ABSTRACTS

Determination of the adenine nucleotide pool in *Acetobacter aceti* NCIB 8554 by high pressure liquid chromatography

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The adenine nucleotide pool has been measured in *A. aceti* NCIB 8554 by a single column HPLC system. The method allows a simultaneous separation of the adenine nucleotides from the acid-soluble cell extract. The chromatogram, carried out at 50 °C and at acid pH, using a linear gradient both of ionic strength and pH [0.01 M $(NH_4)_2HPO_4$/pH 3.0 - 1.0 M $(NH_4)_2HPO_4$/pH 5.0], take about 60 min. A prepacked anion exchange column (25 cm × 4.6 mm i.d., stainless steel) is used in this study. The separated components were identified by their retention time UV-spectra and by an enzymatic test with fire-fly luciferin/luciferase.
During logarithmic growth in a 14-liter glass-fermenter (pH regulated), on ethanol (0.5%) and mineral medium, the culture showed an energy charge (Atkinson, Cellular Energy Metabolism and its Regulation, Academic Press, 1977) of about 0.85, which falls to low values as soon as the substrate is exhausted.
The energy charge was compared with growth parameters such as growth rate, ethanol consumption, acetate production and gas metabolism(Q_{CO_2}, Q_{O_2}, RQ).

Molar extinction coefficients and the UV-photometric assay of cephalosporins

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The molar extinction coefficients ε of several cephalosporins, and the decrease of optical density $\Delta\varepsilon/\varepsilon$ associated with their β-lactamase-catalyzed hydrolysis were determined after prior alkalimetric assay of chromatographically pure samples by the recently described enzymatic methods (J. Konecny and A. Schneider, J. Antibiot. *31*, 776, 1978). For example, the following values were obtained for Na-cephalosporin C, Na-deacetyl cephalosporin C, 7-aminocephalosporanic acid and its deacetyl derivative. λ_{max} (nm): 261, 261, 265, 265; ε (cm^{-1} mM^{-1}): 9.1, 8.5, 8.3, 7.8; titer of sample (% theor.): 89, 93, 96, 96; $\Delta\varepsilon/\varepsilon$: 0.87, 0.86, n.r., n.r. (n.r. = no reaction with lactamase P99).
The molar extinction coefficients may be used for the photometric assay of analytical standards and other samples which display high levels of purity when examined by liquid chromatography with 255-265-nm detectors. This criterion ensures that the concentrations of foreign cephalosporins and other impurities with high molar extinction coefficients are low and that the contribution of impurities to the total optical density of the sample at these wave lengths is negligible. Chromatographic purity at these wave lengths does not ensure the absence of impurities formed by hydrolysis of the labile β-lactam ring, which frequently have low extinction coefficients in this region. Their presence, indicated by differences between the actual carbon content of the sample and the lower value calculated from the alkalimetric or photometric titer, is sometimes revealed by liquid chromatography with 225-230-nm detectors.
The decrease in optical density ΔE, and of the molar extinction $\Delta\varepsilon$, associated with β-lactamase-catalyzed hydrolysis is unaffected by all impurities which are resistant to the enzyme. Measurements of ΔE may therefore be exploited in various analytical applications, such as the measurement of the loss of β-lactam in the thermal decomposition and other reactions of cephalosporins (in preparation), in the assay of cephalosporins in fermentation broths (H.H. Peter, in preparation) and in the determination of the rates of formation of cephalosporins from 7-aminocephalosporanic acid (T. Takahaski et al., J. Antibiot. *30*, S230, 1977).

Pyruvate kinase from *Acetobacter aceti* NCIB 8554

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A. aceti is able to grow on pyruvate as the only source of carbon and energy. The pyruvate kinase (PK) reaction, as the final step of the conversion of triose phosphate into pyruvate, seems to be a control point for glycolysis and gluconeogenesis. With regard to its kinetic data, the enzyme appears to be very similar to the PK isolated from *A. xylinum* by Benzimann (Biochem. J. *112*, 631, 1969).
Monovalent cations, such as K^+ or NH_4^+, do not activate the enzyme. Mg^{++} ions are essential for the activity of 140-fold purified PK (purification procedure: ammonium sulfate fractionation, DEAE-cellulose, ammonium sulfate fractionation, Hydroxyapatite).
The K_m-values for the substrates phosphoenolpyruvate (PEP) and ADP are 0.3×10^{-3} M and 0.13×10^{-3} M. The Lineweaver-Burk plot, representing the velocity-substrate-concentration relationship, suggests a cooperative effect for PEP.
ATP, a reaction product, was found to inhibit the PK activity. The K_i-values, computed from the Dixon plots, are 3.0×10^{-3} M for ADP and 1.23×10^{-3} for PEP. In both cases, the inhibition appears to be competitive.
The equilibrium of the PK reaction lies far to the side of pyruvate formation and is practically irreversible. The enzyme therefore plays an important role in the generation of ATP. This, on the other hand, has a regulatory effect on the PK activity, as free ATP acts as a negative feedback.

Glucose metabolism in *Acetobacter aceti* NCIB 8554

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A. aceti NCIB 8554 is able to grow on ethanol, propanol, acetate, propionate or pyruvate in a mineral medium. Glucose, however, or any other carbohydrate cannot serve as a sole carbon and energy source. The existence of a glucose uptake system and some enzymes involved in glucose metabolism has been demonstrated earlier. It was interesting to see whether the addition of glucose to a mineral medium with ethanol-limitation would result in a modified growth kinetic and cell yield.
The fate of glucose was further investigated by enzymatic and radiorespirometric experiments.
When 5 mM glucose is added to an exponentially growing ethanol culture, about 12% of the carbohydrate is metabolized intracellularly, the rest being oxidized extracellularly to gluconic acid and 2-oxogluconic acid. These sugar acids cannot be further metabolized as no uptake systems for these 2 substrates exist.
Experiments, with specifically labeled glucose showed that nearly 75% of the glucose taken up is metabolized through the pentose-phosphate pathway. The rest is converted directly to fructose-6-P, a precursor of cell wall synthesis.
Addition of 35 mM glucose to an exponentially growing culture with ethanol limitatiom (7 mM) showed no effect on growth kinetic and cell yield during exponential growth (estimated by following optical density, dry weight, cell number, protein synthesis). At the end of exponential growth, a second linear growth phase of the glucose ethanol culture led to an increased optical density (+58%), dry

weight (+43%), cell number (+38%) and protein concentration (+38%).
Incorporation of glucose resulted in a modified cell wall structure as shown by electron microscopy. Radioactivity derived from ^{14}C-glucose could also be detected in ribonucleic acid and protein.
The question as to why *A. aceti* is not able to grow on glucose, despite the presence of all the pentosephosphate pathway enzymes, is under further investigation.

The gene-enzyme relationships of the proline biosynthetic system of *Escherichia coli*

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The conversion of glutamate to proline, in *E. coli*, proceeds via γ-glutamyl phosphate, glutamic-γ-semialdehyde and pyrroline-5-carboxylate (P5C). Proline auxotrophs have mutations in 1 or more of 3 genetic loci, 2 of which, *proA* and *proB*, are contiguous. An assay has been developed for a glutamyl kinase (EI), which is totally inhibited by L-proline (100 nm) and specifically by proline and proline analogues. The assay for γ-glutamyl phosphate reductase (EII) was based on the Pi and P5C-dependant reduction of $NADP^+$. Cycling between EII and P5C reductase (EIII), catalyzing P5C-dependant oxidation of NADPH, prevents EII from being detected, when the much more active EIII is present in crude extracts. A simple, rapid method has been devised to separate EII and EIII. Crude extracts are treated with streptomycin sulphate, and chromatographed on DEAE-cellulose. EII does not bind under the conditions used, while EIII is retained on the column. It was, therefore, possible to survey 18 proline auxotrophic mutants (kindly supplied by R. Curtiss III and H. Condamine) for the specific activities of the 3 proline biosynthetic enzymes. All $proA^-$ and all $proB^-$ strains lacked EII while only $proB^-$ mutants lacked EI. Whereas the reason for the absence of *both* enzyme activities in $proB^-$ strains is not clear, it is possible, from the data obtained, to assign the *proB* locus to γ-glutamyl kinase and *proA* to the γ-glutamyl phosphate reductase. Only $proC^-$ mutants were devoid of EIII. The ColEI hybrid plasmid pLC 44-11 from an *E. coli* colony bank (Clarke and Carbon, Cell *9*, 91, 1976) was transferred from its original host to a $recA^-$, Δ' pro A/B strain. In this host pLC 44-11 was stabily maintained on a glucose/salts medium. These new strains had a 20-fold increase in the specific activity of EII and a 3-fold increase in EI as compared to the values from wild-type strains. It is being used as a source of the enzymes for their purification.
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Genetic recombination in fused protoplasts of *Streptomyces glaucescens*

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Protoplasts could be formed from mycelial fragments of *S. glaucescens* by the glycine-lysozyme technique (Hopwood et al., Nature *263*, 171–174, 1977).
Regeneration of cells from protoplasts on a hypertonic nonselective medium was slow and asynchronous, but efficient. The reversion rate of protoplasts to the normal filamentous state was estimated to be 20–35%. Unprotoplasted mycelium and colonies which develop early from protoplasted cells inhibit colony formation by nearby protoplasts.
The following experiments were performed in order to investigate the usefulness of fused protoplasts for the genetic analysis of *S. glaucescens*.
Fertility in conventional crosses performed by growing parents together on solid complex media and by direct plating on minimal medium is low. Recombination frequencies from 10^{-7} to 10^{-4} compared to the minority parent were obtained, depending on the strains crossed.
Protoplasts from genetically marked auxotrophic or antibiotic resistant mutant strains can be fused in the presence of polyethylene-glycol (PEG 1550 or 1000). The fused protoplasts regenerate easily and quantitatively on nonselective complex media. Stable genetic recombinants can be isolated by this procedure with a frequency of 10^{-4}–10^{2}, i.e. with a 100- to 1000-fold higher frequency than in conventional conjugative crosses. And typically more different recombinant classes could be isolated from post-fusion mycelia than have been found in conventional crosses. Nevertheless the increase in fertility is not sufficient to permit a nonselective analysis of recombinants.

Microcycle conidiation in *Trichoderma harzianum*

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Generally, fungi do not sporulate in liquid shaken culture. By manipulating synthetic media, conidiation can occur, but often takes place after several days of incubation.
An investigation of the nutritional and physical requirements for microcycle conidiation in submerged culture has been undertaken with *Trichoderma harzianum*, an antagonist against phytopathogenic fungi and producer of antibiotics and lytic enzymes. Our purpose was to control and reduce the vegetative growth phase and to induce synchronous conidiation following spore germination.
A culture system involving 2 steps was developed:
1. Rich medium (malt 2%) promoting fast and synchronous enlargement of the initial conidia.
2. Transfer into a synthetic medium containing a small amount (5 mM) of NO_3^- or NO_2^- as nitrogen source, and either low (45 mM) or very high (0.45 M) concentration of glucose as carbon supply.
All experiments were done under continuous light at 23–25 °C, while in full darkness no conidiation occurred.
The first phialide appears at the apex of the germ tube/conidiophore after 15 h and the lateral phialides after 20 h. Conidiophores do not exceed 20 µm, corresponding to 3 times the diameter of the germinated initial conidium. Spore production is synchronous and more abundant in the high carbon medium.

