ABSTRACTS

A comparative evaluation of 2 infectious bovine rhinotracheitis (IBR) vaccines in cattle

M. Lazarowicz, F. Steck, U. Kihm and W. Bommeli
Eidgenössisches Vakzine-Institut, Postfach, CH-4025 Basel, and Veterinär-bakteriologisches Institut, Universität Bern, CH-3001 Bern

With regard to their efficacy and safety, trials with 2 commercial monovalent IBR vaccines were conducted in groups of 10 cattle. Our tests included a live vaccine for intranasal administration, containing a ts mutant, and an inactivated vaccine with adjuvants, administered s.c.
6 of 10 cattle, vaccinated with the modified live virus, were 5-9 months pregnant. Vaccination did not cause abortion, demonstrating the safety of the live vaccine for pregnant cows. After vaccination, we observed respiratory symptoms in some of the animals and a transient febrile response in 2. Similar reactions in the control group indicate that the manifestations can not be ascribed with certainty to the vaccine. The vaccine virus could be isolated from nasalpharyngeal swabs of the vaccinated cattle for up to maximum 3 weeks but was isolated only once from one of the 2 control animals in contact with the vaccinates. This indicates that the vaccine virus does not spread easily. All the vaccinated cattle seroconverted. 12 weeks after vaccination, the vaccinates and 12 susceptible controls were challenged intranasally with a virulent strain of IBR virus. The vaccinated animals showed only slight respiratory symptoms, while all the unvaccinated animals showed clear symptoms of IBR. The challenge virus was isolated from swabs from the vaccinated and the control cattle for several days.
The inactivated vaccine was well tolerated. Average serum antibody titers were high after the 2 vaccinations but were not protective because all animals showed clinical signs of rhinotracheitis after challenge and excretion of virus.

Herpes simplex virus type 1 strains from patients with kidney transplantation have a constant genome over long periods

U. Schilt and T. Seebeck
Institut für allgemeine Mikrobiologie der Universität Bern, Altenbergrain 21, CH-3013 Bern, and Institut für Hygiene und medizinische Mikrobiologie der Universität, Friedbühlstrasse 51, CH-3010 Bern

The source of recurrent herpes simplex virus (HSV) manifestations may be endogenous or exogenous. Discrimination between the 2 possibilites is best accomplished by analysis of the viral genome.
HSV type 1 was isolated from recurrent lesions in 4 individuals which had undergone renal transplantation. All isolates were collected from the throat, in intervals from 2 to 29 months. Viral DNA from all isolates was analyzed by restriction enzyme digestion using Eco RI and Hpa I. The patterns of DNA fragments fractionated by agarose gelelectrophoresis were identical for each patient, but clearly distinct between isolates from different patients. Based on our experiments we can assume that recurrent infection was elicited by reactivation of the endogenous HSV rather than by reinfection by an exogenous virus.

Comparison of the genomes of IBR and IPV virus strains by means of restriction enzyme analysis

M. Engels, F. Steck and R. Wyler
Institut für Virologie der Universität Zürich, Winterthurerstrasse 266a, CH-8057 Zürich, and Veterinär-bakteriologisches Institut der Universität Bern, Länggassstrasse 122, CH-3001 Bern

Although IBR and IPV virus strains produce different clincial pictures these 2 strains cannot be differentiated using biological markers. We suspected that such a difference in pathogenicity might be reflected in a dissimilarity of their genomes. In order to investigate this 4 IBR and 4 IPV virus strains were compared by means of restriction enzyme analysis.
All strains were replicated in bovine fetal lung cells, purified through a 10-25% dextran T10 gradient and DNA extracted by phenolchloroform. The DNAs were cleaved with endonucleases Hind III, Eco RI, Bam HI and Sac I, the digestion products separated by electrophoresis on 0.6% agarose slab gels and the resulting migration profiles compared. Rabbitpoxvirus DNA was run in parallel as a size marker.
The digestion patterns of both viral DNA showed a close similarity. However a few differences between IBR and IPV virus strains could be observed with all the endonucleases used. For comparison of the strains Hind III digestion was most useful, whereas Sac I digestion gave rise to a great number of fragments. Eco RI and Bam HI digestion also resulted in different migration profiles, but the dissimilarity were not as obvious as in Hind III digestion. Additionally differences among strains of IBR have been noted, and to an even greater extend among strains of IPV. The average of mol. wt estimations amounted to 90×10^6 daltons. Conclusions drawn from these results are discussed.

A simple and rapid method for concentration of interferon and removal of concentrated inducing virus

P. Leuthard and A. R. Schuerch
Ciba-Geigy AG, CH-4002 Basel

In standard procedures for production of human leukocyte or lymphoblastoid interferon cells are induced with virus, conditioned medium is treated with acid for several days to inactivative virus and interferon is concentrated by precipitation steps. In the present report we describe the application of an Amicon DC-2 Hollow Fibre system as an easy and rapid method for elimination and recovery of virus as well as the concentration of the crude interferon: an H1P100 Hollow Fibre cartridge with an exclusion range of M_r 10^5 is used to concentrate high mol.wt components including virus; the viral activity for induction of interferon can be recovered quantitatively from such a 25-fold concentrate. The diafiltrate, containing components with an M_r of less than 10^5 including interferon, are transferred to a 2nd Amicon DC-2 system, however equipped with an H1P10 Hollow Fibre cartridge having a nominal exclusion range of an M_r of 10^4. In this 2nd ultrafiltration system, interferon is concentrated 25-fold and dialyzed in the same apparatus with the buffer solution required for the subsequent purification step.
Advantages to this procedure are a) a significant gain of time, since the acid treatment for several days can be omitted or reduced to < 24 h, b) concentration of virus for reuse for further inductions, c) easy concentration of crude interferon with a high yield (80-100%) and a partial purification, since molecules exhibiting an M_r of $> 10^5$ and $< 10^4$ are discarded, d) the possible use of this system for production of interferon with other inducers with an M_r of $> 10^5$, e) the possibility of concentration also of other interferons (e.g. acid labile interferon type II) and f) that the whole system is designed to be scaled up for large scale production of interferon.

Chemiluminescence: an early event in the interaction of Sendai and influenza viruses with mouse spleen cells

E. Peterhans
Institut für Virologie der Universität Zürich, Winterthurerstrasse 266a, CH-8057 Zürich

Myxo- and paramyxoviruses are known to bind to specific receptors on the surface of mouse spleen cells through the glycoproteins which form spikes on the virus envelope. We have observed that influenza and Sendai viruses stimulate a burst of chemiluminescence (CL) in spleen cells prepared from C57 BL/6 mice. The emission of light depends on the presence of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) and is monitored in a liquid scintillation spectrometer. CL increases within seconds of addition of virus to the cell suspension and reaches a peak after 1–3 min with influenza virus and 6–8 min with Sendai virus, respectively. With Sendai virus, the induction of light depends on both the hemagglutinin-neuraminidase and fusion glycoproteins, the latter being responsible for most of the light emitted by the cells.

The analysis of the cell types responsible for CL generation in spleen cell suspensions showed that lymphocytes, rather than macrophages and granulocytes, are the source of CL. Using monoclonal anti-Thy 1.2 antibody and complement and rosetting of Ig^+ cells with sheep antimouse Ig-coupled sheep red cells, it was found that CL is generated by both B and T lymphocytes.

Since CL is triggered by a virus-receptor interaction it provides a tool for functional studies on virus receptors. Moreover, the conditions for the induction of CL by Sendai virus are very similar to those required for the induction of interferon in mouse spleen cells. We therefore consider the study of CL to be interesting from both a virological and immunological viewpoint.

Diagnostic value of anti-HBc-IgM and HBe/anti-HBe in hepatitis B-serology

F. Burkhardt and U. Schilt
Institut für Hygiene und medizinische Mikrobiologie der Universität Bern, Friedbühlstrasse 51, CH-3010 Bern

In order to assess the diagnostic value of anti-HBc-IgM, HBe and anti-HBe in cases where the infection is not self-limited, 2 distinct groups of people with known high incidence of HBs-antigenemia were examined (RIA): 161 Vietnamese boat people and 87 kidney transplant patients. 73% of the boat people and 45% of the renal graft recipients had markers of a past or ongoing hepatitis B infection.

Whereas HBs-antigenemia was comparable in both groups (13.7% of all boat people vs 10.3% of kidney transplant patients), only half of the HBs-positive Vietnamese had also HBe compared to the HBs-positive renal graft recipients who were all HBe positive; this difference probably reflects the fundamentally different epidemiological and immunological situation of both groups: High incidence of healthy carriers from a region where hepatitis B is endemic vs persistent viral replication in a group of immunologically suppressed patients. Anti-HBc-IgM-antibodies were generally not detectable in neither group, this marker obviously being associated with recent hepatitis B infection.

Propagation of human hepatitis A virus in hepatoma cell cultures

G. Siegl, G.G. Frösner, J.D. Tratschin, F. Deinhardt, R. Scheid and V. Gauss-Müller
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern, and Max-von-Pettenkofer-Institut, D-8000 München

Hepatitis A virus (HAV) was isolated directly from human feces and propagated serially in an HB_sAg producing hepatoma cell line. The virus replicated in cultures incubated at 32 °C without cytopathic effect; however, progressive accumulation of hepatitis A antigen (HAAg) could be detected by radioimmunoassay in cellular extracts and by indirect immunofluorescence in the cytoplasm of infected cells. HAAg was first detected in extracts of cultures infected with the original stool suspension 7 weeks after inoculation. After 4 successive passages in vitro, however, significant quantities of HAAg accumulated already within 8–12 days p.i. and extracts of these cultures prepared 6 weeks p.i. contained 10^6 tissue culture infectious doses of HAV per ml. HAAg propagated in vitro could not be distinguished serologically from the MS-1 strain of hepatitis A virus. Moreover, virus purified from infected cultures sedimented with about 160S and banded at a density around 1.34 g/ml in CsCl like HAV particles isolated from human feces.

