Schweizerische Gesellschaft für Mikrobiologie *Berichte der 41. Jahresversammlung*

Société Suisse de Microbiologie *Comptes rendus de la 41e réunion annuelle*

Società Svizzera di Microbiologia *Rendiconti della 41ma sessione annuale*

Swiss Society of Microbiology *Reports of the 41st annual meeting*

Solothurn, 17–19 June 1982

The Society Prize 1982

The Society Prize has been awarded to Dr Othmar Käppeli, Department of Biotechnology, Swiss Federal Institute of Technology (ETH), in recognition of his contribution to technique in the uptake of water-insoluble substrates in microbial systems.

Lectures of the Society Prize winners

1982: Dr O. Käppeli, Department of Biotechnology, Swiss Federal Institute of Technology, ETH Hönggerberg: 'Transfer and uptake of water-insoluble substrates in microbial systems.'
1981: Dr H. Arnheiter, National Institute of Health, Bethesda, Maryland, USA: 'Monoclonal antibodies against synthetic interferon fragments: their application in characterizing the topography of interferon molecules.'

Main lecture

Prof. Dr E.-L. Winnacker, Department of Biochemistry, University of Munich: 'The medical implications of biotechnology.'

Lectures

Medical applications of biotechnology

E.-L. Winnacker
Institute of Biochemistry, University of Munich, Karlstrasse 23, D-8000 Munich 2

Biotechnological developments will be of increasing relevance for medicine during the next 2 decades. These prospects arise mainly through the possibilities of gene manipulation. This field of work requires few experimental elements, the construction of a recombinant DNA molecule, its transfer to an appropriate host, its selection and its reproduction in the new host organism. Since genes thus can be reproduced and expressed in cells in which they are not normally present, a 'synthetic' biology can and will be developed in the near future.
The paper discusses at least 6 possible applications: a) the synthesis of important polypeptides; b) the antenatal diagnosis of human genetic diseases; c) progress in virus research, including interferons; d) determination of the origins of cancer; e) the improved synthesis of primary and secondary metabolites and f) a solution to the problem of sufficient food supply, as exemplified by the production of single cell protein (SCP).

a) Many medically important polypetides (growth hormone, interferons, etc.) are specific for the host organism, e.g. the human. Their isolation can thus present formidable problems. In a few selected cases, e.g. insulin and growth hormone, the transfer of the corresponding genes into microorganisms has been achieved and successful production has been reported. The expression of heterologous genes into proteins requires specific vectors. In general, foreign proteins can be regarded as toxic for a microorganism. Vectors have thus been constructed which permit the inducible expression of the 'foreign' information.
b) Antenatal diagnosis of human genetic defects has in the past been studied in the cases of only selected

hemoglobinopathies by analysis of fetal blood samples. This analysis can now be extended to fetal cells in the amniotic fluid which do not express hemoglobin but which contain, in case of defects, the defect hemoglobin genes. Many diseases such as sickle cell anemia or the thallassemias are now identified through their characteristic DNA defects which can be probed on the level of the DNA by restriction enzymes or direct DNA sequencing. Lesions in the chromosomal DNA do not necessarily have to reside in the defective gene itself but are often found far removed from it. It is likely that specific polymorphisms can in the future be detected in connection with other human genetic defects of even unknown genotype and thus can be diagnosed through biotechnological methods.

c) Considerable progress has been and will be achieved in the field of virus research. These developments include the production of nucleic acid free vaccines as well as the characterization of viruses which cannot be grown in tissue culture or which are too pathogenic to be analyzed easily. The cloning of hepatitis A and B or of the genomes of various foot and mouth disease virus strains are prominent examples in this field.

d) A major progress in the cancer problem has been the recent recognition of tumor genes. It is now assumed that there are rather few tumor genes, and it has already been established that these genes are present in normal cells and, occasionally, in certain tumor viruses. The products of these genes, e.g. specific protein kinases, will have to be studied in considerable detail in order to understand and influence their regulatory influence on cell metabolism.

e) Primary (amino acids, organic acids) as well as secondary metabolites (alkaloids, antibiotics, vitamins) are already produced through biotechnological methods. However, there is considerable demand for improvements and new products. The next decade will bring the discovery of antibiotic operons, the recognition of regulatory mechanisms in their biosynthesis and the transfer of genes of interest to bacteria that are more suitable to metabolite production than fungi.

f) Finally, considerable advances will be achieved in the field of food supply, both veterinary and human. Genetic manipulations have already resulted in improvements of SCP production by the introduction of an *E. coli* gene for glutamate dehydrogenase into *Methylotrophus* m. Formidable efforts are being spent in the elucidation of the mechanism of nitrogen fixation in symbiotic soil bacteria, in vector developments for plants and in the identification of plant genes.

All these developments in the field of genetics will have to be matched by equally important advances in fermentation technology and control.

Transfer and uptake of water-insoluble substrates in microbial systems

O. Käppeli
Department of Biotechnology, Swiss Federal Institute of Technology, Hönggerberg, CH-8093 Zürich

The specific problems related to the utilization of water-insoluble substrates by microorganisms are: a) transfer of the substrate from the medium to the cells; b) uptake of the substrate through the cell envelope; and c) enzymic degradation of the substrate. These problems were investigated using the hydrocarbon-utilizing yeast *Candida tropicalis.* The transfer of hexadecane from the medium to the cell surface was an adsorptive process which is facilitated by the hydrocarbon-induced alterations of the cell surface. The surface is covered with a fibrous layer which contains a polysaccharide-fatty acid complex upon transferring the cells to a hydrocarbon medium. The primary oxidation of hexadecane involves the cytochrome P450-containing monooxygenase system, which was localized in the microsomal membrane fraction. With this fraction, in vitro hydroxylation of hexadecane was possible. The uptake of hexadecane through the cell envelope proceeds most probably by diffusion. The driving forces for a diffusive mechanism are: a) concentration gradient from the surface to the cell interior; b) solubility gradient from the cell exterior to the cell interior. The subsequent degradation of hexadecane, which is converted to palmitic acid by the microsomal enzyme system, continues in peroxisomes via β-oxidation. Endoxidation of the formed acetyl groups takes place in the mitochondria. In the translocation of acetyl groups between organelles, carnitine acetyltransferase is involved.

Interferons from eucaryotic cells: production, properties and biological activities

C. Jungwirth
Institut für Virologie und Immunbiologie der Universität Würzburg, Versbacher Strasse 7, D-8700 Würzburg

The discovery of the family of soluble proteins called interferons arose from investigations of the viral interference phenomenon. Interference occurs when virus growth in cell culture or the whole animal is prevented by infection with another virus. Interferons were discovered in 1957 by Isaacs and Lindenmann during studies of a special case of virus interference among influenza viruses, and the potential medical importance of these compounds was immediately recognized. Since then successful attempts have been made to produce interferons in large amounts, purify them and characterize them proteinchemically. The elucidation of the mechanism of action of interferons should increase our understanding of virus host cell

interaction and of the mechanism of pathogenesis of animal viruses. Most important of course is the question of whether interferon can be used in clinical treatment of viral diseases and human cancer.

Vertebrate cells normally secrete no interferons or only very low amounts. However, cases of spontaneous producers have been known. Interferons are usually secreted by cells after contact with an inducer. Many viral and nonviral inducers are known including members of the influenza-parainfluenza virus group, double stranded RNA, mitogens, etc. Nearly all useful interferon inducers have been discovered accidently. The molecular mechanism of interferon induction is, however, still unknown. The amount and the type of interferon induced can be influenced by use of a specific inductor, by a particular protocol for the induction, and by treatment of the cells with interferon induction promoters such as bromodeoxyuridin, phorbol ester, butyric acid and steroids. Pretreatment of cells before induction with low doses of interferon can also increase the yield of induced interferon. This is referred to as priming. Under comparable conditions several other proteins, called interferon associated proteins, are induced. Their functions are with a few exceptions unknown.

To date, 3 major types of human interferon, named α, β, γ are known and at least 12 subtypes in the α-family have been identified. Using recombinant DNA technology even more interferon genes have been identified. It is not known if different types of avian interferon exist.

Several types of human and mouse interferon have been purified to homogeneity. Highly purified interferon preparations of chick and several other animal interferons are also available. The specific activity of pure interferons lies between 10^8 and 10^9 units/mg. A prerequisite for the purification of interferons produced by eucaryotes was the production of large quantities of crude interferons. Classical purification procedures were usually multistep methods, using different methods of protein purification. Among these, hydrophobic chromatography and preparative sodiumdodecylsulfate polyacrylamid gelelectrophoresis have been particularly useful. The elegant method of affinity chromatography on antibody columns using monoclonal antibodies against interferon is useful for the purification of larger quantities of interferon.

Interferons exert their pleiotropic effects against viral and cellular functions at extremely low concentrations. After contact of interferon with the cell by means of specific cell receptors, the interferon induced effect gradually develops. During this time cellular RNA and protein synthesis must continue and several cellular enzymes are induced during this period. Interferons act intracellularly in the case of cytocidal viruses by specifically inhibiting viral gene expression. Replication and transformation of cells by RNA tumor viruses are also inhibited by interferon. The mechanism of inhibition of replication of these viruses is completely different from that of cytocidal viruses and may be related to interferon induced alteration of the host cell. Among the numerous cellular activities of interferon known, much attention has been given to the interferon induced alteration of the cells of the immune system.

Human interferon prepared from eucaryotic cells or genetically engineered microorganisms is presently being used in clinical studies of viral diseases and human cancer treatment.

Round table discussions

Conclusions from the round table discussions held at the 1982 annual meeting of the Swiss Society of Microbiology.

Hospital hygiene: detection of nosocomial infections

In a review of the methods used for the detection of nosocomial infections, Dr H. Reber (Basel) insisted on the importance of distinguishing between common surveillance and specific investigations of nosocomial infections.

The common surveillance investigation is based on the usual data necessary for individual diagnostic and therapeutic purposes. Therefore it requires only baseline information about the frequency and type of endemic nosocomial infections in order to recognize critical points of hospital hygiene as well as occasional deviations from the baseline. Common surveillance is usually the starting point for specific investigations of peculiar problems and of mini-epidemics.

For the specific investigation, one has recourse to special analytical methods of hospital hygiene, such as typisation, determination of plasmids or R-factors, and investigations of carriers and of the environment.

In order to obtain internationally comparable results, it is recommended to apply the definitions described in the 'Outline for Surveillance and Control of Nosocomial Infections' edited by the Center for Disease Control, Atlanta, or the Algorythmus developed by Haley et al. The detection of a nosocomial infection is based on 2 groups of criteria: the parameters of the infection and the parameters classifying an infection as nosocomial.

As a measure for the frequency of nosocomial infections, one can use their incidence or their point-prevalence; however, the latter requires a population of between 300 and 500 patients and is not suitable for studying small hospitals or special services. The point-prevalence can easily be transformed in incidence values, provided that the mean duration of the hospital stay is not biased by exceptionally long values. The incidence is preferably calculated per 100

hospitalization days instead of per 100 discharges from the hospital, the latter being indicated only if the observation period largely exceeds the length of the hospital stay.

The prospective data collection is generally considered more truthful than retrospective studies. However, a comparative evaluation in the SENIC-Project showed for the retrospective review an average sensitivity of 0.74 and an average specificity of 0.964. Indeed, it is doubtful that this result would be obtained under working conditions.

Common surveillance studies are indispensable to every hospital for providing meaningful data concerning the level of nosocomial infections. However, as the data allows only a restricted interpretation and needs to be completed by special studies, it does not seem economical to spend too much time on it. Instead, continuous surveillance can be replaced by periodical studies made 2 or 3 times a year, each on 500 patients, and the saved time can be used for special studies, such as for the surveillance of working techniques and for instruction.

Drs Reber, Widmer and Gaschen reported the results of a pilot surveillance for nosocomial infections made in 4 Swiss hospitals: 2 university hospitals, 1 cantonal and 1 regional hospital. Of 13,067 discharged patients, 1501 nosocomial infections (11.5%) were discovered, with an average of 15.8% discharges for medical, 11.1% for surgical and 7.6% for gynecological/obstetric services. Urinary tract infections represented 53.1% of all nosocomial infections, 3 quarters of them after instrumentation, followed by 18.9% wound infections, 13.7% respiratory infections and 9.6% septicemias.

The high incidence of nosocomial infections as compared with those in foreign hospitals is due, as H.R. Widmer demonstrated, to the longer hospital stay of patients. The risk of nosocomial infection rose from 4.3 per 100 patients at risk in the first week to 8.7 in the 3rd week, or from 0.62 to 1.24 per 100 hospital days.

The rate of incidence also increases with the age of the patient: over 50 years of age in surgery and over 60 years of age in medicine, the rate of nosocomial infections is proportionally higher.

In order to check the efficacy of the general policy of hospital hygiene, i.e. the preventive measures already established, the Lausanne team headed by Dr Tanner focused surveillance on selected high risk wards, specifically newborn and maternity wards. In fact, every germ that the newborn has not aquired from his mother represents a hospital contamination. The search was limited to 3 tracer germs, i.e. *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae,* present in the newborn's stool before discharge from the hospital. Dr G. Pappalardo (Lausanne) summarized the results of 76 inquiries made in 1980 and of 88 inquiries made in 1981, in which the same serotype or biotype was found in the stool of several newborns. Very few nosocomial infections were evident, but every year about 20 contaminations of the same serotype or biotype in newborn groups were detected in the same nursery, at the same period, and in absence of clinical infections. The most frequently isolated germ was *K. pneumoniae.* In several cases, the search was completed by analysis of the stools of the mothers and of the hands and the throats of the personnel. The greater the number of isolated *Klebsiella* in this group, the greater was the risk of nosocomial infection.

Moreover, a special surveillance was made on blood cultures and CSF. For every positive result, an inquiry was made on the patient, the physicians, and the personnel, often completed by environmental samples of the material in use. From blood cultures that were growing the same type of *Serratia marcescens* not yet observed in the hospital, a cluster of septical shock could be traced to contamination with the soft water used for cooling infusions and to an insufficient disinfection of the stoppers.

Pappalardo concluded that the bacteriological control of high risk patients and of some pathological diagnostic analyses result in the same insights as general surveillance.

Outbreaks of nosocomial infections are caused more and more by bacteria carrying resistance plasmids, reported Dr G. Lebek (Bern). Besides that their overgrowth is favored by the selection pressure of antibiotics, some R-plasmids can apparently increase the ability for transmission.

The plasmids, circular, double-stranded DNA-molecules beside the bacterial chromosome, carry approximatively 300 genes. Whereas chromosomes contain genes for species-specific functions of life cycle and metabolism, plasmid-genes guarantee the adaptability to special environmental situations and the evolutionary flexibility.

The cryptic plasmids, which were found up to 13 per bacteria in nearly all wild strains of Enterobacteriaceae and Pseudomonas, accelerate the multiplication of bacteria. Absorbing a transposon carrying a resistance determinant, it is changed into an R-plasmid (e.g. gonococcus, H. influenzae).

Non conjugative plasmids with phenotypically recognizable genes are transmitted by transformation, transduction or together with helper plasmids by conjugation. Some R-plasmids, present mainly in cocci, and many colicinogenic factors do belong to this group.

The conjugative plasmids are bigger. They are transferred by sexpili from one bacterial cell to another. They exist in all gram-negative species as well as in several cocci and anerobic bacteriae. Their spreading depends principally on the activity of bacterial restric-

tion mechanisms. The genes for antibiotic-resistance determine enzymes which inactivate the antibiotics or inhibit their diffusion into the cell or their intracellular transport.
They are also on the origin of resistance against mercurials, hexachlorophen and UV rays. A virulence-increasing activity can be determined by genes for cytotoxin, for heat-stable and labile enterotoxins, for fimbria and capsular substances for attaching the bacteria to the intestinal epithelia, for enterocholine and hemolysins, for a cardiotoxin and for certain membrane proteins, etc. Plasmids seem to cause a new form of nosocomial infections, due to different species of bacteria, all carrying the same plasmids.
In conclusion, every hospital should be informed of its baseline of endemic nosocomial infections by periodic, but not necessarily continuous surveillance studies. They allow the recognition of critical points of hospital hygiene, which can then be examined by appropriate techniques. They comprise an identification of microorganisms as exact as possible, including the plasmids, which seem to be at the origin of a plasmid form of nosocomial infections.

