hepatitis A antigen (HAAg) over a 20-day period. The so monitored virus/cell relationship could be divided into three distinct phases. Phase 1 extends up to day 5 and is characterized by active, progressive replication of the virus. In phase 2 – between day 6 and 14 – the virus titer has reached its plateau, but de novo synthesis of large amounts of viral antigens continues. Phase 3 – from day 14 onward – is equivalent with persistant infection where virus specific synthetic activity can best be defined as being concerned with conservation of the state reached at the end of phase 2. Labeling experiments indicated that the transition from phase 1 to phase 2 is paralleled by a 'switch off' of virus production. Whether this is due to cessation of synthesis of viral RNA or is brought about by a defect in virus maturation is presently under investigation.

Replication characteristics and genomic variation of different hepatitis A virus isolates

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Hepatitis A virus strains of quite different geographical origin were propagated in cultures of diploid human embryonic lung fibroblasts (MRC-5) and in heteroploid human hepatoma derived (PLC/PRF/5) cell cultures. The ability to replicate in one or both types of these cells and the requirement for distinct culture conditions varied among isolates. The observed differences could not be correlated with variations in antigenic characteristics as current routinely available serologic techniques (RIA) failed to distinguish between strains. To some extent, however, variation was observed at the level of the viral genome. This could be shown by two-dimensional electrophoresis of RNase T1-resistant oligonucleotides generated from the genomes of purified virions. The significance of these findings with respect to the homogeneity of HAV strains originating from various geographical locations will be discussed.

Mouse strain distribution and inheritance of inducibility by interferons of a guanylate-binding protein

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Interferons induce in mouse cells the synthesis of a set of proteins with high binding affinity for guanine nucleotides (Stäheli et al., J. Virol. *47* (1983) 563). These guanylate-binding proteins (GBPs) were analyzed in cells from 34 different inbred mouse strains using GBP-agarose affinity chromatography. We found strain specific differences in inducibility of a major guanylate-binding protein designated GBP-1. In cells of 8 strains, including A/J, BALB/cJ and C3H/HeJ, type I and type II interferons induced the synthesis of GBP-1, while synthesis of this protein was not induced in cells of the remaining 26 strains, including A2G, DBA/2J and C57BL/6J. Analysis of the F_1, F_2 and BC_1 offspring of crosses between A/J and A2G as well as between A/J and DBA/2J mice showed that inducibility of GBP-1 was inherited as a single autosomal locus with two forms of alleles.

Seroepidemiological studies with 'Berne virus', a new RNA virus from a horse

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From a horse a new enveloped RNA virus ('Berne virus') was isolated (Weiss et al., J. gen. Virol. *64* (1983) 1849–1858). A

seroepidemiological study showed that the virus had been active in the Swiss horse population during the last decade, 81% of horse sera collected at random contained neutralizing antibodies against 'Berne virus'. Significant titer rises were noted in 9% of paired serum samples from 273 animals. The antibody incidence had not changed significantly during the last 10 years. Small numbers of horse sera from France, Germany and USA were tested in serum neutralization and ELISA for antibodies against 'Berne virus'; positive reactions were noted. Serum neutralization was compared to ELISA and a correlation of 83% was found. 13% of the samples were neutralization positive and ELISA negative; in 4% the inverse was observed. The neutralizing activity eluted together with IgG in gel filtration. A herd of 20 foals from seropositive mares were followed serologically over a period of 13 months. A decline of maternal antibodies below detection level and a sudden synchronous seroconversion of all foals between the 9th and 12th month of age were observed. No overt clinical symptoms were seen. The inoculation of 'Berne virus' into two foals resulted in a distinct rise of antibody titer but again no clinical symptoms accompagnied the infection. Neutralizing activity against 'Berne virus' was although detected in the sera of other ungulates (cattle, goat, sheep, pig), laboratory rabbits and two species of wild mice (*Clethrionomys glareolus* and *Apodemus sylvaticus*). No antibodies were found in dogs and foxes. Inconclusive results were obtained with feline and human sera.