Studies on regulation of protein synthesis in poliovirus-infected cells using cell-free translation systems

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

Protein synthesis in poliovirus-infected cells is characterized by a switch in synthesis of cellular to viral proteins, shut off of host cell protein synthesis and a decrease in the overall rate of synthesis. These events are paralleled by an influx of Na^+ ions into infected cells.

In cell-free extracts from poliovirus-infected HEp-2 cells, viral protein synthesis and shut off of cellular protein synthesis are similar as in intact cells. Under the controlled ionic conditions of the cell-free translation system however, cell-free extracts made late in infection exhibit a vast increase in synthetic activity. This activity results from multiple cycles per ribosome of reinitiation which is resistant to inhibition by m^7GMP. Hypertonic concentrations of sodium or potassium salts inhibit total protein synthesis in cell-free extracts. Initiation of synthesis as well as the peptide chain elongation rate are affected. Sodium or potassium salts induce identical effects, however chloride salts affect initiation of protein synthesis much more than appropriate acetate salts. Hypertonic conditions result in differential translation of the various cellular mRNAs and in preferential translation of poliovirus RNA over cellular mRNAs. However, elongation of cellular and viral protein chains is affected to identical extens by hypertonic salt concentrations. Combined results implicate, that build up of hypertonicity in poliovirus-infected cells is responsible for the decrease in the overall rate of protein synthesis, but is not the mechanism of virus-induced shut off of cellular protein synthesis.

Intermediates in the maturation of parvovirus LuIII in a subcellular system

D.E. Müller and G. Siegl
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern

An in vitro system prepared by lysis of infected cells with Brij-58 was used to study the maturation of *parvovirus* LuIII. In this system progeny single-stranded viral DNA is synthesized and packaged into viral particles at a constant rate during at least 60 min. Labeled DNA was found associated with several distinct structures which sediment between 70 and 150 S in sucrose gradients and, after fixation, band at densities of 1.46 and 1.37 g/ml in CsCl. Pulse-chase experiments indicated that these structures are precursors in the formation of mature virions (110 S,

1.41 g/ml). They represent nucleoprotein complexes consisting of intermediates in the replication of viral DNA, of viral structural polypeptides, and of so far uncharacterized proteins of host cell origin. Digestion of the complexes with DNase released virus particles and electron microscopy revealed viral capsids associated with nucleosome-like structures.

Isolation of influenza virus ribonucleoproteins by isopycnic centrifugation on metrizamide gradients

S.J. Twigden and M.A. Horisberger
Pharma-Forschung, Ciba-Geigy AG, CH-4002 Basel

The internal structure of influenza virions is composed of ribonucleoproteins (RNPs) formed of segmented, single-stranded viral RNA and of a nucleocapsid polypeptide; 3 minor polypeptides (P_{1-3}) of high mol. wt are associated with the RNPs and may function as components of the viral transcriptase. Preparations of influenza virus RNPs, free of all viral and cellular contamination, have been isolated by isopycnic centrifugation of infected cell extracts on metrizamide gradients. The viral RNPs banded at a density heavier than the messenger RNPs (detected by protein synthesis in a reticulocyte lysate), the ribosomes and the bulk of cellular proteins (detected by staining the proteins separated by SDS-polyacrylamide gel-electrophoresis). Electron microscopy showed structures of approximately 20 nm diameter and of heterogeneous length distribution (50–100 nm), consistant with earlier descriptions of the influenza virus RNPs. However, protein analysis of the metrizamide gradient fractions indicated that the P polypeptides had become separated from the RNP complexes during the centrifugation, suggesting that the P polypeptides are not firmly bound to the RNPs. No transcriptase activity was measurable in purified RNPs depleted of their P polypeptides. The only polymerase activity detected on metrizamide gradients was a polyA polymerase activity which banded at a much lower density than the RNPs; a similar activity was observed in extracts of uninfected cells. However, the addition of glycerol to the metrizamide gradient allowed detection of a virus-specific polymerase activity coincident with the polyA polymerase activity, but did not alter the banding position of the RNPs.

Late proteins mapped on the vaccina genome

M. Chipchase, F. Schwendimann and R. Wyler
Institut für Virologie der Universität Zürich, Winterthurerstrasse 266a, CH-8057 Zürich

Vaccinia DNA was restricted with Sac I and the individual restriction fragments used to select for the corresponding RNAs by hybridization to RNA prepared from cells infected 8 h previously with vaccinia virus. The selected RNAs were translated in vitro and the polypeptide products analyzed by SDS-PAGE.
Each restriction fragment gave rise to a different set of polypeptide bands. About 60 bands were detected altogether and were assigned positions on the Sac I map of vaccinia. Some bands comigrated with virion protein components and may represent structural proteins of vaccinia virus.

Cultivation of *Aedes* (mosquito) cells

P. Pfotenhauer, A. Flaviano and P.J. Späth
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern

According to Späth and Koblet (in press) a cytopathic effect (CPE) can be induced in cultures of a subline of *A. albopictus* (Singh) cells with Semliki Forest Virus. The CPE is related to the pH; the pH seems to be related to the basal medium used to prepare the Mitsuhashi-Maramorosch growth medium (MM-M). To investigate the role of nutritional conditions as a possible basis for these differences in establishing the CPE, cell growth (cells/cm^2) during 2 weeks after seeding was followed. 3 cell lines (A: *A. albopictus,* CCL 126, Flow; D: *A. albopictus,* YARU; C: *A. pseudoscutellaris* [Mos 61], YARU) were used in MM-M with each of 3 identical (catalogues) basal media: a) Flow (Scotland), b) Gibco (Scotland), c) Seromed (FRG). Growth of cells at 28 °C was in the order: line A A > D > C. The influence of the basal media on the growth rate of a given line was insignificant. We conclude that the basal media tested offer similar growth conditions.
Then, the spontaneous changes of pH of MM-M were studied, with growing cells or without (control). 1. Control: Changes within 0.5 pH units are regularly seen with all MM-M's at 28 °C (free gas exchange). 2. With growing cells (free gas exchange): all MM-M's with all cell lines show a cyclic pH fluctuation with a maximum, a minimum and a 2nd increase. The minimum is most pronounced in A with all basal media; the observed absolute minimum is found with the combination Flow MM-M/A.

pH of media, cytopathic effects (CPE) and titers of Semliki Forest Virus (SFV) in *Aedes* cell lines

A. Flaviano, P. Pfotenhauer and P.J. Späth
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern

According to Späth and Koblet (in press) there is a correlation between pH, SFV titers and susceptibility for CPE in a subline of *A. albopictus* cells (Singh, CCL 126), grown in Mitsuhashi-Maramorosch medium (MM-M), composed of a basal medium and fetal calf serum. To find out whether this correlation is a general phenomenon in *Aedes* cells, SFV titers and syncytium formation in function of the pH of the culture medium were investigated. Combinations were performed containing the following: 1. Cells: A: *A. albopictus,* CCL 126, Flow; B: *A. albopictus,* clone $C_{6/36}$, adapted to TCM 199, YARU; C: *A. pseudoscutellaris* (Mos 61), YARU; D: *A. albopictus,* YARU.
2. Media: MM-M with identical (catalogues) basal media from Flow (Scotland); Gibco (Scotland); Seromed (FRG) or TCM 199.
Series I: Spontaneous pH variation; infection of cells 5 (monolayer just established) or 12 days after seeding: Within 38 h p.i. the lowest pH (line A/Flow) was around 6.4. No combination showed a CPE. As a rule, the yield of PFU was higher if infection was performed 5 days after seeding.
Series II: Infection 5 days after seeding, pH fixed at the time of infection: Syncytia were observed 24 h p.i. in line A with all basal media at pH lower than 6 and in line B (TCM 199) at pH equal to or lower than 6.2. With all combinations of MM-M tested a maximal titer was determined around a pH 6.2. In line B/TCM 199 the maximal titer was found around 6.9.

Incorporation of labeled uridine and aminoacids into different *Aedes* cell lines

P. Späth and F. Reigel
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern

To study the replication of insect-mediated viruses, labeling procedures and inhibitors of biosynthesis of macromolecules of arthropode cells should be known; however, systematic studies do not exist. Therefore, we compared the incorporation of labeled precursors into acid-precipitable material of cultures of the following cells: Chick embryo

fibroblasts (CEF) and Vero (Eagle's medium, pH 7.1), *Aedes* (A.) *albopictus* (A, Singh, CCL 126, Flow), *A. albopictus* (B, adapted to medium 199, Igarashi, $C_{6/36}$, YARU), *A. pseudoscutellaris* (C, YARU), *A. albopictus* (D, YARU) in Mitsuhashi-Maramorosch medium (MM-M, prepared with identical [catalogues] basal media from Flow, Gibco, Seromed and 20% fetal, inactivated calf serum; pH 6.9). Cells were either infected (Semliki Forest Virus, MOI = 1) or mock-infected and received 1 µg/ml of actinomycin D (Ac) and/or 2 µg/ml of ethidiumbromide (EB) in some experiments. pH of the media, virus titers (PFU), cell-counts up to 48 h p.i. were also followed.