Virology

An attempt was made to provide virologists of widely divergent interests with an open and to some extent informal forum for discussion. To illustrate basic problems common to all areas of research in virology and, at the same time, to accentuate specific interests, 2 main lectures were presented. Thus, F. Steck (Veterinary Bacteriological Institute, University of Bern) highlighted the virological and epidemiological background of recent successful attempts to prevent further spread of rabies in Switzerland by oral immunization of the local fox population. On the other hand, E. Buetti (Swiss Institute for Cancer Research, Epalinges) reported on the use of the mammary tumor virus genome as a model to investigate gene regulation by steroid hormones. In these studies, the viral nucleotide sequences responsible for hormonal regulation were localized by transfection experiments with cloned genomic and subgenomic fragments of MMTV RNA. Abstracts of both lectures are given below.
The main lectures were framed by brief oral introductions to the individual posters which spanned the field of virology from clinico-pathological observations via diagnostic problems, epidemiological data, and molecular analysis to genetic studies. In contrast to the usual short oral presentations, only summaries stressing aim, methodology, and the most pertinent results were allowed to be given and illustrated by 1 or, at the most, 2 slides. By this means, the audience was rapidly informed on a great number and variety of subjects under study in the various laboratories. Individuals, on the other hand could easily select posters of personal interest for further detailed discussion.
This approach to solve the continuous problem of many meetings - i.e. to accomodate a broad variety and increasing number of presentations in a limited period of time - was mostly well received by the participants. It cannot be denied, however, that improvement at the organizational level of such a session is still possible.

Rabies virology and control

F. Steck
Veterinärbakteriologisches Institut, Universität Bern, Länggassstrasse 122, CH-3012 Bern

Control of rabies consists in:
1. Interruption of the chain of infection from the reservoir to man by prophylactic immunization of animals and persons at risk and in postexposure treatment of exposed persons.
2. Control of the reservoir (reduction, dog control, immunization).
3. Surveillance of the epidemiological situation and of the efficacy of control measures. Provision of diagnostic guidelines for postexposure treatment.
These measures are interdependent and should be part of a strategy well adjusted to the local social and ecological conditions.
The development of purified and efficient vaccines devoid of neuroparalytic accidents has made tremendous progress in the last decades, but their availability is still limited to industrialized countries, particularly because of high production costs. The same holds true for antirabies immunoglobulins of human origin, which should replace hyperimmune horse serum as a very essential component of post exposure treatment. Prophylactic and postexposure treatment of man should only supplement control measures in the reservoir, particularly where there is a high incidence and rate of exposure.
Dog rabies is still rampant in large parts of the tropical and subtropical zones. A working group within WHO is attempting to formulate strategies of dog rabies control, which are adjusted to the local structures and needs in different parts of the world.
Fox rabies in Europe is causing large numbers of rabies cases in domestic animals and frequent exposure to man, which necessitate postexposure treatment. In a large field experiment conducted in the alpine regions of Switzerland, we are attempting to control fox rabies by oral immunization. Approximately 50% of the fox population have developed immunity. Rabies has disappeared from the vaccinated area, but a broad vaccination zone extending from Martigny in the lower Rhone valley to Lake Geneva

and over the Col des Mosses and through the Simmental to the Lake Thoune and Interlaken is at present under challenge of a new epizootic wave.

Mouse mammary tumor virus: a model system for hormonal regulation of gene expression

H. Diggelmann, E. Buetti, N. Fasel, K. Pearson and D. Owen
Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges

Mouse mammary tumor virus (MMTV) is a retrovirus that causes carcinomas of the mammary gland in mice. Tumor incidence and growth are strongly influenced by the hormonal status of the animal. In tissue culture, mammary tumor cell lines or heterologous cells infected with MMTV are stimulated to an increased production of viral RNA by glucocorticoid hormones. Like other steroid hormones, glucocorticoids interact with cytoplasmic receptors and are translocated to the nucleus, where an association with the chromatin occurs. The mechanism by which they regulate transcription is unknown. We have used the MMTV genome as a model gene to study regulation by steroid hormones. Using recombinant DNA techniques, we have cloned a 9000 basepair long DNA copy of an infectious viral genome and have tested its biological activity by introducing it, via transfection, into mouse L-cells or mink lung cells. Our experiments demonstrate that cloned MMTV DNA can be expressed in both transfected cell types to yield normal viral RNA and proteins, and that viral gene expression is stimulated by glucocorticoid hormones. These results strongly suggested that the sequences responsible for the hormone response were located on the viral DNA itself. Using subgenomic fragments of viral DNA for transfection, we were able to localize the DNA sequences responsible for the hormonal regulation within a 1450 basepair long fragment of MMTV DNA that contains the long terminal repeat (LTR), a region that is composed of sequences derived from both ends of the viral RNA and that is present at both ends of the integrated viral DNA. DNA sequence determination showed that the LTR contains signals for transcription initiation and polyadenylation. In addition, we found a long coding region that could give rise to a protein of 36 kilodaltons, whose function is so far unknown. With the aid of an antiserum raised against a synthetic peptide of the deduced protein sequence, we are now searching for the protein in tumor cells and transfected cells.

Medical implications of biotechnology

In 4 presentations, the implications of biotechnological techniques on medicine and their effects on future research were discussed.

During a presentation about mass culture of animal cells, given by Dr Gian Polastri (Behringwerke AG, Marburg), it became apparent that the mass culture technique will be quite useful in future research. It can be applied to various components of the cell, such as interferon, making it also possible to increase the availability of other pharmaceutical drugs.

Dr Peter Brodelius (Department of Biotechnology, ETH Zürich) discussed the present state of the techniques of immobilization of animal and plant cells. Because a large portion of drugs are of plant origin, this technique can be of considerable advantage, especially for industrial application.

Dr H.-J. Tobler (Sandoz AG, Basel) presented data demonstrating potentially effective biotechnological alternatives to animal research. Perhaps plant and animal cell and tissue cultures can be statistically valid for many biotests, in drug development, or for bioassay. More recent mathematical methods allow for improvement in the quality of the tests, which can result in a reduction in the number of animals needed for experimental testing.

Dr Urs Meyer (Institute of Clinical Pharmacology, University of Zürich) outlined the problem of the use of animals in research. It became clear in the discussion by the group attending the round table that the complicated regulatory system of animals makes it impossible to completely eliminate animal experiments by replacing them with laboratory experiments using microbial, animal, or plant cell culture. However, there are many ways to reduce the unnecessary use of animals in experiments. The following resolutions concerning the responsibility of researchers were discussed and passed by the SGM members:

1. Experiments involving animals for the investigation of diseases and their treatment are necessary and in all cases cannot be replaced by other methods.
2. Experiments involving animals should be reduced to an absolute minimum. Whenever possible, experiments using animals should be *replaced* with other methods, *reduced,* or *improved.*

Replacement refers to the replacement of research animals with pain- and stress-free methods such as tissue culture, embryonic tissue application, etc.
Reduction means a reduction in the number of animals needed to be used due to improved planning and performance of experiments.

Improvement refers to maintaining 'respect for animal life' in experimental testing by using techniques that take care to avoid pain and stress in the animals.
3. In agreement with the new law for the prevention of cruelty to animals, the individual researcher should be able to prove the necessity and suitability of experiments involving animals.

ABSTRACTS

A) *Oral presentations*

Salmonella typhimurium in Tessin: an epidemiological study using the 'plasmid pattern'

F. Brunner, A. Margadant, R. Peduzzi and J. C. Piffaretti
Istituto Cantonale Batteriosierologico, CH-6904 Lugano; Centrale Suisse des Salmonelles, Veterinärbakteriologisches Institut der Universität, CH-3001 Bern

During the months of June and July 1981 in Tessin, there was a significant increase in the number of *S. typhimurium* isolated from human feces. The 51 strains isolated during that period and the 23 strains isolated from August to December were collected and compared using the following criteria: biotype (API-50), antibiotic resistance to 14 different drugs, lysotype, and plasmid pattern (plasmid extraction using a rapid method followed by electrophoresis through a 0.75% agarose gel). From an epidemiological point of view, the biotype determination was the less interesting method; the resistance pattern gave better results; the lysotype and the plasmid pattern determinations were by far the most useful methods; in addition, there was a remarkable correlation between these 2 criteria. Our results showed: a) the plasmid pattern determination is a convenient method to be used in epidemiological situations, even in large geographic areas; it has the advantage of being simpler than the lysotype determination; b) the presence of a *S. typhimurium* strain in different intestinal flora does not seem to influence either its plasmid content or its resistance pattern.

Septicemiae due to *Campylobacter* ssp. *intestinalis* in immunocompromised patients

J. P. Grob, P. Francioli and M. P. Glauser
Division des maladies infectieuses CHUV, CH-1011 Lausanne

Campylobacter are well-known causative agents of enterocolitis. Less frequently, septicemiae have been observed, mostly due to the subspecies *intestinalis*. We recently observed 4 cases in our hospital. All patients were over 40 years of age, 3 had an underlying malignancy and 1 had extensive arteriosclerotic disease.

The clinical presentation was nonspecific, with high and persistent hectic fever and abundant perspiration. 2 patients had painful s.c. cellulitis, in the pretibial area in one and in association with septic femoral thrombophlebitis in the other. Another patient developed fatal purulent meningitis. In all 4 patients, *Campylobacter* ssp. *intestinalis* was isolated from blood cultures after 4-9 days of incubation (Institut de Microbiologie, CHUV, Dr D. Roussianos).

An appropriate treatment was initiated only 5-21 days after the onset of symptoms, which were well tolerated and alarmed neither the patients nor their physicians. 2 patients were treated with erythromycin and 1 with doxycyclin, with prompt clinical response (24-48 h). 1 patient improved with a 10 days course of penicillin G, but relapsed clinically and microbiologically. He was subsequently cured with doxycyclin. 1 patient developed septic meningitis before treatment and died a few hours after initiation of erythromycin.

In conclusion, septicemiae due to *Campylobacter* ssp. *intestinalis*, although relatively rare, can be the cause of fever in patients with underlying malignancy, and ought to be suspected if there is an associated cellulitis. These septicemiae are usually well tolerated and respond rapidly to an appropriate antibiotic treatment. Nevertheless they can be fatal.

Characterization of PCL300, a new plasmid coding for multiple antibiotic resistance and for lactose fermentation isolated from *Klebsiella pneumoniae*

C. Lucain and J. C. Piffaretti
Department of Microbiology, University of Geneva, C. M. U., 9, avenue de Champel, CH-1211 Geneva 4

During a survey on the evolution of the intestinal flora of patients undergoing prophylactic antibiotherapy at the Cantonal Hospital of Geneva, multiresistant strains of *K. pneumoniae* were isolated. Among these, 4 strains, originating from 4 stools of the same patient, could transfer by conjugation the ability to ferment lactose. These *Klebsiella* were resistant to ampicillin, carbenicillin, tetracycline, chloramphenicol, streptomycin, sulfonamides and mercuric ions.

All the resistances could always be transferred 'en bloc' together with the lactose fermentation to various *lac*$^-$ *E. coli* strains or to *Salmonella typhi* lysotype A. The transfer was not thermosensitive and the same transfer frequencies were obtained during conjugation from *Klebsiella* to *E. coli* as from *E. coli* to *E. coli* or to *S. typhi*. The plasmids were stable in *E. coli*, where the loss of the lactose fermentation capacity implied the loss of the whole plasmid (0.3-1.0% loss after 20 generations without selection), in contrast to *S. typhi*, in which we could obtain *lac*$^+$ and *lac*$^-$ segregant plasmids.

These plasmids were shown to be *fi*$^+$ and to belong to the incompatibility group FI, as they were unable to coexist in the same cell with the inc FI representative plasmids $R_{IP}162.2$. They were compatible with all the other inc group reference plasmids tested.

After plasmid extraction and purification, the mol.wts were determined by electrophoresis through 1% agarose gels of *Eco*RI digests: a value of 235±20 kb was obtained.

This unusually high value for inc FI plasmids was confirmed by contour length measurements of the plasmids on electron micrographs. By this method, we could also ascertain that there was a unique population of large DNA molecules responsible for the antibiotic resistance and the lactose fermentation.

Investigation to find a selective medium for isolation of *Bacillus cereus* and qualitative research of contamination by *Bacillus* sp. in several spices, aromatics and seasonings

A. Masson and B. Vanos
Laboratoire cantonal, Les Croisettes, CH-1066 Epalinges

Our objective in the present work was: on the one hand, to find the best selective medium among the proposed ones in the market for an easy and clear detection of *B. cereus* in contaminated food products, particularly in spices, and on the other hand, to make a qualitative study of a series of spices for the possible detection of the living species of *Bacillus*.

In the first part, 36 kinds of spices of different origins were examined and 57 different strains of *B. cereus* were isolated and tested biochemically. Among them, 43 strains strictly corresponded to the biochemical characters of *B. cereus*.

3 different selective mediums were used, and we noted that the best results for the identification of *B. cereus* were obtained on the selective medium '*B. cereus* selective Agar CM 617 Oxoid' plus the supplements Oxoid. The efficacity of detection of this medium was 86%.

In the 2nd part, 20 kinds of spices of different origins were examined and 700 different colonies were isolated. They were examined macroscopically, classified in groups, and then a sample of each group was tested biochemically.
We succeeded in identifying the following 5 species of *Bacillus: B. cereus, B. cereus mycoïdes, B. alvei, B. firmus* and *B. coagulans.*

Growth autostimulation in streptococci

J.-P. Monneron, Th. Kuntzer, D. Roussianos and V. Bonifas
Institut de Microbiologie CHUV, Université de Lausanne, CH-1011 Lausanne

Nutritional variant streptococci, or NVS, have already been known to grow well as satellites of colonies of various microbial species, including *Candida.* On Columbia agar enriched with blood (BA), NVS grew well without the addition of either of 2 known supplements (thiol, pyridoxal) and in absence of other bacteria. In all growth conditions, we observed that NVS colonies were either large (up to 0.5 mm diam.), or small. We thus investigated the possible interacting role of some constituents of this population as feeders of the others. In a trypticase soy Agar (TSA) layer in dish, we bored 8 mm diameter holes which we filled with BA maintained at 50 °C. After solidification of the BA plugs, the dishes were inoculated either on the BA plug and the surrounding TSA or on the surrounding TSA only. After incubation at 37 °C in a 5% CO_2 atmosphere, the following was observed: When the BA plug was inoculated, the NVS also grew on the TSA as a surrounding zone with about 10 mm radius. When the BA plug was not inoculated, no growth was observed on TSA. This was easily explained by the diffusion from the outgrowth on BA of growth factors diffusing into the TSA. However, the short distance stimulation indicates that growth alone is not sufficient: growth on TSA is not an actively producing growth factor.

A pedagogical direct action used to sensitize kitchen personnel to hospital hygiene

G. Pappalardo, D. Roussianos and F. Tanner
Division autonome d'Hygiène hospitalière, Unité de Bactériologie médicale, CHUV, CH-1011 Lausanne

A permanent effort is always made to educate the personnel of a hospital kitchen about hygiene; often though, the classical measures are not sufficient and one has to turn to a direct action of sensitization.
Taking advantage of the moving of a hospital kitchen into a new building, a program of bacteriological evaluation was designed with a pedagogical aim. For a few days the personnel could not use sinks equipped with soap dispensers and paper. This allowed us an efficacious demonstration: bacterical cultures of their fingerprints were made on blood agar, with the possibility for each person to examine his own.
The experience was repeated a few days later when the sinks were all equipped and the routine of the washing of hands took place as usual. A comparison of the 2 series of culture was very instructive. The first one showed more than 500 colonies on plates in 30% of the cases, 300 in 45%, 200 in 10%, 150 in 10%, and 60 in only 5% of the cases; whereas the 2nd series showed an average of 100 colonies per plate. The germs were almost all gram-positive: skin flora *(Staphylococcus epidermidis)* and environmental contamination (*Micrococcus* and *Bacillus* sp.). The only exception was in the place where raw vegetables were handled. Here, but only in the first series, was a massive contamination of gram-negative, almost exclusively represented by *Pseudomonas aeruginosa.* This germ certainly came from the vegetables themselves, but there was also one case in which this contamination was due to *E. coli* and *Enterobacter,* due to the fact that one person had not washed his hands after the toilet.
So that the experience could be more complete, we wanted to get environmental samples of the kitchen by means of Rodac plates (trypticase soy agar with inhibitors TLS) before and after daily cleaning with a detergent and a disinfectant product. The results confirmed what had been shown by the fingerprints. They were evaluated according to the standardization used in our hospital for environmental samplings. One exception: the places where only detergent had been used by mistake or negligence. This difference was very helpful, for it showed the necessity for the use of both detergent and disinfectant products.

