for waste disposal currently employed in the chemical industry. He pointed out that in chemical plants up to 50% of the raw materials may end up as waste but that the exact chemical composition of this waste or the biodegradability of its components are not always known. This lack of information often precludes the application of microbiological processes for waste treatment but is of little consequence if waste is disposed of by incineration or other physical processes. Dr Ghisalba therefore strongly advocated efforts on a national basis to improve information exchange and to collect reliable data on the biodegradability of relevant waste chemicals.

The session was concluded by a report of Dr W. Samhaber (Sandoz AG, Basel) on the activities of the Working Party on Environmental Biotechnology of the EFB. This international group has organized a workshop on 'Environmental Biotechnology: Future Prospects' in October 1982. A report on the conclusions and recommendations of the meeting has been published and further meetings to assess the development of this field in Europe are planned. The European Working Party is also in the process of establishing a European expertise database for environmental biotechnology and advocates the formation of national Working Parties on environmental biotechnology to facilitate information transfer in the membership countries.

Foods microbiology and the limits of quality control

W. Schmidt-Lorenz, W. Hauert, G. Kiss, H. Schwab, H. S. Walker und M. Zeller

Einleitend wurden die gesetzlichen Grundlagen sowie der mit den Toleranz- und Grenzwerten verbundene Auftrag des Gesetzgebers, den Konsumenten vor gesundheitsschädlichen Lebensmitteln und vor Täuschung, den reellen Lebensmittelhersteller vor unlauterer Konkurrenz und Wettbewerbsverzerrung zu schützen, ausgeleuchtet. Die Vertreter der Produktionsbetriebe, der Grossverteiler und des kantonalen Vollzuges äusserten sich über ihre Erfahrungen mit und in bezug auf die Anwendbarkeit der Toleranz- und Grenzwerte. Allgemein wurde anerkannt, dass es sich um ein brauchbares, die Lebensmittelhygiene sowie die Rechtssicherheit und Rechtsgleichheit förderndes Instrumentarium handelt. Bis auf wenige Ausnahme sind die festgelegten Werte realistisch und praktikabel. Schwierigkeiten ergibt vor allem die Auslegung des kürzlich revidierten Artikels 1, Absatz 4, der in den Augen der direkt Betroffenen erneute Rechtsungleichheit schaffen wird. Als sehr vordringlich werden weitere, produktspezifische Toleranzwerte angesehen. Ebenfalls Unsicherheit herrscht bei der Anwendung der gesetzlich festgelegten Werte im internen Bereich beim Zukauf von Roh- und Zwischenprodukten.

Die Diskussion konnte weitgehend Klarheit bei den verschiedenen Fragen schaffen. Der Begriff des «normalen Masses» und seine Anwendung wurde erläutert. Ebenso wurde festgestellt, dass die für die Verkehrstauglichkeit ausgearbeiteten Toleranz- und Grenzwerte nur erreicht werden können, wenn unter Berücksichtigung der Prozesskette an die Roh- und Zwischenprodukte betriebsinterne und somit strengere hygienisch-mikrobiologische Anforderungen gestellt werden. Abschliessend wurde festgehalten, dass mit der kommenden Revision des Kapitels 56 des Lebensmittelbuches methodisch die Grundlagen gelegt werden, um den Nachweis der Keimgruppen und die Sicherheit der Beurteilung von Lebensmitteln zu verbessern.

ABSTRACTS

A) *Oral presentations*

Guidelines for characterizing immobilized biocatalysts

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Recognizing the need for better information the EFB Working Party on Immobilized Biocatalysts has drawn up new guidelines for characterizing biocatalysts intended for preparative and industrial applications. These guidelines, which will be published in 'Enzymes and Microbial Technology', are the subject of this communication.

Briefly, it is proposed that investigations should contain answers to questions like the following. 1. What is the quantity of the free enzyme (organelle, cell) preparation needed to prepare a unit volume of wet catalyst? 2. What are the dimensions of the wet particles? 3. Are the observed reaction rates diffusion-limited? 4. In which way are the reaction rates affected by changes in concentrations of the reactants in the concentration range of interest and how do these rates compare with those catalyzed by the same quantity of free catalyst? 5. Does the catalyst hold out promise for practical applications in terms of its mechanical (and other relevant) properties and also its stability under conditions of its intended use?

A new versatile computer system for clinical microbiology

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In September 1982 a minicomputer was installed at our department of bacteriology. The hardware includes a Point 4 Data Corporation Mark 5 central processing unit, 2 disk

drives with 80 megabytes each and 2 matrix printers for reports, bills and various statistical and epidemiological listings. The configuration includes provision for up to 64 visual display units (VDU). MICROBE software, written in business BASIC, had been developed by Saturn Computer, Zürich. The system runs 24 h a day, 7 days a week, without any full time operator attendance. There is online access to all results from the last 12 months (approx. 150,000 specimens). When a sample arrives in the laboratory all previous results are displayed and the relevant ones are printed out. The use of VDUs for data input has several advantages over optical mark reading. The greatest of these is the flexibility and ease of updating the system. A new laboratory method, germ or antibiotic can be entered by the user within seconds. The same programs can be used in clinical bacteriology, virology, immunology, serology or parasitology.

Structural analysis of 2 tandemly repeated acid phosphatase genes in yeast

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The structural genes for repressible *(PHO 5)* and constitutive *(PHO 3)* acid phosphatase in *S. cerevisiae* are located on a 5.1 kb Bam Hl fragment in the order (5') *PHO 5, PHO 3* (3') as shown by DNA sequencing.
Comparison of the nucleotide sequences of PHO 5 and PHO 3 led to the following results:
1. The coding regions of the 2 genes show a high degree of sequence homology (> 80%).
2. Sequence homology is completely lost from the position −14 (numbered) from the ATG of the both genes.
3. The mRNA 5'-leader sequences also differe in the length, *PHO 5* mRNA starts at position −40 while the mRNA for *PHO 3* starts at position −124.
4. The amino-acid sequence for the N-termini of the 2 proteins, as predicted from the *PHO 5* and *PHO 3* coding regions, resemble signal sequences which are frequently found in the exported proteins.
The results suggests that the 2 coding regions with a small part (14 bases) of the promoter region arose by duplication of an ancestral gene for acid phosphatase. Also, the divergent promoter sequences may explain the different regulation of *PHO 5* and *PHO 3.*

Broad host range cosmid vectors for efficient cloning of large DNA fragments

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We have constructed cosmid vectors which are useful for the construction of genomic libraries from a broad host range of bacterial species. The cosmids allow selective cloning of 36 kb DNA fragments generated by partial digestion with *Sau* 3A or *Mbo* I into their single *Bam* HI site avoiding formation of polycosmids. The cloning strategy, which has no dephosphorylation step, uses 2 unique recognition sites within the vectors for endonucleases which generate blunt ended DNA fragments for the preparation of left and right cosmid 'arms'. Ligation of the cosmids with the insert DNA is done under conditions which prevent ligation of the blunt ends of the cosmid "arms" Polymers of the cosmid thus cannot be formed and the only molecules of the ligation which can be packaged by λ will be those consisting of a approximately 36 kb insert DNA fragment flanked by one of each cosmid 'arms'. The DNA is first cloned with either vector pMMB33 or pMMB34 into a *rec–E. coli* strain, where clones can be kept stably. They can then be introduced by conjugal mobilization with a helper plasmid into a wide range of gram-negative species including *Pseudomonas, Alcaligenes, Thiobacillus, Rhizobium, Agrobacterium* and *Rhodopseudomonas* to permit the study of gene expression and complementation. Because of the high efficiency of mobilization compared to transformation, the vector and the cloned genes can be transferred between bacterial species which specify different restriction systems where transformation appears to be inefficient. The cosmids have been shown to propagate very stably without selective pressure in *E. coli* and in *Pseudomonas* strains.
Although, mobilization is one of the advantageous characteristics of these cosmid vectors, recombinant DNA regulations demand for certain experiments the use of plasmid or cosmid vectors which have a low frequency of conjugal transfer or mobilization. For such purposes we have constructed mobilization-defective derivatives of the cosmids described above, by deleting a 500-bp segment which is known to contain mobilization genes. The same strategy as described above can be used for the cloning with the mob^- cosmids pMMB38 and pMMB39 and they show the same good stability in *E. coli* and *Pseudomonas putida* as the mob^+ cosmids.

The carry over of antibiotics, a cause for underestimation of the minimal bactericidal concentration (MBC)

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Because we observed discrepancies between MBC determination and timed-kill curves, we explored the possible pitfalls leading to underestimation of the MBC as determined by standard broth dilution. The accuracy of the MBC may be diminished by using low initial inocula leading to insufficient surviving bacteria, and by the carry over of antibiotics which may inhibit the growth of the surviving bacteria when subculvancomycin, cefamandole and clindamycin 23 strains of *Staphylococcus aureus* isolated from blood cultures. The MIC was determined by broth dilution using a 10^5–10^6 overnight inoculum.
We used 2 different techniques to determine the MBC after 24 h of incubation at 35 °C. 1. We streaked on blood agar plates (BAP) 0.1 ml of clear broth, and 2. we spread over the entire surface of a BAP 0.1 ml of a 10^{-1} dilution of clear broth. The results were the following for the 23 strains (inhibition and 99.9 killing for 90% of the strains).

	MIC 90	MBC 90 streak	MBC 90 dilution
Cloxacillin (µg/ml)	0.5	8	128
Vancomycin	2	16	128
Cephamandole	2	16	128
Clindamycine	0.125	8	128

The results show a large discrepancy between MBC's as determined for clear broth by streaking 0.1 ml or by plating

a 10^{-1} dilution over the entire surface of a BAP. These discrepaning are due to the carry over of antibiotics. We found similar discrepancies when determining the MBC by the Microtiter® assay. Furthermore the results show that when tested after 24 h of incubation, all the strains of *S. aureus* were tolerant, confirming findings made by others (Antimicrob. Agents Chemother. *22* (1982) 364.

Agar diffusion test versus MIC and MBC determination

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With regard to introduce the MIC/MBC determination into the routine of a clinical laboratory the results of the agar diffusion test are compared with those of 2 commercial microdilution systems. The agar diffusion test which has been performed twice was well reproducible. The results of only one of the 2 microdilution tests revealed a good correlation to those of the agar diffusion tests. Considerable variations of results depending on the examined antibiotic and/or species have been observed.

Teichoic acid antibodies in *Staphylococcus aureus* infections: Comparison of a new ELISA method with counterimmunoelectrophoresis (CIE)

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A new enzyme linked immunosorbent assay (ELISA) for ribitolteichoic acid (R-TA) antibodies in serum samples form patients with blood culture positive and negative deep-seated *Staphylococcus aureus* infections is discribed. The results are compared with those obtained by counterimmunoelectrophoresis (CIE) (table). Both tests could be useful in the diagnosis and the management of 'complicated' *S. aureus* infections. The ELISA method is, however, more sensitive and reflects the antibody rise after an infection more acurately and more rapidly than CIE.

Diagnostic groups	A *S. aureus* endocarditis	B Other deep-seated *S. aureus* infections	C Uncomplicated *S. aureus* bacteremia	D Gram-positive bacteremias/ septicemias	E Gram-negative bacteremias septicemias	F Healthy controls
No. of cases	4	20	10	26	12	129
ELISA* mean OD (SD)	1.241 (0.182)	0.386 (0.335)	0.249 (0.115)	0.157 (0.146)	0.109 (0.100)	0.141 (0.075)
Agreement with Cie*	100%	100%	90%	92%	100%	100%

* ELISA cutoff OD ≥ 0.488, CIE positive ≥ 1:2.

Statistical analysis: A–B, C, D, E, F: $p < 0.05 - < 0.001$; B–C: NS; B–D, E, F: $p < 0.05 - < 0.01$; C, D, E–F: NS.