Incorporation of [3H]-uridine (1 µCi/ml): In CEF and Vero Ac inhibits host RNA synthesis; residual labeling is due to formation of viral RNA. A similar behaviour is found mainly in B. EB with Ac gives no advantages. Incorporation levels: infection, no Ac: B > A ≧ D; no infection, no Ac: B > A ≧ D; infection, with Ac: B > A > C > D; no infection, with Ac: A ≧ B > C = D. In all cases, highest levels are incorporated between 15 and 25 h p.i.; Gibco > Flow > Seromed. B cells offer best chances to label SFV macromolecules. Best short-term labeling conditions for B are given.

Incorporation of [^{3}H] aminoacid mixture (1.5 µCi per dish): B > A under all conditions.

Studies on Igarashi's *Aedes* albopictus cell clone $C_{6/36}$

F. Reigel
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern

A clone from *Aedes albopictus* (Singh) cells, grown in vertebrate medium TCM 199 (1), described by Igarashi (J. gen. Virol. *40*, 531, 1978 [$C_{6/36}$, YARU]), was readapted to invertebrate Mitsuhashi-Maramorosch medium (MM-M, pH 6.9) (2). In both media cells show similar growth rates (cells/ml, 14 days) and titers (10^9 PFU/ml) of Semliki Forest Virus (SFV) 14 h after infection.

Cells 1 are homogeneous, whereas cells 2 differ partially in size and shape in the early state after seeding.

In both media a permanent infection with SFV could be established. Then, a secondary infection does not lead to a new virus burst in contrast to the primary infection. In both media monolayers produce similar 'chronic titers' in the range of 10^5 PFU/ml/14 h (observation during 6 months).

The larger the duration of the chronic infection, the more small-plaque virus (in chick embryo fibroblasts). Virus from 91st day p.i. (*Aedes* cells 1) infected Vero cells with cytopathic effects (CPE) after 2 days at 28 °C, resulting in titers of ≧ 10^7 PFU/ml/2 days, whereas at 37 °C CPE's were not observed after 48 h; only discrete CPE's appeared finally after 4 days with titers not exceeding 10^4 PFU/ml/4 days. To judge the nature of the virus produced in the late phases of chronic infection, viral RNA's and proteins from permanently infected cells 1 and 2 were isolated and compared to those of standard SFV.

The morphopoietic core of bacteriophage T4 characterized in vivo

F. Traub and M. Maeder
Department of Microbiology, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel

Electron microscopy of thin sections of *Escherichia coli* B^e cells infected with T4 and T2 mutants blocking the shell formation and fixed according to an improved procedure reveals a new type of an organized head-related particle: the naked cores. These proteinaceous particles are membrane bound and identical to the prehead core in size, architecture and staining properties. Their formation is dependent on the presence of essential core proteins and independent of the proteins involved in shell formation. The number of particles formed corresponds at 30 °C and 90 min post infection to an expected yield of phage precursors. These findings are suggestive for the true precursor relationship of the naked cores: As the first head structure they presumably act as a precise form-giving matrix (= the morphopoietic core) upon which proteins assemble to form the prehead shell of the correct shape and size, as has already been indicated by in vitro results (van Driel and Couture, J, molec. Biol. *123*, 713, 1978).

Physical restriction cleavage map of staphylococcal phage Φ11 DNA

Brigitte Bächi
Institut für medizinische Mikrobiologie der Universität Zürich, Gloriastrasse 32, CH-8006 Zürich

Phage Φ11 is a generalized transducing phage of *Staphylococcus aureus*. Besides as being a useful genetic tool in analyzing the staphylococcal genome it shows some other interesting activities: Φ11 plays an important role in mediating competence for transformation and transfection. It has been shown to condition the acceptor strain for efficient transduction and expression of the methicillin resistance, and also can promote prophage dependent plasmid integration into the chromosome. In order to study some of its various functions, a restriction cleavage map of the phage DNA was constructed first. 11 out of 15 *EcoRI* sites, all 6 *BglII*, the 3 *BglI* and 3 *PstI* sites have been mapped. The total length of the phage genome, determined by gel-electrophoresis, is about 43 kb. The DNA in the phage particles is cyclically permuted and has a terminal redundancy of 10 kb. The preferential starting point for packaging the DNA into the phage head is at map unit 79 and proceeds towards higher map units.

Induced losses of intracellular alkalis and polyamines affect the shape of bacterial nucleoids

M. Moncany, F. Borle and E. Kellenberger
Department of Microbiology, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel

Cell fixation proceeds as follows: 1. A primary (and rapid) action on the cellular permeability. 2. Then the cytoplasm becomes fixed. 3. According to the nature of the fixative, the nuclear material is cross-linked or not. The final result which is observed is the consequence of the balance reached by these events.

Different fixatives have been studied and, e.g. the primary action of glutaraldehyde leads to a leakage of alkalis and polyamines. Because of its high protein content the cytoplasm is then fixed, faster than the nucleoids; their shape depends thus on the time of fixation of the cytoplasm. The shape of nucleoids is determined by the intracellular environment and it is thus altered as a consequence of fixation induced permeability changes prior to becoming fixed. Loss of K^+ and polyamines induce a dispersion (unfolding) of the initially more confined material into the cytoplasm.

The effects of different fixatives (glutaraldehyde formaldehyde, acrolein, OsO_4) have been studied by direct measurements of their induced changes of permeability for small molecules as cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) and polyamines. The effect of these changes on the shape of the nucleoid is studied by the agarfiltration method. Agents which produce disperse nuclei (as UV, X rays, H_2O_2, $KMnO_4$) are checked for a possible induction of leakage.

Fundamental structural differences between eukaryotic and non-eukaryotic DNA containing material

E. Kellenberger, A. Ryter, H. Wunderli, C. Kellenberger and E. Carlemalm
University of Basel, Department of Microbiology, Biozentrum, Klingelbergstrasse 70, CH-4056 Basel, Institut Pasteur, 25, rue du Docteur Roux, F-75024 Paris, France, and ISREC, Chemin des Boveresses, CH-1066 Epalinges

The knowledge gained by in vitro fixation studies of 'naked' DNA and proteins is applied to interprete the completely different behaviour of eukaryotic nuclear material from all others (e.g. prokaryotic nucleoids) in fixation and embedding for the electron microscopy of thin sections. 1. Naked DNA cannot become gelated by aldehydes and pure OsO_4, but by OsO_4 in presence of amino acids by uranylacetate and $KMnO_4$. Bovin serum albumine becomes cross-linked by aldehydes at concentrations above 3%. 2. Isolated, membrane-free eukaryotic nuclei are fixed by aldehydes as indicated by the lack of response towards conditions for swelling or condensation. In general, the aspects of eukaryotic nuclei do not change fundamentally with the conditions or nature of fixation. 3. Non-eukaryotic nuclear material gives a fine stranded aspect only with those fixations gelify 'naked' DNA. In all other cases dehydration produces aggregates of variable sizes.
The results are explainable when long stretches of non-eukaryotic DNA are not associated with proteins. The DNA neutralized by polyamines or Ca/Mg/K is not able to become cross-linked by aldehydes or pure OsO_4.

Spontaneous deletions of the sex factor R68.45

D. Haas
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

The sex factor R68.45, a derivative of the incP-1 plasmid R68, mobilizes the chromosome of a variety of gram-negative bacteria, including *Pseudomonas aeruginosa* and *Escherichia coli* (D. Haas and B.W. Holloway, Mol. Gen. Genet. *144*, 243, 1976). Chromosome mobilization ability (Cma) appears to depend on a 2.1 kb duplicated region located near the kanamycin resistance (Km^r) gene of R68.45 (Riess et al., in preparation). In *P. aeruginosa* strain PAO, R68.45 is unstable, whereas R68 is stable. The instability of R68.45 was observed in *recA* and *recA*$^+$ transconjugants shortly after acquisition of the plasmid, in recombinants from R68.45-mediated matings (with selection for chromosomal markers) and, to a lesser extent, in established R68.45 donor strains. 2 phenotypically distinct classes of R68.45 derivatives were obtained: a) Km^r Cb^r (carbenicillin resistance) Tc^r (tetracycline resistance) Tra^+ (transfer proficient) Cma^-, indistinguishable from R68 by restriction enzyme analysis; b) Km^s Cb^r Tc^r Tra^-Cma^-, due to deletion of the Km gene, the 2.1 kb duplication required for Cma, and a variable portion of a DNA segment coding for transfer genes (the Tra-1 region on the 19.5 kb *Hind*III-*Eco*RI fragment). Deletion formation and Cma did not depend on the *recA* gene product and thus may both be functions of an IS element contained in the 2.1 kb duplication.

Replication mutants of the IncP-1 plasmid RP1

J. Watson
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Mutants of RP1 have been isolated which are thermosensitive for plasmid survival at 43 °C. 2 mutant classes have been identified. One class fails to replicate at 43 °C. This phenotype is expressed in both *Pseudomonas aeruginosa* and *Escherichia coli*. The 2nd class is host-specific since it is host cell-lethal at 43 °C in *P. aeruginosa* strain PAO but not in *E. coli* K12. Temperature shift experiments indicate that this class of mutant arrests host cell division at 43 °C resulting in filament formation. In vitro recombination of the 2 distinct *Hind*III-*Bam*HI fragments of these mutants indicate that the mutations corresponding to the 2 classes are located on different fragments. Double mutants, consisting of complementary *Hind*III-*Bam*HI fragments of the 2 types of mutants, have been constructed.