Effect of chloramphenicol on permeability control in *Escherichia coli* K 12

Saad Abdel Sayed and V. Bonifas
Institut de Microbiologie CHUV, Université de Lausanne, CH-1011 Lausanne

Chloramphenicol (Cm) strongly inhibited active transport of proline by whole cells of *E. coli* K 12 F^-. Whether this was due to the inhibition of proline incorporation into protein or to a leaky membrane resulting from the physical partitioning of Cm in the cell membrane was examined. Isolated membrane vesicles of *E. coli* were prepared by the lysozyme-EDTA Kaback method. These vesicles actively transported proline in the presence of phenazinemethosulfateascorbate as an artificial electron donor system. Membrane vesicles prepared from bacterial cells grown in the absence of Cm but subsequently incubated for 3 min in the presence of Cm had a higher initial rate of accumulation of proline than the normal vesicles but were unable to maintain a proline gradient as effectively as normal vesicles. This, hypothetically, in turn would be expected to impair the ability of whole bacterial cells to retain intracellular solutes. We interpret our experimental data to indicate that Cm impairs the permeability control functions of membranes in *E. coli.*

Intracellular ATP pool and ammonium during growth and differentiation

M. Ojha and G. Turian
Laboratoire de Microbiologie générale, Université de Genève, CH-1211 Genève 4

The differentiation in *Allomyces* is induced by transfer of an actively growing cell from a growth to a starvation medium. At 32 °C this process is completed within 3–4 h. The cellular ATP level followed the exponential growth curve but declined briefly when the cells were transferred to either fresh medium or starvation media. An oscillation of the ATP pool was observed on these replacement media, with 2 peaks of intracellular ATP pools at approximately 1 and 2 h respectively. In starvation media this corresponded to the delimitation of zoosporangial territory and the formation of papilla. In general the level of the ATP pool was higher in differentiating than in growing cultures.
The oscillation of the ATP pool corresponded to an oscillation in the intracellular ammonium level. This level, like the intracellular ATP pools, was also higher in differentiating than in actively growing cultures. The energy charge during the induction period varied between 0.97 and 0.8.

Changed protein pattern during heat shock and conidiogenous shift down in *Neurospora crassa*

D. van Tuinen and G. Turian
Laboratoire de Microbiologie générale, Université de Genève, CH-1211 Genève 4

Conidia that have been heat-treated for 15 h at 45 °C produce prematurely new conidia on their germ tube after shift down to 25 °C (microcycle conidiation in *N. crassa*, M. Cortat and G. Turian, Arch. Microbiol. *95* (1974) 305). During heat treatment, isotropically-enlarged conidia synthesize some new proteins. The synthesis of the most predominant of them can be detected in the first 30 min of the heat shock after electrophoresis, on a sodium dodecyl sulfate-polyacrylamide slab gel (SDS-PAGE), of the proteins pulse-labeled with ^{35}S-methionine. Their apparent mol. wts on SDS-PAGE are 90 k, 78 k and 70 k daltons. These proteins stained with Coomassie Brilliant Blue appear after a 30-min heat shock and are extremely stable during the subsequent 15 h of the heat shock, as determined by pulse-chase experiments.
After shift down to 25 °C, the preshock protein pattern is recovered in about 4 h but can be modified during germ tube outgrowth leading to conidiophores.

B) *Virology posters*

Cytopathology and plaque formation by Aleutian disease virus (ADV)

G. Siegl and G. Kronauer
Institute for Hygiene and Medical Microbiology, Friedbühlstrasse 51, CH-3010 Bern

Aleutian disease is a chronic, slowly progressive infection of mink. It is characterized by systemic plasmocytosis, severe hypergammaglobulinemia and the continued presence in sera of antigen-antibody complexes which leads to severe glomerular and arterial lesions. The causative virus (ADV) is a nonhemagglutinating autonomous parvovirus. It is said to replicate preferentially in a permanent line of feline kidney cells (CRFK) at 31.8 °C and without inducing cytopathologic changes. Virus titrations therefore have to be performed by fluorescent antibody staining and the lack of a rapid, sensitive system for virus quantification has greatly hampered the study of this interesting disease.
Using the Utah-1 strain of ADV we could now show that, under appropriate cultural conditions, ADV is able to replicate and to induce cytopathology in the NLFK feline kidney cell line both at 32 °C and at 37 °C. The accompanying cellular changes - including the formation of Cowdry type A intranuclear inclusions - are indistinguishable from those induced by other well-known parvoviruses. Likewise, formation of CPE depends on the presence of cycling cells. CPE is extinguished as soon as density dependent inhibition of cellular growth ensues.
On the basis of these observations a plaque assay for ADV in NLFK cells has been developed. This assay facilitates quantification of infectious virus, allows the isolation and purification of mutants, and will be helpful in the serologic characterization of this parvovirus.

Presence of lentivirus infections in Swiss goat herds

R. Zwahlen, D. Houwers, M. Walther, M. Aeschbacher, Th. Balcer, M. Weiss and F. Steck
Veterinärbakteriologisches Institut, Universität Bern, CH-3012 Bern, and Central Veterinary Institute, Virology Department, Lelystad, The Netherlands

Serological surveys among goats in Switzerland with ELISA indicate that infections related to Maedi-Visna or the caprine arthritis-encephalitis virus are widespread. Clinical and seroepidemiological observations suggest that these infections are associated with arthritis and other clinical and pathological manifestations, in particular progressive mastitis.
Several virus isolations have been made by direct cultivation of synovial and udder cells from goats. Syncytium formation becomes apparent after several subpassages. Electron microscopy reveals structures characteristic of lentiviruses.

Neutralization of bovine rotavirus infections in cell culture

H. Brüssow, P. Morand and V. Fryder
Research Department, Nestlé Products Technical Assistance Co. Ltd, CH-1814 La Tour-de-Peilz

Bovine colostral milk neutralized bovine rotaviruses in cell culture infections with high neutralization titers. The neutralizing activity was not diminished after heating the colostral milk for 30 min at 56 °C, but was totally abolished after heat treatment at 100 °C. The neutralizing activity was trypsin-sensitive and could be precipitated with 50% $(NH_4)_2SO_4$. The neutralization titers paralleled the anti-rotavirus antibody titers and were markedly reduced when a heterologous simian rotavirus was used. Neutralized rotaviruses showed the same sedimentation coefficient in velocity centrifugation as infectious rotaviruses but demonstrated a different density position in equilibrium sedimentation. Neutralized rotaviruses appeared diffuse with a fuzzy coat in the electron microscope. A covering of the neutralized rotaviruses was demonstrated using the Enzyme-Linked Immunosorbent Assay. Intracellular viral protein synthesis was greatly suppressed in cells exposed to neutralized rotaviruses and rotaviruses were no longer detectable in the supernatant.

Immunoprecipitation studies with rotaviruses

H. Brüssow
Research Department, Nestlé Products Technical Assistance Co. Ltd, CH-1814 La Tour-de-Peilz

Bovine rotavirus NCDV and an isolate from the natural infection of a calf could be distinguished by the genomic RNA pattern. They induced a different intracellular viral protein synthesis pattern in cell cultures infected under identical conditions. The polypeptide composition of extracellular viral particles also varied within these 2 virus strains. No correlation was found between the degree of cytopathic effect induced in cell culture by different trypsin-EDTA treatments and the pattern of intracellular viral protein synthesis. The absence of cytopathic effects correlated with poor virus yields and/or the presence of a high mol.wt protein in these viruses. Immunoprecipitation of intracellular viral proteins of different rotaviruses by heterologous anti-rotavirus antibodies showed that the viral polypeptides 90 K and 42 K are the major cross-reacting antigens.

Murine rotavirus (EDIM): biochemical comparison with monkey, bovine and human rotaviruses

U. Schilt and F. Reigel
Institute for Hygiene and Medical Microbiology, University of Bern, Friedbühlstrasse 51, CH-3010 Bern

Although the murine rotavirus (epidemic diarrhoea of infant mice = EDIM) was the first of the rotaviruses described as a viral agent responsible for gastroenteritis, complete biochemical characterization is lacking. There-

fore, we compared the RNA and protein components of EDIM virus with those of well-characterized virus strains of monkey (SA 11), bovine (NCDV) and human (WA) origin. EDIM virus was extracted from the guts and faeces of infected suckling (BALB/c) mice, human strains were isolated from the faeces of children with gastroenteritis, and SA 11 and NCDV were propagated in MA 104 cells. All viruses were purified in a linear $CsCl_2$ gradient. Typical for EDIM virus were the slower moving genome RNA segments No. 5 and 11 compared to SA 11.

Incidence of rotavirus vs bacterial infections in nonhospitalized children with acute gastroenteritis in Basel, 1981/82

M. Gasser, H. Egger and H. Loeffler
Institut für Mikrobiologie und Hygiene, Petersplatz 10, CH-4003 Basel

Fecal samples from 930 nonhospitalized children suffering from acute gastroenteritis were tested, along with bacterial examinations, for rotavirus antigen by enzyme-linked-immunosorbent assay (Rotazyme).
In 693 children aged less than 2 years the respective incidences were: rotavirus 8.7%, enteropathogenic *E. coli* 14.0%, salmonellae 2.9%, campylobacter 2.0%. No shigellae were observed. Altogether in 25.0% of the patients, an etiologic agent was found. Rotavirus accounted for about one third of the positive cases. Double infections occurred in 15% of the rotavirus positive cases, involving mainly enteropathogenic *E. coli*. With regard to seasonal variations rotavirus infections predominated in winter (26% and 23% in January and February, respectively).
Of 237 children older than 2 years (2–10 years) 4.2% were rotavirus positive.

Detection of hepatitis A virus antibody of the IgM class by bacterial absorption

B. Keller, S. Mühlestein, E. Zoumbou and M. Gasser
Institut für Mikrobiologie und Hygiene, Petersplatz 10, CH-4003 Basel

Laboratory diagnosis of recent hepatitis A is usually made by demonstration of specific IgM antibodies. Löfgren et al. (J. Med. Virol. *6* (1980) 37) have described a bacterial absorption method to discriminate between early and late antibodies in hepatitis A. The method relies on the binding of IgG to protein A bearing staphylococci (Cowan I) and of IgG and IgA to certain streptococcal strains (AR 1, AW 43). We have tested 47 sera for IgM antibodies to hepatitis A virus (HAV) by bacterial absorption and by Havab-M as the reference method.
Procedure. A mixture of 0.4 ml 10% Cowan I, 0.4 ml AR 1 and 0.2 ml AW 43 (kindly provided by Dr Kronvall) was centrifuged at 1600 × g for 20 min. The pellet was resuspended in 0.2 ml serum (diluted 1:10 in PBS). After absorption at room temperature for 1 h, the mixture was centrifuged again, and the supernatant as well as unabsorbed serum 1:10 were tested for anti-HAV by Havab.
Results. 4/47 unabsorbed sera were Havab negative at dilution 1:10 and therefore could not be tested by bacterial absorption. Of the remaining sera, 42/43 (98%) were correctly identified: 14/14 Havab-M positive, and 28/29 Havab-M negative.
Conclusions. Bacterial absorption is a reliable method for discriminating between early and late antibodies in hepatitis A. Furthermore, it may be a useful tool for detecting IgM antibodies in other infections, since the serum need not be used undiluted in the test system.

Virological and serological data of a hepatitis A outbreak in Wasen i.E., Switzerland

U. Schilt, F. Burkhardt and G. Siegl
Institute for Hygiene and Medical Microbiology, University of Bern, Friedbühlstrasse 51, CH-3010 Bern

In the course of a hepatitis A outbreak in Wasen i.E. in fall 1979, fecal specimens and serum samples were collected from patients and contacts. Only one of the stool specimens (KMW 1) yielded hepatitis A antigen which, on the basis of currently available techniques, proved to be serologically indistinguishable from well-known hepatitis A reference antigens. From the same sample, infectious HAV could be isolated and passaged in the PLC/PRF/5 human hepatoma cell line at 32 °C, and the virus adapted to growth in human MRC-5 fibroblasts.
Both the fecal HAV Ag and the antigen extracted from infected cell cultures were successfully used in the anti HAV-IgM (MACRIA). When sera of patients involved in the Wasen outbreak were screened for the presence of anti HAV-IgM, identical results could be recorded independent of whether the original stool sample or tissue culture harvests served as a source of viral antigen. All sera of patients drawn within 2 months after the first HAV case was recorded were anti HAV-IgM positive. Within 4 months, however, only borderline results were obtained in 2 of the 6 sera tested. The advantages associated with the use of cell culture derived HAAg in hepatitis A serological tests are discussed.

Antibody determination in serial sera of patients with cytomegalovirus infection by complement fixation, passive hemagglutination, IgG, IgA- and IgM-ELISA and IgM-ELA-SPIT

T. Krech and W. Vorlaender
Institute of Medical Microbiology, CH-9000 St. Gallen

The laboratory diagnosis of acute CMV infection is complicated by prolonged virus excretion. It was therefore of interest to investigate the possibility of diagnosing the acute stage of infection by the demonstration of CMV-IgM antibodies. The infection can be transmitted from seropositive blood and organ donors. Therefore we investigated whether the passive hemagglutination (PHA) could be a simple screening method for determination of the immune status compared to CF and IgG-ELISA.
From 6 patients with apparent primary CMV infection, subsequent sera were collected up to maximal 30 months. In addition, serial sera from 16 patients, which had in the first serum already detectable antibody levels were investigated exclusively for ELA-SPIT-IgM. (This assay works on the basis of an antihuman IgM coated Solid-phase.)
The following observations were made:
1. All patients showed antibodies in each assay. 2. Antibodies could be measured first about 5–7 weeks after infection and about 1 week after onset of symptoms with the exception of 2 renal transplant recipients, who showed a delayed increase by PHA. 3. Antibodies rose from undetectable to peak levels in all tests within some days with the exception of PHA antibodies, which increased only slowly in renal transplant recipients. 4. For CF, PHA and IgG-ELISA no significant loss of antibodies could be seen over the longest observed time of 30 months. 5. IgM antibodies dropped within about 3–4 months below detectable levels in all patients tested with the exception of one patient, who still had ELISA- and ELA-SPIT-IgM antibodies in the last

sample taken 5 months after onset of symptoms. 6. IgA antibodies followed very closely the IgM-antibody pattern, but they normally developed higher levels in the acute stage and persisted over a longer period of time than the IgM antibodies.
It may be concluded: 1. CMV-IgM antibodies is a more reliable marker for recent CMV infection than virus isolation. 2. Passive hemagglutination may prove to be a simple method to determine the immune status.

A solid phase radioimmunoassay (MACRIA) for the detection of mumps-specific IgM antibodies

F. Burkhardt, U. Schilt and R. Y. Andres
Institute for Hygiene and Medical Microbiology, University of Bern, Friedbühlstrasse 51, CH-3010 Bern

A solid phase RIA for the detection of mumps-specific IgM antibodies is described. In 22 cases of clinically suspected mumps infections (often without parotitis and sometimes associated with meningitis, orchitis and pancreatitis) confirmed by conventional serological methods (CFT, NT) antibodies of the IgM class to mumps virus were detectable by MACRIA. No mumps-specific IgM were shown in 9 cases of clinically debatable mumps infections that could not be confirmed by CFT/NT (seroconversion or significant titer rise).
In order to evaluate antigen specifity and Ig-class specifity of the mumps-MACRIA, sera of 20 cases from a rubella outbreak who had been found positive in a comparable IgM-test as well as 19 sera of RF positive patients and 20 healthy laboratory personnel were also tested in mumps-MACRIA. They were all negative.
All sera from the described patient groups were assessed in parallel in an ELISA-test for anti mumps-IgM. Results obtained were compared with those presented by the MACRIA and specifity and sensitivity of both test systems are discussed.

Rapid electrophoretic differentiation of HSV 1 and 2

N. Pyndiah, L. Glauser, B. Delacuisine and J. F. Anex
Institut de Microbiologie, CHUV, CH-1005 Lausanne

Herpes virus isolates were typed in 18 h by high resolution electrophoresis on acrylamid gel and confirmed in 5 days by neutralization with a hyperimmune guinea-pig serum. Infected cell cultures showing 75–100% CPE were washed twice with 20 mM Tris buffer pH 7.4, solubilized in SDS and applied on a continuous gradient (10–20%) acrylamide gel. A major peptide band with an approximate 23,800 mol.wt is easily detected in HSV 1 patterns: it corresponds to the band numbered 22–23 by Spear, P.G., and Roizman, B., J. Virol. *9* (1972) 143, in HSV 1 purified protein electrophoretic patterns. Cells of different origins (human embryonic lung fibroblasts, Vero, Hep-2, Rita) were infected and similarly treated. The 23,800 mol.wt band was observed in all but never so well as in human lung fibroblasts, where it seems to be more abundantly produced. Thus typing was achieved 24 h after the appearance of a marked CPE in the infected cultures. The reading of the results were more reliable than those of the neutralization test since they were not submitted to statistical evaluation. On the other hand, this technique curtails the difficulties met in high quality immune fluorescence. It is very simple when facilities for performing acrylamide gel electrophoresis exist in the laboratory.