Accuracy of 2 non-staining methods determining the gram reaction of non-enterobacterial rods

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The gram stain may yield false results in older cultures of gram-positive rods or in gram-negative diplobacteria. Nonfermenters in both groups may thus be misidentified. We checked the accuracy of the KOH test (Ryu (1938)) and the vancomycin (VA) disc test (Halebian et al. (1981)) for separation of gram-positives from gram-negatives. First, the Kirby-Bauer test with 30 µg VA discs was used on 93 strains of 29 nonfermentative gram-negative rod species (NFGNR). Since some strains in the genera *Acinetobacter* (A.)., *Flavobacterium* (F.), *Moraxella* (M.) and *Eikenella* (E.) showed zones of ≥ 9 mm diameter, 5 µg VA discs (VA-5) (any zone+) and KOH were used on 330 strains of 36 NFGNR, on 53 strains of 8 non-enterobacterial gram-negative rod species, and on 104 strains of 7 genera of gram-positive rods. VA-5 indicated gram-positivity in some *A.* (27%), *F.* (58%), *M.* (71%), *E.* (62%) and *Gardnerella* (100%), and gram-negativity in single strains of *Lactobacillus, Erysipelothrix* and *Nocardia.* MIC determinations showed that VA does not neatly separate gram-positives from gram-negatives; there may also be differences between disc and MIC data. KOH on 24-h cultures indicated gram-positivity in some *A.* (33%), *Achromobacter* V-D (100%), *Agrobacterium radiobacter* (80%), *Pseudomonas extorquens* (43%), *P. paucimobilis* (33%), and single strains of *M.;* and gram-negativity in some *Listeria monocytogenes* (8%), *Bacillus spp.* (11%) and *Corynebacterium* spp. (5%). These percentages showed small changes (mostly increases) with 48-h cultures. We recommend the use of VA-5 and 24-h KOH in doubtful cases.

A modified method for the identification of *Haemophilus influenzae* and *Haemophilus parainfluenzae* using X and V factor impregnated paper strips

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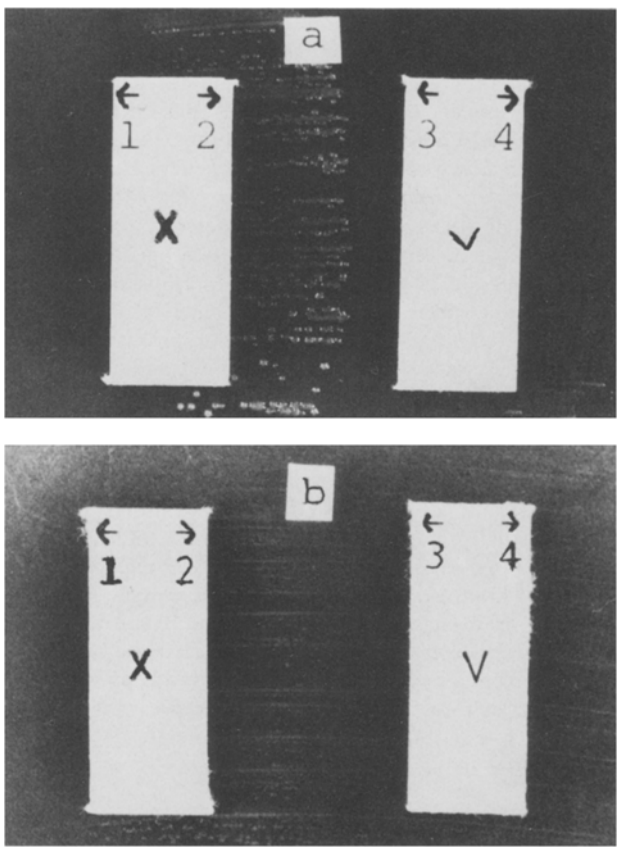
Since use of X and V factor impregnated paper strips are reported to lead to erroneous results, the method was reassessed for possible modifications to ensure its reliability in the identification of *H. influenzae* and *H. parainfluenzae.* A preliminary investigation revealed that the V factor (BBL) diffused much faster than the X factor in Mueller-Hinton agar medium (BBL) generating a characteristic pattern of growth of the *Haemophilus* species (fig. 1). Growth in sector 2 alone of X factor strip (arrow, fig. 1a) and absence of growth in sector 1 of X factor strip and sectors 3 and 4 of V factor strip identified the *Haemophilus* species as *H. influenzae.* These strains failed to grow around a strip supplying either the X or the V factor alone, but they grew around a strip supplying both the factors together. 2% of the isolates examined grew faintly around the V factor strip perhaps with the aid of traces of X factor present in the medium (fig. 1b). However, enhancement of growth progressively towards sector 2 of X factor (arrow, fig. 1b) was discernible by increase in the size of the colonies.

These strains did not metabolize δ-aminolevulinic acid to porphyrins and they conformed to the biochemical reactions characteristic of *H. influenzae*. Empirically, a distance of 15 mm between the strips was ideal to hinder diffusion of V factor past the X factor strip on Mueller-Hinton agar medium streaked with a part of a single colony of the organism from chocolate agar and incubated at 37 °C in CO_2 atmosphere.



Identification of 'atypical' Enterobacteriaceae within 4 hours

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Enterobacteriaceae are often the causative agents of severe of not life-threatening infections. Rapid and accurate identification is, therefore, very important for a laboratory involved in their isolation and identification from clinical specimens. About 5% of the strains cannot unambiguously be identified if tested for 11 biochemical reactions using 6 tubed media. In these cases a gallery API20E is inoculated, requiring a further 18–24 h of incubation and thus causing delay in the reporting of results.

To replace this method by a system which allows identification within only 4 h, 2 systems were evaluated: MICRO-ID (General Diagnostics, USA) and RAPID 20E (API System SA, France). If compared to API 20E both 4-h identification kits exhibited an acceptable identification accuracy of 'atypical' Enterobacteriaceae (85% for RAPID 20E and 77% for MICRO-ID, respectively).

The main difference between the 2 systems was the fact, that MICRO-ID misidentified 17% of the strains tested, whereas RAPID 20E did so in only 1%. On the other hand, no identification was achieved in 14% using RAPId 20E versus 6% with MICRO-ID.

In conclusion RAPID 20E seems to be the more suitable system for the identification of 'atypical' Enterobacteriaceae. However, this judgment must not be applied to the use of the 2 systems for the identification of an average collective of clinical isolates. For this purpose, MICRO-ID has proved very useful.

Organization of nitrogen fixation *(nif)* genes of *Rhizobium japonicum*

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The nitrogenase complex of N_2 fixing bacteria is composed of 2 components. Component 1 is an $\alpha_2\beta_2$ tetramer, and component 2 an α_2 dimer. The 3 consituent polypeptides are encoded by 3 genes: *nifH* for component 2, and *nifD/nifK* for component 1. In *Klebsiella pneumoniae* and 'fast-growing' *Rhizobium* species such as *R. meliloti* these genes are clustered in one operon, *nifHDK*. We present evidence that the organization of the nitrogenase structural genes is completely different in the slow-growing *R. japonicum* strain 110 (the soybean symbiont).

In this organism, the *nifD* and *nifK* genes are on one operon, and are transcribed in the order *nifDK*. Various restriction fragments from this region were cloned and expressed in *E. coli* minicells resulting in the synthesis of the β and α subunits of component 1. Transposon (Tn*5*) insertions in *nifD* are polar on *nifK*. Nucleotide sequence analysis has revealed strongly conserved sections in both genes when compared to respective genes from 2 other N_2 fixing microorganisms. DNA regions on either side of *nifDK* are not coding for functions involved in nodulation and nitrogen fixation. The *nifH* gene is on a separate operon, and is completely unlinked to *nifDK*. Several *nifH*-containing restriction fragments of the *R. japonicum* genome were identified by interspecies hybridization using the *K. pneumoniae* or *R. meliloti nifH* genes as radioactive hybridization probes.

Construction, stability and expression of an artificial yeast tryptophan gene cluster

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The cloning of all 5 tryptophan biosynthetic genes of *Saccharomyces cerevisiae* (*TRP1*, Walz et al., Proc. nat. Acad. Sci. USA *75* (1978) 6172; *TRP5*, Struhl et al., Proc. nat. Acad. Sci. USA *76* (1979) 1035; *TRP2* and *TRP3*, Aebi et al., Curr. Genet. *5* (1982) 39; and *TRP4*, Aebi, unpublished data) made it possible to unify them on a single plasmid pN17 to form an artificial tryptophan gene cluster. The 23-kb plasmid is based on sequences of pBR322 and pJDB207, thus is able to replicate in *E. coli* as well as *S. cerevisiae*. Under selective conditions it is maintained stably in yeast at a copy number of about 10 as estimated from specific activities of the products of genes *TRP2, 3*

and *5*. *TRP1* is expressed only poorly at a twofold increased level compared to the wild type. The regulatory behavior of the TRP-genes is apparently the same regardless if plasmid or chromosomally located: *TRP2, 3, 4* and *5* respond normally to the 'general control'; *TRP1* does not in either case. Yeast strains carrying plasmid pN17 are able to accumulate tryptophan at a considerably higher rate than strains lacking the plasmid.

Functional mapping of a yeast *(PHO 5)* promoter by in vitro mutagenesis

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The promoter of the regulated acid phosphatase gene *(PHO 5)* of yeast *S. cerevisiae* was studied by manipulation of a yeast transformation vector containing the *PHO 5* gene, pBR 322 and the *TRP I-ars I* sequence. After transformation of *pho 5*$^-$, *trp I*$^-$ yeast cells with this plasmids phosphatase activity of the transformants was measured to analyze the effects of specific promoter mutations on *PHO 5* expression. The analysis of various deletions in teh 5'-flanking sequence of the *PHO 5* gene shows, that at least 2 elements in the promoter region are necessary for complete regulation and transcription. A *'upstream element'*, about −200 bp away from the mRNA initiation site modulates transcription, but is not indispensible. A *'downstream element'*, however, which is defined by a 33-bp deletion (−81 to −48) containing the TATA box, is absolutely required for expression.

Developmentally regulated proteases from an aquatic fungus *Allomyces arbuscula*

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Approximately 20–25% of the proteins synthesized in vegetative growth phase are degraded during zoosporangial differentiation. The degradation of proteins begins immediately after the induction of differentiation. 2 types of proteases are induced during differentiation, an extracellular alkaline and an intracellular neutral protease with pH optimum for proteolytic activity at 9 and 7 respectively. These proteases differe in their kinetics of accumulation during differentiation. The accumulation of alkaline protease preceeds the induction of neutral protease and begins immediately after the transfer of the vegetative mycelium to the induction medium. The peak period is attained by 2 h of differentiation at 32 °C when a septum is laid down to delimit the young zoosporangia from the vegetative hyphae. Subsequently the level of the enzyme declines and only a small amount of the activity can be detected by 3 h. The induction of the neutral protease begins after 2 h of differentiation and achieves a peak period by 3 h. Morphologically this period corresponds to the maturation of the zoosporangia, particularly the thickening of their walls. The neutral protease is a zymogen and inhibited in vitro by PMSF (phenylmethyl sulfonyl fluoride), a serine protease inhibitor. Some properties of these enzymes will also be presented.

Sensitivity and specificity of an IgM antibody test (RIA) to ECHO 9 and ECHO 11 viruses

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36 cases from ECHO-virus outbreaks (ECHO 6, 9, 11, 30) were characterized by virus isolation and serum neutralization tests. All sera were then tested for IgM antibodies to ECHO 9 and 11 by MACRIA. Sera from all ECHO 9 and 11 cases were positive in the homologous IgM antibody detecting system when taken 1 week to 2 months after onset of symptoms. Cross reactions in the IgM response between ECHO 9 and 11 sera were observed in many cases. Sera from ECHO 6-infected patients, on the other hand, reacted only weakly if at all, with both ECHO 9 and 11 IgM test systems whereas IgM reactivity of ECHO 30 sera was stronger with the ECHO 9 than with the ECHO 11 system. Furthermore, the varying degree of IgM positivity to ECHO 9 and/or 11 in sera from 31 individual patients with different enterovirus infections is discussed.