Determination of the last 2 steps of the rifamycin B biosynthesis by closely linked chromosomal genes

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A linkage analysis based on genetic recombination has been made for *Nocardia mediterranei*, the producer of the rifamycins. This genetic analysis was used to map the last 2 steps of rifamycin B biosynthesis, the transformations of rifamycin W to rifamycin S and of rifamycin S to rifamycin B. By means of such experiments it should be possible to obtain information on the arrangement of the genes directly controlling rifamycin synthesis.
For our studies mutant strains were used which had lost the ability either to transform rifamycin W into rifamycin S or rifamycin S into rifamycin B. Firstly a series of experiments were carried out in which these mutant strains were crossed with strains carrying different auxotrophic markers and one streptomycin resistant marker whose position on the linkage map of *N. mediterranei* was known. In a second series of experiments the mutant strains with the above mentioned

blocks in the final steps of rifamycin B biosynthesis were directly crossed with each other. The data from this linkage analysis showed that the genes which determine the last 2 steps of rifamycin B biosynthesis are located on the chromosome close to the str-2 marker. They are closely linked to each other, the distance between them being about 1/20 of that between the markers pro-1 and str-2 on the linkage map of *N. mediterranei.*

Transformation of rifamycin S into the rifamycins B and L. A revision of the current biosynthetic hypothesis

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The transformation of rifamycin S into the rifamycins B and L by *Nocardia mediterranei* was reinvestigated in order to establish more detailed pathways.
The rifamycins B and L formed in transformation assays containing washed mycelium, rifamycin S and [U-^{14}C]-glycerol, [U-^{14}C]-, [1-^{14}C]-, [2-^{14}C]-, [3-^{14}C]-pyruvic acids or [U-^{14}C]-L-serine were tested for specific activity. These 3C-precursors labeled the glycolic acid moieties of rifamycins B and L in different ways. Glycerol was strongly incorporated into the glycolic acid moiety of rifamycin B, while serine and pyruvic acids showed only little incorporation. C(2) and C(3) of pyruvic acid labeled rifamycin B better than C(1). In contrast to the results obtained for rifamycin B the glycolic acid moiety of rifamycin L was strongly labeled by the pyruvic acids, while glycerol and serine showed only little incorporation. The specific activity of rifamycin L was identical for C(1), C(2) and C(3)-labeled pyruvic acid. A sample of rifamycin O, labeled only in the glycolic acid moiety (prepared by incubation of wahsed mycelium of the rifamycin nonproducing strain P14 with rifamycin S and [U-^{14}C]-glycerol followed by chemical oxidation of the resulting rifamycin B to rifamyxin O) yielded only labeled rifamycin B but no rifamycin L in the transformation assay.
These results do not support the current hypothesis proposed by Lancini et al. (J. Antibiot. *22,* 369, 1969) considering rifamycin O as the common progenitor in the biosynthesis of rifamycins B and L. Our results exclude rifamycin O as a commonprogenitor and indicate that rifamycins B and L are formed from rifamycin S (SV) by different pathways using different 3C-precursors for the biosynthesis of their glycolic acid moieties.
First investigations with thiamine antagonists such as oxythiamine showed that the transformation of rifamycin S into rifamycin B (L) is completely inhibited. The inhibition is partially reversed by thiamine. We therefore conclude that a thiamine-dependent enzyme (decarboxylase) must be involved in the transformation reaction.

Biosynthesis of L-2-amino-4-methoxy-trans-3-butenoic acid by *Pseudomonas aeruginosa* PAO1

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P. aeruginosa produces L-2-amino-4-methoxy-trans-3-butenoic acid [AMB; $CH_3OCH=CHCH(NH_2)COOH$; Scannell et al., J. Antibiot. *25,* 122, 1972], an antimetabolite irreversibly inhibiting pyridoxalphosphate-linked aspartate aminotransferase (Rando et al., J. biol. Chem. *251,* 3306, 1976). AMB inhibits the growth of *Bacillus subtilis* and *Escherichia coli* on minimal media, a property used to detect and quantitatively assay this antimetabolite. We have tested 3 wild-type strains of *P. aeruginosa* for production of AMB and have found that only strain PAO but not strains PAT and PAC produced, after 3 days of cultivation at 37 °C, 20 mg/l of AMB on a complex medium and 2 mg/l of AMB on a glucose-citrate minimal medium. The identity of the antibiotic substance produced by strain PAO with AMB was confirmed by its chromatographic properties and by NMR-spectroscopy of the purified compound.
The structure of AMB suggested a biogenetic relation of the compound with the amino acids of the aspartate family. The pathway leading to AMB could branch off from the main pathway either at aspartate-β-semialdehyde or at homoserine. In the first case methyl-transfer to the enol-form of aspartate-β-semialdehyde would directly lead to AMB. In the second case formation of AMB would proceed from homoserine by a 2-step sequence of methylation and dehydrogenation.
The involvement of methionine as a methyl-donor was demonstrated by feeding (^{14}C-methyl)-L-methionine to a methionine-auxotrophic derivative of strain PAO, growing on minimal medium. The specific radioactivity of the AMB isolated from this culture amounted to 50% of the specific radioactivity of the (^{14}C-methyl)-L-methionine fed. Removal of the methyl-group from the labeled AMB by reduction over platinum yielded unlabeled α-aminobutyric acid, thus confirming the specific labeling of the methyl-group in AMB. Support for a pathway branching off at homoserine was obtained by feeding experiments. To cultures of *P. aeruginosa* PAO growing on minimal medium 4 mM D,L-homoserine or 4 mM synthetic D,L-O-methyl-homoserine was added at the end of the exponential phase of growth. In both cases a significant (2–3-fold) increase in AMB-formation was observed during the stationary phase. These results support biosynthesis of AMB from homoserine and experiments with mutants of *P. aeruginosa* blocked in different steps of homoserine-biosynthesis are in progress in order to verify the proposed pathway.

Inhibition of the tyrosinase-induction in *Streptomyces glaucescens* by derivatives of tetracenomycin C

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In liquid cultures of *S. glaucescens* the synthesis of tyrosinase (EC 1.14.18.1) can be induced by various amino acids during rapid growth. In old cultures this induction is no longer possible. The reason for this phenomenon is that, shortly after rapid growth begins, the organism produces an antiinduction factor, which is excreted into the medium. It can be extracted from the medium by ethyl acetate. In the extracted medium, the synthesis of tyrosinase can easily be induced. The extract contains different lipophilic substances and some antibiotics, all of which belong to the group of the tetracenomycines. The structure of one of these antibiotics, tetracenomycine C, has been described (W. Weber, Helv. chim. Acta, in press). It is yellow in the visible spectra and shows a blue fluorescence in the UV-spectra. Growth of the producing strain is completely inhibited at concentrations of 100 µg/ml. The antibiotic itself has no influence on the induction of tyrosinase synthesis. However, it is destroyed by daylight and turned into a red-brown product, which is an inhibitor of tyrosinase synthesis. During this process its properties as a growth inhibitor are lost. In shake-cultures, inhibition of tyrosinase production can be observed already at concentrations of 1 µg/ml. The Br-derivative of tetracenomycine C shows also inhibition of tyrosinase production. However, in addition, it inhibits the growth as well. The other tetracenomycines in the extract did not show any inhibition of tyrosinase production.

Investigation of the biosynthesis of β-lactam antibiotics using permeabilized *Cephalosporium acremonium* cells

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At various steps of the so far not well-defined biosynthesis of β-lactam antibiotics substrates occur which cannot penetrate intact cells.
In an attempt to analyze the various enzymes involved in these steps we used permeabilized cells as a tool. Using a well-known enzyme system, the hexokinase/glucose-6-phosphate dehydrogenase reaction, we were able to show that *Cephalosporium* cells are permeable to charged molecules after ether treatment.
With the aid of these permeabilized cells we analyzed the 2 final steps in the biosynthesis of cephalosporin C, namely, the oxidation of deacetoxycephalosporin C and the acetylation of deacetylcephalosporin C. We found that a dioxygenase catalyses the former reaction and the deacetylcephalosporin C-O-acetyltransferase the last step. In the latter reaction, the substrate is S-acetyl coenzyme A, as had been demonstrated with the isolated enzyme. The rate of acetylation in ether treated cells was 60%, compared with that in living cells.

Correlation between cysteine synthase activity and cephalosporin C production in *Cephalosporium acremonium*

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The biosynthesis of L-cysteine by *C. acremonium* can be catalyzed by 2 different enzymes: the cysteine synthase (O-acetyl-L-serine sulfhydrylase, EC 4.2.99.8) or the cystathionine-γ lyase, EC 4.4.1.1. (Liersch et al., Experientia *33*, 1688, 1977). The important function of cystathionine-γ lyase in the biosynthesis of the antibiotic cephalosporin C has been reported earlier (Treichler et al., in: Antibiotics and other Secondary Metabolites, p. 177. Eds Hütter et al. Academic Press, New York 1978).
In this communication we report on our investigations of the function of cysteine synthase in the biosynthesis of cephalosporin C. The experiments have been carried out with ether permeabilized mycelia of the fungus grown in a complex production medium commonly used for antibiotic production. Inorganic sulfate (SO_4) and DL-methionine (met) plus SO_4 were used as sulfur sources. For the experiments the following strains have been used: *C. acremonium* 8650/1326-S^+ and 8650/1326-S^+/OT 28 met rev 20. The last strain was a mutant with a 75% reduced cysteine synthase activity.
In the presence of SO_4 the activity of cysteine synthase in strain 8650/1326-S^+ increased very rapidly between 30 and 50 h and remained constant for the rest of the fermentation period. SO_4 together with met in the culture medium caused the specific activity to drop about 6-fold. In the other mutant 8650/1326-S^+/OT 28 met rev 20 the activity of the cysteine synthase was about 4 times lower. During fermentation its specific activity varied slightly and met decreased it.
With both strains of *C. acremonium* almost the same antibiotic production was achieved when SO_4 was used as S-source and met stimulated antibiotic synthesis about 5-fold. According to these results it may be concluded that cysteine synthase is of little importance for antibiotic synthesis.

Correlation between β-lactam antibiotics production and γ-cystathionase activity

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Regulation of sulfur metabolism plays an important role in β-lactam antibiotic formation because of the existence of multiple pathways. *Cephalosporium acremonium* prefers methionine to sulfate as a sulfur source for optimal cephalosporin C synthesis whereas in *Penicillium chrysogenum* the 2 sulfur sources are equally well converted into penicillin. Treichler et al. (The 5th FEMS Symposium, Basel, Switzerland, p. 177–199) demonstrated that cephalosporin C synthesis depends on a functional γ-cystathionase. Our aim was to show that β-lactam antibiotic production is correlated with γ-cystathionase activity.
We investigated the expression of γ-cystathionase of *C. acremonium* and *P. chrysogenum* in respect to the sulfur source. When grown in inorganic sulfur compounds and harvested after 72 h enzyme activity of *P. chrysogenum* and *C. acremonium* were 4.2×10^{-3} μmoles/mg min and 3.2×10^{-3} μmoles/mg min, respectively. However, when grown on methionine activity of the *C. acremonium*-enzyme increased to 8.6×10^{-3} μmoles/mg min whereas activity of the *P. chrysogenum*-enzyme still remains at the same level, i.e. 3.9×10^{-3} μmoles/mg min.
Furthermore, we investigated the progress of the γ-cystathionase activity of *C. acremonium* (once grown in methionine and once grown in thiosulfate) within a period of 4 days and compared these results with the production of cephalosporin C. γ-Cystathionase activity was proportional to cephalosporin C formation, indicating that a correlation must exist.
Expression of γ-cystathionase and correlation of its activity with cephalosporin C production are in good agreement with the differences in cysteine biosynthetic pathway of these 2 organisms. As in *P. chrysogenum* only the pathway leading from homocysteine to cysteine seems to be operative, its expression should not be influenced by exogenous sulfur source. That's why expression of γ-cystathionase and production of β-lactam antibiotics depends not on the sulfur source.
Considering that *C. acremonium* possess a second alternative pathway for cysteine synthesis (via O-acetyl-L-serine sulfhydrylase) which is repressed by exogenous methionine it becomes evident that the other pathway (via γ-cystathionase) is also controlled by the sulfur source, which leads to different antibiotic production.