The goat herpes viruses: Some biological and physicochemical properties

M. Engels, H. Gelderblom, G. Darai, H. Ludwig and R. Wyler
Institut für Virologie, Winterthurerstrasse 266a, CH-8057 Zürich; Institut für Virologie FU und Abt. Virologie, Robert-Koch-Inst., Berlin West, and Institut für medizinische Virologie, Heidelberg, Federal Republic of Germany

A herpes virus from young goats with a severe generalized infection was isolated in California 1974 (Berrios, P.E., and McKercher, D.G., Am. J. Vet. Res. *36* (1975) 1755). Some years later a similar infection occurred in Switzerland, and a herpes virus was isolated (Mettler, F., Engels, M., Wild, P., and Bivetti, A., Schweiz. Arch. Tierheilkd. *121* (1979) 655).
The californian isolate has been characterized in some detail and found to be specific for goats but serologically related to IBR/IPV-virus (BHV-1). In this report we describe a further characterization of both isolates in comparison to BHV-1. The goat herpes virus is classified as bovid herpes virus 6 (BHV-6).
BHV-6 multiplies without adaptation in a broad spectrum of tissue cultures. Its growth cycle is very fast: BHV-6 reaches a maximum of intracellular virus yield about 14, BHV-1 18 h post infection.
There is no significant serologic relationship with bovine mammillitis virus (BHV-2), pseudorabes virus, equine and canine herpes virus, herpes simplex and herpes B virus, but there is a clear one way cross-reaction between BHV-6 and BHV-1.
Electron microscopic examination of the DNA showed that it is linear, double stranded, with a length of 44 µm, corresponding to a mol.wt of about 90×10^6. Its density in cesium chloride is 1.7295 g/cm^3, the melting point (T_m in $0.1 \times$ SSC) 86 °C, and the G+C content calculated from these data is 72%. These properties are in good correlation to those found for BHV-1.
Restriction enzyme analysis of BHV-6 and BHV-1 DNA showed a homology between the goat isolates which differ clearly from BHV-1. A unique feature was found in digestion of BHV-6 DNA with EcoRi and HindIII which resulted in only 2 fragments. Digestion with other endonucleases such as BamHI gave half molar fragments, indicating that BHV-6 DNA is constructed like BHV-1 DNA (Farley, J.E., Share, I.B., and Skare, J., Int. Conf. Human Herpes viruses, Atlanta 1980) with a flip-flopping short unique sequence flanked by inverted repeats.

Are simultaneous primary and booster vaccinations advisable?

R. Berger and M. Just
Dept. of Microbiology and Immunology, University Children's Hospital, CH-4000 Basel

For economical reasons combined vaccines against different infectious agents are commercially produced. The simultaneous administration of different vaccinations is recommended by public health services. The vaccination programs in Switzerland, FRG and other countries include a primary vaccination against measles, mumps and rubella together with booster vaccinations against polio, diphtheria and tetanus in the 2nd year of age. It has been postulated that the fast response to booster vaccinations may depress the delayed response to primary vaccinations by an immediate stimulation of nonspecific immune defense mechanism. To test this hypothesis 230 seronegative children aged 18–36 months were vaccinated with a triple vaccine against

measles, mumps and rubella (MMR/MSD or Pluserix/SK-RIT). At the same time half of the children received simultaneously with the triple vaccine a polio or diphtheria-tetanus booster, the 2nd half received the booster at the time of the 2nd blood sampling. Antibody titers to measles (HI, IF), mumps (IF) and rubella (HI) were determined 6–8 weeks after vaccination. The number of vaccination failures as well as the mean titers and variations of the immune response were equal in all different groups of vaccinees. A depression of a primary immune response by the booster vaccination was not observed. Therefore the combined primary vaccination against measles, mumps and rubella and booster vaccination against polio or diphtheria-tetanus is advisable.

Public health problem caused by the presence of viruses in water; method, results

F. Lautier and J. Lavillaureix
Institut d'Hygiène, Faculté de Medicine, 4, rue Kirschleger, F-67085 Strasbourg Cedex

Having tried for over 10 years to detect viruses in various waters, we employed first a method of concentration with aluminium hydroxyde precipates and adsorption on Millipore membranes (Wallis and Melnick). Secondly we improved our technique by introducing arcton to correctly eliminate bacteria.
Our results were satisfactory at the outset, but of late we did not find any viruses, and this is in contradiction with the increase of the pathology found in clinics.
Now we employ a method with concentration on glass powder (Schwartzbrod et al.). Instead of 500-ml samples, we have water samples of 12 l each, taken once a month at the entrance and the exit of the sewage treatment plant of Strasbourg, at different hours in the same day.
We note differences depending on seasons (mid-seasons) and on the time of the day (midday). The most frequent viruses are: Polio III, Polio II, Polio I, Coxsackie B5, Echo 7. On an average we have 1–2 positive results in a day of sampling, and the viruses found at the entrance of the plant are also present at the exit.
Our results suggest that the concentration of viruses remains an important technical problem. Besides the treatment plants reject water with the same viruses at the entrance as at the exit.

The detection of surface antigens on living cells by chemiluminescence (CL) measurement

E. Peterhans, L. Weber, S. Degiacomi and R. Wyler
Institute of Virology, Winterthurerstrasse 266a, CH-8057 Zürich

On binding to surface receptors on myeloid and lymphoid cells several particulate and soluble agents stimulate a burst of CL. Agents able to stimulate CL include Zymosan, bacteria, viruses and the calcium ionophore A 23187. Light emission reflects the generation of highly reactive oxygen species by the cells. In this process, NAD(P)H dehydrogenase and the arachidonic acid cascade are known to be important.
We have observed that the binding of antibody to antigen on the surface of living mouse spleen cells results in the generation of CL. Spleen cells were infected with influenza, Sendai, or Newcastle Disease Viruses and suspended in glass scintillation vials. CL was measured after the addition of antibody to the cell suspensions in a modified Kontron MR 300 Liquid Scintillation Spectrometer (LSC). The modifications include a thermostated sample compartment and program alteration allowing the repeated measurement of the samples at fixed time intervals. CPM were recorded on line with a HP-85 computer. Light emission curves were plotted on a HP-7225A graphics plotter and the amount of light emitted calculated by integration of these curves. Both the kinetics and extent of light emission depended on the relative concentrations of antigen and antibody. Compared to antibody assays not involving the use of radioactive label, antibody-induced CL was over 100 times more sensitive. This technique is not restricted to the assessment of anti-viral antibodies and antigens, as suggested by the stimulation of CL by anti-H-2 antibodies.
The results demonstrate the considerable potential of antibody-induced CL in the study of cell surface antigens. In addition to being highly sensitive, the technique requires no radioactive label nor is it necessary to remove unbound antibodies by washing the cells. Finally, conventional LSC can be used to quantitate CL after switching off the coincidence circuit.

The biochemistry of CL induced by Sendai virus in mouse spleen cells

B. Semadeni, E. Peterhans and M.J. Weidemann
Institute of Virology, Winterthurerstrasse 266a, CH-8057 Zürich, and Dept. of Biochemistry, Faculty of Science, ANU, Canberra, Australia

On infection with Sendai virus, mouse spleen cells emit a short-lived burst of CL peaking at 2–5 min post-infection. The generation of light is triggered by the interaction of the spike glycoproteins, HN and F, with the cell surface.
To understand the functional significance of virus-induced CL we are investigating the biochemical reactions leading to this phenomenon.
The omission of glucose from the medium and its replacement by 3-O-methylglucose did not affect Cl while 2-deoxyglucose was inhibitory. This suggests that CL is dependent on external glucose while being dependent on glucose metabolism from internal store(s).
The contribution of lipid metabolism to CL was investigated using inhibitors specific for pathways of the arachidonic acid cascade. Neither indomethazin nor aspirin affected CL, indicating that CL does not reflect the activation of the cyclooxygenase pathway. In contrast, eicosa-5,8,11,14-tetraynoic acid, an inhibitor of both lipoxygenase and cyclooxygenase, strongly inhibited CL. Furthermore, nordihydroguaiaretic acid (10 μM), an inhibitor of lipoxygenase, decreased CL by some 98%. As the activation of the lipoxygenase pathway requires an increase in the concentration of (free) arachidonic acid, we investigated the effect of a phospholipase inhibitor on CL (compound 3585, kindly provided by Dr Wallach, The Upjohn Company, Kalamazoo, USA). This drug inhibited CL induced by the virus while not affecting CL stimulated by exogenously added arachidonic acid, suggesting that the activation of phospholipase is an early step in CL induction by Sendai virus.
Taken together, our experiments point to a prominent role of the lipoxygenase pathway as a source of CL. This interpretation is interesting also in respect to the functional significance of CL. The leukotrienes, products of the lipoxygenase pathway, are important mediators in inflammation. Thus, CL could represent an early event in the inflammatory response to Sendai virus.

Semliki Forest virus assembly in *Aedes albopictus* cells is inhibited at low pH

F. Reigel, J. Stalder and H. Koblet
Institut für Hygiene und Medizinische Mikrobiologie, Universität Bern, Friedbühlstrasse 51, CH-3010 Bern

Semliki Forest virus (SFV) multiplies in *Aedes albopictus* C6/36 cells very efficiently under physiological growth conditions. If the pH of the culture medium was lowered from 7 to 6, SFV infected *Aedes* cells fused and formed syncytia. Formation of progeny virus was drastically reduced at pH 6, while transport and secretion of cellular proteins were less affected. Pulse-chase experiments showed that the precursor protein for the viral structural proteins C, E3, E2 and E1 was only partially processed. Correct processing was observed for the viral coreprotein C on the polysomes and for the viral envelope protein E1 on the endoplasmic reticulum, whereas the 2 remaining envelope proteins E2 and E3 were not separated from each other. Instead, the trimmed precursor protein of mol.wt 62,000 (p62) which is further processed under physiological conditions at the plasma membrane, remained uncleaved. We conclude that processing of p62 in fused membranes is abolished. Lacking of mature E2 and E3 envelope proteins may prevent budding of the virions.

Intracellular nucleoprotein structure of the DNA of Minute virus of mice (MVM)

G. McMaster, N. McKie, C. Degoumois, R. Sahli and P. Beard
ISREC, CH-1066 Epalinges-Lausanne

We have studied the nucleoprotein structure of the DNA of MVM in infected mouse L-cells. To test for nucleosome-like structures infected cell nuclei were digested with micrococcal nuclease and the purified DNA fragments electrophoresed then transferred to nitrocellulose filters. The filters were hybridized with radioactive cloned viral DNA, or with L cell DNA and autoradiographed. DNA fragments with lengths characteristic of nucleosomes were seen with the cellular DNA probe as expected but not with the viral probe. Viral nucleoprotein complexes were extracted from infected cell nuclei. On sucrose gradients these sedimented in 2 peaks at 50S and 110S. Their protein and DNA content were analyzed by gel electrophoresis. Both peaks contained replicative form DNA and virus capsid proteins A and B. Histones were not seen. The complexes were examined by electron microscopy. The 110-S fraction contained DNA attached to what seem to be viral capsids, while in the 50-S peak only DNA was seen. No beaded nucleosome-like structures were observed.

The role of polioviral proteins in host RNA synthesis inhibition

W. Bossart, D. Egger and K. Bienz
Institut für Mikrobiologie und Hygiene, Petersplatz 10, CH-4003 Basel

Poliovirus-induced inhibition of host RNA synthesis depends upon synthesis of viral proteins. In intact HEp-2 cells, poliovirus proteins were shown by EM-ARG and PAGE to migrate into the host cell nucleus. Certain poliovirus proteins were found to accumulate in the nucleus, others were found to be rather excluded from the nucleus, as compared to the pattern of viral proteins in the cytoplasm.

Cytoplasmic extracts prepared from poliovirus-infected cells were shown to inhibit in vitro RNA synthesis in uninfected isolated HEp-2 cell nuclei. The RNA polymerase II activity was preferentially decreased more than the other polymerase activities, as determined by α-amanitin and EM-ARG. Isolated nuclei were used as a sensitive test system to investigate the role of polioviral proteins in the mechanism of host RNA synthesis inhibition. Radioactively labeled poliovirus proteins were shown to enter the isolated nuclei during in vitro incubation. The patterns of poliovirus proteins found in the nuclei after in vitro and in vivo incubation were comparable. Experiments designed to define the possible function of individual poliovirus proteins are reported.

Analysis of polyoma virus early functions by means of viable mutants

H. Türler and C. Salomon
Department of Molecular Biology, University of Geneva, CH-1211 Geneva

The early region of the polyoma virus genome codes for 3 related proteins: large (100 K), middle (57 K) and small (23 K) tumor (T)-antigens. These proteins are responsible for the biological effects of polyoma virus, i.e. induction of lytic infection in permissive cells producing progeny virus, induction of abortive infection in nonpermissive cells pushing quiescent cells into mitosis, and leading eventually to cell transformation, and induction of tumors in susceptible animals. Using various viable mutants affecting structure of either small and middle T-antigens (hr-t mutants) or middle and large T-antigens (ml-T mutants) we measured several parameters to quantitate the mitotic response of the host cell and progeny virus formation in different cells. Some of the mutants have been shown to be transformation defective, but in our experiments we could not define another all or none function of the T-antigens. On the other hand, any structural change in the T-antigens decreased more or less the mitotic response of the host cell and progeny virus production. We propose that a coordinate action of all 3 T-antigens is required for full phenotypic reprogramming of the host cell and that some mutants fail to activate specific host cell genes.

Large T-antigen associated with messenger ribonucleoproteins of cells infected by Simian virus 40

M. R. Michel and M. Schwyzer
Department of Molecular Biology, University of Geneva, CH-1211 Geneva

In monkey or mouse cells undergoing lytic or abortive infection with SV40, about 10% of large tumor (T-)antigen molecules are present in the cytoplasm. Cytoplasmic large T-antigen cosediments in linear sucrose gradients with polyribosomes (100–500 S) and with initiation complexes for translation (20–80 S); in discontinuous sucrose gradients its apparent density (1.2–1.3 g/ml) corresponds to that of ribonucleoproteins (RNPs). In contrast, the bulk of nuclear T-antigen has the sedimentation properties and density of free protein. Reconstitution experiments show that nuclear T-antigen does not bind to cytoplasmic constituents during cell fractionation. After dissociation of polyribosomes with puromycin/KCl or EDTA, large T-antigen cosediments with the released mRNPs containing poly(A)$^+$mRNA. Upon treatment with RNaseA, T-antigen sediments as free monomeric protein (< 10 S). Pulse-chase experiments with [^{35}S]methionine indicate that about 10 min are required until newly-synthesized, complete large T-antigen molecules are detected as mRNP complexes in the cytoplasm. The results point to the possibility that large T-antigen is involved in virus-induced mitotic stimulation of the host cell by regulating RNA maturation or translation.

The mouse gene *Mx* directs the synthesis of a protein in response to interferon

M. A. Horisberger, O. Haller and P. Staeheli
Pharmaceuticals Research, Ciba-Geigy Ltd, CH-4000 Basel, and Institute for Immunology and Virology, University of Zürich, CH-8000 Zürich

The gene *Mx* confers a specific resistance towards influenza virus infection in mice. *Mx*-bearing macrophages or embryo cells cultivated in vitro and treated with homologous interferon (IFN) develop a more efficient antiviral state toward influenza viruses than do non-*Mx*-bearing cells (for review, see Haller, O., Curr. Topics Microbiol. Immun. *92* (1981) 25).
We looked for a *Mx*-gene product by labeling *Mx*-positive and *Mx*-negative cells with ^{35}S-methionine before and after treatment with IFN. The radioactive proteins from cytoplasmic extracts were separated electrophoretically in 2 dimensions and were revealed by fluorography. A protein was identified, which was induced by mouse IFN-α,β in *Mx*-bearing cells only. Its apparent M_r was 72,500 as determined by SDS gel electrophoresis, and its pH was ~ 6.3. Its induction depended on the IFN dose used. The maximal rate of synthesis was attained 6–8 h after treatment with 1000 U/ml of IFN. Actinomycin D blocked the induction of the protein. The action of IFN therefore appeared to be at the transcriptional level. Upon repeatedly backcrossing the gene *Mx* on different genetic backgrounds (BALB/c, C57BL/6J, A/J), a clear correlation between IFN inducibility of the protein and the presence of the *Mx* gene was observed.

C) *Posters*

Testing of disinfectants on an artificially contaminated surface

R. Andenmatten and H. Reber
Bakteriologisches Laboratorium des Zentrallaboratoriums, Universitätskliniken, Kantonsspital, Petersgraben 4, CH-4031 Basel

The evaluation of surface disinfectants 'under in-use conditions' presents difficulties in so far as a great number of applied germs are removed by the cleaning process with water only. In the present study, the effect of usual cleaning methods of several types of disinfectants were compared with water of standardized hardness. The germs were collected by the collector developed by Thran. 10^5 *Klebsiella pneumoniae* dried on clinker (hard bricks) were reduced by 2.7 log with water, 3.1–3.5 log by the surface disinfectants. Therefore the latter gave a supplementary effect between 0.45 and 0.7 log.
After use of the disinfectant liquids, the number of germs in washing water was reduced by 1.8–3.7 log after an exposure of 5 min, according to the type of disinfectant. Similar results were obtained with *Staphylococcus aureus.*
The proposed method fullfills the condition for the evaluation of surface disinfectants in use.