Characterization of the immunosuppressive phase in the murine cytomegalovirus (MCMV) infection

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Cytotoxic T, suppressor T and NK cell activities were measured in the same spleen cell preparations in a MCMV susceptible mouse strain (Balb/c). Specific cytotoxic spleen cell activity against Balb/c embryonic cells infected with MCMV was detected by the ^{51}chrom release assay from day 6 to day 15 after i.p. inoculation of 10^5 PFU of the Smith strain (seedvirus obtained by Mims, London). Maximal cytotoxicity peaked around day 8; these findings are in line with reports of Quinnan et al.
For demonstration of the suppressor activity direct and indirect plaque forming cells (PFC) against sheep red blood cells (SRBC) were measured the 4th day after SRBC challenge. In the hemolysis in gel test PFC were more than 100-fold reduced when the SRBC challenge was given at the 4th day after MCMV infection and if MCMV from a salivary gland homogenate was used. No reduction of PFC was observed using the same virus strain but after passage in vitro 12 times in Balb/c primary embryonic cells. No substantial difference between mock and MCMV infected mice could be shown with Yac-1 cells as targets for NK activity. This mouse model will be used to study selected aspects of immunmodulating therapy.

Some properties of a new enveloped RNA virus (Bern virus) isolated from a horse

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A cytopathogenic virus was isolated from a horse, which could not be classified with any other known equine virus. Roughly spherical particles, measuring about 120–140 nm in diameter, were seen in electron micrographs of negative-

ly stained purified viral preparations. The particles were shown to consist of a core with an apparently helical symmetry and a membrane carrying drumstick-shaped projections about 20 nm in length.
In thin sections of infected equine cells, a rod-, crescent- or ringshaped core could be visualized within the envelope. The core measured about 23 nm across and 104 nm in length. Shapes indicative of virus-budding on the plasma membrane were observed.
The virus banded at a buoyant density of 1.16 g/ml in sucrose gradients. It was inactivated by organic solvents. An RNA-genome was assumed since virus growth was not affected by iodo-deoxyuridine. Replication of the virus in presence of actinomycin D or α-amanitin as well as UV pretreatment of the cells used for virus propagation, resulted in a marked reduction of virus yields, suggesting a dependence of virus replication from host cellular functions.
Serological examination, performed on several hundred horses, showed a widespread distribution of neutralizing antibodies against the Bern isolate in the Swiss horse population. Antibodies were also found in cattle, goats, sheep and pigs, but not detected in dogs, cats or humans.
From all the animal viruses tested, only the Breda virus, a pathogenic agent from diarrheic calves, recently described by Woode et al. (Vet. Microbiol. *7* (1982) 221), showed a serological relationship with the Bern virus.

Function of nonstructural poliovirus protein

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It was shown previously (Bienz et al., Virology *100* (1980) 390) that poliovirus RNA synthesis is associated with membraneous vesicles, the same vesicles which are known to represent virus-induced cytopathology (cytopathic effect, CPE). Sequestring the viral RNA synthesis in vesicles seems necessary in view of the unspecific action of the viral RNA polymerase P3-4, which is able to copy a variety of different viral and cellular RNA's (Tuschall et al., J. Virol. *44* (1982) 209).
Isolation and purification of the virus-induced vesicles by isopycnic sucrose gradient centrifugation followed by PAGE revealed that 5 nonstructural viral proteins are vesicle-associated: P2-5b, P3-4, P3-6a, P3-7c, P3-9. Disruption of the vesicles by treatment with various concentrations (0.04–0.64%) of Na-deoxycholate (DOC) showed that P3-9 is a true intrinsic membrane protein, whereas P3-4, P3-7c and most of P3-6a are found free within the vesicles. P2-5b, together with a small amount of P3-6a remains associated with a DOC-resistant complex of 100–150 S. In order to learn more about the functions of the vesicle-associated viral proteins, posttranslational cleavage of the viral polypeptides was inhibited to various extents, using 3 different concentrations of $ZnCl_2$. It could be shown that virus-induced vesicle formation always coincided with the production of P2-5b. P2-5b is therefore proposed to be the protein responsible for the induction of the vesicles representing both, site of viral RNA synthesis and picornavirus-induced CPE.

Autocontrol in hospital hygiene

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The effectiveness of hospital hygiene depends on a systematic approach. This can be divided into the following three:
1. The general services (kitchen, laundry, cleaning, circulation, etc.).
2. The nursing care units, specialized or nonspecialized.
3. The immediate environment of the patient.
To control the efficiency of the recommended procedures, it is sufficient to take bacteriological samples. The practice of carrying out repeated specimens by the hospital's hygiene service is expensive. Its effectiveness has been discussed.
The introduction of an autocontrol system using a slide agar medium like the one used in food industry permits to control the correct practice by the personell themselves.
The concept of responsible hygiene practice in each service should be developed.
The autocontrol with the slide agar has been compared with the classic Rodac plates. The statistical analyis of the 2 methods has shown a correlation between the two. The are both precise.
An evaluation based on the quantitative result has been proposed:
0–25 col./17 cm^3 = good, 26–50 col./17 cm^3 = fair, 51–100 col./17 cm^3 = unacceptable, 100 col./17 cm^3 = dangerous.

Simplified proof of cross-infections by *Klebsiella* by means of klebsin determination

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A practical method is described in which klebsin production induced by mitomycin-C in klebsiella strains is verified with the help of indicator strains.
The following details have to be considered: The warm overnight culture is diluted with equal parts of mitomycin solutions room-tempered at a concentration of 2 µg/ml. By means of a Pasteur pipette this solution is inoculated in one stroke on the center of the agar (Difco Yeast-Bacto-Tryptone-Agar).
After overnight incubation at 30 °C the grown inoculation stroke is carefully scraped off with a microslide and the culture is wholly destroyed with a chloroform-soaked blotting paper. Subsequently the 16 indicator strains from a log-phase broth culture are added on by means of a loop, in a cross-line to the previous inoculation stroke of the patient strains. After a further overnight incubation at 37 °C an inhibition of growth of the strains serving as indicators for the patient inoculation stroke is observed.
This method is useful for an immediate typification of *Klebsiella*.

Cross-infection by *Serratia marcescens* in a newborn unit

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An infant was transferred to a hospital in Bern because of its cardial disease.
Already in the bacteriological control at the admission we discovered *Serratia marcescens* on the anal swab of this baby. 10 days later the infant died of a generalized *Serratia* infection.
At that time, the *S. marcescens* caused local infections in 9 other cases in the newborn unit. After having typified the *Serratia* by bacteriocin tests, we found identical types in those 9 cases. We also found this type of *Serratia* on hands and aprons of 2 nurses who cared for those babies.
Because we also found other strains of *Serratia* we concluded that only by means of typification a cross-infection and its routes of infection can be ascertained.

A practical method for testing of patient strains of *Escherichia coli* in cross-infection as colicin producer respectively indicator

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E. coli can be a nosocomial infections agent. Therefore it is necessary to have a pratical method for typing. We propose the following steps for the investigation:
First the ability for colicinproduction without and with mitomycin C induction (1 mcg/ml) is proved by testing with the colicin-sensitive ROW strain of Fredericq. The result shows whether the patient strain is producing a colicin constitutively or inductively or whether the strain is colicin-negative.
The strain with a constitutive colicin production can be grouped by testing against different colicin-resistant ROW mutants. This allows the colicin-receptor analysis.
The strain with a constitutive colicin production has to be tested against other ROW-mutants which are resistant for the inductively formed colicins.
The colicin-negative strains are investigated for the spectrum of colicin sensitivity with 13 monocolicinogenic strains received from Šmarda, producing the following individual type of colicins: A, B, D, E1, E2, E3, G, H, Ia, Ib, K, M, V.
By this test a typing of coli germs is mostly possible.

Atypical mycobacterioses. Case reports

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The clinical aspects of infections with *Mycobacteria* other than tuberculosis (MOTT) are well-known but infrequently observed. 4 cases of mycobacterioses with their particular pitfalls are presented. 2 chronic pulmonary diseases caused by *M. kansasii*, one cutaneous infection due to *M. fortuitum-chelonei* with relapsing abscess forming, one osteitis of the sternum after open heart surgery, where contrary to expectations *M. tuberculosis* typus humanus was isolated. It probably originated from a fibrinous pericarditis which was observed but misinterpreted during the operation.

B) *Posters*

1. Virology

Contribution to the characterization of rabies street virus

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A sufficient dose of NYC rabies virus obtained from canine salivary glands killed 100% of experimentally infected cats, approximately 70% of which showed the aggressive form of the disease. Virus titre and cat mortality remained unchanged after 7 passages in mouse brains, but the aggressive form of rabies disappeared almost completely.
Strain NYC virus is poorly neutralized by serum antibodies in dogs, cats and cattle. These antibodies can be induced by various strains of vaccine virus.
As virus neutralization is essential for protection against infection, high antibody titres following vaccination of animals are of great impoartance for effective prophylaxis.

Bovine herpes virus-1 (BHV-1) shedding in the milk during the acute and latent state of infection

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4 lactating, serologically negative dairy cows were infected with IBR (infectious bovine rhinotracheitis, BHV-1) virus by the intranasal route.
All animals became seriously ill and showed typical IBR symptons. IBR antibodies could be demonstrated in both blood and milk from the 8th day post infection (p.i.) onwards.
IBR virus could be isolated from nasopharyngeal mucus up to the 8th day p.i.
The presence of virus in the milk of infected cows could be demonstrated indirectly by showing seroconversion in calves drinking the milk; the calves themselves remained free from symptoms.
During latency (6 months p.i.) all cows were given dexamethasone to reactivate the IBR infection. In order to increase the leucocyte yield from milk, oyster glycogen was infused into one quarter of the mammary gland.
IBR virus was isolated from milk leucocytes over several days.
Furthermore, BHV-1 virus was found in nasopharyngeal mucus for between 4 and 7 days; one animal shed also virus in vaginal mucus.
Our results indicate that milk of BHV-1 infected cows given to serologically negative calves may play a role in IBR epidemiology.

Induction of rheumatoid factor by viral infections and *Toxoplasma gondii*

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There are now several observations, that rheumatoid factor (RF) may be associated with certain infections. This could be for some importance for the understanding of pathogenesis.

We were therefore interested in the following questions: 1. Can RF be induced by infections? 2. Is RF more often induced by some infections than by others? 3. Does RF decline again after infection? 4. Of which immunogobulin class is this RF?

IgM-RF was measured by agglutination of IgG-coated Latex particles (Behring), IgM-, IgG- and IgA-RF by ELISA.

We made the following observations: 1. RF-Activity in sera of patients with acute infections is generally lower than in those of patients with rheumatoid arthritis. 2. RF can be induced by some infections. It is very often induced by Cytomegalovirus (50%) and Epstein-Barr virus (42%), less often by hepatitis-B (25%) and hepatitis-A (22%) virus. In rubella, mumps and *Toxoplasma gondii* RF is as frequent as in blood donors (6%). 3. RF is normally measurable at the same time as specific IgM appear and decline either below detectable levels within 8 months or persist for longer. 4. In RF-inducing infections the ratio of RF-IgM:RF-IgG:RF-IgA is 6:1:1, which is not significantly different from the ratio found in blood donors.