Regulation of cephalosporin biosynthesis in *Cephalosporium acremonium*

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To facilitate the analysis of growth and cephalosporin production by *C. acremonium* a simple synthetic medium containing only one carbon and one nitrogen sources (plus methionine as sulfur donor) was developed. Under batch conditions with glucose as the carbon sources, a maximum yield of 1.1 g/l cephalosporin (cephalosporin C + deacetyl cephalosporin C) could be obtained provided inorganic phosphate (P_i) was present in growth-limiting amounts and nitrogen was in excess. Initial concentrations of more than 2–3 mM P_i accelerated substrate consumption and growth and reduced antibiotic production. A 50% reduction of cephalosporin synthesis was observed at a level of about 12 mM P_i. The negative effect of high P_i concentrations could be overcome by limiting the glucose supply.
Analysis of the batch fermentation kinetics indicated that

most of the antibiotic was synthesized during growth. However, the specific rate of cephalosporin production (Q_p) was at the maximum at the moment when growth was beginning to slow down. Q_p decreased rapidly from its maximum value towards the end of the fermentation. This decrease could be delayed by supplying the culture with additional glucose. As a result the period during which cephalosporin was produced was prolonged and a higher cephalosporin titer was reached. By shifting the culture from batch to continuous growth conditions it was possible to stabilize Q_p at the comparatively high level of 1.8 mg cephalosporin per g dry weight per hour.

Production of methane and carbon dioxide in sanitary land-fills

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It is known that anaerobic fermentation of organic substances generates considerable quantities of methane and carbon dioxide. This phenomenon is potentially of great use as the most economical means of eliminating domestic refuse from modern sanitary land-fills.
Centralization of land-fill sites, very large daily deliveries of refuse, modern methods of depositing it, compacting it in layers and covering it create ideal conditions for the anaerobic fermentation of the organic material it contains.
It has been estimated that Swiss domestic refuse is composed of 45% of organic material which would produce about 200 Nm^3/t in 20 years, of which 40–60% would be methane and 60–40% carbon dioxide (1 Nm^3 of methane is equivalent to about 1 liter of light heating oil). It should not be forgotten that the gases from the anaerobic fermentation process contain small quantities of mercaptan, and are therefore malodorous.
To make a quantitative analysis of this process it is only necessary to know the average annual production of refuse per person per year, which is about 300 kg. In order to make use of this energy potential the people in charge of the land-fills need to take the following steps:
1. Limit or eliminate the danger due to the presence of inflammable gases (CH_4).
2. Limit or eliminate the danger due to the absence of oxygen.
3. Limit evil odours caused by anaerobic fermentation of refuse.
4. Develop a system for the recovery of methane and carbon dioxide.
The sanitary land-fill of Croglio, which is the property of the 'Consorzio Eliminazione Rifiuti del Luganese (CER)' has strong emission of gases and it is estimated that the daily production of methane is in the order of 1000 m^3. Experiments made at the land-fill site of the CER have given encouraging results as far as the possibility of control and utilization of the gas emissions is concerned.

Methane formation from acetate: Isolation of a new Archaebacterium

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A methanogenic bacterium, commonly seen in digested sludge and referred to as the 'fat rod', was isolated. Cells are gram-negative, nonmotile and appear as straight rods with flat ends. They form filaments which can grow to great length. The organism grows on a mineral salt medium with acetate as the only organic component. Acetate is the energy source, and methane is formed exclusively from its methyl group. Acetate and carbon dioxide act as sole carbon source and are assimilated in a molar ratio of about 1.9:1. The reducing equivalents necessary to build biomass from these 2 precursors are obtained from the total oxidation of some acetate. Acetylated compounds (e.g. acetyl phosphate, acetyl chloride, N-acetyl glucosamine, diacetamide, acetoacetic acid) can replace acetate as methane precursor. Hydrogen is not used for methane formation and is not needed for growth. Coenzyme M was found to be present at levels of 0.35 nmoles per mg of dry cells, and F_{420} amounted to 0.55 µg per mg protein. The mean generation time was 9 days at 33 °C, the molar growth yield ($Y_{acetate}$) was 1.1 g biomass per mole, and the half saturation constant K_s for acetate was 0.46 mM.

Relationship of marine fungi to edible *Mytilus* species

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From edible *Mytilus* species collected in the Neapolitan gulf, near Vico Equense, the isolation of fungal flora, present in these animals, was carried out.
This work was aimed at clarifying: a) The relationship of fungi to *Mytilus;* b) the origin of the mycosporine like, found in *Mytilus* itself.
The fungal flora found in these animals, for the greater part, belong to the higher fungi (ascomycetes and imperfecti fungi) and a small amount to the lower fungi (Thraustochytriaceae). The relationship of the higher fungi to these *Mytilus* seems to be an example of a neutral facultative symbiose.
The lower fungi are typical aquatic fungi. Their relationship to the *Mytilus* seems to be an example of an antagonistic facultative symbiose.
Regarding the origin of the mycosporine found probably it is yielded by fungal flora present in this *Mytilus.* In fact we found that a higher fungus, *Graphium* sp., isolated from *Mytilus,* yields the mycosporine like.

Microbiological quality of food pasta

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During the years 1977 and 1978 we have performed the microbiological analysis on 70 samples of food pasta, such as: noodles, ravioli, cannelloni, tagliatelle, tortellini, gnocchi, lasagne, etc. The determination of total germs, staphylococci, yeasts, molds and *Escherichia coli* was carried out in the following media: Tryptone glucose yeast agar, Vogel and Johnson agar, dextrose salt agar and violet red bile agar.
The following results were obtained: 7.14% had less than 1000 total germs, 18.57% had between 1001 and 10,000 total germs, 24.28% had between 10,001 and 100,000, 22.85% had between 100,001 and 1,000,000 and 25.71% exceeded 1 million, the maximum rate being of 60,000,000 total germs per gram. Concerning staphylococci, 62.85% had less than 1000/g, 14.28% had between 1001 and 10,000/g, 12.85% had between 10,001 and 100,000/g and 10% exceeded 100,000 g, the maximum rate being 700,000 staphylococci per gram. 11.42% of the samples contained more than 100 yeasts per gram. Molds as well as *E. coli* were completely absent. This study shows that 'handmade' food pasta is clearly more contaminated than industrial food pasta.

The detection of the degradation of certain β-lactams by β-lactamases by means of a very sensitive method

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On a petri dish, seeded as for an antibiogram, near the β-lactam disk, is put a second disk, containing a cell free extract of a bacteria on study. If the second disk contains a β-lactamase able to destroy the antibiotic which is near, the inhibition zone is altered (Matsuda et al., J. Antibiot. *29*, 662, 1976). This technique is semiquantitiative, if one uses disks containing β-lactamase at various dilutions (by a factor 10). Then the last dilution (LD) which makes a detectable degradation of the antibiotic is scored. This method allows detection of very low β-lactamase activities, or very small amounts of antibiotic degradation.
With a sensitive *Escherichia coli* strain, ampicillin, carbenicillin and ticarcillin are not destroyed, even with undiluted preparations, while cephalothin hydrolysis is significatve at the 10^{-2} LD.
With a cephalosporinase producing *E. coli* strain, the LD-values are respectively 10^{-1}, 1, 1 and 10^{-4}, and azlocillin 10^{-1}, cefoxitin 10^{-2}, cefuroxime 10^{-1}, cefamandole 10^{-2} and HR 756 10^{-1}.
The hydrolysis of the 'β-lactamase resistant cephalosporins' is not an artifact as: 1. The cephalosporinase producing strains present MIC to these antibiotics higher than those of the non-β-lactamase (or low) producing strains. 2. Cloxacillin which is a powerfull inhibitor of the cephalosporinases gives a very interesting synergism with cefoxitin, cefuroxime, cefamandole and HR 756.

Studies on the integration of the drug resistance plasmid R100.1 into the bacterial chromosome

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We have studied some properties of R100.1 plasmids inserted, by integrative suppression of a *dnaAts* mutation, into the bacterial chromosome and of R-prime plasmids (R100 carrying a bacterial chromosome segment) derived from them. We have found that:
1. R100 can integrate, by a 'reverse transposition', by means of the IS10 sequences of its tetracycline resistance transposon.
2. In such integration events there appears a new plasmid-like structure constituted by the r-determinant of R100.
3. An R′-*arg*$^+$ factor, derived from such an integrated R100, continues to produce r-determinant plasmids.
4. An R-plasmid, derived from R′*arg*$^+$ by deletion of the chromosomal segment and of the 2 IS10 sequences flanking it, produces r-determinant plasmids.
5. In all cases the appearance of the r-det plasmid depends on bacterial recombination functions *(recA* and *recBC)*.
6. Integrated R-plasmids have unusual incompatibility properties.

Epidemiological interest of molecular analysis of plasmids: R-factors isolated from *Klebsiella pneumoniae*

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2 patients, A and B, having shared for some days the same room in the Surgical Intensive Care Unit of the Geneva Cantonal Hospital, developed urinary tract infections. Patient A was first infected; 2 colony types of *K. pneumoniae* (types IA and IIA) were isolated from the urine; both types could be found also in the stools. Patient B developed his urinary tract infection 10 days after the departure of patient A from the room; 1 colony type of *K. pneumoniae* (type IB) could be isolated from the urine, although the stools contained 2 types (types IB and IIB).
According to the API 50 E system and other criteria, types IA and IB belong to the same biotype, and types IIA and IIB to a different one.
All these *Klebsiella* strains shared the same resistance pattern: Ampicillin, carbenicillin, chloramphenicol, tetracyclin, streptomycin, kanamycin, gentamicin, tobramycin, sulfonamid and mercuric ions. Conjugation experiments showed that all the resistances are transferred 'en bloc' at low frequency. Moreover, the transfer is thermosensitive and the plasmids, when harbored by *Salmonella*, induce lysotype modification and resistance to phage 0:1. These plasmids are *fi*$^-$, but incompatible with F in the autonomous state. However, they are compatible with IncH1 and IncH2 plasmids. Further investigations will determine to which Inc group they belong. Plasmids were isolated by the method of Hansen and Olsen and cleaved by different endonucleases; comparison of the restriction patterns obtained showed that all the plasmids isolated from the different strains are probably the same. From these experiments, the mol. wt of these resistance factors was assumed to be 148 ± 10 Mdaltons for 224 ± 15 kb.

Transfer of the plasmid pPJ3a by an ampicillin transposon

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pPJ3a is a 9.2-kb long plasmid conferring resistance to streptomycin (Sm) and sulfonamides (Su); its derivative pPJ3b has received the ampicillin (Ap) transposon TnAp (which is strongly related to Tn3): it codes for SmSu Ap resistance and is 14.1 kb long.
Infection of *Escherichia coli* C600 (pPJ3b) with λbb (λb515b519c1857Sam7) followed by 3 cycles of lysogenization and induction results in lysates able to transduce resistance to Sm, Su and Ap at high frequency. SmSu resistance was always associated with Ap resistance. Heteroduplex analysis of the DNA purified from one of these lysates showed that it is composed of λbb infectious particles and of λbb defective particles, the DNA of which contains pPJ3a flanked by 2 Ap transposon copies in opposite orientation, the whole structure being surrounded by 9.0 and 19.2 kb λDNA (corresponding to the extreme regions of λbbDNA).
Analysis of the DNA purified from another SmSu Ap transducing lysate showed that the DNA of these defective particles is composed of pPJ3b surrounded by 14.9 and 8.4 kb extreme regions of λbbDNA. These 37.5 kb DNA molecules circularize in *E. coli* cells and behave like plasmids. Moreover, occasionally, they give rise to smaller DNA circular molecules (13–16 kb long) which confer resistance to Ap, Sm and Su. The mechanism of formation and the properties of these molecules will be discussed.