Preliminary results of the epidemiological study on the *N. gonorrhoeae* isolated in Switzerland

A. Arini, R. Peduzzi and J. C. Piffaretti
Istituto Cantonale Batteriosierologico, Via Ospedale 6, CH-6904 Lugano

The purpose of this study was to make an epidemiological surveillance of the resistant (penicillin producing [PP] or not) *N. gonorrhoeae* (NG) isolated in Switzerland, particularly of their plasmid content. We report here the microbiological results obtained (auxotype determinations, MIC for penicillin, ampicillin, cefuroxime, tetracycline, thiamphenicol and spectinomycin).
From October 1981 to April 1982, we received 149 strains: these could be divided into 3 groups: A: NG sensitive to penicillin (MIC < 0.5 µg/ml; 76% of the strains); B: NG resistant to penicillin but not PP (16%); C: NG resistant to penicillin and PP (8%). The auxotype frequencies presented some differences: group A strains were 55% prototroph; group B strains were Pro^- (proline requiring: 35%), Arg^- (22%) or prototroph (22%); group C strains were Pro^- (42%) or prototroph (33%).
Concerning the antibiotic susceptibility, the NG belonging to the auxotype Arg^- Hyx^- Ura^- were the most sensitive; the PPNG strains were more resistant to tetracycline whereas the penicillin resistant NG (PP or not) were less sensitive to thiamphenicol than the penicillin sensitive strains. No difference was noticed for susceptibility to spectinomycin. A general conclusion for this study is that the NG isolated in Switzerland do not show marked differences with those isolated in other countries.

Reduction of the staphylococcal methicillin resistance level by Tn 551 insertion

B. Berger-Bächi
Institute of Medical Microbiology, University of Zürich, CH-8028 Zürich

The Tn 551 transposon, coding for erythromycin resistance (Em^r), was made to translocate into the host chromosome of a methicillin *(mec)* resistant strain, by heat inactivation of a thermosensitive plasmid carrying Tn 551 and selection for Em^r survivors. 2 independent chromosomal insertions of Tn 551 were obtained, which reduced the levels of the methicillin resistance (Mec^r) in *Staphylococcus aureus* by a factor of 50 to 100, making the strains phenotypically Mec^s. They occupied 2 different insertion sites, about 1 kb apart, on the largest *Eco*RI fragment of the chromosome. They reverted with a rate of 4.2×10^{-8}, respectively of 3.6×10^{-5}, to Mec^r. The majority of methicillin resistant revertants that were segregated, still expressed Em^r. Very few of them lost the Em^r concomitantly with regaining the Mec^r phenotype. Hybridization data with labeled Tn 551 showed complex rearrangements and deletions taking place. In transduction experiments with phage 80 *a*, either of these 2 Tn 551 insertions were able to change a mec^r acceptor strain into a Mec^s Em^r phenotype. Whether these 2 insertions of Tn 551 lie on the same chromosomal linkage group II as *mec* could not be confirmed.

Proteolytic activities of 2 bacteria characteristic of polluted waters: *Zoogloea ramigera* and *Sphaerotilus natans*

E. Balmelli, J. C. Piffaretti and R. Peduzzi
Istituto Cantonale Batteriosierologico, Via Ospedale 6, CH-6904 Lugano

Sewage waters rich in organic materials stimulate the growth of 2 bacterial species: *Z. ramigera* and *S. natans.* In this study, we have examined the proteolytic activity of these bacteria. Both species show chymotrypsic activity which can be connected with the digestion of proteic substrates present in the sewage waters.
The proteolytic activity has been studied using spectrophotometry, electrofocusing and immunochemistry techniques. The optimum of chymotrypsic activity is found after 24 h of growth. The oxygen content of the environment seems to

influence the enzyme synthesis: *Zoogloea* shows more chymotrypsic activity when the oxygen content is diminished.
During bacterial growth, activity found in the media (metabolic extract) is greater than that found in the cells (somatic extract): this supports the hypothesis that the chymotrypsic activity is carried by exoenzymes destined for the biodegradation of proteinic substrates present in the environment. *Z. ramigera* and *S. natans* have pI values of 4.4 and 10 respectively, the identification of the enzyme bands on the electroforegram was performed with a specific coloured substrate. By immunodiffusion techniques and using the same electrofocusing bands, the antigenicity of the enzymes could be established.

Plasmid fingerprints of pig enteropathogenic *E. coli* 0149:91:K88ac with resistance to streptomycin and sulphamides

A. Baumgartner
Institute of Veterinary Bacteriology, University of Bern, Länggassstrasse 122, CH-3012 Bern

Enteropathogenic *E. coli* 0149, associated with diarrhea in piglets, are frequently isolated in our routine diagnoses. It is known that in such strains some important phenotypic markers like colicin, enterotoxin, hemolysin, K88 fimbriae and antibiotic resistance are coded on plasmids. Since the plasmid pattern of a strain can be used as a fingerprint for identification in epidemiological studies, we examined the plasmid pattern of twelve *E. coli* 0149 isolated from diarrheal piglets of 12 different farms, by means of agarose gel electrophoresis, the isolation of plasmids being achieved by the rapid screening method of Birnboim, H.C., and Doly, J., Nucleic Acids Res. 7 (1979) 1513.
10 out of the 12 strains examined showed a similar plasmid fingerprint, suggesting a relatively wide geographical distribution of the related strains. Furthermore it was found that such a strain was constantly responsible for epidemics of diarrhea in one farm without changing its plasmid pattern during the 7 months of observation.
All strains examined showed resistance to streptomycin and sulfonamide. In 10 strains the resistance was coded on a plasmid of 3.9 megadaltons. This small plasmid was characterized by digestion with Hind II, Eco Ri and Pst I and gave the same fragments as described by van Treeck, U., et al., Antimicrob. Agents Chemother. *19* (1981) 371, in a plasmid of a human strain of *E. coli* (pBP_1). This observation confirms the opinion that plasmids may be common both in human and animal.

Isolation of *Coxiella burnetii*, from milk and colostrum of naturally infected cows after parenteral and intramammary treatment with oxytetracycline

L. Corboz and H. Zurgilgen
Institute of Veterinary Hygiene, University of Zürich, CH-8057 Zürich, and Veterinary practice, CH-8303 Bassersdorf

13 cows of a dairy farm, which gave a positive capillary agglutination test for *C. burnetii* (CAT, Veterinaria, Zürich) in milk and blood serum, were treated as follows: on days 1, 4 and 7, they received 20 mg/kg b.wt of Terramycin/LA (Pfizer) as deep i.m. injection. From days 1 to 10, they were given daily in each teat 1 injection of Mastalone, containing 200 mg oxytetracycline (Pfizer). Another CAT positive cow remained untreated as control animal. Placenta of calving cows were examined microscopically for *C. burnetii* by coloration of Koester. Just before treatment, as well as 1 and 2 months after treatment, serological tests of milk (CAT) and blood of the cows (CAT and CFT) were performed, and isolation of *C. burnetii* from milk and colostrum of single udder quarters was attempted by mouse inoculation. For each sample, 2 mice were inoculated i.p. with 0.5 ml inoculum composed of the sediment of 4 ml milk or colostrum (if not too visquous) centrifuged at 15,000 × g/20 min/4 °C and resuspended in 0.15 M NaCl to give about 1 ml. This method gave better results than when whole milk was used. Mice were bled out by heart puncture 4 weeks after inoculation and CFT was performed. Presence of *C. burnetii* in the samples was confirmed when blood titers of mice were 1:5 or higher.
Of 13 experimental cows, 5 were found to excrete *C. burnetii* in milk (4 cows) or in colostrum respectively (1 cow) before treatment. 3 and 4 cows respectively were still excreting 1 and 2 months after treatment. Moreover, *C. burnetii* was found in the placenta of 1 cow, which had calved 3 weeks after treatment. The untreated control cow excreted *C. burnetii* during the whole period. CFT blood titers of excreting cows comprised between 1:10 and 1:640.
These investigations have shown that, in spite of combined parenteral and intramammary treatment with oxytetracycline for 10 days, *C. burnetii* could still be isolated 1 and 2 months after treatment from milk, colostrum and placenta of naturally infected cows.

Hospital hygiene in Tessin: a comparative study

A. De Marta, J. C. Piffaretti and R. Peduzzi
Istituto Batteriosierologico Cantonale, Via Ospedale 6, CH-6904 Lugano

We have evaluated the hospital hygiene in 8 nosocomial institutions of public interest of Tessin. Our purpose was to give usable information to the medical staff and to compare the different hospitals. The following aspects were examined: the architecture, the available infrastructure and the hygiene of staff members' hands, of the premises and of the material; air samples were also taken.
Sampling was done in similar environments (operating theatres, intensive care units, maternity wards, delivery wards, pediatrics wards and sick-rooms).
The bacteriological evaluations were made according to the criteria used by the CHUV in Lausanne, which gave the following results: number of samples taken: 477 (surface: 302, air: 52, hands: 125); number of samples with potential pathogens: 137 (surface: 93, air: 9, hands: 35).
Results are presented in tables in which the different aspects of hospital hygiene have been evaluated using 4 levels of apparaisal. All the hospitals considered in this study presented deficiencies in one or more of the examined aspects. It is interesting to note that the medical staff seemed to be more careful of its own hygiene when working in unfavorable environmental conditions (architecture and infrastructure).

Effects of selection by subbacteriostatical concentrations of tetracyclin on plasmid-free and plasmid-bearing Coli bacteria

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

In clinical observations the question arises again and again whether subbacteriostatical concentrations of antibiotics have any effect.
We have, therefore, investigated the effect of several subbacteriostatical tetracyclin concentrations on plasmid-free and plasmid-bearing (F^+; R^+) Coli bacteria in a chemostat steady state culture. Migration of the population was

recorded regularly. We obtained the following results: In a tetracyclin-free nutritive broth, R^-F^- and F^+ strains of a mixed culture of *E. coli* K12, (F^+; R^+ rep (TSu); F^-R^-) were favored, in contrast to the R^+ strains which showed a slower growth. While 0.1 µg tetracyclin/ml nutritive broth did not cause a visible change of these results, 0.25 µg tetracyclin/ml nutritive broth caused a significant selection of the R-bearing strain. As the MIC of the plasmid-free strain we used amounted to 5 µg/ml, we concluded that even $1/20$ of this quantity can cause a selection effect.

Gentamicin and tobramycin resistance among gram-negative bacteria isolated from hospitalized and ambulatory patients during 1981, in Tessin

M. Franciolli, R. Peduzzi and J. C. Piffaretti
Istituto Cantonale Batteriosierologico, Via Ospedale 6, CH-6904 Lugano

8560 bacterial strains (5956 gram-negative and 2604 gram-positive) were analyzed for gentamicin (Gm) and tobramycin (To) resistance. 4951 strains originated from hospitals and 3609 from ambulatory patients. From the results reported, it could be concluded: a) the levels of Gm and To resistance in Tessin are similar to those found by other laboratories in other towns or countries; at present, there are no problems in Tessin with these antibiotics. b) For some species, such as *Pseudomonas, Proteus, Providencia* and *Enterobacter,* the hospital strains showed more resistance than those isolated from ambulatory patients: this is probably due to the higher amounts of Gm and To used in the hospitals. c) The determinations of cross resistance to netilmicin and amikacin showed that these 3 antibiotics can be used to inhibit Gm and To resistant strains (except in one hospital where these was a small outbreak of netilmicin-amikacin resistant *Pseudomonas*). d) For some of the Gm or To resistant strains, resistance plasmids were identified after conjugation into *E. coli.* Using electrophoresis through 0.7% agarose gels, these plasmids were shown to have W between 70 and 110 kb: these plasmids were thus generally different from one another.

A new antigen fraction from larval *Echinococcus multilocularis* and its use in ELISA for the diagnosis of human echinococcosis

B. Gottstein and J. Eckert
Institut für Parasitologie der Universität Zürich, Winterthurerstrasse 266, CH-8057 Zürich

Up to now, most laboratories have utilized crude *Echinococcus granulosus* hydatid fluid from cattle, sheep, horses or other intermediate hosts as antigen in the ELISA for the serodiagnosis of human echinococcosis. The use of this antigen has many disadvantages: Interference of host factors, heterogeneity of the antigen due to the host-parasite relationship and the age of the cysts.
Therefore, attempts were undertaken to isolate serologically potent antigen fractions from larval *E. multilocularis* (CH-10-isolate). These metacestodes can be maintained continuously by i.p. transplantation in rodents *(Meriones unguiculatus),* and large quantities can be produced.
Rabbit antihydatid fluid *(E. granulosus)*-IgG, covalently linked to CNBr-sepharose 4B, was used to isolate by affinity chromatography proteins from an *E. multilocularis* antigen extract, prepared from mixed (Polytron PCU-2 blender) and sonicated metacestode material. This purified antigen mainly contained proteins common to *E. granulosus* and *E. multilocularis,* as demonstrated by analytical isoelectric focusing. The advantage of the new antigen fraction was its constancy, the reproducibility of its production and the elimination of host factors.
Applied in the ELISA, the new antigen fraction detected 94% of confirmed human cases of cystic or alveolar echinococcosis. In 27% of 40 sera from patients with other parasitoses (toxocariasis, schistosomiasis, cysticercosis, filariasis, trichinellosis) cross reactivity occurred, but only at a low level.
It is concluded that the new antigen fraction from *E. multilocularis* can improve the serodiagnosis of human echinococcosis. Moreover, the use of larval *E. multilocularis* antigen fraction may help to overcome difficulties in the production, worldwide distribution and use of uniform antigens in the serodiagnosis of this disease.

Pneumococci in culture: A comparison of common identification methods

R. Kaeslin, M. Gasser and F. Keusch
Institut für Mikrobiologie und Hygiene, Petersplatz 10, CH-4003 Basel

181 sputum specimens were processed in order to compare the validity of 3 methods commonly used for the identification of pneumococci: optochin susceptibility, bile solubility and coagglutination (Phadebact Pneumococcus Test). If the results of the different methods did not correspond, the API-STREP identification system was used for identifying the streptococcus species, respectively excluding pneumococcus.
217/234 strains tested (92.7%) showed agreement in all 3 tests, either positive (30 strains) or negative (187 strains). 3 strains (1.3%) were optochin positive and negative in the other tests, 2 strains (0.9%) were optochin negative and positive in the other tests, and 12 strains (5.1%) were optochin and bile solubility negative, but coagglutination positive. Biochemical identification profiles of the 17 strains showing discrepancies identified 12 strains as streptococci (5 *S. sanguis,* 3 *S. mitis,* 3 *S. dysgalactiae,* 1 *S. milleri*), while 5 strains remained unidentified by API-STREP (presumably pneumococci).
The results obtained so far are in agreement with the known limitations of the Phadebact Pneumococcus Test, i.e. false positive reactions with some streptococcal strains. On the other hand, in a few strains tested, one or both of the other methods gave false negative results.

Detection of specific IgM-antibodies against *Legionella pneumophila,* by ELA-SPIT and immunofluorescence

T. Krech, S. Pagon and U. Krech
Institute for Medical Microbiology, CH-9000 St. Gallen

The serological diagnosis of infection with *L. pneumophila* is based on the demonstration of a rise of antibodies in 2 serum specimens taken over an interval of several days. The determination of specific IgM-antibodies has been successfully used for the diagnosis of acute viral infections. It was therefore investigated whether a rapid diagnosis could be made in a bacterial infection by testing a single serum for specific IgM antibodies.
The *IgM-ELA-SPIT* (Enzyme Linked Antigen-Solid Phase Immunosorbent Technique) is based on a solid phase (microtiter plates) coated with animal antiserum directed against μ-chain of human IgM. While incubating the patients' serum on this solid phase, antibodies of the IgM fraction are absorbed. Specific IgM are then detected by adding the *L. pneumophila* antigen to which an enzyme is linked. By adding a substrate the reaction can be seen.

Immunofluorescence (IF) was done according to standard procedure, using an FITC-conjugated antiserum directed against human total immunoglobuline or IgM. For both assays *L. pneumophila* type 1 antigen was used, which was propagated on GC agar (BBL) enriched with hemoglobine and Isovitale X (BBL).
67 sera from patients with suspected Legionnaires' disease were compared by ELA-SPIT-IgM and IF-IgM. Although there was a good correlation with most sera, 5 sera were positive by IF only. The results of our ELA-technique were confirmed by a reference laboratory using the IgM Immunofluorescence technique.
Serial specimens were available from 3 patients with Legionnaires' disease. Seroconversion and IgM antibodies were not seen before 10 days after onset of symptoms. In one patient a last serum could be obtained 4 months after onset of symptoms and was negative in the IgM-ELA-SPIT. From another case it is suspected that *Legionella*-IgM can become negative not before 2 years after infection. Incubation time in one patient was probably not longer than 3 days. Cross reactions were seen mainly in *Chlamydia trachomatis* L_2, but occurred also in *Toxoplasma gondii*, but not in Cytomegalo and mumps virus.