Purification of SFV components

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Twice purified Semliki Forest Virus (sucrose and tartrate gradients) was disrupted in a complete denaturing buffer composed of 8 M urea, 0.5% triton X-100, 5% acetic acid and 1 mM dithiothreitol and applied to a column (6×60 mm) packed with the cation exchanger SP-trisacryl and preequilibrated with the same buffer. The RNA, neutral and phospholipids elute from the column with the starting buffer. The membrane proteins elute with sodium chloride (NaCl) gradients of different steepness in the following order: E_3 at 100 mM, E_1^1 at 220 mM, E_2 at 245 mM, E_1^2 at 265 mM end E_1^3 at 285 mM NaCl. The last protein to elute from the column is the core protein which is recovered at 450 mM NaCl. The separation method is very sensitive and offers the opportunity for using a wide range of gradients to achieve dissociation and purification of individual proteins contained in the virion. Furthermore, since it is a 1-step purification with the RNA and the basic core protein forming the 2 extremes of the separation, trace amounts of any protein present in the virion does not escape detection.

Syncytium formation in SFV infected *Aedes albopictus* cells is a 'fusion from within'

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Aedes albopictus cells infected with Semliki Forest Virus can be induced to form syncytia by lowering the pH of the culture medium to 6.0. Fusion of the cell-cell plasma membranes can only occur in infected cells and in addition not prior to 8 h post infection. These observations favor and infer that a 'fusion from within' is responsible for coalescence of the plasma membranes. To verify this, we treated the infected cells with monensin which instantaneously prevented cell fusion. Monensin does not prevent binding of exogenous virus, thus a 'fusion from without' can be excluded as the mechanism for syncytium formation. Similarly, fusion of the cells can be prevented with the inhibitors cycloheximide which suppresses protein synthesis, and also tunicamycin which suppresses glycosylation of viral membrane proteins. Tunicamycin prevents cleavage of the membrane protein precursor p62, although the other viral proteins E_1 and core protein are processed properly. However, the precursor p62 is processed in the presence of the protease inhibitors TPCK, TLCK and PMSF which also prevent fusion. These findings show that syncytium formation is a 'fusion from within' and is governed by an (endogenous) protease and eventually by correct cleavage of p62.

Isolation of a goat retrovirus related to Maedi and Visna virus of sheep

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Several virus isolations have been made by direct cultivation of synqvial and udder cells from goats with clinical symptoms of carpitis/pericarpitis and indurations of udder respectively. Syncytium formation became apparent after one to several subpassages.

Electron microscopical studies revealed viral particles budding on cell membranes or lying extracellularly as C-type particles with a diameter of about 110 nm.

These findings indicated to a retrovirus. Demonstration of reverse transcriptase activity in supernatants of infected cell cultures proved the presence of a retrovirus.

Examination of sera from naturally infected goats in agargel immunodiffusion test resulted in reactions of identity with sera from Maedi and Visna sheep, which on their part are cross-reacting with the caprine-arthritis-encephalitis virus (CAEV) as described by Narayan et al., J. gen. Virol. *50* (1980) 69. Therefore our virus may be related not only to Maedi and Visna virus but also to the CAE virus.

Apparent C1q binding patterns in 3 viral infections and in toxoplasmosis

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C1q, a subcomponent of the first component of complement, is a widely used reactant for the detection of circulating immune complexes. However, C1q has not only a reaction site for complexed IgG but also binding sites for molecules other than complexed igG. In the fluid phase ^{125}I-C1q binding assay (C1q-BA) interaction of C1q with

molecules other than complexed IgG can result in complexes large enough to be precipitated by 2.5% polyethyleneglycol wrongly interpreted as mediated by circulating immune complexes. When a lactoperoxidase and a chloramine T iodinated C1q (LPO- and CT-^{125}I-C1q) are used simultaneously with the C1q-BA (extended C1q-BA), an immediate distinction between binding of C1q to complexed IgG or molecules as heparin, fibronectin, fibrinogen or bacterial endotoxins becomes possible (Spaeth and Buetler, Experientia *38* (1982) 1375). Based on these findings, we tested serum samples from 4 infectious diseases for the presence of various C1q binding activities. Apparent binding patterns in serum samples from toxoplasmosis (TO) or cytomegalo (CMV), mumps (MV) and Epstein-Barr (EBV) viral infections were divided into 4 categories; I: no C1q binding material in the samples present; II: C1q binding material only of the complexed IgG type present; III: C1q binding material of the immune aggregate mixed with the nonimmune aggregate type present and IV: C1q binding material only of the nonimmune aggregate type present. The following apparent C1q binding patterns were observed for TO: 27.1% of samples in I, 35.8% of samples in II, 7.1% of samples in III and 30% of samples in IV; for MV: 50% of samples in I, 6.3% of samples in II and 43.7% of samples in IV; for CMV: 17.6% of samples in I, 23.5% of samples in II, 35.3% of samples in III and 23.6% of samples in IV; for EBV: 4.1% of samples in I, 36.4% of samples in II, 36.4% of samples in III and 23.1% of samples in IV. We conclude that using a conventional C1q-BA a non-immune aggregate mediated C1q binding might be observed frequently in various infectious diseases. This may have led to some misinterpretations in the past. Using the extended C1q-BA this possibility is now eliminated.

The variability of HAV strains isolated in far apart geographical regions

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Circumstantial evidence suggests that hepatitis A viruses isolated in far apart regions of the world are nevertheless antigenically closely related. To investigate this relationship in more detail, 7 HAV strains were propagated in cultures of heteroploid human hepatoma-derived PLC/PRF/5 cells and diploid human lung fibroblasts (MRC 5). Thereby high yields of viral antigens were obtained. Replication of individual HAV strains in one and the same type of cell culture varied considerably. The genetic variation which may be expected for HAV as a member of the picornavirus family was investigated by 1- and 2-dimensional PAGE of RNase T_1-resistent oligonucleotides of the viral genomes. 1-dimensional analysis of ss-RNA labeled in vivo was not found suitable. In contrast, 2-dimensional analysis of viral RNA extracted from highly purified mature virus particles revealed distinct differences between the genoms of isolates from Germany and Costa Rica. Wether these differences are also indicative for variation at the level of the immunochemical properties of viral protein is under investigation.

The replication of hepatitis A virus in a human diploid cell line (MRC-5)

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Hepatitis A virus (HAV) infection of MRC-5 cells doesn't lead to visible cytopathogenic changes but seems to result in a persistant type of infection. There is also evidence that the replication cycle of the virus is more extended than would be expected for a typical member of the picornavirus group. This has made replication studies of HAV difficult and laborious.

In order to characterize the different steps leading to the production of progeny hepatitis A virions, we have elaborated a sensitive RIA test for the rapid detection of de novo synthesized hepatitis A antigen (HAAg). By this means, the dynamics of HAAg production as well as the relationship between infectious virus and viral antigen could be considered.

Furthermore, immunofluorescent studies were undertaken to determine the distribution of HAAg in the cell system as well as within the individual cell over a period of several weeks. The findings were correlated with biochemical events such as synthesis of viral and cellular RNA and protein, respectively.

Enhancement of zoster-specific immune responses in higher age groups by boosting with varicella vaccine

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In an experimental trial elderly volunteers were vaccinated with live attenuated varicella vaccine (OKA strain) with a view to the possible prevention of zoster disease by boosting with attenuated varicella-zoster virus.

In our test persons VZV-specific humoral antibodies and cell-mediated immune reactions (lymphocyte transformation test) were measured before and 1–2 months after the booster.

All subjects aged between 55 and 65 had pre-existing measurable antibodies to VZV, but in 20% no VZV-specific cell-mediated immune response was detectable.

After boosting more than 50% of our vaccinees showed an increase in humoral VZ antibodies as evidence of some vaccine virus replication.

In 28/33 volunteers with a negative cell-mediated immune response the booster vaccination induced a change to a positive CMI response to the VZ antigen.

Mechanism of action of type I interferon on influenza virus replication in macrophages bearing the resistance gene *Mx*

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The gene *Mx* confers a specific resistance towards influenza virus infection in mice. The specific resistance is mediated by interferon (IFN). *Mx*-bearing macrophages cultivated in vitro for 2–3 weeks and treated with homologous type I IFN develop a more efficient antiviral state towards influenza viruses than do non-*Mx*-bearing macrophages.

Type I IFN and the gene *Mx* do not affect attachment, penetration and uncoating of influenza virus (for review, see Haller, O., Curr. Top. Microbiol. Immun. *92* (1981) 25). Therefore we have studied the effect of IFN on the next steps in virus replication, namely primary transcription and translation.

Primary transcription of influenza viruses occurs in the presence of cycloheximide (Ch). Upon removal of the drug the translation of primary transcripts takes place. Therefore it was possible to compare the effect of IFN on these early steps of viral replication in macrophages of resistant A2G (Mx/Mx) mice and of susceptible Balb c (+/+) mice.

Using 40 or 400 NIH U/ml type I IFN the amount of primary transcripts represented 60% and 40% respectively of that found in untreated cells, both in (Mx/Mx) and (+/+) macrophages. In vitro (reticulocyte lysate) these primary transcripts directed the synthesis of viral proteins. The extent of viral protein synthesis correlated well with the amount of primary transcripts. In vivo however, IFN-treated (Mx/Mx) macrophages gave rise to only 6% and 1% translated viral proteins as compared to non-IFN-treated macrophages. In contrast IFN-treated (+/+) macrophages gave rise to 60% and 53% translated viral proteins.

In conclusion, the antiviral state in macrophages carrying the IFN-dependent resistance gene *Mx* affects specifically the translation of influenza virus transcripts.

BHV-1: genome analysis and growth characteristics in cell culture systems

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Bovid herpes viruses type 1 (BHV-1) are causing 2 distinct clinical syndromes, namely a respiratory one (BR, infectious bovine rhinotracheitis) and a genital one (IPV, infectious pustular vulvovaginitis).

With respect to their biological and serological behavior it was not possible to differentiate the 2 virus types. Only on the grounds of restriction enzyme patterns of the DNAs of various IBR and IPV virus strains we were able to differentiate the 2 groups. As a further step the restriction sites on the BHV-1 genomes were mapped using the following strains: IBR'LA', IPV K22, the IPV vaccine strain B IV and its wildtype origin B I. The physical maps (EcoRI and HindIII) of the DNAs show differences in 2 internal regions or a deletion at an endfragment.

Furthermore the growth characteristics of the above mentioned IBR and IPV virus strains in different cell culture systems were examined.

Rotaviral RNA digesting activity in human feces

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During an epidemiological study of rotavirus strain distribution typical dsRNA segment pattern of rotavirus genomes could easily be demonstrated by gel electrophoresis and staining with EtBr in 50% of the ELISA- and RIA-positive stool samples.

Missing dsRNA segments in the remaining antigen positive samples could be explained by either too low a concentration of intact rotavirus, a relative abundance of empty capsids as demonstrated by viral particles sedimenting at lower density in CsCl gradients (≤ 1.3 g/cm^3) or by an activity digesting viral dsRNA. This activity destroyed rotaviral dsRNA in other stools as well as dsRNA of isolated tissue culture grown rotavirus (NCDV).

Rotavirus specific antigens from RIA-positive stools with no detectable dsRNA segments could be demonstrated by the western blot technique.

Comparative electrophoretic analysis of cytomegalovirus-infected cells

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Electrophoretic mobilities of polypeptides extracted from human embryonic lung fibroblasts (HEL) infected with human cytomegalovirus (CMV) were compared on 10% SDS polyacrylamide gel slabs. Low and high passages of isolates were compared with the well-adapted AD 169 strain. The infected monolayers were rinsed 3 times with 0.02 M Tris-HCl pH 7.5 and solubilized in SDS and B-mercaptoethanol; 60 µl were applied to the gel. The AD 169 infected cells showed 3 major bands which were occasionally present as faint ones among the low passage wild viruses. On the other hand these isolates disclose minor differences between their electrophoretic patterns. After 21 in vitro passages the polypeptide profile of one isolate became different from that of its low passage and developed a pattern close to that of AD 169. The electrophoretic analysis of CMV infected monolayers can be made much earlier than that of purified virus since the latter can only be obtained after some degree of cell adaptation and modification.

