Schweizerische Gesellschaft für Mikrobiologie *Berichte der 40. Jahresversammlung*

Société Suisse de Microbiologie *Comptes rendus de la 40e réunion annuelle*

Società Svizzera di Microbiologia *Rendiconti della 40ma sessione annuale*

Swiss Society of Microbiology *Reports of the 40th annual meeting*

Chur, 18–20 June 1981

The Society Prize

The Society price has been allocated to Dr Heinz Arnheiter, Institute for Immunology and Virology, University of Zürich, in recognition of his contribution to the technique of mouse hepatocyte culture.

Main lectures

Dr Ion Gresser, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: 'Interferon-mediated pathogenesis'.

Prof. Dr Rolf Zinkernagel, Division for Experimental Pathology, Institute for Pathology, University of Zürich: 'Cell-mediated immunoprotection and immunopathology in viral infections'.

Round table discussions

Conclusions from the 'Round table discussions' held at the 1981 annual meeting of the Swiss Society of Microbiology.

Biotechnology

The results of the presentations and discussions have shown that the field of Biotechnology is extremely fast developing.

The *USA* (Dr. R. Villet, Solar Research Institute, Golden, Col.) have a large program running at Golden on conversion of cellulosic and hemicellulosic material to ethanol (used as fuel) including genetic engineering methods for the improvement of strains. The SERI Institute is also running a nationwide research program 'Fuel from Biomass'. A number of scientists are concerned about the rather slow development of Biotechnology in USA and efforts are being undertaken to improve the situation. Implementation of national Institutes of Biotechnology is envisaged.

Germany. Dr. Binder, Ministry of Science and Technology, Bonn, outlined the German program. Some 80 million DM are spent annually funding a Grossforschungsinstitut (GBF Braunschweig: 400 collaborators) and numerous research groups at academic and industrial levels. The main subjects are process development for the production of new products (interferons, hormones etc.) and improvement of existing processes like ethanol.

Improvement of Bio-engineering is another subject where substantial progress has already been made. Efforts made in the Federal Republic of Germany have brought the nation to the forefront of the field during the last decade together with Japan and USA.

France (Dr. J. Lebeault, Compiègne) has developed a national program on Biotechnology including 4 Institutions to be promoted in the future. Engineering as well as genetic engineering and also enzyme technology are included in the program.

Finally the situation in *Switzerland* has been discussed. It becomes obvious that the funding of several small projects is probably not sufficient to keep up with the progress made abroad. A more systematic plan for the promotion of Biotechnology in the country would be most useful.

Technical contributions of molecular biology to diagnostic virology

In recent years molecular virologists have developed sophisticated techniques to detect and to identify viruses under carefully controled experimental conditions. These methods could provide powerful tools in clinical virology if they can be used under the conditions prevailing in a diagnostic laboratory. It was the aim of the round-table discussion to define the benefits and the limitations inherent to some of these techniques. Thus, the theoretical background and the practical aspects of immune electron microscopy, restriction enzyme analysis, monoclonal antibodies, methods to enhance the sensitivity of cell cultures for the isolation of certain viruses, as well as of the application of chemiluminiscence resulting from the interaction of viruses and cells were briefly reviewed by experts in the fields. The discussion evolving from these presentations centered mostly on technical aspects. However, they also explored the factors influencing the interpretation of results obtained by means of these methods.

Residues of antibiotics in food

The most important problems of the residues of antibiotics in food were briefly put forward by 6 experts and later on thoroughly discussed together with a larger number of participants.
The results of the discussion can be summarized as follows:

- Antibiotics are prohibited as food preservatives in Switzerland up to date, and this will probably be maintained in the future.
- The use of growth-enhancing 'antibiotics' seems to be indispensable for the animal production. The substances admitted in Switzerland are not used in human medicine and experiments have proved that they do not produce analytically detectable residues.
- The question as to whether residues of antibiotics in food can lead to a selection of resistant bacterial strains, is at least quite controversial. In fact, the evidence in question has so far not been made. The application of antibiotics in therapeutical doses can select such strains in humans and in animals. - The often expressed request to prohibit in veterinary medicine the application of antibiotics, which are therapeutically used in human medicine, seems not only unreasonable but also unrealistic. The uncontrolled use of antibiotics in human medicine as well as the illegal trade of them in animal production conbribute to the unpleasant situation of the present time.
- Only Penicillin or its metabolites respectively, the biologically no longer active Penicilloyl (beta-lactimine), are responsible for allergic incidents. Other antibiotics are usually not recognized as allergens.
- From the allergological point of view, the microbiological assay for detection of residues is not sufficient. Serological methods are well known. Milk is known to be a common 'carrier' of Penicilloyl. Further investigations would have to clarify this problem.
- The four-plate test, proposed by the European Community commission, is unanimously recommended as screening test for the detection of residues of antibiotics in food. The slightly increased expenditure of work and material, compared to the present official test, is justified.

Hospital hygiene

The efficiency of recommended methods in hospital hygiene should be controlled by bacteriological sampling in a precise, reliable and reproducible manner. Three types of bacteriological samples have been discussed during this Round Table.
Dr. P. Hartmann, Nancy (France) recommends the adaptation of the Rodac (R) technique (finger pressure) for surface sampling which is more efficient than the swab technique from the quantitative point of view. Its didactic capacity is very interesting, although its adaptation should be carefully standardized. Regarding the qualitative aspect, the swab method appears superior. The most comprehensive procedure is the washing method, which, however, is limited to specialized laboratories.
Dr. J.-J. Pitteloud, Geneva, believes that air samples give a fair indication of room contamination. In surgery a correlation between the number of postoperative infections and aerial biocontamination of the surgical ward could be established. Among the methods presented the cascade, split and Reuter samplers are most frequently used. The interpretation of their results, however, should be done cautiously, taking into consideration numerous variants.
Dr. D. Roussianos, Lausanne, describes a method of per-operative sampling. This seems to give a fairly satisfactory measure of operation wound contamination at the end of surgical interventions as well as of the operation theatre. The bacterial isolates, however, are not necessarily the ones identified during a postoperative infection.

ABSTRACTS

A) *Oral presentations*

Measurement of the activation of the first complement component C1 by immunoglobulin G (IgG)

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Polyclonal, polyspecific human IgG preparations contain antibody activity against a vide variety of bacterial and viral antigens. Infusion therapy with such IgG is helpful in substitution of patients with antibody deficiency syndromes and in correction of platelet concentration in idiopathic thrombocytopenia and may be useful in prophylaxis and therapy of infections. In IgG preparations the biological effector functions should be preserved, e.g. activation of complement should only arise after antigen binding. So far the overall anticomplementary activity of IgG preparations was measured using titration of residual CH_{50} activities in a reference serum. Such method does not distinguish between classical and alternative pathway activation. The binding of purified Clq to IgG can be used to estimate classical pathway activation. As in vivo, the bulk of Clq is integrated in the $Clqr_2s_2$-complex, interpretation of results have to be made with caution. Our approach to measure the activation of the first component of the classical pathway is a modification of the method of Ziccardi and Cooper (Clin. Immun. Immunopath. *15*, 465, 1980). IgG preparations of our production plant, such as standard and pH 4-treated gamma-globulin were tested, each as a ready-for-use probe and a heat aggregated sample; heat aggregated IgG served as a model system for antigen complexed IgG. Different dilutions of each IgG preparation were incubated with serum obtained from citrated human plasma and disappearance of immunologically detectable Clr was measured. Indeed, such disappearance is due to the activity of Cl-esterase-inhibitor binding activated Clr. Cl activation by IgG was quantitated using the slope of the regression line computed from the amount of detectable Cl-integrated Clr vs the log of the IgG concentration used. It was observed that both, Clq binding and Clr activation were similarly expressed by any given IgG sample. This indicates that depending on their state of aggregation the IgG sampes studied are capable to bind free and Cl-integrated Clq to the same extent and that the binding of native Clq effectively mediates further complement activation.

Experiments for the development of a *Salmonella* ELISA

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ELISA has become a widely used tool in diagnostic and scientific serology. It is ideally suited for mass screening because of its adaptability for automation and miniaturization. Most of the previous authors applied heat or the phenol-water procedure of Westphal and Lüderitz for the extraction of lipopolysaccharides to be used as coating O-antigens. Using 4 strains of *S. typhi-murium, S. choleraesuis, S. dublin* and *S. abortus bovis* - each important in veterinary medicine - we compared the following method for O-antigen preparation: Heat extraction for 60 min at 100 °C and for 30 min at 120 °C; LPS extraction by 1% acetic acid; LPS extraction with phenol-water and with EDTA. In addition we used polystyrene tubes or microtiter plates coated with dried bacterial suspensions. For H serology polymeric flagellin purified according to Fey (Zbl. Bakt. I. Orig. A *245*, 55, 1979) was used. The evaluation of the binding capacity of the different antigen preparations was done using rabbit antisera and protein A-phosphatase as label. Because of its simplicity and specificity we recommend the EDTA extract as O-antigen and polymeric flagellin as H-antigen. The EDTA extraction is done as follows: The bacterial suspension is incubated overnight at 4 °C with 0.05 M HCl, pH 1.5, which solubilizes the flagella. Centrifugation follows for 60 min at 43,000 × g. The sediment is washed 3 times with 0.12 M Tris-HCl, pH 8.0, 0.2 mM EDTA is added and the suspension is incubated for 5 min at 37 °C. 10 mM $MgCl_2$ is added. After centrifugation the supernatant represents the O-antigen which is used as coating agent in the concentration of 10 µg/ml Na-bicarbonate, pH 9.6.

Isolation of *Aeromonas* in stool specimens with different selective media

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Aeromonas have often been described as potential intestinal pathogens. 6 selective media were tested for their suitability to isolate *Aeromonas* and *Plesiomonas*. Xylose-Desoxycholate-Citrate Agar (XDCA), DNAse-Toluidine Blue Agar with 30 µg ampicillin/ml (DNAA), Pril-Xylose-Ampicillin Agar (PXA), *Aeromonas* Agar (AA) of Schubert, *Plesiomonas* Agar (PA) of Schubert, and Rimler-Shotts Agar (RSA). After plating of pure cultures and *Aeromonas*-contaminated stool suspensions on all media, RSA was eliminated for *Aeromonas* selection because of lack of specificity. Only XDCA and PA proved suitable for *Plesiomonas* isolation. The remaining 5 media were inoculated directly as well as after enrichment in Alkaline Peptone Water and Trypticase Soy Broth with 30 µg ampicillin/ml. 254 nonselected stool specimens from different patients with signs of intestinal infections were investigated between November 1980 and March 1981. 2 strains of *A. hydrophila* and 2 of *A. sobria* were isolated and identified by API 20E (1.6%). 3 strains were isolated directly on DNT, PXA and PA; 2 on AA and 4 on PA (the 4th strain, and *A. sobria*, was detected only after enrichment). The quantitative recovery of *A. hydrophila* was identical on all 4 media; *A. sobria*, however, grew better on PA. Both *A. sobria* and one strain of *A. hydrophila* were lactose-positive and alpha-hemolytic and would have been missed by routine stool cultures; one culture of *A. hydrophila* was lactose-negative and beta-hemolytic. *P. shigelloides* was not isolated.

Pyocintyping of *Pseudomonas aeruginosa* strains from nosocomial outbreaks - after the elimination of possible sources of mistakes

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Lysogenic conversion, restriction mechanisms, incompatibility- and repressor-activity of extrachromosomal genetic elements may cause changes of the sero-, lyso- or pyocintype of the *P. aeruginosa* strains. This reduces the reliability of the methods used up to now to detect the infectionary pathes of hospital infections. Since the pyocintyping according to Gillies and Govan is recommended for the routine testing due to its simplicity, we tried to increase its reliability by reducing the variety of pyocintyping results, which appear after medium passages and/or storage. About

⅓ of the freshly isolated strains shows such varieties of the typing results.
After a heat-curing-treatment of the strains in broth at 56 °C the strains have a stable pyocintype. By this procedure the strains lose all plasmids smaller than 25 kbp, in many cases even the larger ones too (detectable in the agarose-gel-electrophoresis).
The pyocintyping of more than 1200 strains showed always stable results after the heat-curing-procedure. Therefore we recommend this method for the routine testing. We also propose a binomial system for the nomenclature of the pyocintypes which is able to cover all types and which facilitates their registration.