Some unknown effects of cryptical plasmids

G. Lebek
Institute of Hygiene and Medical Microbiology, University of Bern, Friedbühlstrasse 51, CH-3010 Bern

Cryptical plasmids in *E. coli, Klebsiella, Serratia* and *Pseudomonas aeruginosa* were heat-cured but not conjugative plasmids, colicinogenous factors and Hly plasmids. After heat treatment it was possible to compare the same strains in plasmid-free and plasmid-bearing states. We found the following properties of cryptical plasmids:
1. Cured, plasmid-free strains showed a 1.2-1.9 times longer generation time. We therefore assume that the effect of generation time on cryptical plasmids induces selection because almost every wild strain bears such plasmids.
2. Many cryptical plasmids in pseudomonas strains changed the pyocin type.
3. Curing agents as well as trypaflavin and ethidiumbromid can eliminate not only the cryptical but also the conjugative and the bigger nonconjugative plasmids. - For this reson the elimination of plasmids by these agents, respectively, by heat treatment must be based on different mechanisms.

Localization of RNA polymerase binding sites and an in vitro transcription map of *Streptomyces* phage SH10 DNA

J. Meyer, J. Gautschi, M. Stålhammar-Carlemalm and S. Klaus
Biozentrum der Universität Basel, Abt. Mikrobiologie, CH-4056 Basel, and Akademie der Wissenschaften der DDR, Zentralinstitut für Mikrobiologie und experimentelle Therapie, DDR-69 Jena

The genome of the temperate *Streptomyces* phage SH10 is a linear molecule of about 48 kb. Electron microscope analysis of preferential binding sites of *Streptomyces* RNA polymerase on SH10 DNA revealed 2 strong binding sites at map units 52 and 84 and probably 3 in the terminal region between map units 97 and 100. *Escherichia coli* RNA polymerase seems to bind to the same regions, although with a lower specificity. A comparison with the denaturation map indicated that the regions around map units 52 and 97 to 100 are fairly A+T-rich, while the region at map unit 84 has a lower A+T content. Transcription R-loops were found in all 3 regions.

Nosocomial infections in a university hospital: results of prospective surveillance in 2 surgical wards and 1 medical ward

J. Munzinger, M. Bühler, S. Gerulanos and R. Lüthy
Abteilung für Spitalhygiene, Chirurgische Universitätsklinik A, und Departement für Innere Medizin, Universitätsspital, Rämistrasse 100, CH-8091 Zürich

The results of a pilot study of surveillance of nosocomial infections for an 11-month period are reported. Prospective surveillance was performed by an infection control nurse by means of daily examination of the microbiology reports and daily visits to the ward for the review of charts and Kardex of all patients; i.e. for detection of 'infection clues'. Work sheets were used to collect all data during the daily rounds. Infection rates were calculated by dividing the total number of hospital-acquired infections by the total number of discharges during the surveillance period. Active surveillance was performed during 6 months in the surgical intensive care unit (SICU), 5 months in the surgical ward (SW) and 9 months in the medical ward (MW).
Of 1527 patients discharged from these wards, nosocomial infections developed in 158 patients (10.3%). The overall infection rate was 14% (214 nosocomial infections). The infection rate varied greatly from ward to ward due to different patient populations, invasive procedures and severity of underlying diseases. Incidence infection rates were 42.5% for the SICU, 19.6% for the SW and 4.1% for the MW. The major sites affected were surgical wounds (42%), urinary tract (23%), respiratory tract (19%) and blood stream (8%). The major etiologic agents associated with these nosocomial infections were *E. coli, Pseudomonas aeruginosa, Enterococci* and *Staphylococcus aureus.* Conclusions drawn from the results of surveillance are discussed. The major benefits of an effective surveillance program for nosocomial infections are: a) estimates of the endemic levels of nosocomial infection, b) identification of the nosocomial pathogens commonly encountered within a given institution, c) identification of risk factors and d) prompt recognition of epidemics.

Activity and efficacy of a stable chlorinated disinfectant-antiseptic product

G. Pappalardo, F. Tanner, D. Roussianos and A. Pannatier
Division autonome d'Hygiène hospitalière, Unité de Bactériologie médicale et Service de Pharmacie, CHUV, CH-1011 Lausanne

A chlorinated product is studied that does not present the classical drawback of hypochlorite solutions: a fast decrease in the amount of active chlorine. The present study concerns the use of electrolytical cloroxydant in hypertonical solution of sodium chloride. Having been submitted in special cells to a partial electrolysis, the sodium chloride is split with the production of sodium hydroxide, hydrogen and chlorine. A part of sodium chloride remains in solution. The tested product is stable: in its concentrated state (experimental value of active chlorine: 11,489 PPM or 1.15%; declared value: 1.1%), as well as in its 5% dilution (573.5±4.5 PPM at time 0, and 566.5±1.5 at time 92 h).
The test in vitro was conducted on the SSM test strains for disinfectants by the membrane filtration method. The neutralizer added was 5% sodium thiosulphate. The M.I.C. for *Pseudomonas aeruginosa* was 3.75% and for *Staphylococcus aureus* 2.5%. With the normally employed dilution of 5% of the disinfectant, a reduction of the vegetative forms from 10^8 to 0 occurred after 1 min of contact, whereas the sporulated forms decreased from 10^8 to 10^5 after 5 min. - In

the clinical trial, the above dilution, employed in the intimate washing of hospitalized pregnant women, was found to be satisfactory. In searching for the number or germs in the washing water, no gram-negative, but a few gram-positive rods were recorded. The mercurial disinfectant previously utilized did not eliminate the germs of the fecal flora completely and showed 10 times more germs when compared with the present product. In addition, this chlorinated disinfectant proved to be a nonirritant to tissues, owing to its marked histophilic property, and was well accepted by the patients.

A semiselective medium for recovery of *Legionella pneumophila* from environmental sources

P. Rathgeb, M. Gasser and H. Loeffler
Institut für Mikrobiologie und Hygiene, Petersplatz 10, CH-4003 Basel

Legionella [L.] *pneumophila,* the causative agent of Legionnaires' disease, is a fastidious bacterium whose natural habitat is soil and water. For the isolation of the slowly growing organism, selective media are required. We have tried, by the addition of antibiotics, to define a semiselective medium for *L. pneumophila.*

First, different nonselective media were tested for growth of *L. pneumophila* serogroup 1, including modified Mueller-Hinton, F-G, CYE, BCYE*α* and Oxoid *Legionella* medium. Optimal growth was obtained on BCYE*α* and Oxoid media. Thus, the selective effect of antibiotic combinations was tested on these 3 media. On Oxoid selective medium no visible growth of *L. pneumophila* was observed within 7 days. On BMPA*α* selective medium (P.H. Edelstein, J. clin. Microbiol. *14,* 298, 1981) *L. pneumophila* grew satisfactorily, but bacteria cultured from soil and water also grew well. Since many of these bacteria were tetracycline susceptible, we modified the BMPA*α* selective medium by replacing anisomycin with tetracycline. While growth of *L. pneumophila* was satisfactory on this medium, growth of bacteria from 24 samples from soil and water was reduced considerably.

The prescription of this semiselective medium for *L. pneumophila* is as follows:

- To 450 ml of distilled water, add 5 g yeast extract, 1 g activated charcoal and 8.5 g Agar Noble (Difco). Autoclave at 121 °C for 15 min.
- Add a solution (sterilized by millipore filtration) of 5 g ACES buffer (Sigma Chemicals) and 0.5 g *α*-ketoglutarate in 20–30 ml preheated distilled water and cool to 50 °C.
- Add one vial of *Legionella* growth supplement (Oxoid) and the antimicrobial agents (sterilized by millipore filtration): cefamandol 4 µg/ml, polymyxin B 80 U/ml, chlortetracycline 8 µg/ml. Adjust pH to 6.9 with 1 N KOH.

Staphylococcus aureus strains resistant to penicillin and oxacillin (P-O/R) but sensitive to cefalotin (C/S): a particular group?

E. Sakellarides and I. Sénéchaud
Institut de Microbiologie, CHUV, CH-1011 Lausanne

By diffusion, some strains of *S. aureus* were characterized as P-O/R but C/S. Independently of the clinical aspect of this finding, we observed the following:

- The C/S character established by diffusion was confirmed by MIC determination. All of our 25 strains showed a MIC value < 1 mcg/ml of cefalotin, despite the fact that 14 strains produced penicillinase and 11 strains did not.
- All 25 strains initially classified as O/R by diffusion were in fact O/S when tested by dilution (MIC values < 0.5 mcg/ml of cefalotin).
- The term of an 'intermediate' category of sensitivity, as determined by diffusion, was not confirmed by MIC determination.
- Additionally, we observed that all 25 strains were sensitive to chloramphenicol, cotrimoxazol and clindamycin (both by diffusion and MIC determination).

The effect of clavulanic acid on some beta-lactam antibiotics

E. Sakellarides and I. Sénéchaud
Institut de Microbiologie, CHUV, CH-1011 Lausanne

In agar diffusion tests, the combination of some beta-lactam antibiotics and clavulanic acid has enhanced the antibiotic effect when tested with some *Staphylococcus* strains. This effect was independent of the overproduction of exoenzyme penicillinase as tested with Oxoid paper bands. Clavulanic concentrations beyond a limit did not provide further enhancement. The results obtained with *S. aureus* and *S. epidermidis* producing or not producing penicillinase are shown below.

Bacterial strain	Penicillinase production	Complementarity with clavulanic acid
S. epidermidis	no	Ampicillin Azlocillin Mezlocillin
S. epidermidis	yes	Penicillin-Ampicillin Carbenicillin-Azlocillin Mezlocillin-Piperacillin
S. aureus	no	Penicillin-Ampicillin Carbenicillin-Azlocillin Mezlocillin-Piperacillin
S. aureus	yes	Penicillin-Ampicillin Carbenicillin-Azlocillin Mezlocillin-Piperacillin

The collaborating substances must diffuse in the agar simultaneously since there is no effect when the compounds act in succession: the cells do not retain the effects of previous contact with either one.

Becton-Dickinson Urine Culture Kit – an excellent transport system for quantitative urine bacteriology

J. Schmidli and I. Heinzer
Institut für Hygiene and Medizinische Mikrobiologie, University of Bern, Friedbühlstrasse 51, CH-3010 Bern

For quantitative urine bacteriology, immediate culture or refrigeration are the recommended methods. 'Urine Culture Kit' (UCK) of Becton, Dickinson & Co is an attempt to replace refrigeration by chemical conservation. This tube contains a mixture of boric acid, glycerol and sodium formate. – Split samples of freshly collected urine were processed directly (reference method) after a simulated transport in the UCK. 100 µl samples, diluted 1:100 and 1:10,000, were plated on 3 different media, and the number of colony-forming units was counted by electronic image processing. The results obtained after storage of urine in the UCK at room temperature for 24 h (n = 112) and for 72 h (n = 70) showed an excellent correlation with the reference method. Even at extreme transport conditions (24 h at 37 °C), a good correlation was found. Antibacterial activity of the urine had no influence and antibiotic sensitivity patterns were not changed. Our results are much

better than those reported on the basis of semiquantitative methods (Lauer, B.A., et al., J. clin. Microbiol. *10* (1979) 42, and Guenther, K.L., et al., J. clin. Microbiol. *14* (1981) 628).

Gardnerella vaginalis as a causal agent of kolpitis

A. Schwank, N. Pavić, R. Renggli and H. Reber
Bakteriologisches Laboratorium der Zentrallaboratorien der Universität, Kantonsspital, Petersgraben 6, CH-4031 Basel

Gardnerella vaginalis as a causal agent of vaginitis is controversial. In the present study, clinical symptoms (first described by Gardner and Dukes, 1954), including greyish vaginal discharge, elevation of pH, the presence of 'clue celles' and a fishy odor, correlate well with the presence of *G. vaginalis*. Beside *G. vaginalis*, anaerobic bacteria also contribute to kolpitis. There is a characteristic pattern in gaschromatographic analysis of vaginal secretion in *Gardnerella* vaginitis. Lactate is decreased, succinate and acetate are increased, and volatile acids are also detectable.
After ornidazole therapy, symptoms and signs of kolpitis regress, succinate disappears, and lactate again becomes predominant. However *G. vaginalis* also persists in several cases.

The Clq-binding assay – a simple method for distinguishing between binding of Clq to complexed IgG and to heparin

P.J. Späth and R. Bütler
Blood Transfusion Service of the Swiss Red Cross, Central Laboratory, CH-3000 Bern 22

Among the tests for detection of circulating immune complexes (cIC), the Clq-binding assay (Clq-BA) of Zubler et al. (J. Immun. *116*, 232, 1976) is one of the best regarding correlation to the activity of infectious and immunological diseases. However, the conditions used in the Clq-BA are very different from those occurring in vivo. For example, purified Clq is used for the detection of cIC. Physiologically Clq exists in a complex with Clr and Cls and the collagen-like part of Clq is masked. During incubation, which takes place in the Clq-BA, the collagen-like part of purified Clq is unmasked. This may lead to false positive results due to unspecific binding of Clq to various components of patient sera such as fibronectin, collagen or heparin.
In this study, extremely simple modification of the Clq-BA is reported. It allows for the distinction between binding of Clq to complexed IgG and binding to heparin in human serum. Results with binding to collagen, DNA, fibronectin and staphylococcal protein A are also discussed.
The modification of the test consists in the substitution of lactoperoxidase with chloramin T for radiolabeling of Clq. Chloramine-T labeled Clq molecules retain their capacity to bind to heparin, but they lose their ability to bind to complexed IgG, whereas lactoperoxidase labeled molecules bind to both. Therefore both labeling techniques are used for the improved Clq-BA.
Labeling conditions with chloramine T are as follows: to 200 µg of Clq (in 200 µl of 0.3 M NaCl, 0.01 M EDTA, pH 7.5), 30 µl of potassium phosphate buffer, 0.5 M, pH 7.2 and 30 µl of chloramine T solution (2.5 mg/ml 0.05 M potassium phosphate buffer, pH 7.2) are added. The mixture is allowed to stand for 30–35 min at room temperature. Thereafter, another 10 µl of chloramine T solution and 20 µl of isotope solution (250–500 µCi) are added. The reaction is stopped after 3 min by addition of 100 µl of sodiumpyrosulfite (2.5 mg/ml potassium phosphate buffer).

Use of the ELISA technique for demonstration of antibodies against *Campylobacter jejuni*

P. Wassmer, J. Karlen, H.J. Bumbacher and M. Gasser
Institute for Microbiology and Hygiene, University of Basel, Petersplatz 10, CH-4003 Basel

We used the enzyme-linked immunosorbent assay (ELISA) to measure antibodies against *C. jejuni* in the sera of 113 patients who had *C. jejuni* positive stool cultures 2–60 days before sera were collected. Specific antibodies of the IgG and IgM classes were determined independently using class specific peroxidase conjugated goat antihuman sera (Cappel Laboratories). As antigen, a commercially available preparation (extraction at high pH of a pool of fresh isolates), originally developed for complement-fixation test (Mosimann, J., et al., Schweiz. med. Wschr. *111* (1981) 846), was used.
About 90% of the patients' sera showed a higher level of specific IgG and/or IgM than 81 control sera. Antibodies were detectable 2–3 days after onset of symptoms.
Our data indicate that the commercial antigen preparation is suitable for investigation of sera by ELISA. In cases where direct demonstration of *C. jejuni* from stool samples is not possible, the ELISA technique permits a simple alternative approach for clinical and epidemiological investigations.

Demonstration of elevated levels of *Candida*-specific IgM antibody in a patient with positive blood culture by ELISA

P. Wassmer, M. Itier, H. Sevestre and M. Gasser
Institute for Microbiology and Hygiene, University of Basel, Petersplatz 10, CH-4003 Basel

We have attempted to develop a clinically useful ELISA technique for serodiagnosis of *Candida* infection. As antigen, whole killed organisms (*Candida albicans* heated at 80 °C for 10 min) were used. Preliminary tests of 140 control sera showed that most individuals had relatively high levels of *Candida*-specific IgG antibodies in the serum. Even at high serum dilutions (1:800), it was not possible to differentiate the serum of a patient with *Candida*-positive blood culture from all control sera by using a peroxidase conjugated antibody to whole immunoglobulin or IgG.
In contrast, low levels of *Candida*-specific IgM were found in the control sera. At serum dilutions as low as 1:320, there was a clear-cut difference between *Candida*-specific IgM antibodies from the infected patient and all controls. IgM antibody, therefore, might be a reasonable indicator of candidosis.

IgG, IgA and IgM antibodies to *S. aureus* purified cell walls (PCW) in normal and *S. aureus* infected individuals

J.A. Wilhelm, L. Matter and K. Schopfer
Section of Immunology, Institute for Medical Microbiology, CH-9000 St. Gallen

The object of this work is the study of the development of the normal immune response in humans to staphylococcal cell wall antigens. Purified cell walls (PCW) were prepared from *S. aureus* H according to previously described methods. Amino acid analysis was done to check for purity. Sonicated PCW were suspended in carbonate buffer (pH 9.6) and allowed to bind on the wells of microtest plates. To measure anti-PCW antibodies within the IgG, A and M class mouse monoclonal antibodies specific for Fc_γ, Fc_α and Fc_μ were conjugated with alkaline phosphatase.