The kanamycin transposon derived from the R plasmid Rtsl and carried by phage P1Km has flanking direct repeats of 0.8 kb

S. Iida, J. Meyer, P. Linder and H.J. Reif
Biozentrum, Abteilung Mikrobiologie, Klingelbergstrasse 70, CH-4056 Basel

The kanamycin (Km) resistance determinant of the R plasmid Rtsl was transposed to phage P1 resulting in phage PlKm (Takano and Ikeda, Virology *70*, 198, 1976). A detailed restriction cleavage analysis of PlKm DNA indicated that the determinant for Km resistance was flanked by direct repeats. We cloned the DNA segments containing the right and left halves of the Km transposon separately into pBR322. Electron microscope heteroduplex analysis documented that the Km transposon studied contains 0.8 kb long, flanking direct repeats. DNA:DNA hybridization did not reveal any sequence homology between these terminal repeats and ISl. Like chloramphenicol resistance transposons flanked by directly repeated ISl (Meyer and Iida, Mol. Gen. Genet. *176*, 209, 1980), this Km transposon can undergo amplification or excision by reciprocal recombination.

The replication of the chromosome and various plasmids in a *dnaAcos* mutant of *Escherichia coli*

J. Frey, M. Chandler and L. Caro
Département de Biologie moléculaire, 30, quai E. Ansermet, CH-1211 Genève 4

The *dnaAcos* mutations are phenotypic suppressors of *dnaAts46* which retain the *dnaAts* allele, are cotransduced with *dnaA*, render the cell cold sensitive, and cause an overinitiation of chromosome replication when the cells are shifted from 42 to 32 °C. We have used pulse labelling and DNA:DNA hybridization to follow the effect of a shift in temperature on the replication of the plasmids pSC101, RTF-TC and λdv in such strains.
The plasmid pSC101 requires *dnaA* for its replication. This requirement is not satisfied when a *dnaAts* mutant is phenotypically suppressed at 42 °C by integration of R100 into the chromosome but is satisfied by the cos suppression. The hybridization results show that after a shift of a *dnaAcos* mutant from 42 to 32 °C, initiation of the chromosome and of the plasmid pSC101 are stimulated while the replication of the *dnaA*-independent plasmid RTF-TC is not affected. The presence of the *dnaA*-requiring plasmid pSC101 does not affect the extent of overinitiation of the chromosome.
The presence of λdv suppresses the cold sensitivity of *dnaAcos* mutants and allows the cells to grow at 32 °C and at 42 °C. It has been suggested that the λ gene P product reduces the initiation potential of both *dnaAts46* and *dnaAcos* mutants, aggravating the initiation defect in *dnaAts* and correcting the overinitiation of *dnaAcos* (Kellenberger-Gujer, Molec. Gen. Genet. *162*, 17, 1978). Hybridization experiments show that the presence of λdv suppresses the overinitiation of the chromosome and of pSC101 replication at 32 °C. λ gene P product also interacts with dnaB. It is therefore interesting to note that while the prophage Pl is

not able to suppress the *dnaAcos* phenotype, efficient suppression is observed using the double mutant P1*bac-crr*. This mutant produces a high level of a *dnaB* analogue. Suppression seems to depend on the level of the *dnaB* analogue since P1*bac* and P1*crr*, 2 phages which produce lower levels of *dnaB* analogue, suppress the cos phenotype to a lower degree.

Discrimination of covalently closed circular and open circular plasmid conformations by 2-dimensional gel-electrophoresis, demonstrated with *Bacillus thuringiensis*

H. M. Fischer, J. Watson and P. Lüthy
Mikrobiologisches Institut der ETH, Haldeneggsteig 4, CH-8092 Zürich

Varieties of *Bacillus thuringiensis* commonly contain a number of different plasmids. To determine the number of plasmids present in a particular strain it is necessary to identify the covalently closed circular (ccc) and open circular (oc) forms of each plasmid species. This was achieved by a 2-dimensional gel-electrophoresis method.
Plasmid DNA from 5 *B. thuringiensis* varieties was examined by this method. 2-dimensional electrophoretic separation was carried out in a horizontal gel apparatus using 17×17 cm 0.8% agarose gels prepared in Tris-acetate buffer. After electrophoresis in the first dimension, the gel was stained with ethidiumbromide and exposed to UV irradiation (254 nm). This resulted in partial conversion of the ccc form to the oc conformation. The gel was then rotated by 90° and electrophoresis was carried out in the 2nd dimension. The oc DNA induced from the ccc species shows the same electrophoretic mobility as the corresponding oc species resolved during the first separation. This allows the ccc and oc forms of a plasmid to be identified. This method is particularly suitable for the analysis of strains carrying several plasmids of different or similar size. The *B. thuringiensis* varieties investigated by this method were found to contain the following numbers of plasmids: var. *thuringiensis*, 3; var. *kurstaki*, 5; var. *aizawai*, 4; var. *israelensis*, 2; var. *finitimus*, no detectable plasmid. The sizes of the plasmids ranged from 2×10^6 to 7×10^6 daltons. In addition, var. *kurstaki* may possess a large plasmid of about 25×10^6 daltons.

Protective mechanisms of plasmid bearing strains against male-specific phages

P. Zünd and G. Lebek
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern

Plasmid bearing strains have 3 possibilities for sex pili synthesis: 1. No pili are coded for by nonconjugative plasmids. 2. Permanent pili by derepressed plasmids. 3. Pili in only 0.1–1% of the population by repressed plasmids.
Since sex pili are the receptors for male-specific phages, host cells with plasmids of the 1st and 3rd type are protected from these phages. Those bearing plasmids of type 2 are unprotected. Sex pili determined by the latter can directly be identified and classified with the help of sex phages. Pili determined by repressed plasmids can also be examined with phages but only by using the HFRT-system. This is done by transferring the R-factor into a new strain by conjugation. The recipient is exposed to sex phages while and directly after receiving the plasmid.
At this moment not enough repressor substance is produced to avoid pilus synthesis. Therefore, the strains are sensitive to male-specific phages and plaques can occur in the overlay-test.
In a series of over 1000 tested R-plasmids a number of Col-plasmids from fecal strains of pigs and cows could not be tested with the HFRT-system. These plasmids caused strong, unspecific adsorption of sex phages from all 4 classes to their host cells. The phages could be released again by EDTA-treatment or by varying the pH, but were not able to lysate the strains.
We believe that this is another, so far unknown mechanism of plasmids to protect themselves against sex phages.

Amplification of an R-plasmid by antibiotics inhibiting protein synthesis

E. Freidig and G. Lebek
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern

According to Rownd amplification is the increase of the copy number of plasmids per host chromosome during cultivation in medium containing chloramphenicol (C). This mainly concerns nonconjugative, so-called relaxed plasmids. Amplification is used to increase the yield of plasmid DNA. The copy number is also of importance, because it determines the time (without antibiotics) necessary for a complete loss of plasmids, which renders host cells sensitive to antibiotics again.
The experiments were performed with the ^{3}H-thymidine-labeled plasmid pBR322 (A, T) in *Escherichia coli* K12 921. Amplification was done by C, tetracycline (T) and Erythromycine (E) under standardized conditions.
For the isolation of plasmid and chromosome DNA and measurement of their quantitiy we used the methods of Bozard and Helinski. Antibiotics which inhibit protein synthesis (C, T, E) showed clear amplification, whereas ampicillin only showed a slight increase of the copy number. Since the tested R-factors pBR322 was amplified by T and E while its host cells were not significantly inhibited, doubts about the conventional hypothesis concerning amplification arise. According to this hypothesis plasmid replication continues after the chromosome replication has stopped.
Testing 27 conjugative wild-R-factors from *E. coli* from medical material we found that 4 of these R-factors occurred in 5 to more than 20 copies, without their host cells being exposed to antibiotics. This means that conjugative R-factors may occur in multicopies in *E. coli*.

Incompatibility testing of R-factors with IKe-like sexpili. Subdivision of Inc-group N

L. Petri and G. Lebek
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern

R-factors from the Bernese area mainly belong to incompatibility groups F and N.
The Inc-group N could so far not be classified into subgroups, which, however, would be useful for epidemiological reasons.
We tested 31 R-factors of the Inc-group N proceeding as follows: Using the HFRT-status we first confirmed the presence of N-pili with IKe-phages. Subsequently incompatibility testing was performed by transferring the test R-factor into an *E. coli* K12 C600 rec A$^-$ strain harboring the indicator plasmid N3. Clones on selective medium were tested for presence of both plasmids. Absence of more than 15% of one plasmid showed incompatibility.
When both plasmids were stable on selective medium, the doubles were incubated in drug-free broth for 12 h, and the eventual loss of one plasmid was examined by replica plating.
Agarose gel-electrophoresis of the plasmids was performed to ensure that no recombination between the 2 plasmids had occurred in spite of the rec A$^-$ host.

From 31 wild-R-factors determining N-pili, only 5 showed incompatibility with the indicator plasmid N3. Therefore, they belong to an uniform subgroup of Inc N, which we propose to call Inc NI. Since the other 26 R-factors were compatible with plasmid N3, we conclude that they belong to different subgroups than Inc NI.
The results show that R-factors coding for N-pili are heterogen concerning incompatibility.