An epidemiologic study of *Campylobacter fetus* subsp. *jejuni* infections in a dogs' kennel

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Campylobacter fetus subsp. *jejuni (C. jejuni)* is recognized as a common human enteric pathogen. Some reports relate to infections in domestic animals, suggesting a potential hazard to human health.
We succeeded in isolating *C. jejuni* in a 3-week-old puppy submitted to necropsy, which died from a parvovirus diarrhea. A bacteriological survey in the incriminated kennel allowed to isolate *C. jejuni* in 5 out of 24 dogs (2 of them just recovering from enteric symptoms), in 5 out of 17 cats and from the 3-year-old daughter of the owner. Oral administration of erythromycin (15 mg/kg b.wt 3 times a day during 5 days) provided a bacteriological cure in all animals 1 week after treatment.
A 2nd survey 5 weeks later allowed to isolate *C. jejuni* in 12 out of 29 dogs (one with clinical symptoms), but in none of the 22 cats examined. Only 2 of the infected dogs were included in the first survey and are, therefore, suspected to be carriers and to have infected animals arrived recently. We further believe that the persistence of *C. jejuni* in this kennel was associated with a parvovirus infection. For comparison purposes we examined 2 other kennels without history of enteric disorders; *C. jejuni* was not isolated.
Since our isolates of *C. jejuni* from dogs and cats are closely related to strains isolated from humans, the transmission from one to the other must be considered. On the basis of our routine analyses it can, however, be stated that the infection in carnivores seems to occur only occasionally.

Human campylobacteriosis: winter 1980/81

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During 4 winter months 1980/81, 400 stool specimens were processed in order to detect enteric pathogens. The overall results are presented thereunder:

Salmonella	24	6%
Campylobacter	24	6%
Shigella	1	0.25%
Yersinia enterocolitica	2	0.5%
Enteropathogenic *E. coli*	2	0.5% (children under the age of 1 year)

In 19 cases, *Campylobacter* was the only pathogen. In 5 cases, there were protozoans in the stool (2 *E. histolytica* cysts, 1 *Giardia lamblia*, 1 *Trichomonas intestinalis*, 1 *Chilomastix mesnilii*). In 23 cases there was *C. fetus* subsp. *jejuni*, in one case subsp. *intestinalis* (terminal diarrhoe in a 82-year-old man).

Signs and symptoms	Presence	Absence	Not mentioned
Fever	16	3	5
Abdominal pain	14	3	7
Nausea	8	8	8
Diarrhoe	23	1	-

Age distribution					
Age (years)	<1	1-10	10-20	20-40	40-60	60-90
NB of isolations:	1	12	0	7	3	1

Serologic results (complement fixation with a mixed antigen) on 12 patients were positive in blood samples taken on days 2, 3, 4, 5, 6, 6, 6, 14 and 21 after the stool sample. The titers oscillated from 1:10 to 1:160, in one group and negative in blood samples taken on days 2 (and 14), 3, 9 (and 21) after the stool samples of another group of patients.

Fermentation von Geruchsstoffen. Biotransformation von α-Ionon

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Die Biotransformation von α-Ionon mit vorgewachsenem Mycel des Fungus imperfectus *Lasiodiplodia theobromae* ergibt eine Anzahl von unerwarteten bicyclischen α-Ionon-Abbauprodukten. Bis auf eine Verbindung sind letztere als Inhaltsstoffe des Tabaks bereits bekannt.
Für die Entstehung dieser bicyclischen Verbindungen wird primär ein Oxygenase-Typ-Enzymsystem vorgeschlagen, das nach der Art einer nach Bayer-Villiger benannten Oxidation auf das α-Ionon einwirkt. Dabei wird dessen Seitenkette um eine C_2-Einheit abgebaut, was zu α-cyclo-homo-geraniol führt. Letzteres wird teils weiter allylisch hydroxyliert zum unstabilen 3-hydroxy-α-cyclo-homo-geraniol, welches leicht cyclisiert.
Die Entstehung verschiedener bicyclischer Metabolite als Folge der Oxygenase-Einwirkung auf das α-Ionon wird diskutiert.

Functional analysis of the *TRP3*-gene of *Saccharomyces cerevisiae* using different deletions made in vitro

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We have cloned the *TRP3*-gene of *S. cerevisiae* on the bifunctional yeast-*Escherichia coli*-vector pLC544 as a 5 kb BamHI-fragment. Subsequently different parts of this fragment were subcloned on the *E. coli*-vector pBR322 and by functional complementation in yeast we could show that the *TRP3*-gene is located on a 2.8-kb BamHI-ClaI-fragment. - To reduce the size of the fragment further we used a procedure which allows for positive selection in *E. coli* of deletions made in vitro, starting outside and proceeding into the yeast DNA-fragment. By this method we could isolate a series of different deletions. - The functional behavior of these deletions in yeast is dicussed.

Conidial germination of the en-am;am mutant of *Neurospora crassa*

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Dry conidia of *N. crassa* have a large pool of glutamate which is rapidly metabolized when these conidia are incubated in Vogel minimal medium or in water. This decrease, presumably through the γ-aminobutyric acid (GABA)

pathway, was supposed to be an essential event of the germination process for which a model was proposed.
In this work, a double mutant en-am;am was used in order to have conidia with a low pool of glutamate. This double mutant is an auxotroph which cannot use ammonium as a nitrogen source. Grown in the presence of 20 or 50 mM of glutamate as sole nitrogen source, conidia were obtained with a glutamate pool of 42 and 98 µmoles/g of residual dry weight respectively, which represent approximately 25 and 50% of the wild type. This could mean that an accumulation of a large pool of this amino acid is not a condition for conidiation. When these conidia are incubated in minimal medium without glutamate, the germ tube formation is greatly impaired, which underlines the importance of the nitrogen metabolism for normal germ tube formation. The levels of the free amino-acid pools were measured at different times of incubation in the same medium and the results will be discussed in the light of the model proposed.

Macromolecular synthesis during the initiation of zoosporangial differentiation in *Allomyces arbuscula*

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The differentiation of zoosporangia in *Allomyces* is induced by transfer of an actively growing culture to a starvation medium or more specifically by amino-acid limitation in a minimal medium. The rate of DNA, RNA and protein synthesis starts to decline and the ATP level starts to increase immediately after transfer to the induction medium, both processes continuing up to approximately 60 min of induction. 3 highly phosphorylated nucleotides (HPN) have been detected in *Allomyces*. The slowest migrating HPN (migrating 1 cm above the base) begins to accumulate immediately after the transfer of the culture reaching a peak by 60 min. The accumulation of ATP and HPN and their possible role as an intracellular signal for nutritional deficiency and the induction of differentiation is discussed.

The initiation of sporogenesis in *Bacillus polymyxa*

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In *B. polymyxa* sporulation cannot be induced by strict substrate limitation but requires exogenous energy supply. NH_4^{++} ions are good source of nitrogen for growth but are inhibitory to spore differentiation. Yeast extract induces limited sporulation although it supports good growth. Any of the 13 amino acids present in yeast extract stimulate sporulation when added singly but the most effective are arginine, aspartate and glutamate. Glutamate is metabolized very slowly and is not a good source energy (only 15% of the total glutamate supplied is metabolized at the end of growth and sporulation) but supports good sporulation in the presence of glucose as carbon source.
The addition of an inhibitor of transaminases isonicotinic acid hydrazide (INH) in the medium containing either arginine, aspartate or glutamate inhibited sporulation more strongly than growth. The inhibition of sporulation by NH_4^{++} ions either in the presence or absence of glutamate can be reversed by (INH).
The intracellular level of glutamate pool increases rapidly during early phases of culture development attaining a peak by 4 h and then starts to decline. This peak is probably related to the intracellular signal(s) for the initiation of spore differentiation. Factors affecting sporulation also affects the intracellular glutamate pools. Spores produced with limiting glutamate pools are not heat resistant.

How many arginine catabolic pathways are there in *Pseudomonas aeruginosa*?

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2 arginine catabolic pathways are known in *P. aeruginosa* PAO: The arginine deiminase pathway (arginine → ornithine) and the arginine decarboxylase pathway (arginine → agmatine → putrescine) (A. Mercenier et al., J. gen. Microbiol. *116*, 381, 1980). Ornithine is converted to glutamate via Δ^1-pyrroline-5-carboxylate (P5C) (R. Voellmy and T. Leisinger, J. gen. Microbiol. *109*, 25, 1978). Genetic techniques were used to construct a PAO strain blocked in arginine deiminase *(arcA)*, catabolic ornithine carbamoyltransferase *(arcB)*, agmatine deiminase (*agu*; H. Matsumoto, personal communication) and P5C dehydrogenase (*pruB*; L. Soldati and L. Meile, personal communication). This multiple mutant did not grow on agmatine, grew very poorly on ornithine, but still used arginine efficiently as the sole C and N source. Thus, we postulate the existence of a 3rd, yet unknown arginine catabolic pathway. No evidence has been obtained for the presence of an arginase, arginine transaminase, an arginine transamidinase or a pathway involving 4-guanidinobutyrate as an intermediate.

Chemiluminescence in bovine polymorphonuclear granulocytes isolated from blood and milk

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Phagocytic cells emit a burst of chemiluminescence (CL) as a result of stimulation with various soluble and particulate agents. Cellular light emission can be quantitated in a liquid scintillation spectrometer operated in the out of coincidence mode. CL originates from the interaction of highly unstable oxygen species generated by the cells with suitable 'acceptor' molecules such as luminol (5-amino-2,3-dihydro-1,4-pthalazinedione). The production of these oxygen species is thought to play a crucial role in the cell's bactericidal activity.
We have investigated CL generation by bovine polymorphonuclear granulocytes isolated from blood (BPMN) and milk (MPMN). Bovine granulocytes from both sources were found to emit a burst of CL when stimulated with paramyxoviruses, mycoplasma or Zymosan. The maximum light emission reached 80 cpm per cell with Zymosan in the presence of luminol. Zymosan-induced CL varied considerably between different animals as well as between cells prepared at different days from individual animals. However, when isolated simultaneously from individual cows, CL generation by MPMN was always less compared to BPMN.
The oxygen species contributing to CL were determined using chemical and enzymatic scavengers. Both BPMN and MPMN were found to produce singlet oxygen ($^1O_2$), hydroxyl radicals (OH) and superoxide anion ($^-O_2$). Catalase did not decrease CL, suggesting that hydrogen peroxide is not an important source of CL in these cells. Scavengers for $^-O_2$ and $^1O_2$ were found to inhibit CL to a similar extent in MPMN and BPMN. In contrast, OH seemed to be more prominent in MPMN than in BPMN, the OH scavenger sodium benzoate inhibiting CL by $60.1 \pm 3.8\%$ in MPNM vs $32.1 \pm 3.0\%$ in BPMN (mean ± SEM, $n = 5$). Together with the less active CL generation observed in MPMN, this suggests that neutrophilic granulocytes may be functionally different in milk and blood.

Inhibition of antibacterial activity of tobramycin by iron

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The effect of divalent cations (Ca and Mg) on the in vitro sensitivity of *Pseudomonas aeruginosa* to aminoglycosides is well documented. That of Fe^3 or Fe^2 is less known. The present work reports on experiments showing the effect of increasing Fe^3 concentrations on the in vitro activity of tobramycin on sensitive strains of commonly encountered species of bacteria. The modulation of this effect by the size of the bacterial inoculum as well as by the normalization of the medium in Ca and Mg were also studied.
The minimal inhibitory concentration (MIC) was measured by a micromethod of dilution in Muller-Hinton broth enriched with $Fe^3(FeCl_3)$. The inoculum size varied from 10^4 to 10^6 cells/ml. The species were *P. aeruginosa, E. coli, K. pneumoniae, P. vulgaris, S. marcescens,* and *St. aureus.* The increase of Fe^3concentration induced a nonlinear augmentation of the MIC value: rapid initially, the MIC increase tended to a stable, inoculum independent value. When effected in MH broth containing the normalized concentration of Ca and Mg, the same experiments showed a reduced effect of Fe^3 on the tobramycin MIC of the gram-negative organisms but a magnification of the effect with *St. aureus* (MIC increased up to 64×). As the same effect was observed with $FeCl_2$ und $FeSO_4$, with gentamicin and amikacin, we conclude that ferrous or ferric ions play an important role on the penetration of aminoglycosides in bacteria. The behavior differences of the various bacteria imply that iron interacts with bacterial cells rather than with the antibiotics.