Genetic analysis of the transfer system of RP1

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A large number of transfer-deficient *(tra)* point mutants of RP1 have been isolated including a number of amber mutants. These *tra* mutants can be subdivided into 3 classes

on the basis of their responses to the male-specific phages PRR1, Pf3 and PR4: 1. $PRR1^s$ $Pf3^s$ $PR4^s$, 2. $PRR1^R$ $Pf3^R$ $PR4^s$ and 3. $PRR1^R$ $Pf3^R$ $PR4^R$.
To facilitate complementation analysis of these mutants, a region of the RP1 molecule carrying a number of transfer genes has been cloned. Using an RP1 *tet, kan*::Tn7 derivative, the region between coordinates 25 and 38 Mdaltons was recombined in vitro into the vector plasmid pBR325.
The resultant plasmid, pED700, was found to be compatible with RP1 and transfer deficient. Complementation tests showed that pED700 is able to complement all the mutants belonging to classes 1 and 2 but only some of the class 3 mutants. pED700 can be mobilized from a $RecA^-$ host by an RP1 derivative indicating that the recombinant plasmid carries the orgin of transfer *(oriT)* of RP1.
These results together with studies of *tra* deletion mutants indicate that the transfer genes of RP1 are located in at least 2 regions of the RP1 molecule as has been previously demonstrated for the plasmid RP4 (Barth et al., J. Bact. *133*, 43, 1978).

The DNA sequence of a small transposon coding for resistance to chloramphenicol and fusidic acid

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The r-determinant of NR1 is a 20-kb segment of DNA flanked by 2 IS1 elements in the same orientation. It carries genes specifying, in this genetic order, resistance to Cm (chloramphenicol), Fa (fusidic acid), Sm (streptomycin), Su (sulphonamide) and Hg (mercury). This determinant has been transposed into the bacteriophage P1 genome and IS1-mediated deletions into the transposon have been selected that retain the Cm marker (W. Arber, S. Iida, H. Jütte, P. Caspers, J. Meyer and C. Hänni, Cold Spring Harb. Symp. quant. Biol. *43*, in press 1978). The smallest transposon obtained in this way contains about 0.9 kb of DNA between the IS1 elements and, surprisingly, still confers Fa resistance.
We have determined the DNA sequence of this transposon for the following reasons: a) The size of the Cm resistance protein is known to be 218 amino acids (W. Shaw, unpublished) and thus requires at least 0.7 kb for the gene and the associated control regions. Thus Fa resistance must either be a function of a small peptide, be a second activity of the Cm resistance protein, or be the product of a gene that overlaps with that for Cm resistance. b) The expression of Cm resistance in gram-negative bacteria is known to be under the control of catabolite repression. We therefore expected to find a binding site for the CAP protein somewhere ahead of the Cm gene. Since the sequence of only 2 CAP binding sites are known at present this would be of considerable interest.
The DNA sequence of the transposon will be presented and its main features will be discussed.

Characterization of multiresistant clinical isolates of *Acinetobacter calcoaceticus*

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During a 3-month period, we observed a hospital epidemic in the intensive care unit of the university hospital caused by multiresistant strains of *A. calcoaceticus*. The isolated bacterial strains were resistant to: ampicillin (Am), chloramphenicol (Cm), sulphonamide (Su), tetracycline (Tc), gentamicin (Gm), kanamycin (Km) and streptomycin (Sm). They were susceptible to tobramycin (Tm) and amikacin (Ak).
3 aminoglycoside inactivating enzymes were detected from representative cultures. These were the 3-N-acetyltransferase I [AAC (3) I], the 3″-O-adenylyltransferase [AAD (3″)] and the 3′-O-phosphotransferase I [APH (3′) I]. The phenotype of resistance to aminoglycosides in the isolates correlates with the substrate profile of the 3 enzymes.
Gentamicin resistance could be eliminated by treatment of cells with ethidium bromide. In some clones simultaneous loss of resistance to Am, Su, Tc and Km was observed. Plasmid DNA analysis by agar gel-electrophoresis of wild-type strains and susceptible variants did not show any difference. Both kinds of strains contained a 6×10^6 daltons plasmid which is probably cryptic. Transfer of resistance to appropriate receptors by conjugation could not be achieved. However, the plasmid RP_4 introduced into a Km^s variant of *A. calcoaceticus*, was able to mobilize and transfer markers for resistance to Cm, Su, Tc and Gm to *E. coli* K-12. Analysis of DNA revealed that transconjugants contained a single plasmid, between 15 and 30 Mdaltons higher in mol.wt than RP_4.
From these data it is assumed that resistance determinants of the wild-type strains are integrated into the chromosome. The DNA sequence carrying the R-markers can be lost from the chromosome rather frequently by excision or can be translocated to other DNA-replicons, both characteristic of transposable elements.

In vitro sensitivity of *Haemophilus* to antibiotics and special problems of the β-lactams

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C. Thornsberry et al. (Cumitech *6*, Am. Soc. Microbiol. 1977) proposed to determine the sensitivity of *Haemophilus* by the disc method using a bacterial density of 10^8 CFU/ml. We have been working with an inoculum of 10^7 CFU/ml. The medium we use is Müller Hinton's agar (BBL) + 5% human blood + 1% IsoVitaleX (BBL). The inhibition zones produced by the discs impregnated with antibiotic of the β-lactam family are turbid; we have even observed 2 concentric turbid zones. The presence of spheroplasts is responsible for this turbidity; we verified that it was not caused by colonies resistant to the antibiotic (absence of β-lactamase, sensitivity of each colony identical with that of the strain itself). With 10^7 CFU/ml, determination of the sensitivity of *Haemophilus* by the diffusion method is not accurate. It may also be noted that by using a bacterial density of 10^7 CFU/ml the MIC determined by the dilution method is $\geqslant 128$ µg/ml. These spheroplasts may be removed by using an inoculum of 10^5 CFU/ml. The zones of inhibition are then clear. This phenomenon is not present by discs impregnated with erythromycin, tetracyclin and chloramphenicol. In accordance with the above details concerning the medium and the density of the inoculum to be employed, we can give our results of the determination of the sensitivity of 250 strains of *H. influenzae* and *parainfluenzae* isolated at Lausanne. All the strains are sensitive to ampicillin; the value of the MIC determined by dilution in agar, in broth and by reporting the diameter of the inhibition zones on the regression line of ampicillin is identical: average MIC 0.5 µg/ml. All the strains tested are also sensitive to chloramphenicol: average MIC 1.3 µg/ml. Tetracyclin has been found to be very active in vitro; only approximately 5% of the strains have a MIC above 2 µg/ml but ≤ 16 µg/ml (maximum value to declare a strain to be resistant). All the others have a MIC ≤ 2 µg/ml. For erythromycin the sensitivity of our strains varies between 6% (1976-1977) and 22% (1978).

Susceptibility testing of *Haemophilus influenzae* and intensity of action of ampicillin and chloramphenicol

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M.I. Marks et al. (Antimicrob. Ag. Chemother. *8*, 657, 1975), C. Thornsberry et al. (Antimicrob. Ag. Chemother. *9*, 70, 1976), I.B. Mayo et al. (Antimicrob. Ag. Chemother. *11*, 844, 1977), and W. Fleming et al. (Antimicrob. Ag. Chemother. *13*, 791, 1978), have described micromethods for the determination of the sensitivity of *Haemophilus* with antibacterial agents. We present the method employed by the Institute of Microbiology of Lausanne: Müller Hinton broth with an admixture of 5% of supplement C; serial dilutions of the antibiotic with a Dynatech® microdilutor; inoculum 10^5 CFU/ml; the time of incubation is 20 h in an atmosphere enriched with CO_2; readings are done by using a mirror to determine visible growth, or by microscopic examination. The MIC is defined as the last dilution of antibiotic showing no growth, or in cases difficult to read, as the last dilution where morphological alterations and the absence of normal coccobacillary forms can be observed. The MBC is determined by inoculating each well showing no growth into an appropriate agar medium. It is defined as the last dilution of antibiotic showing no growth.

In this way we have tested 43 strains of *Haemophilus* with respect to chloramphenicol; the average MIC determined by micromethod is 1.4 µg/ml; the average MBC 2.7 µg/ml. Compared with the MIC and MBC determined by broth dilution method the values are: MIC: 1.3 µg/ml; MBC: 3.6 µg/ml; the agreement of results is satisfactory. As shown by C.E. Turk (J. med. Microbiol. *10*, 127 1977) chloramphenicol cannot be considered as truly bacteriostatic for *Haemophilus*. With regard to ampicillin, the average MIC determined for 39 strains by micromethod is 0.4 µg/ml. For 33 of these strains the average MBC is 0.4 µg/ml; it varies between 8 to more than 32 time for the other 6 strains. The values found with the broth dilution method are: average MIC: 0.3 µg/ml, and average MBC: 0.5 µg/ml.

Multiresistant strains isolated in Lausanne (Switzerland) 1976–1977

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Antibiotic resistance of bacteria is single or multiple. Single resistance is an easy problem but multiple resistance may lead to a difficult choice between more toxic drugs and even preclude antibacterial chemotherapy. Hence epidemiology and prevention of multiple, infectious, plasmide coded resistance is an important research objective. Statistical results of local surveys in 1976 in Lausanne (Vrantchev et al., Revue méd. Suisse romande *97*, 551, 1977) were compared to those of 1977 and were found to be similar. We present data pertaining to hospitalism agents *(Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* sp., *Escherichia coli)* and which represent 58% of the total number of bacterial strains isolated from clinical specimens in the hospital in 1976 and 1977. They were tested with antibiotics which were either the most frequently utilized, or the less frequently utilized or exhibiting different resistance mechanisms. *Staphylococcus aureus:* Methicillin, cotrimoxazol, erythromycin, streptomycin, tetracyclin, sulfonamide and penicillin G. In 1976, 10% of 1823 strains were sensitive, 2% were resistant to all 7 antibiotics. The proportion of inefficient antibiotics was 29%, i.e. 2.04 antibiotics. In 1977 the proportion of inefficient antibiotics was 30,670, i.e. 2.14 antibiotics.

Pseudomonas aeruginosa: Tobramycin, kanamycin, carbenicillin, tetracyclin, polymyxin, gentamicin. In 1976, 4% of 1620 strains were resistant to all 6 antibiotics. The proportion of inefficient antibiotics was 37.4%, i.e. 2.24 antibiotics. In 1977, inefficient antibiotics were 32.7%, i.e. 2.0.

Klebsiella sp.: Ampicillin, streptomycin, tetracyclin, kanamycin, cefalotin, chloramphenicol, cotrimoxazol. In 1976, 2.5% of strains were resistant to all 7 antibiotics.

Escherichia coli: Ampicillin, streptomycin, tetracyclin, kanamycin, cefalotin, chloramphenicol, cotrimoxazol. In 1976, 44% of 3933 strains were sensitive, 1.5% were sensitive to all antibiotics. In 1977, the picture was identical.

Resistance patterns: as an example, *E. coli* exhibits 14.5% strains resistant to 1 antibiotic, 11.8% to 2 antibiotics, 8% to 3. In spite of some large groups of identical resistance patterns, there is very varied distribution of patterns among the other strains representing 48% of double resistants and 75% of triple resistants.

Results from a Swiss multiregional drug resistance prevalence assessment

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Monitoring of drug resistance of bacteria appears necessary at the regional, national and international levels[1,2]. Over a 2-week period in October 1978, 2738 strains of *Escherichia coli* and coliforms were freshly isolated from stool specimens obtained from 19 civilian hospitals (1408 strains) and from 9 Swiss army training settings (1330 strains) distributed all over Switzerland. In addition, 209 staphylococcus-like organisms were isolated from 1416 stools.

The strains, distributed among 7 simultaneously working Swiss army microbiology field laboratories were tested for resistance against ampicillin, gentamycin, tetracyclin, cotrimoxazol and methicillin (the latter against staphylococcus-like isolates only), using conventional filter disc techniques. Criteria for inhibition zone interpretation were adapted from NCCLS guidelines[3]. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 served as control strains in all laboratories.

The overall resistance prevalences of *E. coli* and coliforms and *S. aureus* and staphylococcus-like organisms were 18%, 0.3%, 35% and 4% against ampicillin, gentamycin, tetracyclin and cotrimoxazol, and 12%, 21%, 45%, 3% and 8% against the same drugs plus methicillin, respectively.