Analysis by SDS-PAGE of proteins induced by different strains of bovine herpes virus-1 (BHV-1)

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Infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) are different clinical manifestations of an infection with BHV-1. Attempts at defining differences in biological markers, such as morphology, antigenic relationship and replication in cell cultures have failed. However, Engels et al. (Arch. Virol. *67* (1981) 169) have shown, that strains of IBR and IPV virus could be differentiated on the basis of an analysis of viral DNA with restriction enzymes. We therefore attempted to correlate this observed differences with the proteins induced by IBR and IPV virus strains. 6 IBR and 6 IPV viral isolates were metabolically labeled with ^{35}S-methionine, and viral proteins were analyzed by comparative SDS-PAGE. IBR and IPV viruses induce 25 structural and several nonstructural proteins, ranging in mol.wt from 15,000 to 275,000 daltons. The overall protein pattern among different viral strains was very similar. 5 viral strains from which restriction enzyme data are available could be grouped into IBR and IPV based on small differences of the mol.wt of proteins with approximately 110, 100, 87, 69, 55 and 48 kilodaltons,

respectively. 7 other strains of BHV-1, which have been defined as IBR or IPV depending on their origin (respiratory tract: IBR, genital tract: IPV) do not invariably fit into the differentiating scheme. This observation can be interpreted in 2 ways: 1. some of the observed differences are rather strain then type specific and/or 2. the origin of isolation is not a true key for typing an isolate of BHV-1 as IBR or IPV. Further analysis of both viral proteins and viral DNA will help at defining proteins or protein domains with type-specific reactivity.

The Sendai virus hemagglutinin-neuraminidase (HN) and fusion (F) glycoproteins bind to different surface receptors in mouse spleen cells

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The penetration of Sendai virus is mediated by 2 glycoproteins, HN and F. HN is thought to anchor the virus to the cell surface while F is crucial for the fusion between the viral and plasma membranes. To investigate the receptor structures for HN and F, we have reconstituted liposomes from phosphatidylcholine and HN or F and studied their interaction with mouse spleen cells using the technique of chemiluminescence (CL) measurement. Both the HN- and F-liposomes stimulated CL, indicating that the glycoproteins are able to interact with the cell membrane independently of each other. Monoclonal anti-HN and -F antibodies elicited a CL response in cells that had been pretreated with HN- and F-liposomes, respectively, showing that F does not require HN for binding to the cell surface and vice versa. The presence of F-liposomes on the cell surface was also confirmed by immunoelectron microscopy. Cells pretreated with HN- and F-liposomes revealed a different pattern of CL when challenged with intact virus or the calcium ionophore A23187 indicating that the glycoproteins bind to different receptor sites.

2. Genetics and Biochemistry

Evolution of new bacterial enzyme activities during adaptation to azo dyes

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Longtime adaptation of a *Pseudomonas* sp. (strain KF1) growing on 4,4′-dicarboxyazobenzene (DCAB) to growth on more complex azo dyes of the Orange I and Orange II type resulted in the highly substrate specific strains K24 and KF46. In these organisms azoreductases of different specificity initiate the catabolism of azo dyes by catalyzing the reductive cleavage of the azo linkage with NAD(P)H as a cosubstrate. Purified Orange I azoreductase from strain K24 and purified Orange II azoreductase from strain KF46 reacted only with homologous antisera prepared against each of the pure enzymes. Crude extracts of strains K24, KF46 and of azoreductase-negative strains isolated at different stages of the adaptation experiments, however, contained material crossreacting (CRM) with both antisera. Further studies on the immunological relatedness of azoreductases suggest that the enzymes present in strains K24 and KF46 have evolved from a common precursor.

Further experiments aimed at extending the substrate range of strain KF46. Adaptation in the chemostat of this organism, which grows on carboxy-Orange II (1-(4′-carboxyphenylazo)-2-naphthol), to growth on carboxy-Orange I (1-(4′-carboxyphenylazo)-4-naphthol) led to strain KF808. Strain KF808 utilized both, carboxy-Orange II and carboxy-Orange I, and contained the Orange II azoreductase originally present plus a new type of Orange I-specific enzyme activity. This newly evolved enzyme was oxygen-sensitive and differed in its electrophoretic mobility from the azoreductases characterized so far.

Localization of DNA sequences homologous to the delta-endotoxin gene in 3 different *Bacillus thuringiensis* varieties

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DNA sequences homologous to the delta-endotoxin gene were localized in *Bacillus thuringiensis* var. *kurstaki*, var. *galleriae* and var. *aizawai*. Total cellular DNA and purified plasmid DNA from each variety were used for southern blot hybridization. A cloned DNA sequence from *B. thuringiensis* var. *kurstaki* coding for a biologically active delta-endotoxin protein was used as a radioactive probe.

Cla I digests of total cellular and purified plasmid DNA were hybridized and compared. Var. *kurstaki* and var. *galleriae* showed hybridization in both DNA fractions. In var. *aizawai* hybridization was found only to total cellular DNA. The additional hybridizing bands in the total cellular DNA fractions could be ascribed to chromosomal delta-endotoxin gene copies or gene copies on large plasmids that were lost during plasmid DNA isolation. To have further evidence that the hybridizations in the plasmid DNA fraction of var. *kurstaki* and var. *galleriae* was not due to contaminating chromosomal DNA, undigested plasmid DNA of these 2 varieties was hybridized and shown to contain delta-endotoxin gene homologous DNA sequences on distinct plasmids. An acrystalliferous plasmid-free mutant strain of var. *kurstaki* showed no homologous DNA sequences in the chromosome. 2 strongly crosshybridizing Cla I fragments of var. *galleriae* and one of var. *aizawai* comigrated with Cla I fragments of var. *kurstaki* that are known to be part of the delta-endotoxin structural gene.

Characterization of a plasmid from *Methanobacterium thermoautotrophicum* Marburg

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The archaebacterium *Methanobacterium thermoautotrophicum* Marburg (DSM 2133) was found to contain a plasmid (pME 2001) in covalently closed circular form. It was isolated by CsCl gradient centrifugation of total DNA in the presence of ethidium bromide. Multimers up to the

hexamer were observed upon agarose gel electrophoresis and electron microscopy of a purified plasmid preparation. A restriction map was constructed. The length of plasmid pME 2001 was determined to be approx. 4500 bp. Southern hybridization of plasmid DNA to DNA extracted from *M. thermoautotrophicum* ΔH (DSM 1053) revealed the presence of a plasmid with homologous sequences in the ΔH strain. Total DNA from *Methanococcus vannielii* did not hybridize to plasmid pME 2001. The quality and the yield of plasmid DNA from *M. thermoautotrophicum* Marburg were independent of the growth medium utilized, of the growth phase of the bacterial cells and of the colony type of the inoculum used in the mass cultivation of cells.

Expression of an *argA*$^+$ recombinant plasmid in a recombination-deficient host strain of *Pseudomonas aeruginosa*

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To study the expression of cloned *Pseudomonas aeruginosa* DNA in *P. aeruginosa* we developed a method for the construction of recombination-deficient strains of this organism. The *recA*-like mutation *rec-102* was mapped by conjugation near *argF*, in the 45-min region of the PAO chromosome map. An R68.45 donor *[rec-102]* was used to introduce the *rec-102* locus into an *argA* mutant of *P. aeruginosa*. The resulting strain was used as a host for pME152, a recombinant plasmid carrying the *argA*$^+$ gene of *P. aeruginosa* on a 12-kb fragment inserted into the broad-host range, multicopy vector pKT240 (M. Bagdasarian, unpublished). The plasmid pME152 was highly unstable in both *rec-102* and *rec*$^+$ *P. aeruginosa* and gave spontaneous deletion derivatives having lost part of the insert. One such derivative, which was *argA*$^+$ and stable, was kept and called pME150. The level of the *argA*$^+$ enzyme *N*-acetylglutamate synthase was 25 times higher in strain PAO *rec-102* (pME150) than in the PAO1 wildtype. The recombinant carrying pME150 grow more slowly in minimal medium than did the wildtype, and this growth differential was abolished by the addition of arginine to the medium.

Plasmids of soil bacteria mineralizing azo dyes

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Azo dyes are recalcitrant in aerobic wastewater treatment plants. From bacterial strain KF4 strain KF46 utilizing carboxy-Orange II (1(4′-carboxyphenylazo)-2-naphthol) as the only carbon and nitrogen source was obtained after long periods of adaptation in the chemostat. This adaptive step was paralled by the acquisition of an inducible NAD(P)H-dependent Orange II azoreductase initiating the catabolism of carboxy-Orange II. The following observations suggest an involvement of plasmid-coded genes in carboxy-Orange II degradation: 1. Utilization of carbox-Orange II and azoreductase activity were lost by more than 90% of the cells in cultures of strain KF46 grown at elevated temperature. 2. Strains KF4, KF46 and KF47, a temperature-cured derivative of strain KF46, harbored a 120-kb plasmid as well as a 2nd plasmid whose size was approximately 290 kb in the carboxy-Orange II negative strains KF4 and KF47 and 375 kb in the carboxy-Orange II positive strain KF46. Restriction analysis suggested that the larger form of this plasmid originated by a single insertion into the 290-kb form. 3. Carboxy-Orange II utilization was transferred by conjugation from strain KF46 into strain KF47 at a frequency of 10^{-6} per donor.

Purification of the anthranilate/InGP-synthase complex from *Saccharomyces cerevisiae*

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Anthranilate synthase (AA-synthase, EC 4.1.3.27) and indole-3-glycerol-phosphate synthase (InGP-synthase, EC 4.1.1.48), the first and the third enzyme of the tryptophan biosynthetic pathway, form in *Saccharomyces cerevisiae* an enzyme complex. An elegant way to facilitate purification of this complex was found by the use of a yeast strain with a multicopy plasmid carrying both the *TRP2* and *TRP3* genes, coding for the 2 polypeptides. In this strain the level of both enzymes, and thus also of the complex, is increased about 50-fold compared with the wildtype strain. A 3-step purification (chromatography on ethylaminosepharose, hydroxylapatite and DEAE-sepharose) lead to a further enrichment of the complex by about 40-fold; the total enrichment was thus about 2000-fold. Electrophoresis on SDS-polyacrylamid gel yielded 2 bands with the same intensity (Coomassie blue staining), indicating equimolar ratios of the 2 peptides in the complex; together they contained more than 95% of total protein loaded. The lower band, with a mol.wt of 58 kd, was identified by several methods as InGP-synthase, the upper band with a mol.wt of 64 kd as AA-synthase. The mol.wt of the purified complex was about 130 kd as determined by sucrose gradient centrifugation and gel filtration. Antibodies were raised against the pure InGP-synthase polypeptide.

Interferon-induced guanine nucleotide-binding proteins in cells from different inbred mice

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Interferon (IFN)-induced quanine nucleotide-binding proteins (GBP) of different mouse strains were compared. After labeling of cultured cells from different mice with ^{35}S-methionine, the GBPs were enriched from the cytoplasmic extracts by affinity chromatography, analyzed electrophoretically and visualized by fluorography. 5 GBPs with apparent M_r 55,000, 65,000, 70,000, 71,000 and 78,000, respectively, were induced by IFN in cells from the inbred mouse strains A/J, C3H and BALB/c. In contrast, IFN induced only 4 GBPs with apparent M_r 55,000, 70,000, 71,000 and 78,000, respectively, in cells from the inbred mouse strains A2G, CBA, C57BL/6 and DBA. The major

GBP of M_r 65,000 was lacking completely in cells from the latter group of mice. Depending on genotype, presence or absence of the M_r 65,000 GBP was observed in nondifferentiated/differentiated cells after induction with IFN type I or type II. Endogenous IFN, induced by i.p. injection of poly I:C, led to the synthesis of proteins with guanine nucleotide-binding activity in the spleens of mice. The M_r 65,000 GBP was readily detectable in spleens of A/J, C3H and BALB/c mice, but not in spleens of A2G, CBA, D57BL/6 and DBA mice. Thus, the same strain specific GBP induction pattern was observed in vitro and in vivo.