Reconstitution of a functional Tn*3*-like transposon from a *N. gonorrhoeae* plasmid sequence coding for beta-lactamase synthesis

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The penicillinase-producing *N. gonorrhoeae* (PPNG) strains have their ampicillin resistance (Ap^r) coded by a 7.1-kb plasmid or by its 5.1-kb deletion derivative. The gene specifying the TEM-like enzyme is on a nontransposable DNA segment homologous to about 40% of the Tn*3*-like transposons (Tn) spread among Enterobacteriaceae. We constructed in vitro resistant plasmids originated from 3 PPNG strains isolated in Geneva) the missing complementary sequence (purified from a plasmid carrying Tn*2301*, a Tn*3*-like transposon). When introduced into *E. coli,* these newly constructed plasmids were shown to carry now Ap^r transposable elements, characterized by their similarity with Tn*3* or Tn*2301* (size, presence of the inverted repeats IR, transposition frequency). Therefore, our data show that in PPNG Ap^r plasmids, the DNA sequences homologous to Tn*3*-like transposons retained their functional potentiality for transposition, more precisely part of the repressor gene and one of the IR. This clearly demonstrates that these sequences are originated from Tn*3*-like transposons by deletion.

Viral proteins from SFV infected *Aedes* cells with unusual mol. wt

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In Semliki Forest Virus (SFV) infected *Aedes albopictus* cells (clone C6/36) we detected by fingerprinting and immunoprecipitation and additional viral coreprotein (C2) with a mol. wt of about 26,000. We did not find this protein in isolated virus particles. The mol. wt difference between the normal coreprotein (C1) and C2 is 4000.
Comparing the peptide patterns 2 ^{35}S-Met labeled peptides are lacking and 2 new smaller peptides are found in C2. C2 is immunoprecipitable with antiserum containing antibodies against all structural SFV proteins.
We have seen C2 in small amounts together with C1 in high salt sucrose gradient fractions containing particles sedimenting at 20 S, 40 S and 60 S. 40 S and 60 S correspond to the positions of the ribosomal subunits, to which C1 was reported to be transiently attached. C1 is cleaved off during translation from a polypeptide in statu nascendi containing all structural SFV proteins. Therefore we assume that C2 is the result of an incorrect messenger or incorrect cleavage.
In addition, with respect to the 3 envelope proteins (E_1, E_2, E_3), at least one variant (E_1') may occur intracellularly. The mol. wt is about 7000 smaller than in the case of the normal counterpart. It was identified by comparison of the peptides produced by limited proteolysis with Staphylococcal V8 protease. E_1' is immunoprecipitable with anti-SFV serum.
We ignore whether sugar residues are also involved in the case of the variant envelope protein.

Uncoating of the influenza virus in cultured macrophages from mice bearing the interferon-dependent resistance gene *Mx*

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Mouse peritoneal macrophages cultured for 2-3 weeks are permissive for the replication of the influenza virus A/M-TUR. In macrophages carrying the allele *Mx* lower doses of interferon are necessary to elicit the antiviral state toward Myxoviruses than in macrophages lacking *Mx;* the antiviral state to several unrelated viruses is not influenced by *Mx* (Haller et al., Nature *283*, 660, 1980). This in-vitro model was used to investigate, at the molecular level, the very step at which the replication of influenza virus is arrested in interferonized *Mx* macrophages. The uncoating of the virus was investigated in this study.
The viral membrane protects the RNA genome from degradation by ribonucleases. After attachment and penetration of the virus into the host-cell, and after successful uncoating, the viral RNA is liberated into the cytoplasm and it becomes susceptible to the action of ribonucleases. We have taken advantage of this property to measure quantitatively penetration and uncoating of radioactive virions labelled in their genome with 3H-uridine. The results have shown that: a) uncoating occurred in both types of macrophages (Mx/Mx and +/+) to the same extend and with the same kinetics, and b) the pretreatment of macrophages with various doses of mouse interferon did not influence the normal process of uncoating. The results indicate that uncoating occurred independently of the interferon-induced antiviral state.

Genetic control of antiviral activities of interferon in mice

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Adult mice bearing the allele *Mx* exhibit a high degree of specific resistance to orthomyxoviruses. This resistance can

be abrogated by treating the animals with anti-interferon serum (J. exp. Med. *149*, 601, 1979). Resistance is effective against a variety of influenza virus strains tested and is expressed at the host cell level: studies with macrophages and hepatocytes in culture have shown that interferon is particularly efficient in inhibiting influenza virus replication in cells bearing *Mx*. In contrast, interferon action against several unrelated viruses is not influenced by *Mx* (Nature *283*, 660, 1980). Newborn *Mx*-carriers are generally as susceptible to orthomyxoviruses as newborn mice devoid of *Mx*. This has enabled us to directly demonstrate the efficacy of interferon in protecting newborn mice differing at the *Mx* locus. *Mx*-carriers could be protected against lethal influenza virus infection with doses of interferon that were not protective in the absence of *Mx*. The efficacy of interferon towards other viruses such as EMC or VSV was independent of *Mx*. We conclude that, in vivo, the protective effect of interferon towards influenza viruses depends on the host genotype. We are presently investigating the step at which the replication cycle of influenza viruses is arrested in interferon treated *Mx*-bearing cells. Virus replication seems to be blocked at an early stage following normal attachment and penetration but before viral protein synthesis.

B) *Posters*

The typing of nosocomial *Staphylococcus aureus* strains, which are untypable (NT) by the international phage set

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More and more *S. aureus* strains, isolated from nosocomial outbreaks, appear to be insensitive to the phages of the international standard set; lysotypy is therefore restricted in its significance for these strains. The reverse lysotypy, the so-called lysogenotypy, based on the typing of Mitomycin-induced phages from patient strains (with the help of the host strains of the standard set) is only an unsufficient compensation: the patient phages are host-modified and the host strains of the phage set are lysogenic, which both may cause mistakes. We were therefore looking for another method to type resp. to identify NT-strains of nosocomial cross infections.

With this method we first determine the phage receptors of nosocomial NT-strains with the help of phage adsorption on solid agar medium. We use 6 phages (Nr. 52, 79, 3A, 75, 81, 187 of the international standard set), which we first purified and multiplied on new nonlysogenic host strains. By this we obtain the receptor formula of the patient strains. Since the phage resistance of NT-strains is mainly based on restriction mechanisms, we try to obtain a host modification of one of the adsorbed phages. Single plaques often result from massive infection of the patient strain with such phages. By multiplying this phage in the patient strain we obtain a phage suspension which we dilute to 10 RTD and which can be used for lysotypy. This phage modification by the patient strain allows the identification of the strains from cross infections having the corresponding restriction pattern. This typing method is therefore based on phage adsorption and on the restriction resp. modification pattern of NT-strains.

Application of i.v. metronidazole in cases of septic abdominal surgery: collaboration to 2 multicentric studies

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Anaerobic bacteria present in almost all cases of intraabdominal infections are a source of therapeutical difficulties. The first study 'Klingyl' was intended to prove the efficacy and the safety of the metronidazole i.v. treatment as compared to the clindamycin i.v. treatment. The criteria for including patients into this study were: hospitalized adults with documented intraabdominal anaerobic or mixed infections occurring in surgical and gynecological practice. A 7-day-treatment (500 mg metronidazole or 600 mg clindamycin, each 8-hourly) was allocated randomly to the selected patients; as concomitant therapy, only antimicrobial agents having no action on anaerobic bacteria could be used. Each case was followed clinically and bacteriologically during the treatment according to a very strict protocol. The MIC's of both antimicrobial drugs were established for all isolated anaerobic bacteria.

Among the 15 centres collaborating in this study, our centre accounted for about 5% of the 170 cases and 18 anaerobic strains were isolated, 8 of these were *Bacteroides* sp. gr. *fragilis*. All the isolates were sensitive to both drugs. All patients responded well to either of the therapies and in most cases bacteriological researches were negative after 3 days. No side effects (polynevritis, nausea, diarrhea, etc.) were observed in our patients. On the whole, our results are in accordance with those of the general study. The clinical data recorded with the 170 patients do not show discrepancies between the 2 treatments. The leucocyte count of the cases treated with clindamycin show a more pronounced decrease than those treated with metronidazole.

The 2nd study 'Duomet' is based on the pharmacocinetic simulation showing that a dose of 500 mg metronidazole twice daily should be sufficient. The same criteria of selection of patients and the same protocol as in the first study were used. Our centre provided about 10% of all cases (total of cases = 61), and we isolated 13 anaerobic strains (6 *Bacteroides* sp. gr. *fragilis*). After 7 days treatment all bacteriological researches were negative. Both studies showed the efficacy of the treatment with metronidazole i.v. which was similar to that of clindamycin; futhermore a dose of 500 mg metronidazole, twice daily, should be sufficient, in most cases.

Epidemiological survey on the positive blood-cultures of the CHUV in 1980

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In 1980 an epidemiological inquiry has been systematically made for any germ isolated from a blood culture, and this for the totality of the CHUV. 25,622 patients have been hospitalized. 306 have shown a positive blood culture (1.2%). The laboratory has analyzed 14,996 bottles, out of which 1232 (8.21%) have been found positive. 44.44% of the patients had a gram-positive germ, 50.98% a gram-negative and 4.58% several germs. The most frequently isolated organism was *Streptococcus pneumoniae* (14.05%), followed by *Escherichia coli* (13.40%), *Staphylococcus aureus* (11.11%), the *Pseudomonas*-like (9.15%) and *Pseudomonas aeruginosa* (6.86%).

In order to make the epidemiological inquiry, we contact the physician in charge of the case to obtain the essential informations: fever, infection on admission, other bacteriological samples with the same germ, perfusions, catheters, antibiotic therapy, etc. If found necessary, we contact the specialists in infections diseases. According to the germ found, we take environmental samples, search for health carriers, collect perfusions, sounds and catheters and analyze the whole.

In 1980, out of 306 patients with a positive blood culture, our survey excluded hospital-acquired infection in 222 patients. A probable one has been found in 84 patients (27.45%) where 12 hospital-acquired transient bacteremias, 10 post-therapeutical generalization sepsis, 3 autoinfections and 59 nosocomial sepsis were concerned.
The source of these infections has been found: 12 times in sounds and catheters, 8 times in monitoring devices and blood heaters, twice as complication of an exsanguino transfusion, twice in staff health carriers, once in an Intralipid infusion, 10 times in hematogenic disseminations, 3 times in the patient's own flora, 28 times (*Pseudomonas*-like cases) in the deminaralized cooling water of the autoclaves of the CHUV pharmacy.
In conclusion, it is very important to practice such an epidemiological survey on the positive blood cultures of a hospital, in order to control the sterilization and disinfection of any used material, and to improve inceasingly the technical cares. In addition, such a survey makes us attentive to the outbreaks of severe hospital-acquired infections and to the appearance of new germs.