D) Biotechnology, Environment

Nif mutants of *Rhizobium japonicum*

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The genes coding for nitrogenase (nifDK) and nitrogenase reductase (nifH) are separated in the slow-growing *Rhizobium japonicum*, which is in contrast to the fast growing rhizobia *Klebsiella pneumoniae*, where they are located in one single operon, *nifHDK*. Using the cloned DNA containing *nifDK* and *nifH* we were able to construct *R. japonicum nif:*: Tn*5* mutants by a site-directed mutagenesis procedure. These mutants (*nifD*: Tn*5*, *nifK*: Tn*5*, *nifH*: Tn*5*) induced root nodules on soybeans, but were unable to fix nitrogen symbiotically (Fix^-). In contrast, Tn*5* mutations on either side of *nifDK* and downstream from *nifH* were Fix^+.The ultrastructure of nodules infected with either the Fix^+ wild type or with the Fix^- mutant strains was analyzed by electron microscopy. All contained fully developed bacteroids, but the Fix^- mutants showed massive accumulation of poly-β-hydroxybutyric acid granules. Kan-sensitive derivatives were isolated from Tn*5* containing strains which carried deletions. These could be classified into the following groups: 1. deletions lacking both the *nifDK* and *nifH* regions indicate linkage between the two operons whereby at least 15 kb of DNA separate them; 2. a deletion ending upstream from *nifH* and lacking only *nifDK*, indicates that the *nifDK* operon is located upstream of the *hifH* operon; 3. all deletion mutants are Nod^+ indicating that there are no essential nodulation genes located between and adjacent to *nifDK* and *nifH*.

On the evolution of *nif* genes

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In all nitrogen fixing bacteria the nitrogenase enzyme complex consists a) of the MoFe protein, a $\alpha_2\beta_2$ tetramer, encoded by the genes *nifD* and *nifK*, and b) the Fe protein, an α_2 dimer, encoded by the gene *nifH*. We have determined the nucleotide sequence of the *nifD* and *nifH* genes from the slow-growing soybean symbiont, *Rhizobium japonicum*. The open reading frames code for 545 amino acids (*nifD*) and 292 amino acids (*nifH*) which result in polypeptides of mol.wt 57,918 and 31,525, respectively. There is considerable homology to corresponding sequences from other N_2 fixing bacteria. Based on DNA and amino acid homologies the similarity coefficients (S_{AB}) were calculated which led to some interesting speculations on the evolution of *nif* genes: 1. *nif* genes (nitrogenase genes in the strict sense) from fast- and slow-growing rhizobia are as divergent from each other as they are from corresponding genes of other N_2 fixing gram-negative bacteria. Hence, the placement of both fast- and slow-growing rhizobia within the same genus does not seem to be appropriate. 2) *nif* genes have evolved, to a large degree, together with the bacteria which harbor them. This supports the idea that *nif* genes are ancient genes or have spread very early in prokaryotic evolution. The interpretation is somewhat complicated by the fact that *nif* evolution is probably superimposed by the strict structural requirements of the nitrogenase enzyme complex for catalytic functioning, which allows evolutionary drift only as far as it does not grossly affect enzymatic activity.

Carbon monoxide dehydrogenase in *Methanothrix soehngenii*

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During the anaerobic degradation of organic material acetate is an important intermediate. About 72% of the methane evolved in sludge digestion is formed directly from the methyl group of acetate. The carboxyl group is oxidized to CO_2. If a C_1–X carrier is proposed, in which the C_1 is in the oxidation state of either HCOOH or CO (+2), then a CO dehydrogenase could play an important role in the oxidation of this bound intermediate to CO_2. Cell free extracts of *Methanothrix soehngenii* contain a CO dehydrogenase activity and at 30°C a specific activity of 3.02 µmol min^{-1} mg^{-1} protein was measured. The photometric assays, using methylviologen as electron acceptor, were performed under strictly anearobic conditions in cuvettes sealed with soft rubber stoppers. The reaction rates depended on substrate concentration in accordance with Michaelis-Menten kinetics. Half maximal reaction rates were obtained for CO at 0.44 mM and for methylviologen at 0.18 mM. Neither NADP nor NAD could replace methylviologen as electron acceptor. Factor F_{420}, an electron acceptor unique to methanogens, was reduced in a CO dependent reaction. The reaction rate measured was only 0.9% of the reaction rate obtained with methylviologen. The formation of CO_2 in the above reaction could be measured with a gas chromatograph (TCD detector).

Characterization of whey permeate fermentation by *K. fragilis* NRRL 665

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A comparative study of shaken flask cultures of 10 strains of yeasts capable of fermenting lactose showed no significant dif-

ferences in alcohol yield among the best four strains. Use of whey permeate concentrated 3 times permit to select *K. fragilis* NRRL 665 as the strain having the best capacity to ferment such a concentrated medium. An optimal growth temperature of 38°C was determined. Elemental analysis of both the permeate and the cell mass indicated the possibility of a stoichiometric limitation by nitrogen. Batch cultures in a laboratory fermentor confirmed this finding and revealed in addition the presence of a limitation due to growth factors. Both type of limitations could be overcome by adding yeast extract. The amount of this nutrient required to balance the medium was determined by the pulse and shift technique in continuous culture. A pH of 4.0 did not affect the alcohol yield and sustained the maximum rate of lactose consumption. Unter these conditions the maximum productivity of continuous cultures could be improved to 5.1 g/l h. A dilution rate of 0.200 h^{-1} still permit 100% of lactose conversion which resulted in an ethanol concentration of 23 g/l. The maximum specific growth rate was of the order of 0.310 h^{-1}.