Aspects of the genomic organization in *Streptomyces glaucescens:* a) Analysis of 2 genes with high mutation rate

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Streptomyces glaucescens is a melanin producing actinomycete. The enzyme responsible for the formation of this pigment is a tyrosinase. The strain produces also the antibiotic hydroxystreptomycin and has a natural resistance to streptomycin-type as well as to aminoglucoside antibiotics. The resistance is due to the inactivation of the antibiotic by a streptomycin specific phosphotransferase.
The ability to produce melanin and the resistance to streptomycin are spontaneously lost with a rather high frequence under laboratory conditions (0.1%), which can be dramatically increased by exposure of the growing organism to the intercalating dye ethidium bromide. To understand the mutationel event, the gene coding for the tyrosinase and the gene coding for the streptomycin phosphotransferase were cloned. Hybridization of the cloned sequences to the genome of streptomycin sensitive (strS) and melanin negative (melC) strains of *S. glaucescens* showed that the mutations are caused by deletions of at least 10 kb, comprehending the structural genes and their flanking sequences.

Aspects of the genomic organization in *Streptomyces glaucescens:* b) Occurrence of highly reiterated sequences

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In *Streptomyces* chromosomes heavy DNA reiterations can occur after protoplast regeneration or after some mutagenic treatments. Ono et al. also reported such a reiterated sequence in an ethidium bromide treated *S. glaucescens* strain (Molec. Gen. Genet. *186* (1982) 106). The mechanism and the physiological significance of these phenomena are still not known.
In various ethidium bromide treated streptomycin sensitive and melanin negative mutant strains of *S. glaucescens* different types of reiterated sequences were found. 12 were investigated further. Their repeating units were of 2.9 to ca. 35 kb in length. They were tandemly repeated and occupied up to one third of the total genomic DNA. Several of them were cloned into the *E. coli* plasmid pBR325 and subjected to structural analysis by means of restriction enzymes and cross-hybridization. It was shown that at least 2 hot regions are present in the chromosome of *S. glaucescens* where various head to tail repetitive DNA replications formed these reiterated sequences. Some of the reiterations were found to be accompanied by nearby DNA deletions. The relationship between genetic instability, spontaneous or induced deletion of specific genes (e.g. streptomycin phosphotransferase and tyrosinase) and the DNA reiteration is under investigation.

Biodegradation and utilization of monomethyl sulfate by specialized methylotrophs

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Methylations in organic syntheses are often carried out with dimethyl sulfate (DMS) as the methyl donor. Usually in such reactions, only one methyl group of DMS is transferred to the methyl accepting group (hydroxyl, mercapto, amino or imino group) and monomethyl sulfate (MS) is formed as a byproduct in stoichiometric quantities. Only in rare cases can MS be used for further methylations.
As an alternative to physical or chemical methods of disposal we searched for MS-degrading microorganisms in order to establish a biological waste treatment process for MS-containing mother liquors.
More than 30 methanol and methylamine degrading methylotrophs from strain collections were tested for MS-utilization and the results were always negative. MS-utilization is not a common property of methylotrophs.
We enriched and isolated specialized methylotrophs utilizing MS (5 g/l) as the sole source of carbon and energy from sewage sludge. 5 pure isolates were taxonomically characterized and identified as *Hyphomicrobium* sp. MS 72, MS 75, MS 219, MS 223 and MS 246. All these strains are facultative methylotrophs showing the following properties: a) substrate spectrum (compounds tested as the sole carbon source): MS, methanol, ethanol, acetate, formate, glucose, methylamine, dimethylamine, dimethyl phosphite, trimethyl phosphite, nutrient broth; b) parameters from fermentation studies with MS as the sole substrate (28 °C): $\bar{g} = 9\text{–}12$ h, $\mu_{max} = 0.05\text{–}0.06\ h^{-1}$, $Y_{X/S} = 0.1$.
MS is first cleaved to methanol and sulfate by a sulfatase and then methanol is utilized as the growth substrate.

Biodegradation and utilization of quaternary alkylammonium compounds by specialized methylotrophs

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Dealkylations in organic syntheses are often carried out with trimethylamine (TMA) as the alkyl acceptor. For example, in such a reaction an ethyl group is transferred from an alkyl donor (e.g. a substituted diethyl phosphate or diethyl thiophosphate) to TMA and a trimethylethylammonium salt (TMEA) is formed in stoichiometric quantities. The regeneration of TMA from TMEA is rather difficult to achieve.

As an alternative to physical or chemical methods of disposal we searched for TMEA-degrading microorganisms in order to establish a biological waste treatment process for TMEA-containing mother liquors.

More than 30 methanol, methylamine or trimethylamine degrading methylotrophs from strain collections were tested for TMEA-utilization and the results were always negative. TMEA-utilization is not a common property of methylotrophs.

We enriched and isolated specialized methylotrophs utilizing TMEA (5 g/l TMEA chloride) as the sole source of carbon, nitrogen and energy from sewage sludge. 9 pure isolates were taxonomically characterized and identified as *Pseudomonas* sp TMEA 14, TMEA 81, TMEA 83, TMEA 84, TMEA 86, TMEA 87, TMEA 89, TMEA 199 and TMEA 211. All these strains are facultative methylotrophs showing the following properties: a) substrate spectrum (compounds tested as the sole carbon source): TMEA, methylamine, dimethylamine, trimethylamine, trimethylamine-N-oxide, tetramethylammonium chloride, ethylmethylamine, ethyldimethylamine, triethylamine, ethanol, acetate, glucose, nutrient broth, methanol (only 3 strains); b) parameters from fermentation studies with TMEA as the sole substrate (28 °C): $\bar{g} = 20$–30 h, $\mu_{max} = 0.023$–0.024 h^{-1}, $Y_{X/S} = 0.34$. TMEA is totally biodegraded and no accumulation of organic intermediates, only the formation of NH_4^+, is observed.

Site-directed mutagenesis of *Rhizobium japonicum*

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We have adopted a broad host range gene transfer system developed by Simon and Pühler (submitted for publication) for construction of site-directed Tn*5*-mutants of the slow growing *Rhizobium japonicum* strain 110*spc*4:

R.japonicum nif DNA was cloned into the mobilizable vectors pSUP101 and pSUP201 and mutagenized with the kanamycin transposon Tn*5*. The recombinant plasmids were transferred into *R.japonicum* using *E.coli* strain Sm10 as donor. Derivatives of the pSUP plasmids do not replicate in *Rhizobium*. Therefore, we expected recombination events between the plasmidial and the genomic *nif*-DNA by selection for kan^R *R.japonicum* exconjugants. The position of Tn*5* within their genome was determined by southern blot hybridization experiments.

Although the majority of the kan^R strains contained the entire recombinant plasmids cointegrated into the homologous genomic region, we could also isolate strains in which exchange of the wildtype *nif*-region against the Tn*5*-containing fragment accompanied by abortive loss of the mobilized plasmid had occurred.

We tested the phenotype of these marker-replacement mutants by plant infection tests with soybeans and measured symbiotic nitrogen fixation in the root nodules.

Three *R.japonicum* mutants containing Tn*5* in the nitrogenase genes *nifD* or *nifK* showed the phenotype Fix^-, i.e. they failed to reduce acetylene in the nodules.

Analysis of reisolates from nodules showed that the position of Tn*5* remained unchanged during the infection cycle.

RNA-polymerase from *Rhizobium japonicum*

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DNA-dependent RNA polymerase (EC 2.7.7.6) from *Rhizobium japonicum* was purified. The subunit structure was found to be $\beta\beta'\alpha_2\sigma$, with the following apparent mol.wts (in daltons) determined by electrophoresis: M_r (β and β') 150,000 each, M_r (σ) 96,000, M_r (α) 40,000, M_r (holoenzyme) 490,000, M_r (core enzyme) 380,000. The recovery of σ was 28%. RNA polymerase from aerobically grown *R.japonicum* cells and from nitrogen-fixing cells have the same electrophoretic properties suggesting that no chemical modification of the enzyme occurs when cells undergo this metabolic differentiation.

The enzyme is Mg^{2+}-dependent, rifampicin-sensitive, and has optimal activity at alkaline pH (8–10) and at 35–40 °C. It binds strongly to bacteriophage T7 promoters, weakly to antibiotic resistance genes, and not at all to cloned *R.japonicum* nif DNA.

Nevertheless, the enzyme is able to transcribe linear restriction fragments containing *nif* genes in an unspecific manner. The transcripts obtained in vitro were useful as hybridization probes to determine the coding strand in single-stranded M13-clones from the *nif* region.

The degradative pathway of cyanuric acid

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Pseudomonas spp. strains A and D and *Klebsiella pneumoniae* strain 99 each grew with cyanuric acid as sole and growth-limiting source of nitrogen (3 moles/mole of substrate compared with NH_4^+ as nitrogen source). Strains A and D grew with concomitant degradation of cyanuric acid, whereas strain 99 sometimes excreted biuret. Only strain D utilized biuret as a source of nitrogen (3 moles/mole of substrate) but each of the 3 strains grew with urea as sole nitrogen source (2 moles/mole of substrate). Ring carbon was released quantitatively as CO_2, at least in strain D.

Crude extracts from each strain degraded cyanuric acid but only strain D reproducibly released 3 moles NH_4^+/mole of substrate; strains A and 99 released small amounts of NH_4^+ and almost quantitative amounts of biuret, which was identified by its cochromatography with authentic material (HPLC), UV spectrum and mass spectrum. Separated enzyme fractions from each strain yielded biuret from cyanuric acid: about 1.2 moles CO_2 and 1.1 moles biuret were observed per mole of substrate. Crude extracts of strain D degraded biuret with transient accumulation of urea, which was detected by reaction with jack-bean urease. The biuret-cleaving reaction could be detected in strain 99, where 1 mole urea and 1 mole NH_4^+/mole of substrate were observed in a separated enzyme fraction. All 3 strains had urease activity. Strains A and 99, at least, were found to degrade cyanuric acid (or precursors) anaerobically.

We propose that cyanuric acid is degraded in 3 hydrolytic reactions via biuret and urea to NH_4^+, each reaction releasing CO_2.

Antineoplastic immunoadjuvant agents

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Stimulation with adjuvants obtained from microorganisms is being considered with interest. Several experimental and clinical data already testify to the antineoplastic efficacy.
Aim of this study was to verify the data in literature and then to study in vivo and in vitro the use of these immunoadjuvants in tumoral pathology.
Killed suspensions of *Corynebacterium parvum* (Welcome Lab.), lysate of *Saccaromyces cerevisia* and of other microorganisms were considered. The in-vitro test used was lymphocyte blastogenesis; the in-vivo test was conducted on albino rats infected with a T8 Guerin atypical viral epithelioma.
It was thus possible to verify data described in literature, and to set up a test in vitro and in vivo to research immunoadjuvant substances originating from microorganisms, to be used in tumoral pathology.

3. Bacteriology

Tetracycline R-plasmids in pig enteropathogenic *E. coli* 0149:K 88ac

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Enteropathogenic *E. coli* 0149, associated with diarrhea in piglets, are frequently isolated in Switzerland.
Our study examines the prevalence of tetracycline R-plasmids in *E. coli* 0149 strains of different origin. In these strains, a 50 Mdal plasmid causing resistance to tetracycline is very common. After digestion with EcoRI, 13 fragments are found in a 0.8% agarose gel. The wide geographical distribution of this plasmid in *E. coli* 0149 underlines the epidemic nature of these pathogenic microorganisms.
4 out of 8 strains examined have lost 1–2 fragments of the typical EcoRI pattern but simultaneously, there was an uptake of new DNA sequences. In these cases, the modified digestion pattern was correlated with an additional resistance to streptomycin and sulphonamide. In one plasmid, the new fragments caused a streptomycin resistance only.
These results show the evolution to which plasmids are subjected in nature.