Postantibiotic effects of rifampicin in vitro

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Postantibiotic effects (PAE, i.e. retardation of regrowth of bacteria after removal of antibiotic) are known to occur after incubation of different gram-positive bacteria with penicillin G (H. Eagle and A.D. Musselmann, J. Bact. *58*, 475, 1949).
We studied the PAE's of rifampicin in relation to the drug concentration and the duration of incubation with *Staphylococcus aureus* before removal of the drug.
The bacteria were preincubated with the drug in DST broth OXOID, and the drug in the suspension was diluted by about 1:500 with DST broth at the appointed times, so that not more than $1/100$ MIC was present. The time elapsing until the viable count of the sample and the control culture had increased by one log 10 above the count after dilution was designated Tl and Cl. The difference between Tl and Cl was taken to be the PAE.
Rifampicin at very low concentrations (< 0.02 µg/ml) induced PAE's lasting up to 3 h. Correlations were evident between the PAE's and both the preincubation time and the drug concentration. Penicillin G and cefazolin, by contrast, were only active at higher concentrations, and the duration of their PAE's was not as long as that of rifampicin.
Our summarized data indicate that rifampicin exerts PAE's on a β-lactamase-producing and a non-β-lactamase-producing strain of *S. aureus*; the effects are more pronounced than those of penicillin G and cefazolin, and the PAE's correlate with the duration of preincubation and the concentration of the drug.

Système «spiral» et analyse bactériologique des plats cuisinés – premiers résultats

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Des dénombrements de microorganismes aérobies mésophiles à 30 °C, effectués à partir de suspensions bactériennes et d'échantillons de divers plats cuisinés, ont été réalisés en comparant deux techniques:

1. étalement des différentes dilutions à la surface d'un milieu nutritif = témoin;
2. utilisation de l'ensemenceur «Spiral» = technique à l'essai.

Les premiers résultats, analysés statistiquement, ne montrent pas de différence significative entre les 2 techniques, à 5% de risque d'erreur.
Pour les échantillons de plats cuisinés, avec le système «Spiral», le temps d'incubation à 30 °C le plus satisfaisant est 48 h. Cependant, au bout de 24 h, les résultats ne sont que très légèrement inférieurs à ceux obtenus en 48 h (moins de 0,2 unité logarithme décimal).
Les premiers résultats obtenus sont encourageants; ils permettent d'espérer un gain de temps appréciable lors de l'analyse bactériologique des plats cuisinés.

Regulation of 3-deoxy-D-arabinoheptulosonic acid-7-phosphate synthetase in *Nocardia mediterranei*

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N. mediterranei produces the antibiotic rifamycin B. The biosynthesis of the rifamycin-chromophore starts from an intermediate of the main sequence of aromatic amino-acid biosynthesis (shikimate pathway). The branch point is located between 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) and shikimic acid (SA).
The condensation of phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P) to DAHP is catalyzed by the DAHP synthetase. This is the first enzymatic step in the biosynthesis of the aromatic amino acids tryptophan (trp), tyrosine (tyr) and phenylalanine (phe). – Because of the connection between rifamycin biosynthesis and aromatic amino-acid biosynthesis the regulation of the DAHP-synthetase was investigated.
Using *N. mediterranei N813*, trp, tyr and phe were tested for repression of DAHP-synthetase biosynthesis at concentrations of 1×10^{-2} M and 2.5×10^{-2} M. No repression was found. Kinetic investigations showed that the substrate E4P is a competitive inhibitor of DAHP-synthetase. The following intermediates and endproducts of the shikimate pathway were tested for inhibition of DAHP-synthetase: trp, tyr, phe, SA, chorismic acid (CA), prephenic acid, p-aminobenzoic acid, p-hydroxybenzoic acid and 3-amino-5-hydroxybenzoic acid. Inhibition was found with trp and CA only. With 5×10^{-5} M trp a 15% inhibition and with 5×10^{-4} M CA a 30% inhibition of the DAHP-synthetase activity was observed.
Data on the regulation of the DAHP-synthetase are available for various Streptomycetes, e.g. for *Streptomyces venezuelae, S. aureofaciens Tü 24, S. sp3022a* and *S. antibioticus.* Streptomycetes, as well as *Nocardia mediterranei,* belong to the order Actinomycetales and many of them produce antibiotics. Therefore a comparison of DAHP-synthetase regulation in Streptomycetes and *N. mediterranei* is indicated. In Streptomycetes no repression by the aromatic amino acids has been found. The DAHP-synthetase activity is in some cases inhibited by trp. In the other cases no inhibition by aromatic amino acids is reported. Thus the regulation of the DAHP-synthetase in *N. mediterranei* is identical with its regulation in other Actinomycetales.

R-factors which can pick up lac genes from *Escherichia coli, Klebsiella* and *Enterobacter* and transfer them as transposons into *E. coli* recipients

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Among the R-factors from an Enterobacteriaceae hospitalism outbreak several of these plasmids were able to cotransfer the lac$^+$-property to *E. coli* lac$^-$ recipients. In our poster we demonstrate that the lac$^-$ versions of such R-plasmids can pick up lac genes from plasmid-free wild strains of *E. coli, Klebsiella* and *Enterobacter* and transfer them into new recipients: with pGL 607, pGL 611 and pGL 619 lac genes could be picked up and transferred in 21 of 50 trials. Further examination showed that the lac genes picked up by pGL 611 are parts of a large transposable element with a size of 33.7 kbp. This element gives the same restriction pattern (Eco R1) when it is picked up from the chromosome of *E. coli* 14260/80 (wild strain) of *Klebsiella pneumoniae* 8970 (wild strain). Since the lac regions of the *E. coli* and *Klebsiella* chromosome are smaller than 8 kbp, but the picked up DNA is 33.7 kpb, we tested whether other than lac genes were present on this DNA region. With several multiauxotrophic strains, the following genes could be checked: pro B, pro C (both neighbored to the lac region in the *E. coli* chromosome), leu, thr, arg, his, Bl, ara, lys, trp, gal, tsx, met, recA. None of these properties could be transferred to a corresponding recipient. As the transposable element also does not contain DNA from the pGL 611 R-factor, the question remains, what kind of information the rest of the DNA includes.

Plasmid-coded mechanism of phage inactivation

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Classifying conjugative *Escherichia coli*-R-factors from animal feces we sometimes could not identify their sexpili with the usual RNA- and DNA-sexphages, even by using the HFRT-system.
The failure turned out to be caused by an antiphage activity of the wild strains, which affects not only the RNA- and DNA-phages, but also other Coli-phages, i.e. λ-phages, however not the Salmonella-phage 01 and the Staphylococciphages.
This activity is found in the cell-free supernatant of the cultures. It is mainly produced in the log-phase and acts instantly. The activity can be destroyed by a temperature of over 56 °C, by treatment with Trypsin and by pH beyond 2 or above 10. It is also held back by membrane filter (Ø 0.45 µm, Millipore Typ HA).
The described antiphage activity is plasmid-coded and can be transferred together with certain col-factors into *E. coli*. The inactivation mechanism is not identical with the activity of colicin. The strains can lose this antiphage activity and still produce colicin. This inactivation mechanism is in some properties comparable to bacterial interferon (host and not phage specificity), in other properties it differs from it (inactivation and not restricted multiplying, activity in cell-free environment).

DNA-DNA hybridization studies of the genus *Xanthobacter*

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Hydrogen-oxidizing bacteria are chemolithoautotrophs and were first grouped within the genus *Hydrogenomonas*. Taxonomists realized that the ability to use molecular hydrogen as the sole electron and energy source in an autotrophic metabolism is widespread among different types of bacteria. This classification was reviewed by Aragno and Schlegel (in: The Procaryotes, Springer-Verlag, in press). The genus *Xanthobacter* (Wiegel et al., Int. J. syst. Bact. *28*, 573, 1978) comprises hydrogen-oxidizin nitrogen-fixing nonmotile bacteria. It contains so far 2 species: *X. autotrophicus*, which presents a high phenotypical variability, and *X. flavus*, corresponding to the strains previously known as *Mycobacterium flavum*. The recent isolation of motile strains, of biotine requiring strains and of oxygen-tolerant strains probably related to *Xanthobacter*, prompted us to compare these strains with *Xanthobacter* at the DNA level. All these strains as well as typical *Xanthobacter* strains have a guanine + cytosine content of their DNA between 66 and 68%, as determined by analytical centrifugation. DNA-DNA hybridizations were performed on nitrocellulose filters containing unlabelled, glyoxal-denatured DNA; the input DNA was ^{3}H-labeled by nick translation. We have shown that neither thermal nor alkali denaturation gives a good fixation on nitrocellulose of such high % G + C DNAs. Instead, glyoxal denaturation was successful. Our first results indicate that several different species must be clustered within the genus *Xanthobacter*.

IS1-mediated cointegration contributes to the transduction of chromosomal markers and plasmids by bacteriophage P1

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Transduction of bacterial markers and plasmids is assumed to be due to an accidental packaging of bacterial or plasmid DNA into a phage particle. Bacteriophage P1, widely used for transduction experiments in *Escherichia coli*, carries in its genome one copy of the insertion element IS1. While this IS1 element is not essential for phage propagation, lysogenization and maintenance of the prophage plasmid, it influences the transduction ability of the phage.
In studies of transduction of plasmid and chromosomal markers by P1 and by P1-related phages lacking the IS1 element, we compared a) transduction frequencies, b) the phenotypes of the transductants, and c) the structure of plasmid DNA molecules carried by transductants. The results indicate that a significant portion of P1 transductants are due to specialized transduction. The transducing genomes are cointegrate structures between P1 and the transduced DNA. The cointegration is brought about either by reciprocal recombination between IS1 elements on P1 and on the plasmid or the bacterial chromosome or by transpositional cointegration mediated by an IS1 element. In the recipient cell reciprocal recombination between the direct repeats of IS1 can restore the original plasmid or a plasmid with an additional copy of IS1, respectively. Occasionally cointegration was also accomplished by other structural elements of the P1 genome.

Restriction analysis of the genome of *Streptomyces glaucescens*

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After growing the mycelium of *S. glaucescens* in liquid cultures containing 0.5–1% glycin, DNA was isolated by a standard method including the following steps: lysozyme treatment, lysis with EDTA and SDS, proteinase K treatment, phenol-chloroform extraction, RNAse treatment, NaCl-polyethylene glycol precipitation and ethanol precipitation.
More than 95% of the DNA was isolated by this method (as determined by radioactive labelling) and the mol. wt of the DNA was more then 30 Md.

DNA was restricted in vitro using different restriction endonucleases and the fragments were analyzed on horizontal agarose gels. Best results for the analysis of the restriction fragments were achieved by using the enzyme BamHI and 0.85% agarose gels run at 2.7 V/cm in tris-acetate buffer. The size of the BamHI generated fragments ranged from 16 Md to less than 1 Md and after electrophoresis the fragments showed a typical banding pattern; more than 40 prominent bands could be distinguished from the background.

This banding pattern could be used as a fingerprint for the genome of *S. glaucescens*. It did not depend on cultivation conditions and chromosomal mutations with some rare exceptions:

1. Mutant strains of the Mel C class (no melanin formation) lack a band of about 9 Md in size.
2. An isolated mutant strain Mel$^-$ strS (no melanin formation and increased sensitivity to streptomycin) showed some new highly repetitive fragments of a total size of about 4.8 Md.

All other mutant strains analyzed (auxotrophic, resistant, altered colony morphology) showed the same banding pattern as the wild type.

Analysis of the DNA of different wild type strains of *S. glaucescens* showed some clear variations in the banding pattern; but also some correlation in the distribution of certain bands was recognizable.

Continuous culture of the methanogenic bacterium *Methanobacterium thermoautotrophicum*

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A conventional fermentor system was modified for growth of *M. thermoautotrophicum*, strain Marburg with CO_2 and H_2 as carbon and electron source respectively. After initial growth as a batch culture, the cells were kept under chemostat conditions for several weeks. Growth and methane production have been determined at various dilution rates and growth rates better than ever reported have been obtained. The described modified anaerobic fermentor system allows high growth yields with strict anaerobic methanogenic bacteria and simple techniques similar to the ones used in conventional aerobic fermentor systems.