After a first incubation step with the test sera in the antigen-coated wells, the conjugates were added, followed by the addition of pNO_2/PO_4. The reaction was stopped and the O.D. 405 nm was read.

To study age-dependent development of anti-PCW antibodies, sera from cord blood, from noninfected children of different age groups, and from adults were tested (blood was obtained for other diagnostic purposes). As expected cord blood contained adult levels of IgG anti-PCW, but no anti-PCW antibodies in the IgA and M classes. IgG antibodies to PCW were absent in 1- to 2-year-old children and increased to adult levels only after 10 years of age. On the contrary, IgA and IgM antibody to PCW existed in young children at about the same levels measured in adults.

In patients with bacteriologically proven *S. aureus* infections, only slightly increased mean levels of all classes of antibody to PCW were observed (compared to the normal mean for their age). In patients with the *S. aureus* Hyper-IgE Syndrome (who suffer from recurrent *S. aureus* infections and who have elevated serum IgE levels in addition to IgE to PCW), the mean level of IgG antibodies to PCW was lower than in infected controls, whereas the mean levels of IgA and IgM were higher. This finding again reflectedthe abnormal immunological response of these patients to *S. aureus* infection.

The use of this assay in the serodiagnosis of *S. aureus* and other gram-positive bacterial infections needs to be studied further.

Localization and characterization of the *TRP3* gene of *Saccharomyces cerevisiae*

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

We have recently isolated the *TRP3* gene of *S. cerevisiae*, coding for the tryptophan biosynthetic enzyme indole-3-glycerole-phosphate synthase, by functional complementation in yeast (Aebi et al., Current Genetics (1982) in press). The whole *TRP3* gene is located on a 2.7 kb BamHI-ClaI DNA fragment. Deletions were constructed in vitro, penetrating the yeast DNA fragment from both sides. This allowed for localization of the catalytic activity as well as of the region responsible for the aggregation of the indole-3-glycerole-phosphate synthase with the *TRP2* gene product anthranilate synthase. It was shown that the *TRP3* gene of yeast codes for a single mRNA species and that it is controlled at the transcriptional level.

Distribution of aerobic, hydrogen-oxidizing bacteria and of dissolved molecular hydrogen in an eutrophic lake

M. Aragno and R. Conrad
Laboratoire de Microbiologie, Institut de Botanique de l'Université, CH-2000 Neuchâtel, and Max-Planck-Institut für Chemie, D-6500 Mainz

Aerobic, hydrogen-oxidizing bacteria were counted and isolated by a membrane-filter procedure. Positive colonies were discriminated as 'normal' when larger than 1 mm in diameter, and 'pinpoint' colonies when less than 0.5 mm in diameter. Counts of 'normal' hydrogen bacteria were consistent with counts obtained by the MPN procedure, using liquid enrichment cultures, whereas 'pinpoint' bacteria were more numerous. Most of the 'normal' hydrogen bacteria were associated with a particulate fraction ≥ 3 µm, whereas about half of the 'pinpoint' bacteria were retained on 3-µm filters. The distribution throughout the water column was studied in spring and summer 1981. In spring, the 'normal' bacteria were randomly distributed, whereas the 'pinpoint' bacteria showed higher counts in the upper layers. In summer, the number of 'normal' bacteria, as well as the number of 'pinpoint' ones showed a maximum toward the surface and a minimum in the metalimnic layers. Most of the 'normal' hydrogen bacteria could be attributed to well-known species, e.g. one third were *Xanthobacter autotrophicus*. 'Pinpoint' bacteria probably belonged to species so far unknown, some of them possibly being litho-heterotrophs.

H_2 was randomly distributed in spring, and amounted to 100–200 $nl \cdot l^{-1}$, being much lower in surface waters and just above the sediment. In summer, when a thermal and chemical stratification occurred, hydrogen was mainly confined to the aerobic layer and its concentration showed a distinct diurnal variation, with a maximum in the early morning. H_2 consumption activity was measured in aerobic water samples. It followed Michaelis-Menten kinetics, with K_m value of 67 nM H_2, which is an exceptionally low value when compared to the known K_m values of hydrogenases in 'classical' hydrogen bacteria (about 5000 mM). Moreover, the highest H_2 concentrations measured in the water would not be sufficient enough to allow such bacteria to use it. As the 'normal' hydrogen bacteria has a strictly particulate location, it nevertheless would be possible for them to obtain H_2 from the particle itself (e.g. an alga or decomposing matter) at a concentration higher than could be measured in the free water. The ability of 'pinpoint' bacteria to obtain hydrogen at very low concentration is now under study.

Anaerobic microbial mineralization: depth profiles of natural substrate and product concentrations in freshwater lake sediments

H. Brandl and K. Hanselmann
Institute of Plant Biology, Dept. of Microbiology, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich

Microbial mineralization and fermentation processes in sediments were followed in situ with the equilibrium diffusion technique (Hesslein, R.H., Limnol. Oceanogr. *21* (1976) 912). Depth profiles of soluble substrates and products of anaerobic microbial metabolism were recorded in sediments at different locations and during different seasons. Diffusion fluxes of CH_4 through the sediment-water interphase were calculated from the depth gradients (Lerman, A., A. Rev. Earth Planet. Sci. *6* (1978) 281). Winter profiles in shallow water showed a constant acetate concentration of approx. 20 µM. No gradient and no other volatile fatty acids (vFA) could be detected. Saturation of the pore water with CH_4 was reached at a sediment depth of 25 cm. In sediments at a water depth of 5 m, we observed an elevated acetate concentration (150 µM) 3–6 cm below the sediment-water interphase but CH_4 saturation was reached below 36 cm. Summer profiles in shallow water showed higher concentrations of a variety of vFA (acetate 0.5–2 mM) in the top 3–12 cm. Saturation of the pore water with CH_4 was reached at sediment depths of 3 to 4 cm. Diffusion fluxes for CH_4 increased from 1.1 in winter to 6.6 mmoles $m^{-2}d^{-1}$ under summer conditions. This indicated temperature-dependent mineralization processes.

At 170 m a distinct acetate and propionate horizon of 100 µM and 120 µM respectively could be located 9–12 cm in the sediment. The vFA concentration above and below this horizon varied between 5 µM and 20 µM. At this depth CH_4 saturation of interstitial water (approx. 38 mM) was not reached within the top 40 cm of sediment. The rate of microbial methanogenesis at this location and time seems

to be slower than the diffusive flux of CH_4 through the sediment-water interphase. Sulfate, which is present in the hypolimnion at a concentration of 0.5 mM, disappears within the first 6 cm in the sediment. This indicates that sulfate reducing bacteria can be active in this horizon. The sulfide produced is precipitated as black iron sulfide; no free or dissolved H_2S could be detected at most locations. Fermentation and mineralization processes are the cause of these chemical gradients. Various microbial activities can be associated with different sediment depths from which characteristic bacteria could be isolated.

Measurement of the membrane potential in *Methanobacterium thermoautotrophicum*

B. M. Butsch and R. Bachofen
Institute of Plant Biology, Zollikerstrasse 107, CH-8008 Zürich

As reported previously (Butsch, B.M., and Bachofen. R., Experientia *37* (1981) 1225) we measured the membrane potential ($\Delta\psi$) by means of an electrode sensitive to tetraphenylphosphonium (TPP^+). Cells of *M. thermoautotrophicum* strain Hveragerdi (Butsch, B.M., and Bachofen, R., Res. Inst. Nedri As *36* (1981) 21) were grown in a fermenter at pH 6.5 rather than 7 to prevent foaming at 58 °C and at a gassing rate of 180 ml per l of growth medium (Schönheit, P., Moll, J., and Thauer, R.K., Arch. Microbiol. *123* (1979) 105). Cells were harvested anaerobically at the end of logarithmic growth ($t_d = 5.5$ h) in a density of 1.2 g dry weight per l culture medium when the cells produced 0.76 l methane per h per g dry weight. After 15 min of anaerobic centrifugation at 1600 × g to a density of 14 g dry weight per l, the cells were gassed with H_2/CO_2 (80:20) and placed in a water bath (58 °C). After flushing the reaction vessel containing the electrode with the same gas mixture as described earlier, calibration was performed by injecting TPP^+ in progressive steps, followed by anaerobic addition of the cells. The concentration of the TPP^+ remaining in the medium after equilibration with autoclaved cells was considered as unspecific adsorption and was similar to that of viable cells after treatment with air, indicating a 100% elimination of $\Delta\psi$ under aerobic conditions. Using Nernst's equation and the cell volume calculated by Jarrell, K.F., and Sprott, G.D., Can. J. Microbiol. *27* (1981) 720, $\Delta\psi$ was estimated from the uptake of TPP^+ into the cells. TPP^+ slightly diminished $\Delta\psi$: values for $\Delta\psi$ were 173, 167, 158, 155 and 155 mV at TPP^+ concentrations of 1, 2, 5, 10 and 20 µM, respectively (52 °C). $\Delta\psi$ was rather stable over a period of time: in another experiment $\Delta\psi$ dropped from 130 mV to 123 mV during 1 h.

Influence of different physiological states on carnitine-acetyl-transferase activity in *Saccharomyces cerevisiae*

R. Claus, O. Käppeli and A. Fiechter
Department of Biotechnology, Swiss Federal Institute of Technology, Hönggerberg, CH-8093 Zürich, and Department of Biological Sciences, Karl-Marx-Universität Leipzig, Talstrasse 33, DDR-7010 Leipzig

Carnitine-acetyl-transferase (CAT) plays an important role in the metabolism of fatty acids or other substrates leading to the formation of acetyl groups, The enzyme is involved in the transport of these acetyl groups through biological membranes.

In *Candida tropicalis* growing on n-alkanes, the CAT was detected at high activity and the enzyme was found to be localized both in peroxisomes and in mitochondria. In this way the enzyme connects the metabolic pathways of fatty acid β-oxidation and citric acid cycle and provides cytoplasmic pathways with acetyl groups.

We studied the carnitine-acetyl-transferase activity in *Saccharomyces cerevisiae* at different growth conditions in continuous culture. During glucose-derepressed growth, a low CAT activity was measured in cell-free extracts. A change from glucose to ethanol as the growth substrate led to an increase of CAT activity, indicating the catabolism of ethanol via acetate and the translocation of acetyl groups into the mitochondria via the CAT reaction. A subsequent substrate shift back to glucose was accompanied by a decrease in CAT activity corresponding to the glucose derepressed growth level. By initiating glucose-repressed growth where ethanol is formed by the cells, a total loss of CAT activity occurred. These results allowed the formulation of a hypothesis on the transport of acetyl groups in yeasts.

Mixotrophy and litho-heterotrophy by *Aquaspirillum autotrophicum*

M. Fasnacht and M. Aragno
Laboratoire de Microbiologie de l'Université de Neuchâtel, CH-2000 Neuchâtel

Aquaspirillum autotrophicum, a spirillum isolated from a small freshwater lake, is a hydrogen bacterium. Hydrogen bacteria are facultative chemolithoautotrophs. They can grow with H_2 as an electron and energy donor, CO_2 as a carbon source, and O_2. Their physiological adaptations and enzymatic responses to different conditions of growth are especially interesting. We first studied the induction of *A. autotrophicum* hydrogenase in a Warburg vessel; this induction followed a parabolic curve and was influenced by many organic substrates. Certain of them activated the induction, whereas others inhibited it.

However, when cells were submitted to a shift from autotrophic to mixotrophic conditions in a chemostat (growth limited by N source), the final level of hydrogenase depended on the organic substrate concentration. If the substrate was in excess, the level of hydrogenase fell after the shift to one fourth of its initial value. In contrast, if the substrate concentration was limiting, the level of hydrogenase was barely lowered. Then, growth and induction of hydrogenase were followed in a chemostat (growth limited by pyruvate), before and after a shift from heterotrophic (pyruvate, O_2 and CO_2) to mixotrophic (pyruvate, H_2, O_2 and CO_2) conditions. After the addition of H_2, hydrogenase was immediately and quickly induced, and an increase of bacterial population occurred in 2 successive phases. We suppose that the first phase corresponded to a litho-heterotrophic growth, whereas in the second phase, the cells achieved an actual mixotrophic metabolism, using CO_2 as a carbon source in addition to pyruvate.

In another experiment, we submitted the cells to a shift from heterotrophic (pyruvate, O_2) to litho-heterotrophic (pyruvate, H_2 and O_2) and then to mixotrophic (pyruvate, H_2, O_2 and CO_2) conditions in a chemostat (growth limited by pyruvate). After the first shift, induction of hydrogenase occurred, but only the first phase of population increase. A second phase appeared only after the addition of CO_2. This result seems to confirm the previous hypothesis. Further studies will involve doing measurements of ribulose-bisphosphate carboxylase activity during the 2 phases of population increase.

Mechanism of dehalogenation of dichloromethane by cell extracts of *Hyphomicrobium* DM2

R. Gälli, G. Stucki and Th. Leisinger
Mikrobiologisches Institut ETH, ETH-Zentrum, CH-8092 Zürich

The conversion of dichloromethane to formaldehyde and hydrochloric acid by cell extracts of *Hyphomicrobium* DM2 is dependent on reduced glutathione (GSH) (Stucki et al., Arch. Microbiol. *130* (1981) 366). If this reaction is catalyzed by a glutathione S-transferase (EC 2.5.1.18) the protons of CH_2Cl_2 are conserved and should be detected in CH_2O. In order to verify such a mechanism, we have converted dideuterodichloromethane (CD_2Cl_2) to formaldehyde by a cell extract of strain DM2. The formaldehyde formed was allowed to react with 2,4-pentanedione and ammoniumacetate to form 2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridine by the Hantzsch reaction. The derivative was collected on a filter, dried and recrystallized in ethanol. The product obtained from CH_2Cl_2 and that from CD_2Cl_2 were analyzed by nuclear magnetic resonance (H^1-NMR) and mass spectrometry. It was verified that, under the conditions used, the protons originating from formaldehyde did not undergo exchange with protons of the medium. The product formed by the enzymatic reaction from CD_2Cl_2 was identified as CD_2O. This result excludes oxidation-reduction or elimination-addition reactions in the formation of CH_2O from CH_2Cl_2. It supports a mechanism involving an enzymically catalyzed nucleophilic substitution by GSH, yielding an S-chloromethyl glutathione conjugate which probably is nonenzymically hydrolyzed to S-hydroxymethyl glutathione and subsequently decomposes to CH_2O and GSH. Dehalogenation of CH_2Cl_2 by the glutathione S-transferase from *Hyphomicrobium* DM2 may thus be summarized by the following scheme: a) $CH_2Cl_2 + GSH \rightarrow GS\text{-}CH_2Cl + HCl$, b) $GS\text{-}CH_2Cl + H_2O \rightarrow GS\text{-}CH_2OH + HCl$, c) $GS\text{-}CH_2OH \rightleftarrows CH_2O \pm GSH$.

Anaerobic digestion of urban wastes. Preliminary research and perspectives

M. Glauser, P. Messner and M. Aragno
Laboratoire de Microbiologie de l'Université, CH-2000 Neuchâtel

In the region of Neuchâtel (Switzerland), urban waste is presently treated in an incineration plant that consequently produces electricity. Due to the dramatic increase in the cost of treatment and to the tendency toward energy and matter recuperation and biomass conversion, another principle is now under study. It requires at first a separation process, allowing recovery of glass, metals and plastics, and the obtaining of an organic fraction which may be submitted to anaerobic, methanogenic digestion. The residue could then be partly dehydrated, composted and used in agricultural soils. We developed preliminary research in order to study the adequacy of organic waste to anaerobic digestion, the stability of the process and its yield. We designed a 20-l glass digester with mechanical stirring, and developed a strategy of control.

The organic fraction of waste is poor in nitrogen. Thus, we combined ground organic waste with sewage sludge to obtain a substrate devoid of N-limitation. Digestion experiments were conducted semicontinuously, at 35 °C and 60 °C, with retention times of 54, 18 and 9 days. Yields were almost identical at 60 °C and 35 °C. Once the microflora was established, chemical and physical parameters remained stable, and it was not necessary to correct them by additions. About 40% of the organic solids were converted into gas with 18 days retention time. No distinct increase of this yield was observed with 54 days retention time, whereas gas production was lowered only by ca. 10% with 9 days retention time. Gas composition was 55–60% CH_4 and 40–45% CO_2.

Reduction of nitrates must occur before methanogenesis can take place. Addition of 10 mM NO_3^- in the digester resulted in complete blocking of methanogenesis: Nitrate reduction was complete in less than 24 h, and only a small part was converted to N_2, most being reduced to NH_4^+. Methanogenesis began soon after NO_3^- exhaustion, but original activity was restored only after 4–5 days. Counts and isolation of nitrate reducers were performed.