Antibacterial effects of cefotiam in an in vitro pharmacokinetic system

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The pharmacokinetic system, by means of which all possible time courses of the concentrations of antibiotics in the plasma of treated individuals can be exactly simulated in vitro without diluting the test organisms and affecting the growth curves, has been described elsewhere (J. Antibiot. *35* (1982) 843).

Cefotiam, an injectable broad-spectrum cephalosporin, was tested in the system at concentrations corresponding to the plasma concentrations produced in man by doses of 125, 250 and 500 mg i.m. The standard deviation of the concentrations from 6 experiments in the system was similar (approx. 10% of $\bar{x}$) to the reported standard deviation from 3 volunteers (Chemotherapy *27* (1979) 172). The antibacterial effect of cefotiam against *E. coli* 205 was most pronounced at the concentrations corresponding to the dose of 500 mg; no bacteria were found over the limit of detectability of 10^2/ml after the 3rd h until the end of experiments at 6 h. But the lowest concentrations (125 mg) likewise decreased the viable count (3 log after 6 h). *S. aureus* 10B was less affected (1–2 log) than *E. coli*, as also by penicillins and other cephalosporins (unpublished results). The lowest concentrations (125 mg) were more effective than the concentrations corresponding to 250 and 500 mg (Eagle effect).
Thus in our system, cefotiam was effective against *S. aureus* and *E. coli*, even at concentrations corresponding to the low dose of 125 mg.

A novel site needed for the expression of methicillin resistance in *Staphylococcus aureus*. Mapping and characterization

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A novel site, not linked to the methicillin-resistance determinant *(mec)* of *Staphylococcus aureus*, and mapping between *tyrA* and *thrA* was found by transposon mutagenesis. Inactivation of this site by Tn551 renders methicillin resistant strains phenotypically methicillin sensitive. Various other Tn551 insertions have been described in this region of the chromosome, but none of them has any influence on *mec*.
A finer mapping of the relative position of these various Tn551 insertion sites by restriction analysis and DNA-DNA hybridization was attempted.
Methicillin resistant strains have higher levels of penicillin binding protein-2 (PBP-2), than sensitive strains. The Tn551 insertion mutant, now Mec^s, showed a higher level of PBP-2 than its Mec^r parent. Transfer of this Tn551 insertion to an originally Mec^s strain, results also in an increase of PBP-2 levels. A methicillin sensitive mutant obtained by novobiocin induced 'curing' of a *mec* strain, also showed greatly increased levels of PBP-2. Attempts to map this mutation in the same region as the Tn551 insertion have failed. We assume that the Tn551 insertion affect a regulatory gene and that the structural gene maps elsewhere or vice versa. The role of PBP-2 in methicillin resistant strains remains unclear.

Technical problems in the determination of staphylococcal tolerance to β-lactam antibiotics

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Tolerance of bacteria to β-lactam antibiotics is defined as resistance to the lethal action, but inhibition by normal

concentrations of these drugs. It has been reported that tolerance in staphylococci does occur in 1-100% of isolates. We observed that bactericidal activity ($\geq$ 99.9% killing) was considerably influenced by the growth medium, by the duration of incubation, by the growth phase of the inoculum, by the accuracy of plate counts, and by such variables as storage and passage of cultures or shaking of cultures before and/or after incubation. Clones, which had survived the lethal activity of β-lactams, were either equally, but usually more susceptible to killing than the parent population. Many strains showed the paradoxical phenomenon (Eagle effect) of having more survivors in higher than in lower concentrations. By carefully adjusting the conditions of bactericidal tests, no strain with stable tolerance to β-lactams among 87 staphylococci was observed. Thus, the high frequency of occurrence of tolerant staphylococci reported in some areas might be only a laboratory curiosity. An accepted reference procedure for determination of bactericidal endpoints to establish clinical correlates is urgently needed. For the time being, routine MBC tests on staphylococci with β-lactam drugs should be interpreted with great caution.

Susceptibility of *Neisseria gonorrhoeae* to spectinomycin and thiamphenicol

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Penicillinase producing *N. gonorrhoeae* (PPNG) is spreading throughout the world. A good surveillance system may increase the cases reported if the laboratory test all isolates for penicillinase production.
We report the results of the antibiotic-resistance study of 18 strains isolated locally. They fall into 6 groups, as shown in the table. In group V, one strain was penicillinase producer as was the strain of group VI. There was no other penicillinase producer.
The 18 strains were susceptible to spectinomycin and thiamphenicol.

Distribution of 18 strains of *N.gonorrhoeae* into 6 groups of resistance against 14 antibiotics.

Groups	Resistance to	Number of strains	%
I	0 antibiotic	2	11.1%
II	1 antibiotic	5	27.7%
III	2 antibiotics	2	11.1%
IV	3 antibiotics	6	33.3%
V	5 antibiotics	2	11.1%
VI	6 antibiotics	1	5.5%

Presence of *Cyanidium caldarium* and other microorganisms in the mephitis of Ansanto valley (AV), Italy

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Preliminary results of an investigation on the microflora present in the mephitis of Ansanto valley (AV), Italy, are referred to.
The mephitis is characterized by the production of pseudovulcanic phenomena such as gaseous exhalations of boric acid, hydrogen sulphurated and carbon dioxide. Actinomycetes, bacteria (lithotrophic bacteria) and a unicellular alga, *Cyanidium caldarium*(?) (Tilden) Geitler, have been found in this particular environment.
The morphological and physiological properties of the microalga and its ecological significance were studied with particular interest. The microorganisms found are those which have survived and have adapted to this particular environment.
An exciting hypothesis on the biological role of these microorganisms on the genesis of the geochemical phenomena of Ansanto valley is also discussed.

Marine fungi in the saline Margherita di Savoia (Bari), Italy

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Preliminary results of an investigation of marine microflora in the saline of Margherita di Savoia (Bari), Italy, are referred. The recorded fungi belong to the marine lignicolous fungi *Ascomycetes* and *Deuteromycetes*.
The presence and the frequency of the collected fungi are related to NaCl concentration.
An increase of NaCl concentration from 30‰ to 300‰ provokes a wide selection in number and in species.
A 50‰ concentration of NaCl seems to be a limiting factor for the growth of marine fungi.

Microcycle conidiation of *Neurospora crassa* by partial nitrogen deprivation

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Sporulation of many microorganisms is known to be under the control of nitrogen metabolism and can be induced by nitrogen limitation. In order to induce conidiation of *Neurospora crassa* (FGSC No.354A) in liquid shaken culture and test the importance of nitrogen for this process, conidia ($5 \cdot 10^6$ per ml) were incubated at 37 °C in 500-ml flasks containing 150 ml Vogel's minimal medium supplemented with 2% sucrose and 2 different nitrogen concentrations in a water bath with reciprocal shaking (110 strokes per min). When incubated in the presence of 25 mM or 2.5 mM NH_4NO_3 (respectively control and nitrogen-limited cultures), more than 90% of conidia had a germ tube after 4 h in both media. However, after 6 h, conidiation appeared in the nitrogen-limited culture and almost all the hyphae had conidiated after 12 h. During the same period, vegetative growth was observed in the control culture. The results obtained by modifying different parameters - such as nature and concentration of nitrogen source, sucrose concentration, temperature - and using other wildtype strains wil be presented. These results indicate a triggering effect of a partial nitrogen deprivation for conidiation under the specified conditions of culture and a best response in the wildtype strain 354A. The reduction of the vegetative growth phase allows to consider this differentiation process as a microcycle conidiation.

Methods for the isolation and identification of thermophilic methanogens

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Techniques are reported for the rapid growth and detection of methanogenic bacteria by using liquid and solid mediums. Petri plates are prepared and stored in an anaerobic 1024 Forma Scientific cabinet. For isolation the antibiotics ampicillin, clindanycin and cephalothin are added as proposed by E.M. Godsy: Petri plates are incubated in modified Oxoid model HP 11 pressurized anaerobic jars (A. Zehnder, personal communication). All incubation are peformed at 60 °C. Analysis of methane production and volatil fatty acid used as substrat are determined by gas chromatography. Identification of methanogenic bacteria is made by exposing a replica of the isolation Petri plate to long ware UV light. One type of vulture vessel used for growth experiments in liquid medium is a flasks described by G.T. Taylor and J. Pirt. These techniques have been employed to isolate novels thermophilic methanogenic bacteria from sewage sludge and rumen fluids. This methods have been also used to determine anaerobic plating efficiences (viable count/total count) and to study physiological growth of *Methanobacterium thermoautotrophicum.* The system above described presents a lot of pratical advantages in isolation, identification and physiological growth study of methanogenic bacteria. Special culture techniques are not required and additional sensitivity and working speed are gained.

IgE-EIA: a simpler and cheaper alternative for the quantitation of IgE in serum

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Most laboratories use commercial reagents, which are available either as a RIA or an EIA, for the quantitation of IgE in patient sera. The test procedure is time consuming, the RIA produces significant quantities of radioactive wastes, and the cost is between 8 and 20 Swiss francs per test, depending on whether duplicates are done and on the number of tests per standard curve.

I have developed an EIA for IgE performed in microtest plates. In brief, affinity purified goat antihuman IgE (Tago, Inc.) is used as catching antibody at a dilution of 1:1000. The test sera are diluted 1:10 and 1:100 in PBS, 1% BSA and 2% polyethylene glycol and incubated 1 h at room temperature (RT). The detecting antibody is affinity purified goat antihuman IgE coupled with alkaline phosphatase (Kirkegaard and Perry Labs, Inc.) used at a 1:400 dilution and incubated 2 h at RT. After a 1 h (37 °C) incubation with the substrate, the absorbance is read at 405 nm in a Titertek Multiskan spectrophotometer.

A serum pool calibrated against known standards is diluted 2-fold from 50 to 0.8 units/ml to make a standard curve, which is linear from 0.2 to 1.5 absorption units. A reference control is included in each test series. The range of the test is 5-2000 units/ml. 16 sera can be tested per plate including the standards at a cost of about $^1/_2$ Swiss franc per test serum. In summary, the advantages of this EIA for the quantitation of IgE include: 1. reduced working time and the completion of the test in one day, 2. avoidance of radioactive reagents, 3. increased reliability as a result of 2 dilutions tested in duplicate, 4. the use of EIA equipment now available in most diagnostic and research laboratories, and 5. a cost reduction of more than 10-fold.

Quantification of human serum C-reactive protein (CRP) by a new zone immunoassay (ZIA) technique

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Quantitative determination of human C-reactive protein, an acute phase serum component, was performed by zone immunoassay (ZIA), a new immunoelectrophoretic method. Serum samples were loaded on small vertical glass tubes partly filled with agarose gel containing anti-CRP-serum. Standards or patient specimen of C-reactive protein transported into the gel during electrophoresis formed immunoprecipitates directly proportional to the quantity of CRP. The method is simple, rapid, unexpensive and has a sensitivity of about 0.7 mg/l. ZIA was applied in determination of CRP concentration in newborn babies (term and preterm infants). A significant correlation between elevated CRP-levels (> 5 mg/l) and neonatal infections was observed. It was concluded that the rapid quantitative determination of C-reactive protein can be used as a valuable preliminary evidence and follow-up of newborn infections in addition to clinical and hematological signs.

An enzyme-linked immunosorbent assay (ELISA) for the detection of antitetanic antibody IgG among blood donors

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This communication reports the initial step of an inquiry on the duration of acquired immunity (IgG) after antitetanic vaccination and delayed booster injections (injection de rappel). With ehe ELISA method, we could objectivize the limited duration of actively acquired immunity and the uselessness of late booster injections if the individuals have been immunized more than 10 years ago or not vaccinated. The results suggest that the response to a booster injection is variable (is the immune state propitious to a secondary response variable?) and that the duration of IgG immunity is also variable among individuals of the investigated blood donor group.