Photosynthetically active radiation (PAR) transmitted through sandy sediments

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Light is one of the regulating factors in sandy salt marsh sulfureta. Natural blooms of Chromatiaceae attached to sand grains 7-18 mm below the surface are possible because sulfide concentration and the quality and quantity of light transmitted produce selective conditions for certain organisms. In a model system PAR transmitted through broken quartz sand is determined with the spectroradiometer QSM-2500 (Techtum Instruments). The net radiation available to the organisms ($\bar{R}_n$) is defined as

$$\bar{R}_n = \bar{S} \cdot (1 - \rho) - \bar{R}_{na},$$

where ρ = reflectivity, $\bar{S}$ = radiation received as direct or diffuse solar irradiance, $\bar{R}_{na}$ = radiation absorbed in the zone above the layer with phototrophic bacteria. Radiation balances are determined from measurements of incident, reflected and transmitted radiant flux densities. Transmittancy through dry sand of $0.102 \leq$ grain size ≤ 0.25 mm·mm at 2 mm depth is approximately 40 times higher for radiation of $\lambda = 750$ nm than for that of 450 nm. 1‰ of the incident radiation of 750 nm reach a depth of 7 mm through moist sand of the same grain size distribution, while only 0.01‰ of the blue light arrive at the same depth.

Grain size, surface reflectance, absorbing pigments in top layers and moisture content of the sediments regulate the amount of photosynthetically active radiant energy available to organisms in the deep layer. Red light is specifically enriched with depth. This leads to a selective advantage for bacteria with bacteriochlorophyll a and b over those with bacteriochlorophyll c and therefore to natural enrichments of Chromatiaceae several millimeters below the sand surface.

Light determines coexistence of phototrophic bacteria competing for sulfide in sandy sediments

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In certain areas of salt marshes stratified sulfureta are formed in which phototrophic bacteria predominate in some layers (Farbstreifen-Sandwatten). The variety of color patterns that develop and the fragility of the stratifications indicate fine regulation of these ecosystems by environmental parameters. Chlorophyll a, bacteriochlorophyll a and c and carotenoids are the predominant pigments, giving a first indication of the kinds of microorganisms present. Chromatiaceae could be isolated from distinct deep pink layers above a zone of active sulfide production. Chlorobiaceae and diatoms appear at all depths that can be reached by actinic light but do not naturally forme distinct layers. However, Chlorobiaceae can be induced experimentally to do so. Green surface mats consist mostly of Cyanobacteria. Chromatiaceae in pink deep layers adsorb to surfaces and their polysaccharide coatings cause sand grains to cluster. Activities within these ecosystems are regulated mainly by light and sulfide. Cultured Chromatiaceae tolerate sulfide concentrations from 0.5 to ≤ 4 mM; Chlorobiaceae grow within an even wider range of sulfide concentrations. Red light ($\lambda \geq 800$ nm) penetrates sand layers of up to 20 mm thickness, thereby creating selective conditions for Chromatiaceae in deep layers. Chromatiaceae outgrow Chlorobiaceae in mixed cultures under full light, while Chlorobiaceae dominate in cultures behind thick sand layers. Brown coloration in nature and in culture is indicative of more balanced green and pink populations. Spatial separation of phototrophic bacteria competing for sulfide is sustained by the quality and quantity of the transmitted light and the kind of chlorophyll present.

Mikrobiologie sulfatreicher Mineral- und Heilquellen der Schweiz

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Schweizerische Gips- und Bitterwasserquellen haben schon früh als Heil- oder Mineralquellen Bedeutung erlangt. Sie entspringen aus Sulfatsalzgesteinen (Gips, Anhydrit) im Alpengebiet und im Jura. Einige sind nie gefasst worden und bilden Nassbiotope, andere wurden in jüngster Zeit vernachlässigt, beides Bedingungen, die zur ungestörten Entwicklung von mikrobiellen Sulfureta führten. Mikrobiologisch dominieren sulfatreduzierende und phototrophe Bakterien, wenn neben einem hohen Sulfatgehalt organische, abbaubare Substrate unter anaeroben Bedingungen im Licht vorhanden sind. Solche Ökosysteme sind meist in

stehenden Gewässern anzutreffen. Weisse, häufig fädige Sulfidoxidierer (Beggiatoaceae, Leucotrichaceae) dominieren unter aeroben Bedingungen, oft im Dunkeln in gefassten Quellen und an Wasseroberflächen. Diese Extrembedingungen schaffen ideale Voraussetzungen, um mikrobielle Wechselwirkungen in einem verhältnismässig gut definierbaren Ökosystem zu untersuchen.
Das God-dal-Fuorn-Sulfuretum im Nationalpark zeichnet sich aus durch einen hohen Sulfatgehalt (15 mM). Es sind nur sehr geringe Gehalte an Stickstoff, Phosphat und organischem Material feststellbar (F. Schanz, Verh. int. Verein. Limnol. *20*, 2188, 1978). Der relative Anteil an schwer abbaubarer, organischer Substanz (Holz, Nadeln) ist ausserdem hoch. Für den Teich kann eine Verdünnungsrate von 0,67 h^{-1} errechnet werden. Unter diesen Bedingungen sind für die Bakterien nur strömungsarme Nischen als Lebensräume geeignet, da beim oligotrophen Nährstoffangebot keine Generationszeiten möglich sind, die eine erfolgreiche Besiedlung des freien Wassers ermöglichen würden. Bakterien wachsen deshalb vorwiegend auf Oberflächen von Pflanzenteilen und auf Steinen, um Auswaschung zu verhindern. In stehenden Gewässern (Zeglingen, Cadagno di fuori, Seeberg) bilden sich natürliche Anreicherungen von Mikroorganismen in Schichtungen als Konsequenz der Umgebungsbedingungen (v.a. Licht und O_2) und der physiologischen Abhängigkeit (C-Substrate, Sulfid). Aus verschiedenen Sulfureta konnten mehrere Arten anaerober, phototropher Schwefelbakterien (Chlorobiaceae und Chromatiaceae) sowie einige sulfatreduzierende Bakterien angereichert und teilweise isoliert werden, die möglicherweise in syntropher Wechselbeziehung leben.

Methane production and degradation of organic material in an anaerobic filter type reactor

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Degradation of sucrose containing substrates and methane production was followed in an upflow anaerobic filter in laboratory scale. The reactor had a total volume of 11 l and a fluid volume of 5 l and contained porous clay particles as support media for the organisms which were taken from a sludge digester from a wastewater treatment plant. The incubation temperature was held at 30 ± 2 °C.
In a first period of 4 months a synthetic medium containing sucrose was used, later the anaerobic degradation of wastewater from a sugar refinery plant (Frauenfeld) was investigated. Without the need of a pH control reasonable degradation was achieved with organic loadings of 1.5, 3.5 and 6 kg COD/m^3·day. The reduction of the COD was 87, 86 and 76% and the methane production 1.2, 2.0 and 3.4 m^3/m^3 reactor volume for the 3 loadings. Loadings above 10 kg COD/m^3·day were degraded with substantially less efficiency.
The results presented will be the basis for the construction of a pilot plant for a prepurification of wastewater from the sugar refinery.

Measurement of the membrane potential in whole cells of *Methanobacterium thermoautotrophicum* and other bacteria by use of an electrode sensitive to the tetraphenylphosphonium cation (TPP^+)

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Methanogenic bacteria are believed to build up a proton motive force (pmf) for synthesizing ATP from ADP and phosphate[1]. This suggestion is supported by the fact that in *M. thermoautotrophicum* the uptake of H^+ changed over to a release of H^+ after the addition of valinomycin[2]. It is therefore of interest to determine the contribution of the membrane potential $\Delta\Psi$ to the pmf. An electrode, sensitive to the lipophilic cation TPP^+, has been used to measure $\Delta\Psi$ in mitochondria[3]. We have adapted the system to measurements with whole cells of anaerobic bacteria. We measure simultaneously $\Delta\Psi$ and the redox potantial E_0' under a stream of oxygen-free gas. With the conditions used (pH 7.2–7.5, initial concentration of TPP^+ 10 µM) we observed an uptake of TPP^+ into the cells of the methanogen and a release of TPP^+ after addition of valinomycin (17 µM). In case of methanogens, success depends mainly on holding E_0' deep enough (−350 mV) during the measurement and on the conditions under which the cells grew.

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Anaerobic degradation of benzoic acid derivatives by bacterial communities from fresh water lake sediments

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Few organisms are known to be able to cleave the aromatic ring under anaerobic conditions (W.C. Evans, Nature *270*, 17, 1977). We find that ring fission is facilitated in plant derived aromatic monomers with certain substituents. Enrichment cultures under methanogenic, fermentative and sulfate-reducing conditions yielded bacterial communities capable of growing with various hydroxy- and methoxy-derivatives of benzoic acid as their sole carbon and energy source. Methanogenic communities mineralize syringic acid (3,5-dimethoxy-4-hydroxy-benzoic acid) at a rate of 1.5 mM per day to a minimal threshold concentration of 0.2 to 0.3 mM. Primarily acetic and butyric acid, but also propionic, isobutyric and isovaleric acid, are formed as intermediates. Acetic acid serves as a long lasting source of methane formation. Under sulfate-reducing conditions acetic acid accumulates as endproduct; fermentative communities accumulate acetic and butyric acid. The degree of degradation of the aromatic substrate reaches 90% under these conditions. Communities adapted to syringic acid also degrade gallic acid (3,4,5-trihydroxy-benzoic acid) and vanillic acid (3-methoxy-4-hydroxy-benzoic acid). Benzoic acid is metabolized only after several weeks of adaptation and then very slowly. Enrichments with vanillic acid as sole carbon and energy source convert the substrate without ring fission into a product with a UV-absorption maxima at 276 nm. No volatile fatty acids are formed, sulfide cannot be detected and only in the first few days are traces of methane detectable. We propose that benzoic acids with substituents in the 3,4 and 5 position serve as effective inducers for aromatic ring fission enzymes.

An equilibrium diffusion technique for measuring concentration gradients of small soluble molecules applied to in situ determination of anaerobic mineralization processes in lake sediments

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Depth profiles of methane concentration in lake sediments give an indication of mineralization processes under anaerobic conditions. Depth represents time periods of several years. Undisturbed lake sediments contain therefore stable microbial ecosystems. We determined substrate and product profiles in situ with a plexiglas sheet containing small dialysis chambers filled with distilled water and covered with a dialysis membrane. The system is allowed to equilibrate in situ for several days before the contents of the compartments are retrieved and analyzed for methane and volatile fatty acids. The great number of dialysis chambers allows a fine resolution of substrate pools within a depth gradient. Since only small molecules diffuse, clean samples are available for further processing. Depth profiles from sediments in shallow water at winter conditions show a distinct gradient of methane from 0 µM at 1-2 cm below the sediment-water interface to saturation (2290 µM) at 30 cm depth. No methane could be detected in the overlying water. We have applied the technique to determine methane, sulfide and short chain fatty acids and are comparing the results with direct measurements of these constituents from interstitial water. Acetate is present in very small concentrations under winter conditions (20 µM), but we find no concentration gradient within the sediment. Although we can isolate sulfate reducing bacteria from these sediments, we have not been able to detect any sulfate reducing activity in situ with this technique. Part of the organic matter in the sediment is very slowly biodegradable; therefore, it constitutes a long lasting substrate source. Addition of small amounts of exogenous substrate leads to an increase in microbial activity as determined in vitro. Increased temperatures do not stimulate breakdown of endogenous substrate to full capacity of the degrading community. We conclude that the microbial populations can thrive under these substrate limited conditions due to the slow bioerosion of the available organic matter. The structure of the sediment makes possible the formation of gas bubbles and the maintenance of gassaturated conditions.

Behavior of fecal bacteria during activated sludge process and in a river

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The effect of natural and artificial biological epuration processes on bacterial populations of fecal origin was investigated in an activated sludge plant, in a river and in a laboratory scale fermenter.
Counts of coliforms, fecal streptococci, salmonellae and saprophytic bacteria were performed in the in- and output waters of an activated sludge basin (STEP Neuchâtel), along the first 80 km of the Doubs river and during incubation of a sample of water, aerated and stirred in a 2 l fermenter. The results show a strong decrase (90-99%) of the fecal bacteria during the biological epuration processes. In the fermenter experiments, the fecal populations did not change significantly during the first day of incubation, and then slowly decreased during the following days, whereas the saprophytic population strongly increased during the first day.
Bacterial suspensions and contaminated waters were introduced in dialysis bags and dipped into the sludge basin or in the river. The evolution of bacterial numbers in these bags suggest that sedimentation, caused by floculation, was the main cause of the strong decrease of fecal bacteria. Chemical precipitation of phosphate by addition of iron chloride could enhance this effect by increasing the amount of floculated sludges.
A strong decrease in fecal bacteria counts was noted in the Doubs after introduction of sewage treatment in the neighboring cities.