Taxonomical study of the genus *Xanthobacter*

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The genus *Xanthobacter* as described by Wiegel et al. (Int. J. Syst. Bacteriol. *28* (1978) 573). comprises hydrogen-oxidizing, dinitrogen-fixing bacteria. Two species were described so far: *X. autotrophicus*, type species of the genus, and *X. flavus* (the type species of which was previously known as *Mycobacterium flavum* 301). We performed phenotypic comparisons and DNA:DNA hybridizations between 18 strains of yellow-pigmented, nitrogen-fixing hydrogen bacteria, among which the type strains of these species, reference strains previously attributed to them, as well as biotin requiring strains and motile strains resembling *Xanthobacter*. Our results showed that some motile strains are well clustered and form a distinct species. Biotin requiring strains, though more dispersed, could belong also to a distinct taxonomic unit. The dendrogram obtained by phenotypical analysis was in good agreement with the one resulting from DNA:DNA hybridizations. Nevertheless, some differences emphasized the usefulness and complementarity of both approaches. The genus *Xanthobacter* could be related to the Azotobacteraceae and further investigations are needed to establish its genotypic relationships with other genera of dinitrogen-fixing aerobes.

Auxotrophic and analogue-resistant mutants of *Methanobacterium thermoautotrophicum*

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The death rate of *Methanobacterium thermoautotrophicum* strain Marburg upon exposure to N-methyl-N'-nitro-N-nitrosoguanidine (NG) under anaerobic conditions was of the same order of magnitude as the death rates that have been reported for *Escherichia coli*. Cultures of the methanogenic bacterium, mutagenized by NG-treatment and grown under nonselective conditions, yieled mutants resistant to DL-ethionine (30 mM) or to 2-bromoethane sulfonic acid (3.8 mM). No mutants were observed in untreated controls. Among 1500 clones obtained from a NG-treated culture there were 6 mutants requiring a single growth factor each, namely L-leucine, L-phenylalanine, thiamine (2 mutants) or adenosine (2 mutants). Three mutant-strains were studied in more detail. They were genetically stable (no revertants among 10^9 cells), and wild-type growth rates were restored by 1 mM L-leucine, 0.4 mM adenosine and 0.03 mM thiamine, respectively.

New directions in methanotrophy

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Throughout the literature it is generally stated that two types of methanotrophs (methane-utilizing microorganisms) exist: obligate and faculative methanotrophs. However, facultative strains, growing not only on methane (and methanol) but also on complex organic substrates with more than one carbon atom (e.g. glucose, ribose, succinate or ethanol) seem to be very rare and were found in the United States only. The facultative strains described so far are: *Methylobacterium organophilum, Mb. Hypolimneticum, Mb. ethanolicum, Mb.* R6 and *Mb.*sp. As we orginally intended to set up a microbial process for the bioconversion of methane to methanol we screened for mesophilic and thermophilic methanotrophs in order to isolate a suitable facultative methanotroph. During this screening program we made the following observations: 1. *Mb. organophilum* obtained from culture collections (ATCC 27866, NRRL B-11222, NCIB 11483) as lyophilized preparations did not grow on methane but only on methanol and complex organic substrates. 2. Enrichments with methane (shake flasks) and samples from many different environments (lake sediments, sewage sludge, mud, hot springs, etc.) yielded a large number of methanotrophic cultures at 28, 37, 45, 50 and 55°C. Isolation and purification was achieved by repeated plating (on mineral salt agar) of resuspended single colonies (incubation in an atmosphere of CH_4 and air 1:1). After the first and second plating step most of the cultures behaved like facultative methanotrophs (growth on glucose, ethanol, acetate, nutrient broth, methanol, methane). However, after four to five plating steps all the methane-utilizing isolates turned out to be obligate methanotrophs (growth only with methane and methanol). We were thus not able to find true facultative methanotrophs so far. 3. It seems that an imperfect purification procedure yields 'syntrophic associations' of obligate methane-utilizers and facultative methanol-utilizers. Quite recently *Mb. ethanolicum*, originally described as facultative methanotroph, was identified as a syntrophic association of *Methylocystis* sp. and *Xanthobacter* sp. (J. gen. Microbiol. *129*(1983) 3139). 4. Obligate methanotrophs are very sensitive to lyophilization. In our hands, lyophilized preparations (our own preparations as well as preparations obtained from strain collections) cannot be reactivated to grow on methane. Facultative methanol-utilizers are not sensitive. Assuming that also *Mb. organophilum* was originally a mixed culture (syntrophic association) we would then explain the lack of methane growth with the damage of the methanotrophic component (by lyophilization) and survival of the facultative pink methanol-utilizing component. Some authors have also tried to explain the loss of the methaneritilizing capacity with the loss of a plasmid coding for methane monooxygenase. In this case, however, a 100% curing must be assumed what we consider as rather unlikely. We conclude that the existence of facultative methanotrophs is very unlikely and that the strains described so far were in fact masked stable mixed cultures. Besides the well-known strains of *Methylococcus capsulatus* (optimum at 45°C) only very few descriptions of thermophilic methanotrophs are found in the literature: *Methylococcus thermophilus* (56°C) and strain H-2 (50°C). Our own enrichments at elevated temperatures yielded obligate methane-utilizing cultures up to 55°C. Growth of such cultures above 60°C seems to be possible, but only at very low growth rates. Studies on methane mono-oxygenase (MMO) from *Methylococcus capsulatus* strain Bath and *Methylosinus trichosporium* OB3b have shown that this enzyme exists in a soluble and in a membrane-bound form within the same organism (Stanley et al., Biotechnol. Lett. *5* (1983) 487). Recent studies on methanol dehydrogenase (MDH) indicate that this enzyme exists in two molecular forms as well. At the