Mobilization of nonconjugative R-factors by helperplasmids. Influence of fi-type and incompatibility group of the involved plasmids

P. Zünd and G. Lebek
Institut für Hygiene und medizinische Mikrobiologie, Friedbühlstrasse 51, CH-3010 Bern

For the spread of nonconjugative R-factors in the medical field, conjugal transfer by helperplasmids is important. Additionally, it can be necessary to transfer nonconjugative R-factors into specific host cells for scientific reasons. This can also be done by helperplasmids. We investigated, what kind of helperplasmids could transfer a sample of 14 nonconjugative fi^+ (Inc F6) wild-R-factors from *Klebsiella pneumoniae*. For this purpose conjugative fi^+ and fi^-plasmids of the incompatibility groups F1, F2, F4, Iα, Iγ, H and M were transferred into the strains harboring the nonconjugative R-factors. Subsequently conjugation with *Escherichia coli* K12 921 Na^{res} was performed. It became apparent that the nonconjugative fi^+ R-factors could be transferred only by fi^+ helperplasmids, but not by fi^- ones. Hereby the incompatibility of these fi^+ helperplasmids had no influence.
The results show that nonconjugative R-factors (approximately 25% in the Bernese region) can easily be mobilized by tra^+ plasmids and then be spread by conjugal transfer.

Evidence that the C-terminal of chloramphenicol acetyl transferase (Cm^r gene) is involved in resistance to fusidic acid

R. Marcoli, S. Iida and T.A. Bickle
Microbiology Department, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel

At last year's meeting we presented evidence that the genes for resistance to chloramphenicol (Cm) and fusidic acid (Fa) carried by the plasmid NR1 were either identical or overlapping. We have now characterized a deletion derivative of NR1 first described by Dempsey and Willetts, which in our hands is sensitive to both antibiotics. Restriction cleavage analysis of this plasmid revealed that ISIb mediated deletion had removed most of the r-determinant. Mutants of this strain selected for restored Cm resistance are also resistant to Fa. DNA sequence analysis of these strains indicated that the original mutation was due to an ISl-mediated deletion of the 6 C-terminal codons of the structural gene for Cm resistance. However, since the deletion left an open reading frame running into the ISIb element, the protein produced in this mutant strain would be 20 amino acids longer than the wild type protein. Reversions to Cm and Fa resistance were due in 2 cases to insertions of single base pairs resulting in frame shifts to reading frames in which stop codons were immediately encountered resulting in the production of proteins shorter than the wild type by 5 and 8 amino acids, respectively. These results show that the extreme C-terminal region of the Cm^r gene is also involved in resistance to Fa.

Analysis of plasmids specifying ampicillin resistance derived from *Neisseria gonorrhoeae* strains isolated in Geneva

O. Fayet, J. Cl. Piffaretti and Y. Froment
Département de Microbiologie, 22, quai Ernest Ansermet, CH-1205 Genève

Since 1976 several beta-lactamase producing (Ap^R) *Neisseria gonorrhoeae* strains were isolated in several countries. This resistance was always found associated with the presence of a small nonconjugative, but mobilizable, plasmid of varying size (5.1-7.1 kb).
We have analyzed 3 Ap^R strains, isolated recently in Geneva. Analysis by electron microscopy and agarose gel-electrophoresis revealed in each strain the presence of 3 plasmids: the 3.9 kb 'phenotypically cryptic' plasmid and the 36 kb conjugative plasmid which are frequently encountered in *N. gonorrhoeae*, and a 7.1 kb plasmid responsible for the Ap^R phenotype. The association of Ap^R with the 7.1 kb plasmid was shown by transformation of *Escherichia coli* strains with plasmid DNA from the 3 *N. gonorrhoeae* strains. Further analysis by electron microscopy of heteroduplex molecules and by the Southern technique demonstrated that the beta-lactamase gene present on the three 7.1 kb plasmids is identical to the *bla* gene of the widespread Tn3 transposon. All these results therefore seem to indicate that the Ap^R plasmids from the 3 *N. gonorrhoeae* resistant strains isolated in Geneva have the same origin than those previously isolated in other countries.

Transduction of resistance plasmids mediated by Tn A transposons

J. Cl. Piffaretti and Y. Froment
Département de Microbiologie, 22, quai Ernest Ansermet, CH-1205 Genève

pPJ3a is a nonconjugative plasmid (8.7 kb) conferring nontransposable resistance to Sm and Su. pPJ3b and pPJ3c are derivatives of pPJ3a, carrying respectively Tn2301 and Tn3; the former is an Ap transposon close to Tn3. pPJ3a and Tn2301 originated from an *Escherichia coli* clinical isolate. When phage λ is grown on *E. coli* $recA^+$ or $recA^-$ harbouring pPJ3b or pPJ3c, it gives rise to lysates able to transduce Ap resistance (10^{-4} transd. units/Pfu) and Sm resistance (10^{-7} tu/pfu) into *E. coli* sensitive cells. Ap and Su resistances are always cotransduced with Sm resistance. When SmSuAp resistant lysogens obtained from such lysates are for plasmids (cleared lysates), they show the presence of pPJ3b or pPJ3c molecules, as confirmed by restriction and heteroduplex analysis.
In a similar way, when λ::Tn3 or λ::Tn2301 are grown on *E. coli* containing pPJ3a, lysates able to cotransduce SmSuAp resistances are found (10^{-7} tu/pfu). SmSuAp resistant lysogens obtained from such lysates show the presence of pPJ3a::Tn3 or pPJ3a::Tn2301 molecules. These results show that pPJ3a can be transduced by phage λ, provided the presence of a Tn3 like transposon, either on the plasmid or on the pahge; therefore this type of transduction appears to be related to transposition mechanisms. We are currently investigating this phenomenon with other plasmids and different transposons in order to establish whether this type of transduction could represent a general way for plasmids, in particular for nonconjugative plasmids, to be transferred from one bacteria to another.

Drug resistance among pathogenic bacteria from animals in Switzerland

J. H. Penseyres and J. Nicolet
Institute of Veterinary Bacteriology, University of Bern, CH-3012 Bern

936 clinical isolates of *Staphylococcus aureus, S. hyicus, Escherichia coli, Salmonella spp., Klebsiella pneumoniae* and *Pseudomonas aeruginosa* collected from 4 veterinary laboratories in Switzerland during 1977-8 were examined for their drug resistance to 22 antimicrobial agents on the base of MIC-determinations.

Penicillin G, streptomycin, tetracycline, chloramphenicol and sulfonamides showed the highest prevalence of resistance. Nearly 70% of the *S. aureus* isolates were resistant to one or more of the tested drugs, correspondingly 60% in *E. coli*, 35% in *Kl. pneumoniae* and 21% in *Salmonella* spp. 85% of the resistant *E. coli* isolates were multiple-resistant, against 64% in *S. aureus*.

52% of the *S. aureus* isolates were resistant to penicillin G. 28% of *S. hyicus* isolates were resistant to macrolides against 3% in *S. aureus*. Of the *E. coli* isolates 52% were resistant to streptomycin and sulfonamides, 43% to tetracycline and 26% to chloramphenicol, the extremes being found in the calf with corresponding values of 100%, 90%, 76% and 62%. 4% of the *Salmonella* spp. isolates were resistant to chloramphenicol. The most common resistance patterns in bovine *S. aureus* isolates were penicillin G-monoresistance (18%) and penicillin G-streptomycin-tetracycline-sulfonamide-three-fold-resistance (10%).

20% of the multiple-resistant *E. coli* isolates showed resistance to streptomycin, tetracycline and sulfonamide altogether.

42% of the *Ps. aeruginosa* isolates of the dog were resistant to gentamicin. No resistances were found to rifampicine in staphylococci, to gentamicin in staphylococci and enterobacteria, to polymyxin B in enterobacteria and *Ps. aeruginosa*.

Resistance to antibiotics of the common bovine udder pathogens in Switzerland since 1977

D. Schifferli, J. Nicolet and M. Schällibaum
Institute for Veterinary Bacteriology, University of Bern, CH-3012 Bern, and Swiss Dairy Research Station, CH-3097 Liebefeld-Berne

MIC determinations were used to study the resistance of bovine udder pathogens in Switzerland. This investigation comprises 3 periods (1977-8; autumn 1979; spring 1980) during which *Staphylococcus aureus* (N = 153; 232; 110), *Streptococcus* sp. except *Streptococcus agalactiae* (N = 92; 144; 102) and *E. coli* (N = 142; 122; 93) from clinical mastitis were collected by 4 regional laboratories. The strains were analyzed by an agar dilution method during the first period and an automated broth microdilution technique (MIC 2000 Dynatech) during the other 2 periods.

We found 43-52% of the *S. aureus* to be penicillinase producers; one single strain was resistant to oxacillin (MIC 32 µg/ml). 1% of the strains were resistant to neomycin, spiramycin and erythromycin, and none to cotrimoxazol. Other resistance rates were: streptomycin (17-48%), tetracyclin (7-26%), chloramphenicol (3-11%) and sulfonamides (3-8%).

No penicillin- or ampicillin-resistant streptococci (MIC > 4 µg/ml) occurred, whereas 3-15% were intermediate (MIC > 0.125 µg/ml). Few strains were resistant to erythromycin, spiramycin and chloramphenicol (0-5%); a MIC > 16 µg/ml was obtained by 88-89% of the strains with streptomycin and neomycin and by 3-15% of the strains with tetracyclin.