High resolution electron microscopy of stained vs unstained bacterial cell envelopes in CTEM and STEM

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The envelope of *Escherichia coli* consists of several structurally defined layers. Starting from the outside one first finds the outer membrane composed of lipids and (glyco-) proteins. The following layer is the peptidoglycan perhaps separated from the outer membrane by an intermediate layer of unknown nature. Between the peptidoglycan and underlaying cytoplasmic membrane is what is called the periplasmic space.
Cell envelopes of *E. coli* were prepared by Mickle disintegration and then subsequently fixed solely with glutaraldehyde followed by dehydration/embedding at low temperatures in HM20, a nonpolar resin (Carlemalm et al., Experientia *36*, 740, 1980). In CTEM, staining with uranyl acetate shows that outer membrane with underlying peptidoglycan remains. 3 parallel, darkly straining bands of particulate material are observable, with a possible arrangement of these particles into a regular sequence in some areas. However, the size of these particles is similar to deposits of uranyl acetate grains (approx. 20 Å).
To overcome the limitations imposed by staining, unstained cell envelopes were viewed in STEM. The 3 parallel bands, seen previously in CTEM, were observed in STEM and appeared 'particulate' in some areas, with a possible regular arrangement. Future development of STEM, linked to tilt studies, will lead to more information concerning the nature of bacterial envelopes, and more particularly the matrix protein of *E. coli* which is arranged as a hexagonal lattice in the outer membrane (A.C. Steven et al., J. Cell Biol. *72*, 292, 1977).
From our measurements the hypothesis arises that the native peptidoglycan is much looser than presently assumed and thus could fill the periplasmic space completely. Tests of this hypothesis are being continued.

Bacterial mesosomes: facts or artifacts?

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Mesosomes are found along the cytoplasmic membranes and at sites of septum formation in gram-positive bacteria. They are defined as pocket-like membrane invaginations containing so-called vesicles, lamellae and tubules. They can only be visualized by electron microscopy following fixation of the bacterial cells. In the past immobilization of the cellular structures was achieved by chemicals such as glutaraldehyde and/or osmiumtetroxide as well as by freezing with cryoprotectants. The fixed preparations were then processed either as freeze-fractures or thin sections. Several reports have been published stating in general that the size and structure of the mesosomes varied with the conditions used for fixation. This led even to some doubts if mesosomes are integrated and functional organelles of the bacterial cell, especially since nobody was able to assign a definite function to them although it has been suggested that they might be involved in membrane synthesis, in the transport of enzymes and in the endospore formation.

In the present study, we showed that mesosomes are absent if the cells were processed by the cryofixation and freeze-substitution technique. In contrast to chemical fixation, cryofixation leads to an immediate immobilization of the intracellular structures and the subsequent substitution of the ice by an organic solvent with concomitant fixation at low temperatures prevents artifacts also during this step. For cryofixation the bacteria, placed on a gold grid between 2 low mass copper platelets were frozen in the propane jet. Substitution and fixation was carried out in methanol containing osmiumtetroxide (1%), uranyl acetate and glutaraldehyde (3%). The frozen samples were held in the substitutent solution at 183, 210 and 343 K for 8 h at each step, followed by a final incubation for 1 h at 273 K. After exposure to anhydrous acetone, the specimens were embedded and processed as thin sections. Cryofixed cells could also be prepared as freeze-fractures using a Balzers BAF 300 at a pressure of 10^{-5} Pa.
Mesosomes never occurred if cryofixation was used, a pure, high-speed physical method for immobilization which does not allow time for membranes to rearrange. With the same organism, *Bacillus cereus* (ATCC 10,702), mesosomes appeared abundantly if the classical chemical fixation was used.

Polioviral proteins enter the host cell nuclei and also accumulate in confined structures of the cytoplasm

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Poliovirus (type I, Mahoney) specific proteins undergo extensive migration within the host cell. To demonstrate this, HEp-2 cells were pulse-labeled with ^{3}H-leucine during maximal viral protein synthesis, i.e. at 2.5 h p.i., while being kept under high salt conditions to minimize cellular protein synthesis. They were then processed for EM autoradiography which showed protein synthesis to occur randomly distributed in the cytoplasm. If the same ^{3}H-leucine pulse is followed by a 0.5-h chase, however, viral proteins tend to accumulate in newly formed clusters of vacuoles (i.e. the structure of cpe and viral RNA synthesis, as reported earlier), located in close vicinity of the Golgi fields. In the still functional nucleus viral proteins are randomly distributed. With a chase of 1 h and longer, the viral proteins are definitely contained within the central field of cpe-vacuoles. In the nucleus, which at that time only synthesizes rRNA and no more hnRNA, viral proteins are found preferentially in or around the nucleolus. PAGE of isolated, purified nuclei showed an accumulation of partially cleaved viral precursor proteins as compared to the corresponding cytoplasmic extract. From our data and those of the accompanying paper by Bossart et al. on in vitro experiments it can be concluded that viral proteins enter the host cell nucleus and that they may be involved in shutoff of cellular RNA synthesis.

Studies on in vitro RNA synthesis in nuclei isolated from poliovirus-infected HEp-2 cells

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Infection of host cells with enteroviruses results in an inhibition of cellular RNA synthesis. The kinetics and specificity of this inhibition, however, was shown to be determined by the host cell line. The presence of poliovirus proteins in the host cell nucleus was demonstrated by EM autoradiography and protein analysis by PAGE (see communication: Bienz et al.).
In order to investigate a possible role of viral proteins in host cell RNA synthesis inhibition, an in vitro transcription system for isolated nuclei was established. Isolated nuclei prepared from uninfected HEp-2 cells synthesized rRNA and hnRNA as determined by EM autoradiography, sucrose gradient analysis or inhibition by α-amanitin. Experiments using heparin or sarkosyl also indicated a limited extent of de novo initiation of RNA synthesis. In nuclei isolated from HEp-2 cells infected with poliovirus (type I, Mahoney), the capacity to synthesize RNA decreased with time after infection. In addition, hnRNA synthesis was selectively inhibited more than rRNA synthesis as confirmed by EM autoradiography or by using α-amanitin. Virus-specific proteins shown to be present in infected nuclei were not released by incubation of isolated nuclei in the in vitro reaction mixture. The virus-induced inhibition of host cell RNA synthesis, therefore, could not be overcome by the in vitro assay conditions. The kinetics and specificity of inhibition in vitro was comparable to the in vivo situation.

Early events in the replication of parvovirus LuIII

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LuIII virus, a nondefective parvovirus, was labeled with either ^{3}H-thymidine or ^{3}H-amino acids and purified by a sequence of centrifugations in sucrose/CsCl gradients. 2 types of infectious particles banding at around 1.45 g/ml ('heavy' particles) and at 1.42 g/ml (mature virions) were used to study the process of adsorption, penetration and uncoating of the parvovirus in NBK cell cultures.
In a first experiment it was shown that 'heavy' particles and mature virions differ in their ability to bind to susceptible cells. Under otherwise identical conditions, the number of 'heavy' particles adsorbed per cell during incubation at 4 °C for 60 min was only one third that observed with mature virions. In both cases, however, adsorption was completed within 30–60 min. At this stage of infection virus particles could still be removed from the cell surface by washing with EDTA but the virus/cell complex became resistant to such treatment during incubation at 37 °C.
The bulk of mature virions appeared in the cytoplasm of infected cells within 15 min after rising the temperature to 37 °C. With prolonged incubations both DNA and viral proteins were rapidly transported into the nucleus. Experiments in which the cytoplasmic and nuclear fractions were digested with DNase provided evidence that uncoating of parvovirus particles starts in the cytoplasm and is continued in the nucleus. Finally, SDS/polyacrylamide gel-electrophoresis suggested that the uncoating process is characterized by a distinct degradation pattern of the viral structural polypeptides.

Herpesvirusnachweis mit Hilfe des Enzymtestes und der Anzüchtung in der Gewebekultur

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Der Nachweis von *Herpesvirus hominis* in klinischem Untersuchungsmaterial mit Hilfe der Anzüchtung in der Gewebekultur und dem Nachweis im Enzymtest wurden bezüglich ihrer Empfindlichkeit untereinander verglichen. Dabei wurden folgende Ergebnisse erzielt:
1. Die Gewebekultur ist bezüglich des Nachweises von *infektiösem* Herpesvirus empfindlicher als der Enzymtest.
2. Der Enzymtest eignet sich im Gegensatz zur Gewebekultur aber auch zum Nachweis von *nicht mehr infektiösem* Virusantigen.

3. Der Nachweis von Herpesvirus mit dem Enzymtest gelingt innerhalb von 5 Stunden bei gleichzeitiger Typenbestimmung des Herpesvirus.

Herpes varicella-zoster IgG antibody screening of a Swiss blood donor population

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Because of the severity of herpes varicella-zoster infections in immunocompromised patients the demand for specific high titre immunoglobulins for prophylactic use has increased. Such high titre anti-herpes varicella-zoster IgG is usually isolated from plasma collected by plasmaphoresis from convalescent individuals. In order to make available plasma from regular blood donations, 3 methods, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and passive hemagglutination inhibition assay (PHIA), respectively, were adapted to use in mass screening of donor plasma.

Plasma of 4883 Swiss blood donors was screened by ELISA and RIA, respectively. Good correlation between these 2 techniques was observed. In 1.2% of the samples antibodies with high titres, of the same order as observed in convalescent plasma, were detected by both methods. Intermediate ('normal') titres, which are supposedly due to residual immunity, were observed in 93.1% (ELISA) and in 91.9% (RIA), respectively. The average titre in this category was $1/_{17}$ of the one in reconvalescent plasma. On the other hand, no specific IgG anti-varicella-zoster antibodies could be detected in 5.7% (ELISA) and in 6.9% (RIA), respectively.

PHIA was performed on a Groupamatic C-360 automatic blood grouping system. Anti-varicella-zoster antibodies purified by affinity chromatography were coupled onto human group 0, rhesus negative red cells by means of $CrCl_3$. Strongly positive plasmas were capable to inhibit the passive hemagglutination. Among 465 donor samples 10 (2.2%) inhibitors, apparently with high titre antibodies, could be detected, whereas 97.8% of the samples did not react. Therefore it was not possible to distinguish intermediate ('normal') titre antibodies from negative specimens with this technique. We conclude that ELISA and RIA are more reliable techniques than PHIA for mass screening of herpes varicella-zoster antibodies in plasma of blood donors.

Charakterisierung von Epstein-Barr-Virus-(EBV-)stamm- und -gruppenspezifischen Antigendeterminanten durch monoklonale Antikörper

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Durch Immunisierung von BALB/C-Mäusen mit gereinigten EBV-Partikeln (Stamm QIMR-WIL) und Anwendung der Hybridoma-Technik wurden monoklonale Antikörper gegen virale Polypeptide erhalten. Diese monoklonalen Antikörper konnten mit Hilfe der indirekten Immunfluoreszenz erfasst werden. Immunpräzipitationen von ^{125}J-radioaktiv markierten viralen Polypeptiden unter Verwendung dieser monoklonalen Antikörper und der Protein-A-Methode zeigten, dass eine Vielzahl von Antikörperklonen gegen die 4 bedeutendsten Oberflächen- und Envelop-Polypeptide des Epstein-Barr-Virus erhalten wurden. Diese monoklonalen Antikörper präzipitierten die Polypeptide p340, p340/p240, p140 und p80. In einer Reihe von weiteren Versuchen wurde geklärt, inwieweit monoklonale Antikörper, gerichtet gegen Polypeptide des QIMR-WIL-EBV-Stammes, auch mit analogen Polypeptiden der EBV-Stämme P3HR-1 und B95-8 reagieren. Es zeigte sich, dass eine Reihe von anti-p340-(QIMR-WIL-)Antikörpern nur die stammspezifischen Antigendeterminanten erkennen, während anti-p340/p240-(QIMR-WIL-) sowie anti-p140-(QIMR-WIL-)Antikörper auch Antigendeterminanten erfassen, die B95-8- und P3HR-1-Virusstämmen gemeinsam sind. Diese Ergebnisse zeigen, dass verschiedene EBV-Stämme serologisch mit Hilfe von monoklonalen Antikörpern typisiert werden können.