These results allow us to predict that methane production from urban wastes and sludge in the region of Neuchâtel would amount to about $\frac{1}{3}$ of the actual natural gas consumption in the same area.

Characterization of the *TRP2* gene of *Saccharomyces cerevisiae*

F. Jucker, M. Aebi and R. Hütter
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

We have isolated the *TRP2* gene of *S. cerevisiae* by functional complementation in yeast on a 6.2-kb BamHI DNA fragment (Aebi et al., Current Genet. (1982) in press). The gene product anthranilate synthase, the first enzyme of the tryptophan biosynthetic pathway, is active only in aggregation with the *TRP3* gene product (indole-3-glycerole-phosphate synthase).

A restriction map was established and the *TRP2* gene was localized on a 2.7-kb BamHI-PvuII fragment by subcloning the original BamHI fragment. With a set of deletions made in vitro, a further localization of the gene was achieved.

The *TRP2* gene was not expressed in an *E. coli* strain carrying a mutation in the analogous *trpE* gene.

Vesicles from *Acetobacter aceti*

W. Jucker, D. Studer and L. Ettlinger
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Membrane vesicles are usually prepared by the standard method of H.R. Kaback (Meth. Enzymol. *22* (1971) 99) involving the conversion of the cells into an osmotically sensitive form followed by a controlled osmotic lysis. However, this method is not applicable for many gram-negative bacteria, including acetic acid bacteria.

We present a method for preparing spheroplasts and membrane vesicles from *A. aceti*. 2.7 mmoles/l glycine and 10 mmoles/l magnesium sulphate were added to exponentially growing cells and further incubated for 2 or 3 doubling times. Cells were harvested by centrifugation and washed twice with an aqueous solution of 150 mmoles/l sodium chloride and 10 mmoles/l magnesium sulphate. The washed cells were osmotically shocked as described by Neu and Heppel (J. biol. Chem. *240* (1965) 3685). The pellet was resuspended in 0.1 moles/l potassium phosphate buffer pH 7.4 with 10 mmoles/l ammonium sulphate as a stabilizer. By adding 0.2 mg/ml lysozyme the degradation of the peptidoglycan layer was begun. 8 min later the spheroplasts were stabilized by the addition of 20 mmoles/l magnesium sulphate. Osmotic lysis was performed according to Kaback (l.c.). The transformation of cells into membrane vesicles is verified by electron micrographs.

Anaerobic degradation and conversion of methoxylated and hydroxylated benzoic acid derivatives by bacterial communities

J.-P. Kaiser and K. W. Hanselmann
Institute of Plant Biology, Dept. of Microbiology, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich

Stable microbial communities consisting of 4–5 different populations were enriched with lake sediments under strictly anaerobic conditions with syringic acid as sole energy source.

The aggregate populations degrade syringic acid completely to CH_4 and CO_2 with acetate as the intermediate. Several other aromatic compounds with hydroxy or methoxy substituents at 3 consecutive ring carbons are also completely mineralized anaerobically. Benzoic acids which carry hydroxy or methoxy substituents in the 3 and 4 positions only (protocatechiuc acid, vanillic acid and veratric acid) are initially degraded completely. But when subsequently fed low concentrations of these acids, no ring fission takes place. These aromatic substrates are demethoxylated and decarboxylated; catechol accumulates as a conversion product.

High concentrations of catechol (25 mM) do not inhibit those populations that demethoxylate vanillic acid. Ring fission of 3,4,5-trisubstituted benzoic acids (syringic-, gallic-, 3,4,5-trimethoxy benzoic acid) can still proceed at high catechol concentrations. We assume that a ring fission product of catechol degradation inhibits further catechol metabolism without affecting the metabolic route of 3,4,5-trisubstituted benzoic acids.

Separation of the community by the agar shake technique yields populations that grow on methoxylated benzoic acids but not on hydroxylated ones, while those that can be isolated on gallic acid do not grow on the methoxylated compounds. This indicates that the ring cleaving organism(s) can function without the aid of a hydrogen-producing or a substrate-scavenging companion organism.

Slow-growing, rod-like, acetate-utilizing methanogenic organisms represent the rate limiting population within the community.

Plating efficiency of methanogenic bacteria

A. Kiener and Th. Leisinger
Mikrobiologisches Institut ETH, ETH-Zentrum, CH-8092 Zürich

Methanobacterium thermoautotrophicum Marburg, *Methanobrevibacter arboriphilus* AZ and *Methanococcus vannielii* were grown on standard media in a series of serum bottles pressurized to 1 bar with H_2:CO_2 (80:20). At appropriate intervals after inoculation, flasks were removed for determining methane production, absorbance of the medium, total bacterial counts and viable counts. Viable counts were obtained by plating serial dilutions in 2 ml of soft agar (0.7% agar) on anaerobically prepared agar plates in a Freter-type anaerobic chamber. The plates were incubated in pressure cylinders with 1 bar of H_2:CO_2 (80:20). A petri dish containing a few pellets of palladium catalyst and a small amount of anhydrous calcium chloride was included in each pressure cylinder. The overlay method of plating proved advantageous because smearing of colonies from condensation of water was avoided and cells were protected against traces of oxygen during incubation. With the 3 strains tested, methane production, absorbance, total counts and viable counts increased in parallel at an exponential rate. The plating efficiency (i.e. viable counts/total counts) was 0.74 for *M. thermoautotrophicum*, 0.82 for *M. arboriphilus* and 1.00 for *M. vannielii*. Based on these results, the oxygen sensitivity of the 3 strains was determined under standardized conditions. While a 2-h exposure to oxygen killed > 99% of the cells of *M. thermoautotrophicum* and *M. vannielii, M. arboriphilus* survived this treatment without significant decrease in the number of viable cells.

Replication of pSC101: analysis of plasmid encoded functions

P. Linder, G. Churchward and L. Caro
Department of Molecular Biology, University of Geneva, CH-1211 Geneva

The plasmid pSC101 is the only known naturally occurring plasmid whose replication has been shown to be dependent upon the activity of the product of the *dnaA* gene of *E. coli*. This gene product is absolutely necessary for the initiation of chromosome replication in *E. coli*, and so the analysis of pSC101 replication should provide valuable information concerning the role of the *dnaA* gene in replication. We have therefore attempted to determine what plasmid encoded functions are required for pSC101 replication. A hybrid plasmid consisting of a 2.9-kb fragment, containing all the necessary information for pSC101 replication, joined to a colE1 replicon was constructed. Mutant derivatives of this hybrid plasmid caused by the insertion of the transposon Tn1000 into the pSC101 portion of the hybrid were isolated and analyzed. These mutants allowed us to map a function that is required for pSC101 replication and that can be complemented in trans. We also mapped a function responsible for pSC101 specific incompatibility. The hybrid plasmid, the mutants and various fragments of DNA cloned from the mutants were used as templates for in vitro protein synthesis. These experiments show that the trans-acting function required for replication is a protein of 35,000 D. We have yet been unable to demonstrate the synthesis of this protein in vivo in a maxi-cell system.

We have also carried out in vitro transcription experiment, which shows the synthesis of a small RNA species. This RNA of about 170 bp is absolutely required for replication. It is also synthetized by plasmids defective for pSC101 specific incompatibility which distinguishes it from other RNA species known to be involved in the control of plasmid replication.

Mapping of genes for proline and ornithine utilization in *Pseudomonas aeruginosa*

L. Soldati, Th. Leisinger and D. Haas
Mikrobiologisches Institut ETH, ETH-Zentrum, CH-8092 Zürich

The enzymological analysis of mutants blocked in the utilization of proline (*pru* mutants) revealed 3 phenotypic classes with the following properties: a) absence of proline dehydrogenase activity, b) absence of both proline dehydrogenase and 1-pyrroline-5-carboxylate dehydrogenase, c) presence of both enzyme activities. Transduction analysis using the generalized transducing bacteriophage G101 showed that the *pru* mutations carried by representatives of the 3 phenotypic classes were linked. The *pru* loci were mapped by FP2 and R68.45 mediated conjugations in the very late region of the chromosome between *pur-67* and *cys-59*. *Orul* mutations, which affect the catalytic activity or the inducibility of N^2-acetylornithine-5-aminotransferase and thereby lead to a block in the utilization of ornithine as a carbon source, were mapped by the same technique between *pur-67* and *pru*. By linkage analysis of recombinants from 3 factor crosses the following marker order was established: *pur-67, orul, pru, cys-59,* FP2-origin, *proB*.

Identification of *Lactobacillus bulgaricus* by free solution DNA hybridization

M. Smiley and M. C. Zwahlen
Research Department, Nestlé Products Technical Assistance Co. Ltd, CH-1814 La Tour-de-Peilz

10 strains used for yoghurt manufacture, and previously identified by classical microbiological procedures and CH 50 API tests to be similar, and *L. bulgaricus* were analyzed for their DNA buoyant densities and DNA sequence homologies by free solution hybridizations. Type strains *L. bulgaricus, L. casei, L. acidophilus, L. helveticus* and *L. delbrueckii* were also included in this study for comparison and identification of strains. Density and GC base composition analyses were done with the Beckman Prep. UV Scanner and hybridizations using a Gilford 2600 microprocessor controlled spectrophotometer and thermoprogrammer, after the method of De Lay et al., Eur. J. Biochem. *12* (1970) 133. Since by this method all hybridization combinations could be tested easily, strain interrelationships were also determined.

Although density determinations segregated strains into only 2 distinct groups with GC values of 50–53% and 38–40% hybridizations segregated strains clearly into 3 distinct groups (species), each having only approximately 35% sequence homology to other groups. Only one of these groups of 5 strains (with 70–90% homologies and GC values of 50–53%) was found to be *L. bulgaricus*. A 2nd group of 4 strains was found to be *L. helveticus* (with 93–100% homology and GC values of 38–40%). A single strain of a third group has not yet been identified.

Consequently, at least 50% of the strains had been incorrectly identified by classical microbiological procedures and API tests. Since legislation in most countries requires that *L. bulgaricus* is the lactobacilli to be used in the manufacture of yoghurt and microbiological procedures presently available for their identification can no longer be considered reliable (neither for *L. helveticus*), we propose free solution DNA-DNA hybridization be chosen as the most suitable method of identification.

Also this work casts doubt on other *L. bulgaricus* studies as to whether they have been correctly identified. Notably these studies claiming *L. bulgaricus* to survive gastric digestion must be reappraised.

Localization of an intracellular antigen in a bacterial cell by use of protein A-gold complex

M. Wyss, H.-R. Ebersold and L. Ettlinger
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

A low temperature embedding procedure with the resin Lowicryl K4M (Roth et al., Histochem, Cytochem, *29* (1981) 663) was used to localize the tyrosinase (E.C. 1.14.18.1) in *Streptomyces glaucescens*.

To retain the antigenicity it was necessary to use a very high freezing rate. The bacterial suspension was placed onto a gold grid between 2 low mass copper platelets and then frozen in the propane jet (Müller et al., Balzers Report BB 800011 DE (1980)). The substitution medium at first consisted of methanol containing glutaraldehyde and uranyl acetate at the temperature of 185 K. This was then replaced by anhydrous methanol, after which the sample was stepwise embedded in Lowicryl K4M at 243 K. The polymerization was catalyzed by UV-irradiation. Thin sections were cut and mounted on a carbon-collodium film and processed for the protein-A-gold technique. The immunochemical procedure as described by Roth, Histochem, Cytochem. *26* (1974) 1074 was used. The sections were flooded with 10% H_2O_2, washed in buffer and incubated in rabbit antiserum containing antibodies specific for tyrosinase. The pAg-complex (Romano et al., Immunochemistry *11* (1974) 711) was added, rewashed and stained with aqueous uranyl acetate and lead citrate. The result showed that a significant amount of tyrosinase was found in the region of the cell wall and membrane.

Characterization of melanin mutant strains of *Streptomyces glaucescens*

M. Wyss and L. Ettlinger
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

2 classes of melanin (mel^-) negative mutants have, until now, been found in *S. glaucescens* (Hütter et al., Proc. int. Symp. Actinomycete Biol. 551 (1981); Crameri et al. J. gen. Microbiol. *128* (1982) 371). Mutant strains of class I completely lack tyrosinase activity, whereas those of class II produce active tyrosinase but are defective secretors of the enzyme. In class I 3 subclasses, $melA^-$, $melB^-$ and $melC^-$ have been found which map on the chromosome and may correspond to 3 different genes.

With purified tyrosinase (E.C. 1.14.18.1) from *S. glaucescens* GLA 0, the wild type, we produced rabbit antiserum and used it to test several mutant strains of *S. glaucescens* for cross-reacting material in the Ouchterlony double diffusion test. Representatives of all 3 subclasses $MelA^-$–C^- of class I produce material that fully cross-reacts with the tyrosinase antibodies. Using this test it was observed that a representative of class II mutant excreted a material not differing from the intracellular enzyme, although once excreted it showed no activity. Both of these compounds were seen to have reactions that differed from those shown by wild type tyrosinase.

Differences in the diffusion test were also observed between *S. glaucescens* and strains of other melanin forming *Streptomyces* species. *S. bikiniensis* and *S. antibioticus* produced a tyrosinase that cross-reacted with only a part of the antibodies in the *S. glaucescens* antiserum, and tyrosinase from *S. lavendulae* and *S. galbus* showed no cross-reaction at all.

Purification and properties of Orange II-azoreductase from *Pseudomonas* KF46

Th. Zimmermann, H. Kulla and Th. Leisinger
Mikrobiologisches Institut und Technisch-Chemisches Laboratorium ETH, ETH-Zentrum, CH-8092 Zürich

Pseudomonas sp. KF46 utilizes the 4′-carboxylated Orange II derivative 1-(4′-carboxyphenylazo)-2-naphthol as the sole source of carbon and nitrogen. The initial step in the degradation of this compound is mediated by an enzyme termed Orange II-azoreductase, which catalyzes the NAD(P)H dependent reduction of the azo-linkage. We purified this enzyme 130-fold with 25% yield to electrophoretic homogeneity by dye-ligand chromatography on Blue Sepharose 6B (Pharmacia) and Matrex gel Red A (Amicon). The enzymatic activity of the pure preparation was stable in buffers containing 50% (v/v) glycerol. The enzyme had a broad pH optimum in the neutral range and a temperature optimum at 30 °C. Orange II-azoreductase may exist as a monomer since estimates of 30,000 were obtained for the mol.wt by gel filtration on sephadex G-200 and by polyacrylamide gel electrophoresis under denaturing conditions. Orange II (1-(4′-sulfophenylazo)-2-naphthol) was a good substrate. The ability of a variety of related azo dyes to serve as substrates for the enzyme depended on the position of the hydroxy group in the naphthol moiety. For all substrates tested K_m values of approximately 1 μM were observed whereas the maximal reaction velocities differed strongly for different substrates.

DNAGEL: a computer program for determining DNA fragment sizes using a small computer equipped with a graphics tablet

T. Kieser
John Innes Institute, Colney Lane, Norwich NR4 7UH, England

The program DNAGEL is used to determine the size of DNA fragments run on agarose or polyacrylamide gels. The positions of the bands are read from gel photographs by means of a digitizer. Standard curves are calculated by the method of Southern (1979). The bands, as they are measured, are reproduced on the screen so that erroneous input can be recognized and corrected immediately. Similarly the estimated fragment sizes are printed in a table in the same relative positions as the bands on the gel. This makes it especially easy to relate fragment sizes with the bands on the gel picture. As an additional function the calculated positions of bands can be displayed on the screen. The program DNAGEL is written in APPLESOFT BASIC, suitable for APPLE II computers with 48 K memory connected to a monitor, printer and a HOUSTON graphics tablet.

Announcements

Federal Republic of Germany

29th international congress of pure and applied chemistry

Cologne, June 5–10, 1983

The 29th IUPAC congress, organized by the Gesellschaft Deutscher Chemiker, will present main lectures on 'New advances in inorganic chemistry', 'New advances in organic chemistry', 'New advances in physical and theoretical chemistry', 'Progress in the production of chemical basic materials', and 'Education in chemistry'. Plenary lectures on 'Removal of chemical wastes' and 'Removal of wastes of origin other than chemical industry'.
Further information by the General Secretariat, c/o Dr W. Fritsche, P.O. Box 900440, D-6000 Frankfurt a.M. 90/FRG.

3rd international symposium on invertebrate reproduction

Tübingen, August 22–27, 1983

The symposium is organized by the International Society of Invertebrate Reproduction (ISIR), with 5 sessions with invited speakers covering: 1. Cellular differentiation and cellular events in invertebrate reproduction, especially gametogenesis and fertilization; 2. endocrine control of invertebrate reproduction; 3. environmental adaptations of invertebrate reproduction; 4. population dynamics, reproductive strategies of invertebrate reproduction and their genetical background; 5. manipulation and control of invertebrate reproduction.
Further information by Prof. Dr W. Engels, LS Entwicklungsphysiologie, Auf der Morgenstelle 28, D-7400 Tübingen/FRG.

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