Clearly bimodal MIC distributions of *E. coli* in histograms showed the following resistance rates: ampicillin (13-26%), cephalothin (6-15%), streptomycin (24-35%), neomycin (11-20%), tetracyclin (22-35%), chloramphenicol (13-26%), cotrimoxazol (1-3%). All strains were susceptible to polymyxin and gentamicin.

Few differences in the resistance pattern were observed between the collection periods. Most deviations of the MIC repartitions are thought to be due to the selection modalities of the strains in the different laboratories.

Cefsulodin, a cephalosporin with potent antipseudomonal activity virtually insusceptable to Ca^{++} and Mg^{++} in the medium

W. Tosch, H. Fehlmann, J. Gysin and O. Zak
Forschungslaboratorien der Ciba-Geigy AG, Departement Pharma, CH-4002 Basel

Depending on the composition of the medium, tests of the sensitivity of *Pseudomonas* to gentamicin can yield varying results. Data are presented to show that cefsulodin does not suffer from this disadvantage. The experiments were carried out with media supplemented either with no Ca^{++} and Mg^{++} or with concentrations up to twice the physiological amounts of these ions. In the disc diffusion test with *Pseudomonas aeruginosa* ATCC 12055 in a medium of saline, 0.4% glucose and 1.4% agar (Difco) an inverse relation was demonstrable between the concentration of Ca^{++} and Mg^{++} and the diameter of the zone of inhibition. The inhibition-zone diameter of cefsulodin (CEF), however, was less reduced (approx. 3 mm) than those of carbenicillin (CAR) and piperacillin (PIP) (4.5 mm), tetracyclin (TET) (7 mm) and rifampicin (RIF) and gentamicin (GEN) (10 mm). Similar findings were made with Mueller-Hinton (MH), DST and Iso-Sensitest (ISO) agar, the inhibition zone around cefsulodin being diminished by only 2 mm, as compared with 4 mm for CAR, PIP and RIF, 6 mm for TET and approximately 11 mm for GEN. In the microtiter system upon addition of Ca^{++} or Mg^{++} to the MH, DST or ISO broth, the MIC's of GEN increased by a factor of 10-100, those of the other substances, however, by a 2-fold dilution step at the most. In the agar dilution test, the mean MIC's of GEN for 34 clinical isolates of *Pseudomonas aeruginosa* increased by about 5, that of CEF only by less than 2. In tests with strain ATCC 12055 in human serum, the MIC values of GEN were as high as in the media supplemented with Ca^{++} and Mg^{++}, the MIC's of CEF, however, were scarcely increased. We conclude that tests of the sensitivity of *Pseudomonas* to gentamicin in the usual media give deceptively good results, whereas the generally good MIC values of cefsulodin much more accurately reflect its activity under physiological conditions.

Epidemiological control and evolution of the sensibility to disinfectants

G. Pappalardo, D. Roussianos and M. Heitz
Division autonome d'Hygiène hospitalière, CHUV, CH-1011 Lausanne, and Institut Universitaire de Microbiologie, Bugnon 44, CH-1011 Lausanne

The epidemiological control of the surgical intensive care unit, made us typize 54 strains of *Pseudomonas aeruginosa*, $^1/_3$ of which were of serotype 0:13 ONPG- (18 strains), when in other respects this group only contained 8 strains belonging to other serotypes. These 18 strains have the same profile in relation to the antibiotical activity (sensitive to POL-GEN-TOB-AKN, resistant to KAN), but 3 are resistant to CAR. The epidemiological inquiry allowed us to exclude the transmission of the germ from one patient to another in most of the cases. However, the persistance of this serotype during the course of the year was a worry and consequently required a research program:

1. Detection in humid spots: 8 *Pseudomonas*, but none of them was 0:13. 2. Lysotypic study (Dr. Vieu, Inst. Pasteur): unique lysotype 5.6 ± 0.12. 3. Sensitivity to disinfectants used in hospital: no difference with other serotypes isolated

during the same period in relation to phenols, aldehydes, chlorhexidine has been established, whereas a difference appeared in relation to phenylhydrargyri boras.
In vitro (streak test): sensitivity of isolated strains others than 0:13 = $^1/_{10,000}$; sensitivity of 0:13 strains = $^1/_{9000}$.
In vivo (skin impressions on volonteers artificially infected by 10^8 germs): phenylmercury borate emulsion and hexachlorophene (Remanex®): diminution from 10^8 to 5.10^4; chlorhexidine solution (Secalan®): diminution from 10^8 to 8 colonies.
The acquisition of organomercurial resistances determined by plasmids in *Pseudomonas,* is an event that has to be taken into consideration in hospital hygiene: it is fortunately put in evidence by an epidemiological control that includes typization.

A case of blood-tinged, recurrent enterocolitis due to *Campylobacter fetus,* ssp. *jejuni*

E. Engelbrecht and L. Vlasveld
Bethel Hospital, NL-2600 GA Delft, The Netherlands

A boy of 8 years was admitted to the hospital during a period of blood stained diarrhea, accompanied by abdominal pains and a slightly raised temperature. In the preceeding 12 months he had already suffered from 3 of such episodes of several weeks duration and marked by feelings of ill-being. The anamnese and the physical examination revealed no abnormality except for a vivid peristaltic activity. The results of the analyses of the blood, the liver functions and the urine were all normal. The radiological examination only showed a spastic ascendent colon, and excluded the possibility of an intestinal invagination. Under a provisional diagnosis of ulcerative colitis an arrangement was made for colonoscopy, which, however, was cancelled when the result of the stool culture became available. In fact, the anaerobic stool cultures repeatedly showed an abundant growth of *Campylobacter fetus* ssp. *jejuni.* Treatment with erythromycin was then started, leading rapidly to clinical and bacteriological cure. The techniques for the isolation and the identification of *Campylobacter fetus* ssp. *jejuni* are discussed as well as the epidemiological aspects of this infectious disease.

Sensitivity of *Candida* spp. towards 5-fluorocytosine, amphotericin B and imidazole

E. Sakellarides
Institut de Microbiologie de l'Université de Lausanne, 44, rue du Bugnon, CH-1005 Lausanne

A study of 119 *Candida* strains isolated from pathologic specimens from the CHUV (Centre Hospitalier Universitaire in Lausanne) during the fall-winter period of 1979–80 has shown that the proportion of resistant strains is lower than that reported in France, USA or Great Britain. Amongst 100 *Candida albicans,* only one was a 'primary resistant' whereas 3 showed a partial secondary resistance to 5-fluorocytosine. The highest resistance proportion was observed within the species *C. tropicalis* and *C. pseudotropicalis* (28.5%) whereas no resistance was observed in *C. krusei* (5 strains). All strains but one were sensitive to amphotericin B and all strains were sensitive to the imidazole group.

Restriction in *Streptomyces glaucescens*

J. M. Sanchez and R. Hütter
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Experiments with the broad host range actinophages P10, P17, P23, and P27 indicated, that P17 and P27 might be restricted in *Streptomyces glaucescens.* The efficiency of plating (e.o.p.) of P17 and P27 was investigated in more detail in several different wild type isolates of *S. glaucescens, S. albus* G, *S. albus* GR^-M^- and *S. achromogenes* ATCC 12767. *S. albus* has been described to contain 2, *S. achromogenes* 3 different restriction endonucleases (see Roberts, Gene *4,* 183, 1978). Phages grown in the original hosts (*S. viridochromogenes* A 3026 for P17; *S. coelicolor* for P27) showed e.o.p.s $< 10^{-2}$ in *S. glaucescens* A 3174, A 3080 and A 3065, as compared to the original host. The e.o.p. of both phages was $< 10^{-7}$ in both *S. achromogenes* and *S. albus* G, but increased notably in *S. albus* GR^-M^- (0.8 for P17; 10^{-2} for P27), suggesting that the restriction activity is operative in vivo against the phages utilized. It was confirmed, that the low e.o.p. on *S. glaucescens* strains was not due to inefficient adsorption.
When phage P27 was grown in *S. glaucescens* A 3174 or A 3065 the same e.o.p. was observed in these strains and in *S. coelicolor.* Passage through *S. coelicolor* lead again to a low e.o.p. on *S. glaucescens* strains, suggesting the presence of a modification system in the glaucescens strains.
Additional data suggest, that the 2 strains *S. glaucescens* A 3174 and A 3065 contain the same R-M-system. The system has been shown to be different, however, from the ones present in *S. albus* G or *S. achromogenes.* Experiments testing DNA restriction activity in vitro with the isolated enzyme(s) are under way to support the above hypothesis.