4th C_1-Symposium it was reported (J.A. Duine et al., TH Delft) that *Methylcoccus capsulatus* contains two MDH (N-MDH, PQQ- *and* NAD-dependent). N-MDH exists in a complex with NADH-dehydrogenase and NAD-dependent formaldehyde dehydrogenase.

The proton motive force in *Methanobacterium thermoautotrophicum*

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Measurements of the membrane potential were performed under various conditions using the tetraphenylphosphonium chloride distribution technique and monitoring the probe by means of an electrode sensitive to it. $\Delta\Psi$ was strictly dependant on anaerobic conditions and correlated to the concentrations of the electron donor, H_2, and of the electron acceptor, CO_2, both of which are involved in methanogenesis. Na^+ but not K^+ exhibited a pronounced effect on $\Delta\Psi$. Correspondingly, $\Delta\Psi$ could be weakend by small concentrations of valinomycin, but was abolished completely by monensin. While valinomycin had no effect on the methane production rate, monensin abolished methane formation parallel to the membrane potential. For details see B.M. Butsch, Thesis, University of Zürich, 1984. The internal pH of the cells was determined using the pH indicator carboxyfluoresceindiacetate (J.A. Thomas, P.C. Kolbeck and Th.A. Langworthy, in: Spectrophotometric Determination of Cytoplasmic and Mitochondrial pH Transitions Using Trapped pH Indicators, p. 105–123. Eds A. Nuccitelli and D.W. Deamer. Alan R. Liss, Inc., New York 1982). Cells were loaded with the indicator and its absorbance measured spectrophotometrically. The calibration was achieved with cells deenergized by Triton-X-100 or by N_2 instead of H_2. The internal pH is close to the external pH of the growth medium. When the external pH is changed to the acidic or alkaline side, the internal pH varies accordingly but to a much smaller extent.

Methane production from cow manure at low temperatures

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Methane production from animal manure in mesophilic digesters has become a common procedure in over 400 farms all over Europe. However, todays technique is too expensive for smaller farms. One of the possibilities to reduce the installation costs and hence improve the economy is the fermentation at ambient temperatures in continuous-fed digesters or in combined digester/storage-systems (accumulation-systems). For this purpose, the influence of temperature and hydrolic retention time (HRT) on the activity of the mixed population naturally occurring in cow manure was determined in lab-scale batch cultures (2 l) as well as in pilot-scale continuous cultures of 5 m^3. The gas yield in function of the temperature was primarely influenced by the pretreatment of the inoculum. Batches which were inoculated by 20% with cultures grown at 35°C produced at 15°C only as little as 32% of methane per kg volatile solids (VS) after 50 days compared to batches inoculated with cultures pregrown at 15°C. The adaption of mesophilic cultures to psychrophilic temperatures may take as long as 50 days. On the other hand demonstrated batches of cow manure with a 20% (v/v) psychrophilic inoculum gas yields as high as those with mesophilic inocula when incubated at 35°C. Gas yields after 50 days of cultures adapted to psychrophilic temperatures showed a linear correlation with temperature in the range of 10 to 20°C. However, only during the first 20 to 30 days the daily gas production varied significantly. Beyond that time it remained fairely constant at a low level independent of fermentation temperature. In other words, within a reasonable HRT of less than 50 days mainly the easily degradable substances determine the final gas yield. In fact in a experiment with the continuous-flow pilot plants at 18°C we found that only at HRT's of less than 25 days the VFA's started to increase and at on HRT of 18 days the process broke down.