The findings concerning multiple drug resistance patterns will be presented and possible effects of origin, sex, age, profession and medical history of stool donors on drug resistance prevalences in Switzerland will be discussed.

1 S.S. Schneierson, J. Lab. Clin. Med. *40*, 48 (1952).
2 P.E.G. Chemotherapie, Infection *6*, 35 (1978).
3 National Committee for Clinical Laboratory Standards: Performance standards for antimicrobial disc susceptibility test. Villanova, USA, 1975.

R-factor prevalence in animal feces 6 years after prohibition of R-selecting antibiotics as feed addition

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After years of discussing the danger of antibiotics in animal feeds, above all regarding the prevalence of R-factors, the Federal Council enacted in 1972 a prohibition of R-factor selecting, therapeutic antibiotics as feed addition.

Now, 6 years after this legal measure, we wanted to control its effect by testing the feces of 42 cows and 21 calves from 5 different farms in regards to the R-factor content: All calves and half of the cows showed R-factors in 20–100% of their colibacteria. Thus the R-factor prevalence among the tested animals was about the same before and after the prohibition, and the expected regression of R-factors had not taken place. The reason for this failure is discussed.
Since the abuse of antibiotics in animal feeds obviously cannot be avoided, and since R-selection also results from veterinary antibiotic treatment, the following question arises: Would it be possible to introduce a strict separation between antibiotics used in human medicine and antibiotics used in veterinary medicine, whereby the latter use necessitates non-R-factor-selecting antibiotics?

Action value of the sampling of the bacteriological environment in hospitals

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The standardization of bacteriological samples has been done within the framework of environmental controls and was based on published data as well as on experience acquired since 10 years in Lausanne.
The samplings of the bacteriological environment are used to control the efficacy of the daily procedures used to remove all bacteria due to patients and clinical personnel. These procedures are prophylactic. The elimination of bacteria from the environment is achieved by a once daily cleaning with a disinfectant with detergent effect producing a systematic disinfection. The samples give an objective index of the value of the total procedure applied against hospital infection.
The swab rinse method or better the Rodac plate method with media containing compound neutralizing the disinfectant give qualitative (presence of pathogens) as well as quantitative results (colony counts).
The results are classified and numerically coded from 0 to 4. Class contents can vary according to the site of sampling as well as to the relationship of qualitative and quantitative results. As an example, a valuation 0 (satisfactory) may cover results of 0 to 6 colonies obtained on the wall of an operating room whereas it may cover up to 30 colonies obtained on the floor of a patients room. However, if pathogens occur within these 30 colonies, the valuation is pushed back to 2. The implication of this code for the hospital is that action is to be taken if codes from 1 to 4 are presented. The gradation works as follows:
1. Control of the effective application of the current procedure.
2. Control and correction, if needed.
3. Corrective measures are necessary.
4. Emergency action is to be undertaken.

Epidemiological control of *Staphylococcus aureus* cross-infection by serotyping. Its relations with resistance to antibiotics

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The serotyping of staphylococci can be systematically utilized for the control of cross-infection in a hospital, as it allows investigation of a large number of strains. The typing was done with the method of Cowan, modified by Pillet, using his sera received from Pasteur Institute, Paris.
S. aureus strains were isolated from the following sources:
1. Pathological samples received for bacteriological diagnosis.
2. Isolation of carriers in the hospital personnel by nasal smears.
3. Weekly fecal cultures from newborn and operated children.
541 strains were serotyped.
The prevalence of the different types is as follows:
39.37% for type I; 6.28% for type II; 5.36% for type III; 7.76% for the combined type II/I; 3.33% for the combined type III and II/I; 1.11% for type 14; 1.11% for type 18; 3.14% for the combined type I–II.
26.62% of the strains are not typable and 5.89% represent the uncommon combined serotypes.
Antibiotic resistance to 12 different antibiotics was studied for 219 strains: 87.21% of these strains are resistant to penicillin; none of the strains are found resistant to the 12 antibiotic tested.
There is no correlation between serotyping and antibiotic resistance. Nevertheless, the multiresistance of the hospital types becomes evident: 80% for type 14; 60% for type 18 and even 27.27% for type III.
We consider cross-infection in a hospital environment when the identical serotype is found in 2 or more individuals at the same time and at the same place (same ward or same personnel).
In these cases, we look for the source of the infection, by detecting the carriers and the germs in the environment, controlling the techniques of care, the hygiene rules and when the occasion arises, the isolation techniques.
In the above studies, the prevalence of 39.37% of type I – which is not a hospital serotype – confirms the very weak contamination transmitted in the hospital and consequently the low incidence of cross-infection. This is also supported by the findings of 26.62% of non typable strains, to which can be added 5.89% of uncommon combined serotypes.

Survey of the occurrence of streptococci of groups A, B, C, G from 1972 to 1978 in Lausanne

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From 1972 to 1978, all streptococcus isolates were submitted to the Lancefield or to the Fuller's technique in order to extract the C-polysaccharide and to identify them with the specific precipitating sera from the Czech Institute of Sera and Vaccines.
The prevalent streptococci belonged to the groups A, B, C and G. Those which could not be assessed to a particular group were considered as non groupable. Group D was excluded. In each group, for each 3-month period (winter – January, February, March –, spring, summer and fall), the total number, the number of throat isolates and the number of pus isolates were plotted.
Group A. Throat streptococci were present in all seasons, but their frequency was highest in fall and/or winter.
Air-borne infections were not alone since a deep pathology runs throughout the years at a fairly constant rate and seems rather independent from air-borne mechanisms of transmission.
Group B. Essentially deep pathogens, the B-streptococci sometimes behave as air-borne pathogens (one epidemic peak in winter 1978 for instance).
Group C. As for A, most infections are air-borne in epidemic periods. The deep infections run at their own rate.

Group G. Infections due to the streptococci occur as throat or deep infections with alternating frequencies.
Streptococcus infections due to all 4 principal groups are thus truly endemic with seasonal, epidemic peaks.

Hospital infections by *Pseudomonas cepacia*

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Several episodes have been described, which are all caused by medicaments (Phillips et al. 1971, D.C. Speller et al. 1971, De Mol 1979, Zech 1979). The salient feature of these infections is the sudden onset, the big number of patients affected in a short lapse of time, the spectacular appearence of the hyperthermia with shivers, and the sedation of every symptom once the administration of the drug has been stopped. In most cases, it could become evident that the source of illness was the contamination of a commercial pharmaceutical preparation (anesthetics, anticoagulants or even disinfectants in aqueous solution) by *P. cepacia.*
Our experience with the epidemiology of *P. cepacia* has been a great puzzle as at the time of an infectious episode we could not implicate it with any patent drug.
11 patients, within a course of six weeks, had a septicaemia by *P. cepacia,* showing the same clinical picture: high and abrupt hyperthermia with shivers. From every patient we received blood cultures, which were positive for *P. cepacia* in the half of the samples. The only common factor to these 11 patients had been the blood transfusion. But, on one occasion only, we could obtain the remaining of the transfused blood, from which we were able to isolate *P. cepacia.*
The department of blood transfusion was fully investigated, but this germ could not be found. However, it was isolated from the water-baths used for warming the blood.
The profil of this strain reveals: It grows on nutrient agar, Mac Conkey and cetrimide agar media. It is motile, grows well at 30 °C and is strictly aerobic. OF-test (0)+; it oxidizes glucose, lactose, maltose, mannitol and cellobiose. H_2S −; reduction of NO_3 −; oxydase weakly +; L-lysine decarboxylase +; L-arginine dihydrolase −.
Antibiotic spectrum: Ampicillin R; carbenicillin R; gentamicin R; tobramycin R; polymyxin R; co-trimoxazole S; chloramphenicol S.

Occurrence and drug-resistance pattern of air-borne gram-negative bacilli in 2 hospital units

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The aim of this work was to determine the level of air-borne bacterial contamination in 2 distinct hospital units and to precise the drug-resistance pattern of these strains. The samplings have been done with a Joubert apparatus on TSA petri dishes. The identification of species has been done by the API 20 system and some other complementary biochemical tests. Drug-sensitivity tests have been executed according to the method of Chabbert (Institut Pasteur, Paris) and the interpretation of the level of drug-resistance estimated according to the manufacturer's proposals. 2 periods were concerned: December 1977 to July 1978 and February to May 1979. During the first period, the samplings have been done every 14 days, during the second period every week. The drug-sensitivity pattern has been determined for the most frequent antibacterial drugs used in the Geneva hospital (i.e. β-lactams, aminoglycosides and other important antibacterial drugs).
The results of these investigations led us to the following conclusions:
a) The level of air-borne contamination is quite constant in both period (77–78 and 79); the mean value is lower for the second period;
b) no significant difference can be detected between the 2 hospital units (1 opened, 1 closed);
c) the most frequent bacterial species are: micrococci (up to 80% of total bacterial count), diphteroids (40%), gram-positive bacilli (30%), gram-negative bacilli (20%);
d) among the gram-negative bacilli, *A. calcoaceticus (lwoffi* and *anitratus). Pseudomonas (maltophilia* and *fluorescens)* and *Enterobacter (agglomerans* and *cloacae)* are the most frequently encountered;
e) it must be pointed out that *Klebsiella, Serratia* and coliform bacteria are found very rarely in our samples;
f) the results of drug-sensitivity tests for 12 antibacterial agents concerning *E. agglomerans, E. cloacae, Citrobacter* sp., *P. maltophilia, P. fluorescens, A. calcoaceticus* and other nonfermentative gram-negative bacilli will be discussed.

Resistance of *Pseudomonas aeruginosa* to high levels of nalidixic acid

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In populations of *P. aeruginosa* strain PAO, mutants resistant to high concentrations (≥ 2000 µg/ml) of nalidixic acid (NAL) occurred at frequencies of 10^{-8} to 10^{-9}. The mutations conferring high level NAL resistance were found to be closely linked and probably fall into one locus, designated *nal.* The *nal* gene has been mapped by conjugation and transduction; it lies between *hex-9001* (30–40% cotransduction) and *leu-10* (1% cotransduction) in the 60-min region of the chromosome.
The minimal inhibitory concentrations (MIC) of NAL were about 50 µg/ml for nal^+ strains and ≥ 2000 µg/ml for *nal* mutants on nutrient agar. All *nal* mutants examined exhibited cross-resistance to 2 NAL analogs, pipemidic acid and piromidic acid. However, the MIC of pipemidic acid was only about 5 times higher for *nal* mutants (MIC = 50–400 µg/ml) than for the parent strains.

Protein synthesis in cells of *Escherichia coli* permeabilized with ether

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In recent years, several attempts have been made to assay complex bacterial enzyme reactions (replication, transcription, synthesis of peptidoglycan) under conditions resembling as closely as possible those in intact living bacteria. One approach has been to render whole cells permeable to molecules such as nucleoside triphosphates by various methods.
The present work describes a method to measure protein synthesis in *E. coli* MRE 600, RNase I⁻ permeabilized with ether. Permeabilization was performed by a simplified version of the method described by Vosberg and Hoffmann-Berling (J. molec. Biol. *58,* 739, 1971). Viability of the bacteria was completely lost by this treatment. Protein synthesis was measured as incorporation from a mixture of radioactive labeled amino acids into hot trichloroacetic acid insoluble material. The complete synthesis mixture consisted of tris-acetate buffer, salts, all 4 nucleoside triphosphates, phosphoenolpyruvate, a mixture of ^{14}C-amino acids and about 5×10^7 cells per sample (100 µl). Polypeptide

synthesis was completely dependent on the addition of ATP and phosphoenolpyruvate and also required optimal concentrations of magnesium ions. Addition of chloramphenicol to the synthesis mixture resulted in a concentration-dependent inhibition of protein synthesis (50% inhibition at 10 µg/ml). Rifampicin did not influence the system and incorporation was stimulated by the addition of gentamicin. Storage of the ether treated cells in 50% glycerol at −20 °C for 4 weeks resulted in only negligible loss of activity.