Carbon balance studies with 2 *Cephalosporium acremonium* strains of different cephalosporin producing capacity

A. Jaramillo and M. T. Küenzi
Ciba-Geigy AG, Werk Klybeck, CH-4002 Basel

The fermentations of 2 *C. acremonium* strains with different specific cephalosporin production rates were compared. The result indicate some aspects for general consideration when optimizing fermentation processes with new strains.
Strain J18215, which was the higher cephalosporin producer and AS5510 were cultivated under the same conditions in a simple synthetic glucose medium. When optimizing the medium, both strains showed a phosphate requirement of 3 mM to obtain maximum cephalosporin yield, which was 1800 mg/l for J18215 and 220 mg/l for AS5510.
In batch fermentation the maximum growth rates were the same for both strains but AS5510 produced 10% more biomass. Carbon balances made at the point of maximum biomass production showed the following distributions of the initial carbon for strain J18215: 34% of carbon as CO_2, 34% as biomass, 1% as cephalosporin and 31% as by products which could only partially be accounted for as proteins and amino acids. For strain AS5510 these values were 29%, 42%, 0.1% and 29% respectively.
Fed batch fermentations in which glucose was added continuously after all the glucose in the start medium had been consumed, confirmed the increased cephalosporin synthesis and reduced biomass formation of strain J18215. Only 14% of the additional carbon was utilized for biomass production and 7% for cephalosporin synthesis whereas in strain AS5510 these values were 22% and 1% respectively. The maximum cephalosporin titres were 2500 mg/l for J18215 and 220 mg/l for AS5510, signifying an overall yield of 36 mg cephalosporin per g of glucose as against 5 mg cephalosporin for AS5510.
The results show that increased cephalosporin production of J18215 is achieved at the expense of biomass and not by product formation. J18215 also autolysed more rapidly after consumption of the carbon source and secreted more amino acids into the medium. In addition, an increased sensitivity to lyophilization and storage on agar was observed.

These results suggest that certain mutations which strain J18215 has undergone have not only given rise to an increased cephalosporin production capacity but it might also be proposed that the cell permeability has been altered.

Biochemical investigation of cystathionine-γ-lyase (EC 4.2.1.15) and O-acetyl-L-serine sulfhydrylase (EC 4.2.99.8) in *Cephalosporium acremonium*

A. Scheidegger and H.J. Treichler
Research Laboratories of Ciba-Geigy Klybeck, CH-4002 Basel

The fungus *C. acremonium* produces the β-lactam antibiotic cephalosporin C, which is derived from the 3 amino acids, γ-aminoadipic acid, cysteine and valine. As the cysteine moiety itself is a product of the sulfur metabolism, the enzymes involved are important in the formation of the antibiotic.
The 2 important enzymes in the sulfur metabolism, cystathionine-γ-lyase and O-acetyl-L-serine sulfhydrylase (OASS) were examined in 2 *C. acremonium* strains producing varying amounts of cephalosporine C. OASS is involved in the anabolic pathway of cysteine formation whereas γ-cystathionase mediates the last step of the reverse transsulfuration from methionine to cysteine.
It could be shown that the specific activity of γ-cystathionase in the highest producer, strain I 18.2.15, which was 2.6 nmoles/mg min, was about 10 times higher than in the lowest producer, strain 8650. This difference in specific enzyme activity would support the assumption that the cleavage of cystathionine by the enzyme γ-cystathionase mediates the incorporation of the cysteine moiety into the β-lactam molecule and that this enzyme is more active in strains of superior productivity.
The measured specific activity of OASS in strain I 18.2.15 of 20.2 nmoles/mg min is again about 10 times higher than that found in 8650. From this one might conclude that a more intensive anabolic cysteine formation is taking place. However it was shown genetically that in I 18.2.15, serine could not be acetylated to O-acetyl-L-serine (OAS). Under these conditions the lack of OAS would render in vivo sulfhydrylation impossible. It can only be assumed that the supply of cysteine is obtained from the alternative pathway by O-acetyl-L-homoserine sulfhydrylase via homocysteine and cystathionine.

Tryptophan starvation causes the synthesis of an altered tryptophan-tRNA in *Saccharomyces cerevisiae*

P. Stäheli and R. Hütter
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Yeast cells prevent their amino acid pools from decreasing beyond certain levels by enhancing the flux through the corresponding amino acid biosynthetic pathways. This can be achieved by the restauration of the maximal activity of the feedback regulated enzymes or by a derepression of some biosynthetic enzymes. We investigated the nature of the regulatory signals, which lead to this derepression, by a study of the influence of tryptophan limitations on the tryptophan-tRNA.
Tryptophan limitation always led to a more or less pronounced decrease of the degree of charging of the tryptophan-tRNA, depending on the efficiency of the residual tryptophan biosynthesis. DL-5-methyltryptophan (5×10^{-4} M) caused the degree of tryptophan-tRNA charging to decrease in the wild strain X2180-1A from 95% to about 55%. In the leaky tryptophan auxotrophic strain RH 570 the degree of tryptophan-tRNA charging fell from 95% to less than 10%, when the strain was shifted from a tryptophan supplemented medium to minimal medium. Under conditions of tryptophan limitation the synthesis of a new tryptophan-tRNA species, which showed an altered chromatographic behaviour on RPC5-columns could be observed. The portion of this new species of the total tryptophan-tRNA varied depending on the intensity of the limitation and could reach as much as 60%. The degree of charging in vivo of the new and the normal tRNA species were found to be similar and both species were equally chargable in vitro. In intact cells, the new tryptophan-tRNA species was converted to the normal form rather quickly after addition of tryptophan to the medium.
We postulate that the new tryptophan-tRNA species differs from the normal tryptophan-tRNA by some base modification, which can be introduced only after the charging of the tRNA molecule with tryptophan is performed and that newly synthesized, uncharged tryptophan-tRNA molecules can not be modified completely. It still has to be proven, that this new tRNA species has regulatory functions.

The role of the 'general control of amino acid biosynthesis' of *Saccharomyces cerevisiae* in vivo

P. Niederberger and R. Hütter
Mikrobiologisches Institut ETHZ, Universitätsstrasse 2, CH-8092 Zürich

Yeast cells encounter in their natural environment (e.g. grape must) amino acid mixtures highly variable in composition and concentration. We compared growth behaviour of our wild type strain X 2180-1A and of 2 mutant strains with a defective 'general control' RH 375 (*ndr 1-1*) and RH 487 (*ndr 2-1*) in the presence of a) all 20 essential amino acids except one and b) each of the 20 amino acids alone (conc. 2 mM).
The omission of arginine, phenylalanine or tryptophan from the medium containing the amino acid mixture led to a decrease in growth rates in the Ndr-strains but not in the wild type strain. Omission of other amino acids did not significantly affect growth in any of the strains.
In minimal medium with or without ammonia, the addition of leucine, isoleucine, methionine, threonine, tyrosine, ornithine or lysine respectively led to considerably decreased growth rates in the Ndr-strains. The growth rate of the wild type strain was not or only weakly affected. In all cases investigated, the reduction in growth rate was due to amino acid limitations, that could be relieved by the addition of other suitable amino acids.
The experiments show, that the wild type strain with a properly functioning 'general control' is able to cope with amino acid imbalances in the medium much more efficiently than the Ndr-mutant strains.

Tryptophan metabolism in *Saccharomyces cerevisiae*

P. Kradolfer and R. Hütter
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Degradation of tryptophan by *Saccharomyces cerevisiae* starts immediately after addition of the amino acid to the growth medium. The degradation rate increases 2- to 4-fold during the first 2 h after tryptophan addition, depending on the concentration of tryptophan added and on the growth medium. In order to get an insight into the regulation of the degradative pathway(s), we studied the occurrence and behaviour of tryptophan degrading enzymes.
Tryptophan degradation occurs in the strain X2180-1A nearly exclusively through deamination by tryptophan aminotransferase activities; 2-oxoglutarate, pyruvate or phenylpyruvate can serve as aminoacceptors. While the activity with 2-oxoglutarate remained constant, the activities with pyruvate or phenylpyruvate increased after tryptophan

addition. DEAE-cellulose chromatography of crude extracts of *S. cerevisiae* grown on minimal medium supplemented with tryptophan separated 2 aminotransferase activities. Aminotransferase I was active with 2-oxoglutarate, pyruvate or phenylpyruvate as aminoacceptors, aminotransferase II was only active with pyruvate or phenylpyruvate. In cells grown on tryptophan-free minimal medium aminotransferase II was not detectable. The apparent K_m for tryptophan of aminotransferase I was 6×10^{-3} M, of aminotransferase II it was 4×10^{-4} M. Both aminotransferases were also active with phenylalanine and tyrosine as aminodonors.
We postulate that aminotransferase II is a catabolic enzyme whereas aminotransferase I is the anabolic enzyme needed for phenylalanine and tyrosine biosynthesis.

Proline catabolism in *Pseudomonas aeruginosa*

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

L-proline is converted to glutamate in *Pseudomonas aeruginosa* strain PAO by 2 enzymes: the FAD-dependent L-proline dehydrogenase specifically oxidizes L-proline to Δ^1-pyrroline-5-carboxylate (P5C), which is metabolized by the NAD (P)-dependent P5C dehydrogenase to glutamate. Both enzymes were induced by L-proline and were not subject to catabolite repression. In strain PAO ornithine is transaminated to P5C, an intermediate of proline catabolism. Cells grown on ornithine as the sole source of carbon and nitrogen showed a partially induced level of P5C dehydrogenase, which was considerably lower than in proline grown cells. Proline dehydrogenase was not induced by ornithine and arginine did not induce either enzyme.
Different types of mutants which cannot grow on proline as the sole carbon and nitrogen source (*put* mutants) were isolated. These mutants had either a defective system for proline uptake or were blocked in the proline dehydrogenase or in both enzymes of proline catabolism. No mutants were found with a block in the P5C dehydrogenase activity only. All mutants lacking proline dehydrogenase activity had a hyperinduced level of P5C dehydrogenase when grown on ornithine. 2 *put* mutants were unable to grow on ornithine; they lacked proline dehydrogenase and P5C dehydrogenase activity.
All put mutations were linked to the *hisI* locus, which is located at 12 min on the chromosome map of *P. aeruginosa.*

Anaerobic growth of *Pseudomonas aeruginosa* in the absence of respiration

D. Haas and Marta Kley
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Members of the genus *Pseudomonas* are characterized by a 'respiratory, never fermentative' metabolism (M. Doudoroff and N.J. Palleroni, in: Bergey's manual of determinative bacteriology, 8th ed., p.217, 1974). We now find that *P. aeruginosa* can grow in the absence of terminal electron acceptors (e.g. O_2, NO_3^-, NO_2^-) by using arginine as an energy source plus a carboxylic acid (e.g. fumarate or pyruvate) as an H acceptor. 1 mole of ATP is formed per mole of arginine in the arginine deiminase pathway. The enzymes of this pathway, arginine deiminase, catabolic ornithine carbamoyltransferase and carbamate kinase, are strongly induced by oxygen depletion (D. Haas et al., J. Bact. *139,* 713, 1979). Cofermentation of arginine and a reducible carboxylic acid generates sufficient energy to permit slow growth in a semisynthesic medium supplemented with casamino acids and vitamins. Mutants blocked in the deiminase pathway do not grow in this medium. The capacity of *P. aeruginosa* for fermentation is cryptic during aerobic or anaerobic respiration.