Phototrophic bacteria in the redox transition zone of Lago Cadagno, a meromictic, alpine lake

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Lago Cadagno is a stable sulfuretum with sulfate reducing bacteria and phototrophic sulfur bacteria dominating. It is embedded in gipsum containing dolomite rock of the southern alps at 1950 m above sea level. It is covered by ice and snow for 5 to 7 months every year. The sedimentary pigment pattern indicates the great stability of this lake ecosystem. Oxygen concentration, light and sulfide concentration create optimal conditions for phototrophic sulfur cycling in a narrow redox transition zone (RTZ) at a depth of 13 m. Although the sulfate concentration (2 mM) is not limiting for carbon mineralization processes, sulfate reducing bacteria and methanogens coexist in the sediment and in the monimolimnion. Sulfide oxidation in the light by purple sulfur bacteria takes place near the lower, oxygen-free boundary of the RTZ. Methane oxidation is localized at the upper, microaerobic boundary of the RTZ. In summer ammonium and phosphate are continuously cycled between the RTZ and the monimolimnion. The RTZ acts, thus, as a barrier which limits diffusion of nutrients into the aerobic water layers preventing sediment borne eutrophication of the mixolimnion. The anaerobic conditions below 13 m, created by the microbial activities, the local geological situation and the use of the lake as a reservoir for hydroelectric power generation put a limitation on the survival and propagation of the animal populations in this lake.

Isolations of *Yersinia* in lake waters (Lago Maggiore)

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The search of yersinias has been carried out on the coastal waters of Lake Maggiore, partly close to bathing areas along the coasts of Piemonte, Lombardy and Switzerland.
Altogether 561 samples have been tested, collected monthly during the years 1982–83. 99 samples, which means 17.6% of the tested samples, have made it possible to isolate stocks of *Yersinia*. The isolation of *Y. enterocolitica* has been more frequent (9.8% of the samples), while the other species have reached lower values: *Y. intermedia* (1.9%); *Y. frederiksenii* (1.8%); *Y. kristensenii* (0.4%); atypical stocks (2.1%).
Through the simultaneous determination of the colimetrical values (coliforms and fecal coliforms) as well as of the salmonellae it has been possible to ascertain that the possibility to find yersinias is more frequent in waters which are not exceedingly polluted. The fact that the highest frequency of isolation occurs in May (temperature of the waters between 12°C and 21°C) while the lowest frequency occurs either in the very hot periods (in July when the temperature of the water varies from 24°C to 30°C) and in the very cold ones (winter months, with temperatures sometimes lower than 6°C) clearly follows the psychrotrophic characteristics of the tested species.

Acetate formation in anaerobic sediments through synergistic interaction of fermenting and sulfate reducing bacteria

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Acetate is one of the intermediates of anaerobic mineralization which accumulates temporarily to measurable levels in sediments. Its main production horizon coincides with high activity of sulfate reduction. Acetate formation from carbohydrate with sulfate as oxidant could proceed according to

$$6\ (CHOH) + SO_4^{2-} \rightarrow 2\ CH_3COO^- + 2\ HCO_3^- + HS^- + 3\ H^+.$$

From sediments of the waste water oxidation pond of a sugar beet processing plant we isolated a two-membered community which is capable of catalyzing the above reaction using glucose as the model carbohydrate. Although the community always appears as a single colony in agar shakes with glucose and sulfate, no mandatory symbiosis exists between the two organisms. With appropriate substrates each can grow in pure culture. First *Klebsiella pneumoniae* breaks down the carbohydrate via a butanediol variation of the mixed acid fermentation pathway. Acetate, ethanol and formate are the main products, with lactate, butanediol, acetoin, pyruvate and succinate as minor ones. The accompanying sulfate reducing organism resembles *Desulfovibrio vulgaris*. Except for acetate and succinate it uses the fermentation products for growth under sulfate reducing conditions. It oxidizes them incompletely to acetate and bicarbonate. No measurable levels of the fermentation intermediates can be detected in sediments which indicates that the two processes must be coupled efficiently in nature. They create thereby habitat structures which allow certain chemical reactions to proceed in specialized microenvironments.

Microstructure of lake sediments: Sediment aggregates as microbial habitats

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Stable aggregates are formed from autochthonous and allochthonous materials when they are buried in sediments. Aggregates are most stable in sediment layers approximately 10 cm below the surface where nutrients slowly become scarce. Aggregate sizes between 10 and 1000 µm can be prepared by flotation fractionation of sediment contents through PE-sieves or in flotation columns. The aggregates are mechanically stable and are a conglomerate of predominantly diatome skeletons, calcite crystals, clay platelets and organic detritus. They can be disintegrated by sonication and by oxidation of the organic content by H_2O_2. Reaggregation does not occur spontaneously, indicating that these aggregates are formed through microbial action. SEM analysis of aggregates reveals a dense network of bacterial adhesion threads and patches of bacteria attached to certain surfaces. These bacteria cannot be removed easily for enumeration on agar plates. The attached bacteria are, however, more numerous than the free living ones in the interstitial water. Perfusion of aggregates with nutrients leads to rapid enrichment of surface attached bacteria. Bacteria also attach rapidly to artificial surfaces incubated in sediments in situ. We use glass fibers as models for the silicium oxide surface. The surfaces are first coated with patches of detritus before single bacteria attach to the organic coating. The bacteria then multiply under favorable conditions and colonize the surfaces irregularly. It seems that sedimenting particles scavenge numerous organic and inorganic molecules which form a layer on their surfaces. These films attract bacteria and act as nutrient source for them. The bacteria produce stalks, slimes and adhesion threads which tend to cluster even other particles.