Adhesiveness, hemagglutination and piliation of *Escherichia coli* grown in sub-MIC concentrations of antibiotics

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The adhesion of microorganisms to host epithelia is generally considered to be an important factor for their capability of colonizing epithelial surfaces and of subsequently causing disease. Hemagglutination techniques have often been proposed as convenient in vitro methods for the assessment of bacterial adhesiveness. Bacterial pili are thought to mediate hemagglutination as well as bacterial adhesiveness. We have compared the adhesiveness of 9 *E. coli* strains to the human epithelial cell line, Intestine 407 (ATCC CCL6), with their ability to agglutinate human erythrocytes, and with their piliation.

Several adhesive strains showed a decreased adhesiveness, when grown in the presence of sub-MIC concentrations (usually ¼ the MIC) of either tetracycline or clindamycin. In general, a loss of adhesiveness was paralleled by a decrease in the bacterial hemagglutination titer and in the piliation of the bacteria. There were, however, notable exceptions: 1. Only 4 of the strains (45%) agglutinated human erythrocytes at all. 2. In 1 strain neither tetracycline nor clindamycin influenced adhesiveness or hemagglutination, although piliation was clearly reduced. 3. An unpiliated strain adhered well to tissue culture cells, but did not hemagglutinate. 4. 2 additional strains did not adhere to tissue culture cells, but were piliated and hemagglutinated. Piliation and hemagglutinating titer were reduced after growth in the presence of tetracycline.

Our results show that adhesiveness of *E. coli* to the tissue culture cell line, Intestine 407, hemagglutination and piliation are independent properties, which may be affected differentially by antibiotic agents. Their relationship to bacterial adhesion in vivo remains to be demonstrated.

Synergism of 2 β-lactam antibiotics, benzylpenicillin and cefoxitin, in inhibiting growth and function of peptidoglycan DD-carboxypeptidase/transpeptidase in *Proteus mirabilis*

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P. mirabilis survives in the presence of a variety of β-lactam antibiotics added singly at high concentration by growing as spheroplast-type L-form. Synthesis of cell wall peptidoglycan continues in this state. Thus, main target enzymes of β-lactam action, peptidoglycan transpeptidase and DD-carboxypeptidase (CPase) must remain functional during growth with β-lactams. 2 isofunctional CPases L and H (comparable to CPase 1A and 1B of *Escherichia coli*) were purified from *P. mirabilis*. These were also characterized as penicillin binding proteins (PBP) 5 and 4, among 7 PBPs present. Sensitivity to benzylpenicillin (PenG) and stability of the enzyme-inhibitor complex EI^+ are low for CPase L and high for CPase H. Half-lives of EI^+s are 3.5 and 300 min, respectively, at 37 °C. Also, PenG is degraded to benzylpenicilloic acid during breakdown of EI^+ with CPase L. This explains why CPase L alone is able to continue to function during L-form growth with 120 µg/ml PenG and why PBP 5 remains free in L-form cells. – In a search for a permanent inhibitor of CPase L among a variety of β-lactam antibiotics cefoxitin was unique in forming a stable complex with this enzyme (half life 900 min) and also in inhibiting growth of *P. mirabilis* in combination with PenG. With cefoxitin alone (150 µg/ml) *P. mirabilis* grew well as L-form containing a functional CPase H. With this enzyme cefoxitin forms a short-lived complex, only. Thus, in *P. mirabilis* and probably in other problem bacteria of antibiotic therapy no single β-lactam antibiotic can effect permanent inactivation of all targets essential for viability. But application of suitable complementary pairs of β-lactams should achieve this aim.

Animal experiments for the evaluation of topical antimycotic agents

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In the evaluation of potential antimycotic agents the usual growth inhibition tests in vitro are of only limited value. This is obvious with respect to systemic activity which is essentially influenced by distribution and metabolism taking place in the host, but is also true for topical activity in superficial mycoses where appropriate penetration of the antimycotics into the tissues harboring the fungus, e.g. the cornified layer of the epidermis, must be guaranteed. Any activity in vitro is, furthermore, not only determined by the inherent antifungal power of the agent, but also by the methods used, i.e. by variables such as the inoculum size, incubation period, composition and pH of the culture medium and last but not least by the parameter (MIC or others).

The screening procedure in our laboratory is to apply the compounds topically to 2 superficial animal mycoses representing the 2 main superficial mycoses in man, candidiasis and dermatophytosis. 1. *Vaginal candidiasis in the rat:* Female rats, which are ovarectomized and kept in permanent estrus by means of estrogens, are infected vaginally with *Candida albicans* yeast cells. The compounds contained in an ointment base are applied vaginally twice daily for 3 days, starting 1 day after infection, when the mycosis has fully developed. The degree of infection is expressed by the colony count in cultures from the vagina, and significant reduction of this count (particularly a 50% reduction of its logarithm) is the parameter of activity. 2. *Experimental dermatophytosis in the guinea-pig:* Spores of *Trichophyton mentagrophytes* are rubbed into the shaven skin of the animals, where an inflammatory mycotic lesion develops with an incubation period of 3–4 days. Ointments with the compounds are applied to the same skin area for 11 consecutive days, in the 'simultaneous' treatment schedule starting 6 h, and in the 'therapeutic' schedule 3 days, after the infection. The main parameter of activity is complete prevention of the lesions.

The usefulness of the 2 models is exemplified by: *nystatin* and *gentian violet* which are active in model 1; *griseofulvin* and *tolnaftate* active in model 2, and by the imidazolyls *clotrimazole, miconazole, econazole* and *oxiconazol* which distinguish themselves by definite activity in both models.

Identification of leukocytic factors interfering with gentamicin bactericidal action in purulent exudates

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Purulent exudates have the property of binding aminoglycoside antibiotics avidly, leading to a dramatic reduction in the concentration of the bioactive drugs (Bryant and Hammond, Antimicrob. Ag. Chemother. *6*, 702, 1974). Purulent material is a complex cell suspension of live and lysed leukocytes, attracted into an infected focus in order to destroy invading bacteria. In an attempt to study the interaction mechanisms of the purulent material with antibiotics, a simplified experimental model was devised by incubating purified polymorphonuclear leukocytes (PMNL), isolated from human blood, in vitro with aminoglycoside antibiotics at physiological pH and ionic strength (Dulbecco's phosphate buffer saline solution). This simplified system allowed to identify 2 leukocytic factors interfering strongly with gentamicin bactericidal action:

1. 10^7/ml live PMNL actively engulfing a test strain of *Staphylococcus aureus* (Wood 46), were able to protect ingested bacteria from gentamicin concentration equal to 80 times its minimal bactericidal concentration in cell free medium. As only a minimal amount (< 100 ng) of ^{14}C-gentamicin was found to be associated with PMNL, we postulate that intact leukocytes preclude the aminoglycoside antibiotic to accumulate inside the cells and to interact with the target bacteria. Thus, permeability barrier of live PMNL is one factor interfering with gentamicin bactericidal action.
2. 10^7/ml freeze-thawed PMNL on the other hand bound a 100-fold larger amount (10 µg) ^{14}C-gentamicin than live cells, as defined by the amount of antibiotic cosedimenting ($3000 \times g$, 10 min) with particulate material from lysed PMNL. The sedimented antibiotic showed no detectable loss of biological activity, when eluted from the pellet and assayed microbiologically (Bennett et al., Appl. Microbiol. *14*, 170, 1966). Pretreatment of the particulate material with either RNase, proteinase K or Triton X-100 did not reduce the binding of ^{14}C-gentamicin. In contrast, pretreatment with pancreatic DNase I abolished the binding of ^{14}C-gentamicin. Similar results, showing extensive binding of gentamicin were obtained by using isolated chromatin from PMNL as the binding substrate. In conclusion, chromatin of lysed PMNL as well as permeability barrier of live PMNL are 2 identifiable factors interfering markedly with gentamicin bactericidal action in purulent exudates.

Studies of the bactericidal activity of antibiotics

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The membrane-transfer method permits the determination of the minimum bactericidal concentrations (MBC) of the antibiotics. The principle of membrane transfer described by Chabbert (1965) and Lorian (1974) was modified by us for use in the agar dilution test. Plates of DST agar in which the antibiotic dilutions are incorporated are dried at 40 °C for 20–30 min. Before inoculation with the bacteria to be tested, the surface of the agar is covered with a membrane (millipore filter 0.45 µm, HAWG 00010). Up to 36 bacterial strains per plate are deposited on the membrane with a Steers multipoint replicator, and the plates are then incubated for 24 h at 37 °C. The minimum inhibitory concentrations (MIC) are determined in the usual manner and the membranes transferred to plates of agar containing no antibiotic. The plates are left to stand for 1 h to allow the antibiotics to diffuse from the membrane into the agar; the membranes are thereafter transferred once again to plates of agar containing no antibiotic, which in turn are incubated for 24 h at 37 °C. The concentration at which the number of colonies is reduced by a factor of 3 $\log_{10}$ ($\pm$ 0.5 $\log_{10}$) in relation to the original inoculum is taken as being the MBC. Tests conducted in triplicate in which established laboratory strains and clinical isolates were exposed to 10 antibiotics showed that the MBC-values were just as readily reproducible as the MICs determined both by the membrane method and the conventional dilution method. On applying the membrane-transfer method in tests on 106 strains of *Staphylococcus aureus* originating from 3 clinical units and institutes in Switzerland, we found that rifampicin was the only drug that exerted a bactericidal as well as a bacteriostatic effect at all strains. By contrast, 10–30% of the strains displayed tolerance, i.e. MBC/MIC $\geq$ 32 (Sabath), upon exposure to the penicillins, including oxacillin, which is stable against penicillinase, and the cephalosporins, including cefoxitin and cefotaxime, which are stable against β-lactamases.

Bacteriophage P1 DNA has a distinct denaturation pattern

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Electron microscopy of bacteriophage P1 plasmid DNA partially denatured by high pH in the presence of formaldehyde and mounted by the protein monolayer technique revealed a unique denaturation pattern. Alignment with the physical map (Bächi and Arber, Molec. gen. Genet. *153*, 311, 1977) was obtained by comparing the melting pattern of plasmid DNA linearized by a single cut introduced by the restriction enzyme *Pst*I with those of the large *Hind*III, *Bgl*II and *Bam*HI restriction fragments. 5 major AT-rich regions are located at map units 0 to 6, 21, 46, 52, and 97. The DNA-segment melting first and therefore having the highest AT content spans the restriction-modification region between map units 0 and 6 of the P1 genome.

Membrane damaging effect of the δ-endotoxin of *Bacillus thuringiensis*

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The δ-endotoxin of *B. thuringiensis* is known to act primarily on the gut epithelium of susceptible host insects. The cell organelles such as microvilli, mitochondria and endoplasmatic reticulum are damaged within minutes following application of endotoxin. The effect of the δ-endotoxin can also be demonstrated on insect cell lines. In the present study an established cell line derived from the spruce budworm *(Choristoneura fumiferana)* was used. It has been developed by Dr S.S. Sohi, Insect Pathology Research Institute, Sault Ste Marie, Canada.

The experiments were carried out with approximately 10^6 cells/ml in the medium of Grace. 10 µg/ml of trypsin activated endotoxin were used. The endotoxin was derived from a strain of *B. thuringiensis* var. *thuringiensis*. The incubation time at room temperature was varied between 0 and 60 min. At different intervals samples were taken for the preparation of freeze-etched specimens and thin sections. Furthermore, the intracellular activities of the lactate dehydrogenase and of the acid phosphatase were determined.