Enzymatic cleavage of cyanuric acid by a hydrolase

P. Beilstein and R. Hütter
Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich

Cyanuric acid is the last s-triazine ring system in the degradation pathway of s-triazine herbicides. The pathway of cyanuric acid degradation is still uncertain. It is assumed that the degradation occurs via biuret to urea, which is then hydrolyzed to NH_3 and CO_2 by urease. The purpose of our study was to confirm or reject the proposed pathway and to elucidate the enzymatic mechanism of ring cleavage.
The strain used in our study was *Klebsiella pneumoniae* 99B. This strain is capable of growing on cyanuric acid as a sole nitrogen source. The yield of protein on cyanuric acid was the same as on ammonia (about 65 g protein $\times$ mole^{-1} N). Maximal growth rate on ammonia was 0.73 h^{-1} and on cyanuric acid 0.58 h^{-1}. We were able to show that CO_2 and NH_3 are the end products of the degradation. Cell-free extracts (desalted on sephadex G-25) were able to degrade cyanuric acid efficiently without any additions. The specific activity in the cell-free extract was 1.6 µmoles cyanuric acid degraded $\times$ h^{-1} $\times$ mg protein^{-1} and is comparable to the specific degradation rate of growing cells (2.7 µmoles $\times$ h^{mg} protein^{-1}). The reaction was also catalyzed under anaerobic conditions. Based on the lack of any cofactor requirements and on considerations of the structure of the cyanuric acid molecule, we postulate that ring cleavage occurs by enzymatic hydrolysis.
The cell-free extracts were also active with biuret (0.5 µmoles biuret degraded $\times$ h $\times$ mg protein^{-1}) and with urea (5.6 µmoles urea converted $\times$ h^{-1} $\times$ mg protein^{-1}) as substrates. These findings agree with the assumed degradation pathway of cyanuric acid. Further experiments are necessary to support the hypothesis.

Degradation of 2-chloroethanol by a *Pseudomonas* species

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

A bacterium capable of utilizing 2-chloroethanol as its sole source of carbon and energy was isolated from sewage and identified as a *Pseudomonas* sp.
Common carbon sources such as lactate ($\mu = 0.36$ h^{-1}), succinate (0.36 h^{-1}), ethanol (0.22 h^{-1}) and halogenated compounds such as 2-chloroacetate (0.08 h^{-1}), 2-bromoacetate, 2-iodoacetate, 2-chloropropionate and 2-2-dichloropropionate supported growth.
Growth on 2-chloroethanol proved to be linear. During further experiments a mutant was isolated which was able to grow exponentially ($\mu = 0.08$ h^{-1}). Cell-free extracts of 2-chloroethanol-grown cells did not release chloride ions from 2-chloroethanol, but 2-chloroacetate was dechlorinated effectively. 2-Bromoacetate, 2-iodoacetate, 2-chloropropionate and 2-2-dichlorpropionate were also dehalogenated in vitro, but at a lower rate. The dehalogenation reaction showed a broad pH optimum between 9.5 and 10.5. It was inhibited by p-chloromercuribenzoate and p-hydroxymercuribenzoate.

Energy production of *Bacillus stearothermophilus* by denitrification processes

H.E. Reiling and H. Zuber
Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, CH-8093 Zürich

Using batch calorimetry at 65 °C the anaerobic growth and energetics of a prototrophic, facultative anaerobic strain of *Bacillus stearothermophilus* have been studied. For good growth this thermophilic bacterium requires a minimal

medium supplemented with an inorganic terminal electron acceptor such as nitrate or nitrite. Depending on the growth-limiting factor (glucose or nitrate) the calorimetric heat-flux profile exhibits different metabolic activities. If glucose is limiting and nitrate is in excess, the heat production of the growing population yields a rather symmetrical, bell-shaped thermogram, and an almost quantitative reduction of nitrate to nitrite is observed. If, on the other hand, glucose is in excess and nitrate is growth-limiting, a heat-flux curve with 2 maxima is obtained, where the nitrate supplied is reduced to nitrite about stoichiometrically during the first activity period, and the latter is reduced completely in the 2nd phase under further energy gain. Nitrate reduction as well as nitrite reduction must be coupled to phosphorylation sites, because in both cases growth proceeds. The reduction of the accumulated nitrite after exhaustion of nitrate suggests an inhibitory effect of nitrate on the operation of nitrite reductase.

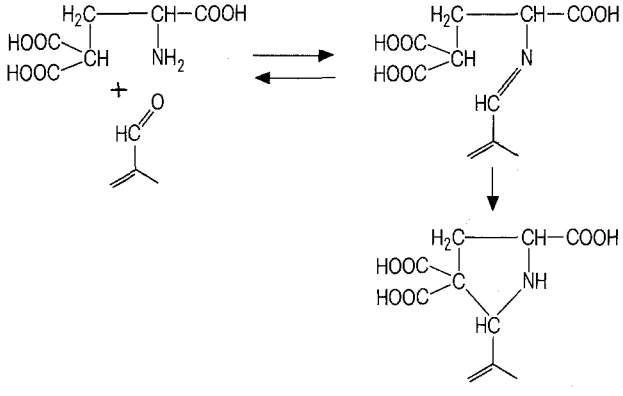
Investigation into the microflora of Sarno river

V. Cuomo and F. Vanzanella
Ciba-Geigy S.p.A., Research Lab., Via Provinciale Schito 131, I-80058 Torre Annunziata (NA), Italy

In this note we report a study on a tract of lower river in which the Ciba-Geigy Antibiotics Factory is located. The fungal flora recorded belongs to terrestrial fungi (Ascomycetes, Fungi Imperfecti and Basidiomycetes) and marine fungi (Ascomycetes, Fungi Imperfecti). Some lower fungi, Thraustochytrid fungi, are also found. The distribution depends on the physical-chemical properties of the water, especially on the salinity variation. The greater part of fungal populations is typical of detriticus habitat and consequently is saprobic. In fact the physical chemical tests show that this tract is characterized by waters typical of a polysaprobic environment.

CORRIGENDA

Laura Pecci, S. Duprè, A. Antonucci and D. Cavallini: Reaction of pyridoxal-5′-phosphate with γ-carboxyglutamic acid, Experientia *36*, 910 (1980). The reaction scheme (p. 911, left column) should correctly read:



J. Carr, I. Carr, B. Dreher and C. R. Franks: Lymphatic metastasis of tumour; persistent transport of cells, Experientia *35*, 825 (1979).
Due to a miscalculation of the volume of 1 cm length of polythene tubing, which was 0.628 cm^3 and not 0.0628 cm^3 as stated, the cell counts in the text (though not in the figure) are 10 times too high and the output of cells from the tumour therefore ranges from 10^2 to 10^5 cells per 10 min.

E. Berlin, L. Hellgren, O. Thulesius and J. Vincent: Prostaglandin-like substances in *Propionibacterium acnes.* IV. Effect of isolated human vessels, Experientia *36*, 197 (1980). The results from 10 experiments are summarized in the following table (not included in the paper).

Responses of isolated human blood vessels towards noradrenaline, PLS and PGE_2. Values of agonists given in molar concentrations (M). n = number of experiments, $\Delta T/\Delta t$ = kinetic parameter, characterizing velocity of contraction. Molar concentration of PLS is expressed as PGE_2-equivalent in gerbil colon bioassay.

	Drug	n	Threshold (M)	ED_{50} (M)	Maximum response (10^3 N/m^2)	$\Delta T/\Delta t$ (10^2 $N/m^2 \cdot min$)
Saphenous vein	Noradrenaline	3	$2.5 \cdot 10^{-9}$	$5.0 \cdot 10^{-7}$	3.8	
	PLS	3	$2.5 \cdot 10^{-8}$	$1.3 \cdot 10^{-7}$	1.0	
Umbilical artery	Noradrenaline	8	$9.5 \pm 7.0 \cdot 10^{-7}$	$1.3 \pm 1.0 \cdot 10^{-5}$	0.6 ± 0.2	0.8
	PLS	6	$6.7 \pm 3.3 \cdot 10^{-9}$	$4.3 \pm 2.7 \cdot 10^{-7}$	1.9 ± 0.6	4.4
	PGE_2	8	$6.0 \pm 4.0 \cdot 10^{-9}$	$9.0 \pm 8.6 \cdot 10^{-7}$	2.0 ± 0.5	3.4