Microbiology of deep, pelagic sediments in Lake Geneva

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Lake Geneva is a large, holomictic, eutrophic lake with a maximum depth of about 300 m. The oxygen concentrations near the sediment-water interface decrease to 1 to 2 mg/l towards the end of the stagnation period. The sediments in the central basin have a pillow-like appearance. The soft elevations containing the major portion of the recently sedimented detritus are separated by trenches of 5 to 15 cm depth in which the top sediment layers seem to be missing. Bottom dwelling fishes (*Lota lota*) prefer the trenches as their habitat and might partly be responsible for the turbation of the 'trench sediment' layers. Thus, within distances of 10 to 30 cm two sediment types can clearly be distinguished. They differ with respect to morphology and chemical stratification. Concentration depth profiles of NO_3^-, Mn^{2+}, Fe^{2+}, SO_4^{2-} and CH_4 dissolved in the interstitial water reveal the location within the sediment of microbially catalyzed redox processes. The redox transition zone (RTZ) from aerobic to anaerobic is located only a few millimeters below the sediment surface in the 'pillow sediments' which contain the bulk of the organic detritus. The RTZ is at a depth of 6 cm in the 'trench sediments' which are poor in oxidizable organic matter. The same thermodynamic sequence of microbially catalyzed redox reactions can be observed in both sediment types, the difference being that they are located at different depths and that they are less pronounced in the 'trench sediments'. As a consequence diffusion fluxes of dissolved substances in and out of the different sediment regions vary greatly. This leads to horizontal differences in the sediment's abilities to supply nutrients to the bottom water which is probably a major controlling factor for sediment borne eutrophication of this lake.

Aeromonas lysotyping: epidemiologic applications

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In aquatic and medical microbiology, the genus *Aeromonas* has acquired an increasing importance. In the water environment, most of the epidemic-like infections, particularly those of fishes, are caused by this bacterial genus; in medical microbiology, its pathogenicity for the human being is more and more recognized, either in hospital infections or as an enteropathogen agent. In this study, we have examined the correlations between the *Aeromonas* isolated from the water environment (piscicultures, free waters, sewage water plants) and the hospital environment.

440 *Aeromonas* strains have been isolated from 296 samples collected from aquatic and hospital localizations; 46 bacteriophage strains able to lyse *Aeromonas* have been isolated from the same samples. Our results show that the clinical and environmental bacterial strains share the same lysotypes; more particularly, we could hypothetize the aquatic origin of some clinical infections. From a taxonomic point of view, 50% of the strains characterized by their biochemical properties could be grouped into different lysotypes. The same strains are at present analyzed using immunochemical methods to establish the eventual correlations between their lysotypes and their antigenic properties.

In conclusion, the results presented show that lysotyping of *Aeromonas* can be used as an epidemiologic and taxonomic tool.

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Influence of cadmium on growth, α-amylase production and activity in *Bacillus licheniformis*

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Bacillus licheniformis was chosen as a model soil microorganism to study the influence of cadmium on microbial activity. Growth rate was already affected by Cd concentration as low as 0.25 ppm. At higher concentration, growth was delayed and resumed after 1–2 days, with a growth rate similar to that of the control without Cd. This was due to the selection of a Cd-resistent mutant. This mutant grew unaffected up to 80 ppm Cd in mineral medium with D-glucose as carbon source. Yet, with starch as sole C-source, growth was inhibited above 10 ppm. The activity of the α-amylase in the supernatant of cultures grown in absence of cadmium was unaffected by Cd concentrations up to 200 ppm. Thus, it appears that an undefined step in α-amylase synthesis, rather than the activity of the enzyme itself, is affected by cadmium.