Purification by chromatofocusing of an antigen fraction with high species specificity for *Echinococcus multilocularis* in ELISA

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In a first step we isolated by affinity chromatography and immunosorption an antigen fraction Em2 from larval *Echinococcus multilocularis.* This fraction demonstrated a high specificity for sera from patients with *E. multilocularis* infections in ELISA. Sera from patients with *E. granulosus* infections could be discriminated from the other species at

a rate of 95%. No sera from patients with other parasitoses showed any significant cross-reactions with antigen fraction Em2. Examinations of the antigen fraction by isoelectric focusing and western blotting technique resulted in the identification of a single specific immunoreactive band with a pI of 4.8, out of 26 bands on the whole. Using the same techniques most remaining bands were identified as host components, predominantly host albumin.
In a 2nd step we separated the specific component from nonreactive components by a preparative way. Chromatofocusing as a new chromatographic method allowed us to separate the antigen fraction Em2 on the basis of differences in isoelectric points. Using an amphoteric buffer for elution we obtained a linear pH gradient in the range pH 6.9–4.0. The fraction at the pH 4.8 only showed high immunoreactivity in ELISA uniquely with *E. multilocularis* sera. The method offered us the possibility to separate the specific component from nonimmunoreactive components by a high resolution, as revealed by examination of the purified fraction with analytical isoelectric focusing.

A diagnostic kit for the detection of staphylococcal enterotoxins (SET) A, B, C and D (SEA, SEB, SEC, SED)

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Food poisoning due to the ingestion of staphylococcal enterotoxins is most frequent next to salmonella food poisoning. However, its diagnosis is practised in relatively few laboratories only due to laborious food extraction methods and expensive antisera. These are mostly used in immunodiffusion techniques. It has been shown, that 1 µg SEA is causing clinical disease in man. Assuming that this amount is ingested with 100 g of incriminated food a diagnostic test should detect at least 10 ng SET per g. After a comparative evaluation of 4 different versions of the ELISA we prefered the sandwich method. Briefly it is done as follows: The food is extracted in a simple manner and 20 ml of the extract is used for the test. Polystyrene balls coated with rabbit anti-enterotoxin IgG are added and gently agitated over night. Control balls are coated with normal rabbit IgG (NRS). After washing, the balls are individually reacted with the respective conjugates anti SEA-phosphatase, anti SEB-PH, anti SEC-PH and anti SED-PH for 6 h. Tey are washed again and then reacted with NPP as substrate. From the extinction values of the 4 extinction values $\bar{y}+3s$ is calculated and taken as threshold value for positive/negative reaction (99% confidence limit). The calculations are done with a programmable calculator TI 59 coupled to the photometer Vitatron (Fey and Gottstein). An artificially contaminated food samples we regularly detected 0.1 ng SET/ml which is 10–100-fold below clinical relevance. Culture supernatants are examined at a dilution 1:10. The Diagnostic kit shall be available by Dr W. Bommeli, Diagnostische Laboratorien, Länggassstrasse 7, CH-3012 Bern.

The bacterial symbiote of the olive fruit fly *(Dacus oleae)*

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The olive fruit fly *(Dacus oleae)* is the key pest of olive cultures in Mediterranean countries. A bacterial symbiote is closely associated with this insect during all the development stages. In the adult fly the bacteria are contained in a special kidney-shaped organ, called esophageal diverticulum or cyst, which is located in the head and connected with the gut. In the larvae the symbiote is present in the caeca near the anterior end of the midgut. Larvae do not develop if the bacteria are absent. A mixed culture with *Pseudomonas savastanoi,* the causative agent of the olive knot disease, as the predominant organism was believed to be responsible for the symbiosis.
To test this hypothesis we analyzed the organ of adult insects containing the symbiote by scanning electron microscopy. The interior lamellae of the cysts were found to be densely colonized with a pure bacterial culture. The presence of more than one bacterial species could thus be excluded. No flagella could be observed.
To isolate the microorganism cysts were removed from the head capsules and homogenized in 0.85% NaCl solutions. These suspensions were used as inocula for in vitro cultivation studies. Parallel isolations resulted in identical cultures. A medium supporting good growth had the following composition (g/l): peptone 20; glucose 10; NaCl 5. On solid media translucent and yellowish colonies with a diameter of 3–5 mm were obtained. Motility was never observed. No growth took place in the absence of oxygen. The optimum growth range lay between 20 and 25 °C while already at 30 °C no cell mutiplication was obtained. The gram reaction was negative. A comparison between in vivo and in vitro grown organisms revealed complete identity. The bacteria measured 0.6×2.8 µm. It has to be added that in vitro cultivation at temperatures above 25 °C favored the development of shorter, nearly coccoid cells.
A direct comparison proved that the symbiote of the olive fruit fly is not identical with *Ps. savastanoi* which differs in growth rate, temperature range, colony form and motility. The identity of the bacterium is under investigation.

Toxicity expression by dissolved crystals of *Bacillus thuringiensis* var. *israelensis* (H-1[4])

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Bacillus thuringiensis var. *israelensis* (Bti) is a promising microbial insecticide against mosquitos (Mulla and Federici, Proc. int. Col. Invertebr. Path. Brighton, England (1982) 466) and blackflies (Gaugler and Finney, Misc. Publ. ent. Soc. Am. *12* (1982) 1). It may be preferable to chemical insecticides due to its specificity, lack of adverse impact on environment and probably absence of resistance development in insects.
Based on analogous situations with other *B. thuringiensis* serovars and their action against lepidopterous insects, it can be assumed that the crystalline inclusions are not

directly toxic but only after dissolution and proteolytic activation to toxic fragments. It is of central interest to know the nature of the smallest toxic unit. But direct bioassays of dissolved fragments of Bti crystals with mosquito or blackfly larvae are hindered by the behavior of these larvae consuming only particulate matter. Therefore we developed a method to retransform the dissolved material into a particulate form.

Washed sediments of a sporulated Bti culture (strain ONR-60A/WHO 1897) or purified crystal inclusions (ultracentrifugation at 99,000 × g, 180 min in 64% sucrose solution) were dissolved in 50 mmoles l^{-1} carbonate buffer, pH 9.5, containing 10 mmoles l^{-1} dithiothreitol. The solubilized material was precipitated with 12% citric acid, pH 4.5. The precipitated material was taken up in 2 mmoles l^{-1} citrate buffer, pH 4.5. The material was tested using *Aedes aegypti* larvae (3rd instar).

The procedure yielded a good recovery of (80% or more) of input toxic units.

4. Hygiene

Genetic epidemiology of the *N. gonorrhoeae* isolated in Switzerland: characterization of the plasmids

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We have analyzed 177 *Neisseria gonorrhoeae* (NG) strains, including 47 PPNG (penicillinase producing NG) for their plasmid content. All these strains have been previously characterized (MIC for different antibiotics, auxotypes). From our results, we conclude: 1. No plasmid of a new type has been detected. 2. The plasmid content cannot be correlated with the auxotype, with the exception of the auxotype PRO^- $ARG^{\circ-}URA^-$ and the complete absence of plasmids. 3. 16% of the strains contained no plasmids: this is due to the high rate of the auxotype $PRO^-ARG^{\circ-}URA^-$ among the strains analyzed. 4. The conjugative plasmid is significantly more frequent among the PPNG containing the 7.4-kb plasmid (originated from the Far East). 5. No correlation has been found between the auxotype and the resistance plasmid type (7.4 or 5.1 kb).

Preliminary results of the epidemiological study on the *H. influenzae* and *H. para-influenzae* isolated in Switzerland

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The purpose of this study is to make an epidemiological surveillance of the resistant (penicillinase producing [PP] or not) *H. influenzae* and *H. para-influenzae* isolated in Switzerland, particularly of their plasmid content. We report here the microbiological results obtained (serotypes, biotypes [according to Kilian, M., 1976], MIC for ampicillin, tetracycline, chlorophenicol, trimethoprim-sulfamethoxazole, ceftriaxone). 208 strains of *H. influenzae* (20 PP) and 56 strains of *H. para-influenzae* (21 PP), mainly isolated from the respiratory tract, were analyzed. From our results and from other epidemiological data, we conclude: 1. At present, in Switzerland, the emergence of resistant invasive *H. influenzae* strains is not a problem. 2. The *H. influenzae* and *H. para-influenzae* strains isolated in Switzerland do not show marked differences with those isolated in other countries.

Utility of detection of *Staphylococcus aureus* healthy carriers among the staff of the university hospital in Lausanne (CHUV)

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Systematic detection of *Staphylococcus aureus* healthy carriers is conducted among newly-engaged staff and pin-point screening is performed in the course of epidemiological inquiries following the apparition of hospital infections due to this germ. The results of a 4-year survey (1979-82) are reported here.

As regards the examinations carried out on *newly-engaged members of staff,* an average number of 1125 nasal swabs were taken each year and 298 were found to be positive, that is 26.5%, which corresponds to the carrier rate for *S. aureus* in the normal population. Each carrier of this germ was treated with a disinfecting gel and checked afterwards. The treatment proved efficient in 46.1% of the subjects that applied it, i.e. in 38.6% of the positives. This result remained unmodified when a different disinfectant was introduced in the middle of 1982. All the isolated strains were submitted to serotyping according to Cowan's method modified by Pillet (Institut Pasteur).

Serotype I was most widely represented over those 4 years (28.6%); far behind came serotype II (8.7%) and serotype 66438 (5.8%). Hospital serotypes 14, 18 and III were much rarer. Combined serotypes, on the other hand, represented 25.3% of the strains that were isolated and 21.7% could not be agglutinated by means of the serums available. The remaining strains were self-agglutinating.

As the regards the *pin-point examinations* carried out in the course of epidemiological inquiries - leaving out the year 1979 during which the detection of healthy carriers was not conducted systematically - an average number of 200 nasal swabs were taken annually between 1980 and 1982 and 16.7% were found to be positive. The difference between the number of carriers among newly-engaged members of staff (26.5%) and the number of positives in the wards (16.7%) shows that the disinfecting treatment applied to the staff that were found to be carrier is effective. Moreover the rules of hygiene implemented in the hospital reduce the total number of carriers which is significantly inferior to the number of carriers among the normal population.

We are reporting an example of epidemiological research in which the source of infection was found among some healthy carriers belonging to the staff. This clearly reveals the effectiveness of such detections which form an integral part of the hospital hygiene policy pursued at the CHUV with a view to reducing the total number of germs.

Presence of viruses in sludge and water: Public health problem

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Continuing our detection of enteroviruses in various waters, as we described last year, these last months we have detect viruses in sludges. Many authors examined digested and dried sludges; we took only raw sludges in the sewage treatment plant of Strasbourg. The viruses were obtained from the samples by elutriation with 10% fetal calf serum in normal saline (pH 7.2). The most frequent viruses are: Polio I, II, III and ECHO viruses 7, 17 and 23.

Besides, in the water samples, 44/200 are positives and we found often mixtures of viruses, the most frequent are: Polio I, II, III, Coxsackie B1, B3, B5 and many ECHO viruses (E1, 7, 12, 14, 17, 22, 29, 30, 31).

The method we employed is the concentration on glass powder (Schwartzbrod et al.) and the identification method is the technique of Melnick.

Announcements

England

1st international conference on Biointeractions 84

London, January 4–6, 1984

The conference 'Biointeractions 84' on materials/interactions will be held at the City University, London. Information may be obtained from Mary Korndorffer, Conference Organizer, Butterworth Scientific Ltd, Journals Division, P.O. Box 63, Westbury House, Bury Street, Guildford, Surrey GU2 5BH, England.

EXPERIENTIA's first two issues in 1984 will feature a special multi-author review on 'Cadmium as a Complex Environmental Problem'.

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