In vitro persistence of canine distemper virus: generation of viral progeny with altered host cell spectrum

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In vitro isolation of virulent canine distemper virus (CDV) is accomplished most successfully by either inoculating primary dog macrophage cultures or culturing infected tissues. It has been shown, however, that once adapted to grow in a variety of cell culture systems other than canine macrophages, the carried virus loses its virulence for susceptible puppies. In an attempt to establish an in vitro state of viral persistency with virulent CDV, infected canine glial cells and a bovine cell strain, carrying the virus were cocultivated with VERO cells (African green monkey kidney cell line). The induction of a stable state of in vitro viral persistence could repeatedly be accomplished. In such cell lines the generation of progeny with an altered in vitro host cell spectrum was observed. In vivo virulence of progeny virus was maintained during more than 1 year in cultures.

Isolation of *Orthomyxovirus* influenza in MDCK cells in the winter 1980/81 in Lausanne

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The isolation of influenza viruses from clinical specimens, obtained from patients suspected of having an acute infectious respiratory disease, was performed in canine kidney cells (MDCK) and in primary patas monkey kidney cells (PPMK). 22 specimens were found positive for *Orthomyxovirus* influenzae, which were grouped by haemagglutination inhibition test as the following: 10 strains of subtype A H1N1, 5 strains of subtype A H3N2 and 7 strains of type B similar to B-Hongkong /5/72. 7 specimens were inoculated both on MDCK and PPMK and produced a positive HAd on MDCK only, thus not on PPMK. They were 5 strains H1N1, 1 strain H_3N_2 and 1 B. 5 specimens produced a positive HAd on both cell types (1 A H1N1, 3 A H_3N_2 and 1 B). 4 specimens (B-Hongkong /5/72) produced a strong HAd on MDCK 24 h postinoculation, whereas HAd was weak on PPMK. 4 strains A H1N1, 1 A H3N2 and 1 B-Hongkong /5/72 were isolated on MDCK only, for lack of PPMK. Using the MDCK cell results one can see that the winter 1980–81 influenza epidemics started early in the winter with both A subtypes. H1N1 strains were isolated from school children and young adults. At the end of the winter and early in spring, the B-Hongkong /5/72 were isolated from all age classes patients. Several severe cases were then observed.

Infection of the *Aedes albopictus* cell clone C6/36 with Semliki Forest Virus

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Semliki Forest Virus (SFV), an α-Togavirus, multiplies in both vertebrate and invertebrate cells. Infection of a variety of vertebrate cells, where SFV grows to high titers, leads to extensive cytopathic effect (CPE) and cell death. In contrast, propagation of the mosquito cell line *A. albopictus* (Singh) appears to be unaffected by SFV infection; cells show no CPE, while SFV multiplies to low titers. Generally, such cultures survive and grow out to a permanently infected cell line. However, clone C6/36 (A. Igarashi, J.gen. Virol. *40*, 531) derived from *A. albopictus* (Singh) produces high titers and shows CPE with plasma bridges between neighboring cells.

To investigate the behavior of clone C6/36 after SFV infection cultures at different growth stages were infected with SFV at high multiplicities (MOI $\sim 10^3$). During acute infection, irrespective of the growth stage, 90–95% of the cells died as monitored by the following criteria: growth kinetics of the cell cultures, DNA content per culture and DNA and protein synthesis. Infection of the cells with low multiplicities (average MOI < 1) resulted in a delay of the observed cell death by 20–24 h. This is probably due to a secondary infection of the noninfected cells since at that time virus production of infected cells is maximal. A small portion (5–10%) of the cells survived and became permanently infected producing low amounts of virus.

In parallel experiments permanently infected cells exhibited resistance to SFV superinfection and showed no alteration in their growth behaviour.

We conclude that our line of the clone C6/36 became inhomogenous: the majority of the cells being high virus producers, showing CPE and perishing upon infection with SFV; and a small portion of the cells surviving, producing low titers, showing no CPE and becoming permanently infected.

Comparison of various canine parvovirus (CPV) strains with both wildtype and live attenuated vaccine strains of feline panleukopenia virus (FPV) and mink enteritis virus (MEV)

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Since 1978 outbreaks of a so far unknown enteric disease in dogs were observed almost simultaneously throughout the world. The causative agent was found to be a parvovirus, now referred to as canine parvovirus (CPV) which antigenically resembles feline panleukopenia virus (FPV) and mink enteritis virus (MEV). The latter virus frequently is referred to as a strain of FPV. The origin of CPV as well as the mode by means of which it was spread so effectively still remains unknown.

In order to obtain some information on the relationship between CPV, PFV and MEV, we have compared the genomes of various strains of these viruses by restriction site mapping of their double-stranded replicative form DNAs. The analysis of the genomes of 4 CPV strains isolated in Switzerland, Belgium, West Germany and the USA, respectively, revealed no significant differences between the various isolates. However, the CPV strains could be distinguished clearly from wildtype FPV and MEV. For instance, the comparison of MEV with a CPV strain isolated in Switzerland showed that 11 (14%) out of 79 restriction sites were present on only one type of DNA. These results support the idea of a common origin of CPV and further indicate that CPV is closely related to FPV and MEV. The restriction site mapping of the genomes of various vaccine strains of the latter viruses as well as a detailed serological comparison are now in progress. So far, we cannot decide whether CPV, FPV and MEV evolved from a common precursor or if CPV is derived from a distinct strain of FPV or MEV.

Coproantibodies against rotavirus in calves

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The excretion of coproantibodies of calves naturally and artificially infected with rotavirus was examined. Daily fecal samples taken from calves of a dairy operation were diluted, clarified and individually checked for antibody against rotavirus by ELISA. High values of IgG_1 binding to rotavirus antigens were detected in feces of all colostrumfed calves during their first few days of life. A 2nd peak of antibody excretion was observed at the end or just following episodes of rotavirus diarrhea. Again IgG_1 was the predominant class of antibodies. IgA occurred in lower quantities.

Diarrhea and massive virus excretion after artificial inoculation was observed only in colostrum deprived calves. These calves were protected against septicemia by an immunoglobulin preparation given i.v. on their first day of life. Coproantibody excretion at the end of virus diarrhea was the same as in naturally infected calves. IgM and IgG_2 directed against rotavirus were not found in the examined feces.

Comparison of serum neutralization and the ELISA-technique in the detection of antibodies to IBR-IPV-virus in cattle

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The influence of virus dose, temperature and length of the preincubation period on the sensitivity of a microneutralization test was studied. The standard procedure uses 30–300 TCD_{50} and 3 h at 37 °C for preincubation. A higher sensitivity can be obtained by increasing the preincubation period to 18–32 h. The epidemiological evaluation indicates that all inhibitory activity of undiluted serum has to be considered as specific.

In the ELISA-test problems are encountered in determining the limiting value for negative versus positive reactions. This value can be arbitrarily fixed by the use of a positive serum with a low minimal titre. Sera reacting equal or higher than this reference serum are considered positive. Or, several negative sera can be included in the test and their mean value plus 2 standard deviations used for determining the negative background level.

Comparative studies have been conducted in herds with known epidemiological history using both tests on blood serum and in addition in some herds the ELISA-test with milk sera in order to evaluate the significance of results in the control of the disease.

Serologische Bestimmung der Immunität gegen Mumps (Parotitis epidemica) – Vergleich von 5 verschiedenen Methoden

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Von 32 Kindern im Alter von 1½ bis 2 Jahren, die gegen Mumps geimpft worden waren, standen Serumproben vor und nach Impfung für die Bestimmung von Antikörpern zur Verfügung. Mumps-Antikörper wurden mit folgenden Techniken bestimmt: Serum-Neutralisation, Hämagglutinationshemmung (HAH), ELISA, radiale Hämolyse und Immunfluoreszenz. Für die Auswertung der Resultate wurden die Ergebnisse der sehr aufwendigen Serum-Neutralisation als Leitlinie verwendet. Die Immunfluoreszenz erwies sich als die zuverlässigste Methode, gefolgt von ELISA und HAH, während die radiale Hämolyse die meisten Abweichungen von der Serum-Neutralisation zu verzeichnen hatte.

Immunological study of the glycophorin from swine erythrocytes and a glycoprotein extracted from the membrane of *Mycoplasma hyopneumoniae*

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Membrane glycoproteins have been described with regard to some pathogenic mycoplasmas. Their biological function, however, remains unclear. The detection of cold agglutinins in patients suffering from infection with *M. pneumoniae* suggests some immunological cross-reactions between glycophorin from human red blood cells and membrane glycoproteins of the involved mycoplasma. The aim of our study was to demonstrate similar reactions between *M. hyopneumoniae* and swine erythrocytes. For this purpose, antisera against glycophorin from swine erythrocytes and the membrane glycoprotein of *M. hyopneumoniae* were produced in rabbits. Neither by agargel precipitation nor with an enzyme linked immunosorbent assay (ELISA) a clear cross-reaction could be shown. Furthermore, no cold agglutinins have been found in pigs affected with enzootic pneumonia. On the other hand, pigs experimentally infected with *M. hyopneumoniae* show antibodies reacting with glycophorin extracted from swine erythrocytes, as detected with an ELISA.
The development of such antibodies occurs parallelly, but to some extent independently from the specific immune response to *M. hyopneumoniae* infection, pointing out an autoimmunological aspect in the pathogenesis of this disease. Since no cross-reactions could be demonstrated between glycophorin and *M. hyopneumoniae* glycoprotein, it seems uncertain that the mycoplasma glycoprotein is directly involved in the observed immunological phenomenon.

Isolation and purification of equine IgE

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Homocytotropic antibodies capable of sensitizing equine skin were demonstrated in equine serum 3 weeks following antigenic stimulation with o-DNCP-BSA. The heat labile antibody had a mol.wt of about 200,000 daltons. Positive Prausnitz-Küstner reactions demonstrated that homocytotropic antibodies persist in equine skin for at least 10 days. This reaginic immunoglobulin class was separated from appropriate antisera by ammonium sulfate precipitation and purified by chromatography on DEAE-cellulose and gel-filtration on Sephacryl S 200 columns. 10 µg protein of the final preparation was capable of eliciting a positive PCA. In immunodiffusion tests the immunoglobulin class associated with the skin-sensitizing activity did not cross-react with antisera against the known equine immunoglobulin classes. The results indicate that homocytotropic antibody activity in the equine is associated with an immunoglobulin class analogous to human IgE.

Production of hybridoma secreting monoclonal antibodies against lymphocyte activating factor (Interleukin 1)

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In recent years a whole set of new molecules synthesized and secreted by lymphoid cells called lymphokines have been discovered. Such lymphokines are playing an important role in various aspects of the immune response. A mitogenic activity that appears in supernatants of stimulated human and murine leukocyte cultures, termed lymphocyte-activating factor LAF (Interleukin 1, IL-1), was first described by Gery in 1972. IL-1 increases DNA-synthesis, mitosis and response of thymocytes and T-lymphocytes to mitogens. Because of its small concentration in serum fluids, isolation and further characterization of human IL-1 is difficult up to now.
BALB/c mice have been immunized several times with a protein fraction (OZ 46 III) of partially purified human IL-1 obtained from supernatant of Con-A stimulated lymphocytes (U. Otz, S. Lazary, L.B. Schook and A.L. de Weck, in: Biochemical characterisation of lymphokines, p. 15. Academic Press, New York and London 1980). Spleen cells of immunized mice have been fused with Sp 2/0 myeloma cells according to the method described by Fazekas (S. Fazekas de St. Groth and D. Scheidegger, J. Immun. Meth. *35*, 1, 1980).
A first screening of hybrid culture supernatants for specific anti-IL-1 antibodies has been performed by solid phase radioimmunoassay using 125-I-*Staphylococcus* Protein A and crude IL-1.
From 250 cultures 94 showed more than twice background levels. These have been tested in a 2nd round for inhibition of 3-HTdR-uptake in an IL-1 dose-dependent lymphoproliferative assay, where some of them showed inhibition of IL-1 activity. For this screening a fraction > 5000 D of a standard human IL-1 preparation obtained from Con-A stimulated human buffy coat cells has been used. Attempts to establish cell lines producing anti-human IL-1 antibodies are in progress.
The production of monoclonal anti-human IL-1 antibodies provides an excellent tool for further isolation and characterization of this lymphokine and its biological function.