Thermophilic, hydrogen-oxidizing bacteria in geothermal manifestations

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In geothermal manifestations (solfataras, hot springs, fumaroles ...), underground gases and waters bring at the surface reduced compounds, some of them (e.g. NH_4^+, H_2S and H_2) being potential electron donors for aerobic, chemolithoautotrophic organisms. In most cases, these manifestations reach high temperatures: they are thus typical habitats for thermophiles. Due to the presence of compounds (e.g. H_2S) whose oxidation generates strong acids, the pH of some manifestations may be very acidic, whereas other are near neutral. We studied the H_2-oxidizing microflora in a solfatara near Lago, province of Grosseto (Italy), in the geothermal area of Tuscany. The dry fraction of gases contained 3 to 5% H_2, along with minor concentrations of CH_4 and H_2S, the bulk being CO_2. Organic compounds were very scarce, less than 1% the dry matter. Two main types of H_2-lithoautotrophic organisms were found. The first one was isolated from acidic ponds subjected to strong variations in temperature and water level. It consisted of gram-positive sporeformers, with oval, subterminal endospores swelling the sporangium. They grew best autotrophically, although they could also use a limited amount of short chain fatty acids, amino acids and alcohols. They grew optimally at 55°C and pH 4.5. They were characterized by a soluble hydrogenase, which dit not reduce pyridine nucleotides. Such a pattern was not known so far in hydrogen-oxidizing bacteria. The other type was isolated from rather neutral, hot waters, with more constant temperature and level. They were gram-negative rods, without spores, neutrophilic and more thermophilic (optimum 70–75°C) than the former. They were obligately litho-autotrophic. As ribulose-1,5-bisphosphate carboxylase and phosphoribulose-kinase could not be detected, they appear to fix CO_2 through an unidentified way differing from Calvin cycle.

CO_2 as electron acceptor in anaerobic oxidation

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In oxidant limited lake sediments CO_2 serves as electron acceptor from H_2 in chemolithoautotrophically growing methanogens and acetogens and in organisms with homoacetate fermentation. We find in lake sediments other microbially catalyzed reactions which use CO_2 as electron acceptor for the oxidation of reduced fermentation products (alcohols, acetone, short chain fatty acids) to acetate. Organisms capable of catalyzing these reactions have been found in lake sediments rich in organic matter at depths where thermodynamaically more favorable oxidants (O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}) have been depleted. An organism isolated from a syringic acid degrading community is capable of utilizing each, methanol, ethanol, isopropanol, butanol, acetone and methylgroups of methoxylated aromatics as sole source of carbon and electrons in presence of bicarbonate as the only electron acceptor. Metabolism of these substrates leads to an enrichment of organically bound carbon in the fermentation product. Dense populations of this organism fed with methanol are also capable of catalyzing the thermodynamically unfavorable conversion of butyrate to acetate. When fed methanol it accumulates butyrate as a temporary electron sink. It oxidizes butyrate after methanol has been used up. Since acetate is one of the products that accumulates temporarily in lake sediments, we believe that CO_2 reducing reactions are one of the main sources of acetate. Microbes can still catalyze acetate formation in sulfate depleted sediment zones from oxidizable small organic molecules. A generalized redox equation for alcohols is:

$$\langle C_nH_{2n+2}O\rangle + \tfrac{1}{2}n\ HCO_3^- \rightarrow \tfrac{3}{4}n\ CH_3COO^- + \tfrac{1}{4}n\ H^+ + H_2O.$$

Thus, microorganisms capable of internal electron disposal onto CO_2 do not depend on companion organisms for interspecies hydrogen transfer.

Homoacetate fermentation of aromatic compounds

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A three-membered microbial community isolated from the oxidant limited zone of lake sediments is capable of fermentatively mineralizing syringate to CH_4 and CO_2. Separation of the community with the agar shake technique yields organisms with limited catalytic abilities. Organism I demethylates syringate and leaves acetate and gallate as products; organism II opens the aromatic ring and produces acetate and bicarbonate as the sole products and organism III is an acetotrophic methanogen. Cleavage of the aromatic ring in the absence of elemental oxygen is accomplished by a nonspore forming organism. It converts, for example, pyrogallol to acetate according to the equation:

$$C_6H_6O_3 + 3\ H_2O \rightarrow\rightarrow\rightarrow 3\ CH_3COOH.$$

Several as yet unidentified intermediates, separated by HPLC, are present in media of growing cultures. Following the hypothesis of the 'reductive ring cleavage mechanism' (Evans, Nature *270* (1977) 17–22) one would expect 1,2,3-trihydroxycyclohexane as the product of the initial hydrogenation of the aromatic ring. Primary ring fission products could be 5,6-dihydroxyhexanoic acid or 6-hydroxy-2-oxohexanoic acid which would be oxidized to 5-hydroxyadipinic acid and 2-oxoadipinic acid, respectively. These dicarboxylic acids could undergo β-oxidation to yield 3 moles of acetate each. Methoxylated aromatics cannot be metabolized by this organism unless organism I removes the methyl functions first. The hydroxyl functions destabilize the aromatic ring thus allowing the ring fission reaction to proceed exergonically. No hydrogen scavengeing companion population is required for the fermentation of these substituted aromatics.