Generally, the cells derived from *C. fumiferana* exposed to the δ-endotoxin showed the same morphological changes as the gut epithelium under in vivo conditions. The principal alterations comprised the desintegration of the cell or-

ganelles and the characteristic ballooning of the cells. Freeze-fracturing studies concentrated on the morphology of the cell membranes.
The first changes can be observed after 15 min. The membraneous protein particles begin to form agglomerates, thus increasing the pure lipid portions. 30 min following the application of δ-endotoxin the whole regular fine structure of the membrane is destroyed. According to these results, it may be assumed that the primary site of action is the cell membrane and the intracellular changes are secondary effects. A similar mode of action on cell membranes is known from the delta-toxin of *Staphylococcus aureus.*
The lactate dehydrogenase is present in an unbound state in the cytoplasm. Leaking of this enzyme through the plasma membrane was detected after 10 min following exposure to δ-endotoxin. After 1 h, 80% of the lactate dehydrogenase was found in the supernatant. The acid phosphatase is an enzyme located in the lysosomes. Its intracellular activity increased during endotoxin incubation by at least 50%. No enzyme activity was measured outside the cells.
The results obtained with the 2 enzymes support the findings of the electron microscopical investigation. The efflux of unbound enzyme and the increased permeability for p-nitrophenol phosphate as substrate for the acid phosphatase indicate a severe damage of the cell membranes by the endotoxin.

Involvement of insertion sequences IS1 and $\gamma\delta$ in the formation of hybrids between phage P1 and R plasmid NR1

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The RTF and the r-determinant domains of the R plasmid NR1 are separated by 2 IS1 elements in the same orientation. Bacteriophage P1 carries one IS1 in its genome. We have isolated P1-NR1 hybrid phages (P1Cm, P1CmSm, P1CmSmSu, P1CmSmSuHg, P1CmSmSuHgTc, etc.) and characterized them by electron microscope heteroduplex, restriction cleavage and DNA-DNA hybridization studies.
IS1 plays an important role in the formation and stability of P1-NR1 hybrids. In particular, we observed IS1-mediated cointegration, excision, transposition, deletion, duplication and inversion.
The NR1 used in these studies was now found to carry a 5.7 kb long $\gamma\delta$ element. A class of P1 hybrid phages carrying r-determinant and the replication origin region of RTF must have arisen by $\gamma\delta$-mediated deletion formation from P1-NR1 cointegrates. In independent experiments, the $\gamma\delta$ element was shown to transpose to a P1 plasmid.

An economic ELISA reader combined with a programmable calculator

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The enzyme-linked immuno assay (ELISA) has become a very important serological tool in bacteriology, virology, parasitology and toxicology. It is very useful for mass screening tests and suitable for automation. There are already different semiautomatic and fully automatic devices for the read-out of tubes and microtitration systems in the market. These instruments either just print out the extinction values or have a sophisticated but fixed program available for the evaluation of a given serological method. These systems cost at least sFr. 20,000.- or more. - We report on an instrument for half that price which is programmable and therefore fully adaptable to any serological modification of the test. We asked Paul Bucher, Laupenring 150, 4054 Basel, to link a Vitatron digital concentration photometer (DCP) by an interface log 2 (Kontron Analytik, CH-8000 Zürich) to a programmable calculator TI 59 with a printer PC 100A (Texas Instruments). The photometer can be used directly with the polystyrene tubes in the ELISA, or with a 0.5 ml or a 100 µl quartz cuvette (to be emptied by suction), one measurement is done within seconds. We set up different programs for instance for the measurement of tetanus antitoxin and staphylococcal enterotoxins: The extinction values of the respective standards are printed, their mean values and the regression are calculated. The samples's extinction values are then printed, their means compared with the regression formula and finally units or ng/ml, respectively are printed out. The degree of sophistication of the different programs is only limited by the possibilities of the TI 59 calculator. Evidently the TI 59 can be used without the photometer for any kind of calculations. There are also 2 different micro sampler systems attachable to the Vitatron photometer usable for the Micro ELISA Technique. The whole system is therefore an extremely versatile tool.

A solid phase immunosorbent test for the demonstration of IgM by hemagglutination inhibition and passive hemagglutination

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The rubella hemagglutination inhibition test has been modified for the detection of rubella-specific IgM. The test is performed on microtiter plates, in which Ig from the patient has been selectively retained by antihuman IgM, bound to the polystyrene surface. This technique can also be applied to test IgM-antibodies by passive hemagglutination.
1. In contrast to most techniques for the demonstration of IgM-antibodies, the solid-phase immunosorbent technique (SPIT) requires no expensive equipment. It can be used in all laboratories which are equipped for the demonstration of rubella antibodies by the hemagglutination inhibition test.
2. The technique is very sensitive and the results are in good agreement with the highly sensitive radioimmunoassay, and results compare also well in the density gradient centrifugation technique.
3. The results of the IgM-titers are available at the same time when values for total antibodies are known. That means normally within 24 h.
4. There is no limitation to use this technique on a large scale. The number of sera tested each day is practically unlimited.
5. The rheumafactor does not interfere with the results.
6. The SPIT test has been reproduced by a number of different laboratories with similar results.

Immunoperoxidase technique for the rapid diagnosis of methanol-H_2O_2 labile viral antigens in clinical material

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The use of the immunoperoxidase technique (IPT) as an alternative method for the rapid diagnosis of viral antigens in clinical material is hampered by the presence of endogenous peroxidase activity (EPA). EPA can be irreversibly eliminated by treating the specimens with methanol followed by very dilute H_2O_2, but unfortunately some viral antigens are also destroyed by this treatment.

In the course of experiments undertaken to establish a rapid diagnostic method for Newcastle disease virus (NDV) by immunoperoxidase and immunofluorescence technique (IFT) we have demonstrated by gel-electrophoresis-derived enzyme-linked immunosorbent assay (GEDELISA) that the predominant NDV antigen HN-VGP 75 is destroyed by treatment with methanol-H_2O_2 and, moreover, is no longer detectable by IPT and IFT in the NDV-infected tissue culture system. When the acetone-fixed infected tissue cultures were overlaid with specific antibody before exposing the antigen to methanol-H_2O_2 for the inhibition of EPA we observed that the antigen-antibody complex was stable and that a strong antibody reaction persisted which could be detected by indirect IPT or IFT. This simple modification of the procedure greatly improved the detection of methanol-H_2O_2 labile antigens (glycoproteins) by indirect IPT in the absence of interfering EPA in NDV-infected chicken organ kryostat sections. The antigen could also be easily and regularly detected in lung, spleen and kidney sections by this method. This modified IPT method may also be extended to the detection of other virus antigens in clinical material.

Transfer, in natural-like host environment, of drug resistance of *Escherichia coli* isolated from septicemic calves

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314 serologically grouped strains of *E. coli* isolated between 1968 and 1978 from calves with septicemia were tested for resistance to the following drugs: tetracycline (Tet), streptomycin (Str), neomycin (Neo), gentamycin (Gen), ampicillin (Amp), chloramphenicol (Cmp), polymyxin B (Pol), sulphonamide (Suf), furadantin (Fur), trimethoprime-sulphamethoxazole (Tsu), nalidixic acid (Nal). All strains were sensitive to Gen, Pol and Nal. 7.6% of the strains were fully sensitive, 4.8% had a single resistance and 87.6% had a multiple resistance to 2 to 8 drugs. The occurrence of each drug resistance in all resistant strains was as follows: Suf 86.5%, Str 83.8%, Tet 79.1%, Amp 46.2%, Cmp 41.1%, Fur 40.8%, Neo 25.2%, Tsu 1.6%. Transferability of drug resistance was determined in 110 of 168 strains (65.5%). Total transfer of resistance to 1 to 6 drugs was observed in 50% of the strains with an R-factor. The occurrence of transfer of each drug resistance in all strains with an R-factor was as follows: Cmp 100%, Str 94.5%, Tet 90.5%, Suf 88.2%, Neo 88.1%, Amp 39.3%. Resistance to Fur was not transferred. Transfer of drug resistance was also established a) in or on various substrates from the environment of the calves, i.e. cloth, blotting paper, sawdust, wood, straw, hay, gras, soil, calf-manure, tape water, milk, urine, liquid manure, and b) under stable-like conditions, although with a lower frequency. In the later experiment, the conjugation was performed by mixing substrates coming from the environment with substrates coming from the animal, after the donnor and receptor strains respectively had been preincubated in these substrates over night. This suggests that transfer of drug resistance outwards the host could be of importance.

Induction of interferon with psoralen cross-linked double-stranded RNA in mouse cells

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Double-stranded (DS) but not single- or multiple-stranded RNA molecules are potent inducers of interferon. For this activity thermal and helical stability is required. Using 4′-aminomethyl-4,5′,8-trimethylpsoralen hydrochloride (AMT) and long wave UV the influence of covalent interstrand cross-links on interferon induction capacity of poly(I, C) in mouse cells was investigated. So far all chemical modification studies of DS-RNA designed to reveal the structural elements of the RNA molecule which are required for induction activity have been based on modifications of individual residues. The extent of cross-linking was varied by use of different amounts of AMT and followed by melting analysis. Standard interferon induction assays showed that a) the use of such cross-linked poly(I, C) yields about equal amounts of interferon as using the nonirradiated control, b) amphotericin B methyl ester (AME) augments both yields by a factor of about 30 and c) interferon yields were independent of the number of covalent cross-links. Thus, cross-linking of poly (I, C) is not advantageous for interferon induction; a slight decrease in induction capacity of cross-linked RNA may reflect impaired flexibility of the molecule. On the other hand the fact that cross-linked poly(I, C) is still able to induce interferon indicates that strand separation may be unnecessary.

Experiments in mice with cross-linked and control poly (I, C) lead to similar conclusions.

In conclusion, these studies indicate that most probably covalent cross-links (e.g. also in VSV DI-O11; Marcus and Sekellick, Nature *266*, 815, 1977) are not the sole requirement for the best inducer; rather transport (e.g. AME) and particular DS-RNA structures are essential.

Is cytomegalovirus (CMV) antibody required for in vitro transformation of lymphocytes from CMV-seropositive donors?

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There is clinical and experimental evidence that primary infections with herpes viruses may be followed by latent persistence of the virus in the infected host. The mechanisms responsible for producing latency and for activating the latent infection to produce clinically manifest disease are not known. The role of humoral and/or cellular immunity in these processes remains to be elucidated.

We have studied the role of CMV antibodies in vitro on the proliferative response of lymphocytes to CMV antigen. Peripheral lymphocytes are cultured in the presence of crude freeze-thaw antigen preparations from CMV-infected and normal human embryo fibroblasts. We have observed that:

1. Lymphocytes from CMV-seropositive donors respond to CMV antigen when cultured in the presence of autologous plasma or an AB serum pool containing CMV antibodies (measured by complement fixation).
2. If AB serum *without* CMV antibodies or another CMV-seronegative plasma is used in the same assay system, no or greatly reduced lymphocyte transformation is observed.
3. Lymphocytes from CMV-seronegative persons do not respond to the CMV antigen irrespective of the presence or absence of CMV antibody in the assay system.
4. The use of purified CMV virions in place of the infected cell extract does not affect the above results.
5. Lymphocytes respond normally to mitogens in the presence of any plasma or serum.

These results are in disagreement with those of other investigators who do not report a dependence on CMV antibody in the CMV-specific lymphocyte transformation assay. At present we have no explanation for this discrepancy. It appears at this time that our assay system may lend itself to the study of the interactions among antibody,

antigen, immune complexes and lymphocytes in the cellular immune response to CMV. It is also possible that the presence of CMV antibodies plays a significant role in the establishment and/or activation of latent CMV in vivo.