Yeast outgrowing buds with more acid pH than their mother cells

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Intracellular pH of resting cells of *Saccharomyces cerevisiae* is in the range of 5.8–6.0 (Conway and Downey, 1950). We confirm such values in the cytoplasm below the buds from 4-h cells growing on malt-agar by its staining red with chlorophenol red and greenish-blue (blue mitochondria) with bromocresol green. Emerged, still amitochondrial buds showed their homogeneous hyaloplasm stained yellow (slightly orange) with the best penetrating vital indicator, chlorophenol red (2.10^{-5} aqueous) and greenish-yellow with bromocresol green (idem), as confirmed on color photomicrographs and indicating an average pH of no more than 5.0. An even lower pH (~4.0) was reached in buds from high glucose-KCl grown cells, as suggested by their yellow staining with bromophenol blue or bluish with Congo red. That the pH was at least higher than 5.5 in the

cytoplasm of the mother cells and sharply lower in their buds was confirmed by the greenish-grey fluorescence (UV 350 nm) of 4-methyl-esculetin in the mother cells (maximum in scars) contrasting with its near extinction in the buds. The acid buds may behave as sinks of protons vectorially extruded from the mitochondria of the mother cells; being then positively charged, they could attract the negatively charged precursor and enzyme-loaded vesicles to the expanding, softened bud wall. The spindle plaque thought to orient the bud emergence site (Byers and Goetsch, 1975) may therefore simply be positioned electrically in the axis of the primary − to + gradient which will be the direction of the subsequent mitotic figure.

Kinetics of bacterial adhesion in vitro

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We have recently described a tissue culture system for measuring the adhesion of radiolabeled *Escherichia coli* cells to monolayers of the human epithelioid tissue culture cell line, Intestine 407 (Vosbeck et al., Rev. Inf. Dis. *1*, 845, 1979). An analysis of the adhesion of *E. coli* SS142 in this system suggests that bacterial adhesion follows Michaelis-Menten enzyme kinetics, and that it therefore can be characterized by simple kinetic constants, such as the dissociation constant (K_s) and the maximum velocity (v_{max}) of the reaction, even though these constants may be expressions of complex interactions.
Adhesion of *E. coli* SS142 was shown to be irreversible in this system. It was linear up to about 10^9 bacteria/ml, after which saturation was approached. A double-reciprocal plot (Lineweaver-Burk) of these data yielded a straight line. Adhesion of bacteria to the monolayers was concentration, rather than volume dependent, as expected for an enzyme-substrate-like interaction. Raffinose was shown to act as a competitive inhibitor of the adhesion of *E. coli* SS142.
The kinetic treatment of adhesion data enables one to determine both, the affinity of the bacteria to the monolayer cells, and the maximum rate of irreversible adhesion. It may be possible to differentiate between at least 2 types of inhibitors of adhesion, the first affecting the affinity of the bacteria for the monolayers, and the 2nd inhibiting the irreversible adhesion step.

The role of acetylornithine 5-aminotransferase in ornithine and arginine catabolism of *Pseudomonas aeruginosa*

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22 ornithine nonutilizing mutants (Oru mutants) of *P. aeruginosa*, strain PAO were isolated and genetically and biochemically characterized. They fell into 3 transduction groups (Oru I, Oru II, Oru III). Ornithine and arginine transport as well as the enzyme levels in the arginine deiminase- and the arginine decarboxylase-degradative pathways were normal in all mutants. Whereas no biochemical lesions have been detected so far in Oru II and Oru III mutants, the Oru I mutants differed in the following properties from the wild type: 1. No growth on media containing ornithine or arginine as the sole carbon source. Restoration of ornithine or arginine utilization by lysine. 2. Inhibition of growth by ornithine or arginine (arginine sensitivity). 3. Increased or decreased specific activity or changed catalytic property of acetylornithine 5-aminotransferase (ACOAT).
Determination of ACOAT-activity and of the level of ACOAT-crossreacting material in crude extracts suggested that the Oru I class comprises mutants with an altered ACOAT structural gene (*argD* mutants) as well as mutants affected in a regulatory region or a regulatory gene governing expression of *argD*. The inability of Oru I mutants to utilize ornithine or arginine is thus not due to a block in ornithine/arginine catabolism but rather to their sensitivity to ornithine and arginine which arises in parallel with changes in the quality or the quantity of ACOAT. Ornithine and arginine may deplete the lysine pool or interfere with lysine catabolism or biosynthesis in Oru I mutants.

Propionate metabolism in *Acetobacter aceti*

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To determine which of the several known pathways of propionate metabolism in microorganisms might be operative in *A. aceti*, a suspension of propionate adapted cells was incubated in growth medium containing propionate 1-^{14}C, 2-^{14}C or 3-^{14}C, and respired $^{14}CO_2$ was trapped and quantitated. The radiorespirometric pattern obtained (1>2>3) is consistent with the oxidation via pyruvate. Because there is no experimental evidence for the condensation of propionate with glyoxylate or oxaloacetate, oxidation of propionate via acrylate and lactate to pyruvate seems to be the only pathway operative. The following enzymes of this sequence were present in cell-free extracts: propionate CoA-transferase (EC 2.8.3.1), Acyl-CoA dehydrogenase (EC 1.3.99.3), lactoyl-CoA dehydratase (EC 4.2.1.54) and 2 NAD-independent lactate dehydrogenases (EC 1.1.2.3 and 1.1.2.4).
Glyoxylate cycle enzymes (malate synthase and isocitrate lyase) are not active in extracts of propionate adapted cells. C_4-acids will therefore be formed, as already known for pyruvate grown cells, by pyruvate, orthophosphate dikinase and phosphoenolpyruvate carboxylase.

Ametryne, prometryne and methylsulfonic acid as sulfur sources for bacteria

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Bacteria were isolated that could utilize quantitatively the s-triazine herbicides prometryne and/or ametryne as sole and limiting source of sulfur for growth. The success of enrichments depended on previous exposure of the soil inoculum to s-triazine herbicides. Deaminoethylametryne and methylsulfonic acid could also be used as sulfur sources.
Utilization of a compound was quantified as the growth yield of protein per mole of sulfur supplied. Inorganic sulfate and the 4 organic sulfur compounds each allowed yields of about 6 kg protein per mole of sulfur.
The s-triazines were stable in sterile control experiments; the compounds were pure, as assayed by mass spectrometry and elementary analysis. The product of desulfuration of an s-triazine was the corresponding hydroxy-derivative. This derivative was identified by co-chromatography with authentic material, by UV-spectrophotometry, and, for hydroxyprometryne and hydroxyametryne, by mass spectrometry.

The degradative pathway of the s-triazine melamine

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Pseudomonas sp. strain A grew with melamine, ammeline, ammelide, cyanuric acid or ammonium ion as sole and

limiting nitrogen source and the growth yield was about 55 g protein per mole of nitrogen. Growth and utilization of nitrogen source were concomitant for ammeline, cyanuric acid and ammonium ion. Growth with melamine was biphasic. A compound was excreted and subsequently utilized during growth. This compound was isolated and identified as ammeline by co-chromatography with authentic material, UV-spectrophotometry and mass spectrometry.

Cell extract of strain A was applied to a DEAE-cellulose column and eluted with a gradient of phosphate buffer. The capacity to deaminate melamine to ammeline was eluted as a very broad peak. A narrow peak of activity converted ammeline to ammelide, and another narrow peak converted ammelide to cyanuric acid. Ammelide and cyanuric acid were isolated and tentatively identified by co-chromatography and UV-spectrophotometry. Each reaction was stoichiometric.

Melamine is thus degraded in 3 reactions, each of which has its own deaminase, to cyanuric acid. Degradation of cyanuric acid is being studied (Beilstein and Hütter, Experientia *36*, 1457, 1980).

Copurification of the enzymes of proline catabolism from *Pseudomonas aeruginosa*

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The activity ratio of proline dehydrogenase and Δ^1-pyrroline 5-carboxylate (P5C) dehydrogenase from *P. aeruginosa*, strain PAO remained constant during a 3-step purification procedure leading with 5% yield to a nearly homogeneous preparation (>90% pure) containing both activities. The enzyme activities were very unstable during purification. In the first step of the purification, chromatography on DEAE-cellulose, the enzymatic activity dissociated into 3 peaks with an identical elution volume on sephadex G-200. Each of them, rechromatographed on DEAE-cellulose, dissociated again into 3 peaks. Further purification was achieved by affinity chromatography on Matrex Gel Red A (Procion Red HE3B) and by gelfiltration on sephadex G-200.

The enzymatic activities of the final preparation were stable in buffer containing 50% glycerol. Its mol.wt was estimated at 240,000 by gelfiltration on sephadex G-200. It catalyzed the 2 consecutive steps of proline catabolism: L-proline was oxidized to P5C with FAD and 2 nonphysiological electron acceptors (phenazine methosulphate and p-iodonitrotetrazolium), P5C was converted to glutamate with NAD(P). Electrophoresis on polyacrylamide gels in the presence of FAD resulted in one protein band containing both enzymatic activities. Electrophoresis in the absence of FAD led to 2 protein bands, each catalyzing both enzymatic reactions. Preliminary experiments with electrophoresis under denaturing conditions suggest the existence of a bifunctional protein catalyzing the 2 consecutive reactions of proline catabolism.

Resistance of *Pseudomonas aeruginosa*, to nalidixic acid

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Spontaneous mutants of *P. aeruginosa* PAO resistant to different levels of nalidixic acid (NAL) have been investigated genetically and physiologically. 3 types of mutations have been found.

nalA mutations were mapped in the 60-min region of the chromosome and gave high level NAL resistance which was also manifest in permeabilized cells.

nalB mutations were mapped in the 30-min-region of the chromosome and gave low level resistance to NAL, to β-lactam antibiotics and to novobiocin. In permeabilized *nalB* mutants the DNA-synthesis was as sensitive to NAL as in the wild type strain.

A *pip-3* mutation was mapped in the 60-min region, close to *nalA*; *pip-3* conferred low level resistance to NAL and pipemidic acid and resulted in increased sensitivity to novobiocin.

Oxygen as a regulator of tyrosinase formation in *Streptomyces glaucescens*

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In the wild type strain of *S. glaucescens* tyrosinase (E.C. 1.14.18.1) can be induced by different substances. Without an inducer the strain does not produce the enzyme in a measurable amount. The amino acid L-leucine is used by us as an inducer.

In liquid medium oxygen has a negative influence on the level of the enzyme. If we induce the enzyme in a chemostat, the level of specific activity is only a fraction of that in shake cultures. The reason is that the dissolved oxygen levels are not the same in both cases. During rapid growth in shake cultures the content of oxygen decreases from initially 100% (1.16 µmoles/l at 30 °C) to less than 5%. In a chemostat stirring is more effective and so in this case the content of oxygen can be held at 100%. If the organism grows in the chemostat with a mixture of 99% nitrogen and 1% oxygen instead of air, the specific activity rises to a higher level than in shake cultures. In the case of the tyrosinase constitutive mutant strain 429 the specific activity of the enzyme in the presence of pure oxygen can be reduced to less than 10%. Experiments with purified enzyme have shown that oxygen does not decrease the activity of tyrosinase. However, the formation of tyrosinase by growing cells is inversely proportional to the content of dissolved oxygen in the medium. It is not known whether the influence of the oxygen is a consequence of inhibited synthesis or due to an inactivation or a degradation of the enzyme by the cells.