Anaerobic digestion of urban wastes: optimization, yield and perspectives from laboratory-scale experiments

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Anaerobic digestion experiments of wastes of urban origin (solids and activated sludge) were performed semi-continuously in 20 liters, laboratory digesters with a retention time of 18 days. They were conduced either at 35°C or 60°C. Anaerobic digestion of the organic fraction of urban waste was limited by the amount of disponible nitrogen. Under such limitation, volatile fatty acids (VFA) accumulated, thus lowering the pH which in turn slowed down methanogenesis. Anaerobic digestion of concentrated (14% dry weight) sewage sludge presented similar difficulties, although it had nitrogen in excess. The symptoms were identical: decrease of gas production, methane content and pH, accumulation of VFA. The causes of this phenomenon are not yet determined. On the contrary, a mixture of both types of waste products, with equal dry matter content, provided an equilibrated culture medium, allowing a stable digestion process. It was possible to maintain for years, depending on the conditions, a production of 39–45 l gas/100 g organic matter introduced day, and 2–4 l gas/l digester volume. Except a higher of organic matter destroyed, pretreatments before methanogenesis (resp. aerobic, thermoic and alkaline, thermophilic acidogenesis) didn't allow increasing yields in gas production, compared to the one-step digestion. Heavy metals in wastes could be a problem, either for the digestion process itself or for the utilization of residual sludge. In our experiments with a mixture of solid waste and activated sludge, the heavy metal content of the residual sludge was not only below the limits of toxicity for the organisms of the methanogenic syntrophic association, but also below the norms edicted by the Swiss Federal Office of Environment Protection. This enables the residual sludge to be processed in view of their utilization as soil fertilizers. These results are encouraging enough to allow the research to proceed with an industrial-scale pilot plant.

Treatment of wastewater from a sugar beet factory with an anaerobic filter

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Today the biological treatment of industrial wastewaters represents an important section of the biotechnology. In most cases however, the usual aerobic degradation is not possible because of the high load of the effluents and a pretreatment such as an anaerobic fermentation is required. The advantages of the anaerobic process are low sludge production, high process stability and the yield of directly utilizable energy. The wastewaters of the sugar beet factory Frauenfeld has COD-values between 14 and 30 g/l and cannot be treated in the clarification plant of the city. Laboratory scale experiments were carried out with an anaerobic filter of 11 l filled with clay balls. Organic loads up to 9 kg COD/$m^3 \cdot d$ were treated with an efficiency of 70–90%. A pilot plant of 600 l was installed in the factory in Frauenfeld and remained in operation over 3 years during the months of sugar production from October to December. The organic load was increased from 10 up to 40 kg COD/$m^3 \cdot d$. The COD reduction was always greater than 70%. The average biogas amounted to 0.45 m^3/kg COD removed with a methane content of 61–74%.

Hydrogen production by immobilized cells of the photosynthetic bacterium *Rhodospirillum rubrum*

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The photosynthetic, nonsulfur bacterium *Rhodospirillum rubrum* was immobilized in agar, agarose, Ca-alginate, Ba-alginate, k-Carrageenan and pectine. Spheres of 2–4 mm diameter of the immobilized cells were given into a glass reactor of 40 ml volume and 20 mM lactate pumped through as the substrate at a rate of 1.2 ml/h. High rates of H_2 evolution in the light were obtained with agar, agarose, Ba-alginate, k-Carrageenan and pectine, while Ca-alginate was solubilized and washed out from the reactor. The highest rates obtained with agar as immobilizing agent were 90.8 µl H_2/h·mg d.w. and 87 µl H_2/h·ml immobilized culture. The activity to produce hydrogen from lactate in the light was similar for cells in suspension and under immobilized conditions, however immobilized systems showed activity over a quite longer period of time. After 10 weeks a cell suspension produced only 2–4 µl H_2/h mg d.w. while under immobilized conditions the rate was about 10 times higher. Best rates of hydrogen production were obtained with cells cultured in the light for 80–90 h up to a cell density of 1.2 mg d.w./ml before immobilization.

Eglin c from the leech *Hirudo medicinalis:* chemical synthesis of the gene, isolation and characterization of the polypeptide synthesized in *E. coli*

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The coding sequence of the specific and potent proteinase inhibitor eglin c (M_r 8100, 70 amino acid residues), a protein occurring in the leech *Hirudo medicinalis*, has been chemically synthesized by a combined biochemical assembly of the gene segments. The gene consists of a 232-basepair fragment containing initiation and termination codon signals with conveniently placed restriction enzyme sites (EcoRI, BamHI) for cloning into a bacterial plasmid vector. The chemical synthesis involved preparation of only six single-stranded oligodeoxynucleotides which share a stretch of complementary regions at their 3'-termini. After annealing the single-stranded strands were enzymatically converted into double-stranded DNA fragments with DNA polymerase I (Klenow) and assembled by ligation with T_4 DNA ligase. The complete synthetic gene was expressed in high yield in *E. coli* under the transcriptional control of the tryptophan promoter. The proteinase inhibitor synthesized in *E. coli* has been purified by affinity chromatography and was shown to have an electrophoretic mobility and an in vitro biologic activity similar to the native protein isolated from the leech.