A versatile plaque assay for feline panleukopenia virus

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Multiplication of feline panleukopenia virus (FPV) in primary and permanent cultures of feline cells is associated with only weak and frequently uncharacteristic CPE. Titration of FPV infectivity therefore is based on the detection of virus specific intranuclear inclusion bodies by means of hematoxilin and eosin or immunofluorescent staining. To overcome the difficulties inherent to these techniques, a plaque assay has been developed which is about 100-fold more sensitive than is screening of cultures for inclusion bodies and which exceeds the sensitivity of the immunofluorescent assay by a factor of 10. Both the size and number of plaques induced by FPV in NLFK cell cultures (a permanent line of feline kidney cells) proved to be a function of cell density. In monolayers grown from an initial concentration of 2.5×10^5 cells per 60-mm petri dish plaques showed a diameter of 4–6 mm whereas they were almost invisible in dishes seeded with $1–1.5 \times 10^6$ cells. The number of plaques reached a maximum in monolayers established from 3 to 3.5×10^5 cells. Under the latter conditions, the diameter of plaques varied between 1 and 4 mm and 50–60 plaques could be readily distinguished in a standard dish. Plaques became detectable as early as 2 days after infection. They increased in size and number up to day 5. Later on, focal degeneration of monolayers frequently confused the results. Since the relationship between virus concentration inoculated and the plaque numbers recorded proved to be linear, the plaque assay offered an efficient means for cloning FPV. Besides, a rapid and sensitive test for specific neutralizing antibodies to FPV has been developed.

Structure and stability of hepatitis-A virus

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Virus particles for which a close serologic relationship to the MS-1 reference strain of hepatitis-A virus could be established were purified from stools of patients in 2 epidemics of hepatitis A. After repeated centrifugation in CsCl and sedimentation in sucrose gradients particles banding at a density of 1.34 g/ml and sedimenting with 160S were analyzed for their polypeptide constituents. Electrophoresis in polyacrylamide gels revealed 3 major polypeptides the mol.wt of which were in accordance with that of the 34,000–29,500, 25,500–24,000 and 23,000–22,000 daltons polypeptides reported by Coulepis et al. (Intervirology *10*, 24, 1978) and Feinstone et al. (personal communication). Besides, a 4th minor polypeptide with the electrophoretic mobility of VP4 of poliovirus (7000 daltons) was regularily detected and an additional polypeptide with a mol.wt of about 50,000 was present in varying amounts. The significance of the latter polypeptide is still under investigation. The protein composition of the capsid as well as the presence of linear single-stranded RNA within the virion suggests HAV to be a member of the enterovirus group. Enteroviruses, however, are destabilized by heating to 60 °C and, upon sedimentation in sucrose gradients, resolve into a 80S nucleoprotein complex, free viral RNA, and substructures of the capsid which sediment below 15S. In contrast, purified HAV particles heated to 60 °C for 10 min sedimented homogeneously with 160S. Only after heating to 80 °C they lost both their structural integrity and their antigenicity. This outstanding resistance to heating distinguishes HAV from the known enteroviruses.

Rotavirus infections in calves

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The epidemiology of rotavirus diarrhea in a conventional dairy operation and in a calf fattening operation was investigated. Daily fecal samples were examined for excretion of rotavirus with fluorescent antibody technique (FAT), enzyme-linked immunosorbent assay (ELISA), and electron microscopy. The fluorescent antibody was made from serum of a calf hyperimmunized with rotavirus grown on fetal calf kidney cells. For ELISA-polystyrene tubes were coated with hyperimmune serum from rabbits, then serial dilutions of fecal material were applied. After that the tubes were incubated with the rabbit antirota hyperimmune serum, then with staphylococcal protein A-peroxydase conjugate, and finally with H_2O_2/ABTS-substrate. Of 416 fecal samples examined by FAT and ELISA 307 were negative and 72 were positive by both methods. Positive by FAT and negative by ELISA were 3 samples, while 34 ELISA-positives were negative by FAT. Antibody screening was done by Ouchterlony gel precipitation technique.
In the fattening operation 31 calves were brought together at an age of 2 to 3 weeks in a single room shed, and observed there. Only one excreted rotavirus for the first 2 days. Most showed rising precipitating antibodies, so probably had previous contact with rotavirus.
In the dairy operation, 28 calves were born during the observation period from December 1977 to April 1978. Of the 27 examined, 24 excreted rotavirus for a single period of 1 to 7 days each during the first 2 weeks of life. Virus excretion was almost always associated with diarrhea. In all calves affected the ELISA-antigen titer dropped suddenly, while the clinical disease often persisted for several days.

Comparison between 2 methods for the detection of IgM-antibodies to hepatitis-A virus (HAV)

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Due to the rapid elimination of HAV from the stool of infected patients, virus-serology is the only way to detect acute HAV-infections.
Antibodies against HAV are usually demonstrable already during the icteric stage and thus, even under optimal blood sampling conditions, a rise in titer can only be shown in less than 50% of all cases (Deinhardt et al., International Symposium on Viral Hepatitis, 5–7 April, Munich) in the routinely used HAVAB-test (RIA, Abbott). Therefore, the only reliable serological proof of a recent HAV-infection is the measurement of specific IgM-antibodies to the agent: As a rule, antibodies of this class appear first in the course of the infection, decline fairly rapidly and are no longer detectable 6 months after onset of the disease at the very most (Frösner et al., ibid.).
In this trial, 138 HAVAB-positive sera have been checked for IgM-antibodies to HAV by 2 different methods:

1. Absorption of diluted sera with a standardized suspension of *Staphylococcus aureus* cells (Cowan strain) in order to remove IgG from the sera and subsequent testing with HAVAB-RIA.
2. A new solid phase radioimmunassay for the detection of specific anti-HAV-IgM, originally developed by Frösner et al. (J. med. Virol., accepted, in press).
The results show a marked difference in reliability between the 2 tests: Whereas the new test mentioned above yielded exclusively clearcut values, borderline values in the staphylococcal test, however, especially 'false positive' results, have often been encountered.

Comparison of subviral particles from Semliki forest virus infected vertebrate and invertebrate cells

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Cytoplasmic subviral particles from Semliki forest virus (SFV) infected primary chick embryo fibroblasts (CEF), Vero cells and *Aedes albopictus* cells were isolated with different methods with or without detergents. In CEF and Vero cells, particles containing the viral 26S RNA or the viral 42S RNA and the core protein sedimented with about 40S, 60S, 80S and 110S independent of the isolation method used. These particles are not attached to the ribosome or ribosomal subunits and seem to be free in the cytoplasm. They could be precursors of polysomal messenger ribonucleoproteins or of viral core particles.
In infected *Aedes albopictus* cells we found differences according to the isolation method. The method using detergents (method A) revealed particles sedimenting at about 30S, 40S and 60S containing 26S RNA (no 42S RNA) and particles sedimenting at 80S, 100S and 110S containing 26S, 33S, 38S and 42S RNAs. Method B without detergents didn't yield these distinct peaks and the intact viral RNAs could not be isolated from the fractions. However, if the sediment, which is produced in the first step of the isolation without detergents, was extracted with method A (with detergents), viral particles with the viral RNAs could again be found.

Factors influencing Semliki forest virus (SFV) induced fusion of *Aedes albopictus* (mosquito) cells in culture

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In cultures of a subline of Singh's *Aedes albopictus* cells the initial pH of 7.4 falls to around 6.4 as cultures become confluent and then begins to rise again to values over 7.6. Infection of cultures with SFV at a time of low pH is followed by massive cell fusion. Syncytia are evolving within the first 2 days post-infection. If the pH is high at the time of infection, there is no observable cell damage.
Intentional pH-modification at the time of seeding reveals a wide pH-range compatible with *Aedes* cell growth. The cyclic pH-changes are observed with all initial pH-values, whereby the lowest pH reached is related to a given initial pH. Therefore it is possible to study the influence of pH on syncytium formation. The upper pH-limit for syncytium formation is about 6.7–6.8 at the time of infection. Experiments with modification of the pH at the time of infection confirm these data. In addition, with decreasing pH-values, production of infective units increases.
Repetition of these experiments with a medium from another source (composition as above according to catalogue) yields in part different results. The pH-changes, too, but lowest values are never below 6.6 and a virus-induced cytopathic effect is never observed. In principle, attempts to enforce formation of large syncytia by intentional lowering of the pH fail. At a pH of 6.35 (time of infection), however, discrete syncytia can be observed but not earlier than 5 days post-infection. Again, the amount of infective units increases with decreasing pH.

Investigation of various effector mechanisms on mengovirus-infected target cells

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It is well established that infection with picornavirus only leads to humoral but not to a detectable cellular immune response of the host. In contrast other viruses, such as those of the herpes, influenza, parainfluenza groups, etc., induce a cellular as well as a humoral immune reaction. Several reasons can account for the failure of a cellular immune mechanism following a picornavirus infection. The induction of cellular effector mechanisms might be prevented or, alternatively, they might not be effective because suppression is induced by these viruses.
In order to get more information about this question, 2 effector mechanisms were studied in vitro using either mengovirus (picornavirus) or sendaivirus(parainfluenzavirus)-infected EL_4-target cells. We tried to lyse the target cells with either specific antivirus-antibodies and complement, or with antibodies and K-cells (antibody dependent cell-mediated cytolysis).
The results obtained showed that sendaivirus-infected EL_4-cells were easily lysed by all 2 effector mechanisms in contrast to mengovirus-infected EL_4-cells which remained unaffected. This lead to the hypothesis that mengovirus-infected cells cannot be lysed because they do not express the relevant surface antigens recognizable for antibodies or effector cells.
To demonstrate that the failure of lysis is not due to antibodies which might not be cytotoxic or otherwise defective, EL_4-cells were incubated with mengovirus at 0 °C. This prevents the penetration but not the binding of the virus to the cells. Such cells treated with anti-mengovirus-antibodies at 0 °C for $\frac{1}{2}$ h were lysed after subsequent incubation at 37 °C in the presence of complement.
Thus once mengovirus have penetrated cells they are completely protected from the hosts defence mechanisms.

Suppression of poxvirus growth by ouabain

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Ouabain, a specific inhibitor of the plasma membrane Na^+-K^+-Mg^{2+}-activated ATPase (EC 3.6.1.3) is known to inhibit cellular protein synthesis and cell proliferation. It also inhibits the appearance of late cytopathic effects and the production of infectious virus during rabbit poxvirus infection in HeLa cells over a wide range of concentrations. This effect of ouabain is due neither to an inhibition of virus adsorption nor to a direct inactivation of infectious virus by the drug. To test whether the suppression of poxvirus growth by ouabain was due to its known action on the Na^+-K^+-Mg^{2+}-activated ATPase, we compared the effects of ouabain on virus growth in wild-type and ouabain-resistant mutants of HeLa cells. These mutants had been isolated by selection from the wild-type population after mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine. Rabbit poxvirus proliferates in the mutant cells, even

in the presence of ouabain in concentrations which inhibit virus growth in wild-type cells. However virus growth is inhibited in both cells by the K^+ ionophore valinomycin. The described suppression of poxvirus growth is reversible if ouabain is washed out 6 or 24 h after infection.
These results indicate that ouabain inhibits rabbit poxvirus replication in HeLa cells by its known inhibition of the plasma membrane Na^+-K^+-Mg^{2+}-activated ATPase. It is very likely that the primary effect of this inhibition is a depression of overall protein synthesis due to a disturbance of the intracellular ionic milieu. We are currently trying to use this system to study rabbit poxvirus replication under various defined intracellular ionic conditions.

Interaction between interferon and host genes in antiviral defense

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Using antibody to mouse interferon (IF) we have recently been able to show that IF plays an important part in the inborn resistance to influenza virus determined by the single dominant gene *Mx* in mice (O. Haller et al., J. exp. Med. *149*, 601, 1979). This finding was unexpected since resistance is highly specific for a group of closely related viruses, whereas IF is not considered to be virus specific. In the present virus-host system, IF might be envisaged either as an auxiliary factor increasing the efficiency of a genetically determined cellular resistance or, alternatively, as the main factor inducing an antiviral state, but modulated by host genes. Our present data support the latter view: 1. Cells from *Mx* bearing mice were found to be equally susceptible to influenza virus in vitro as cells from control (+/+) mice under certain experimental conditions. 2. Graded doses of IF were more effective in protecting Mx/+ cells than +/+ cells against infection with influenza virus (but not VSV). 3. Better protectability was associated with the presence of the *Mx* gene in cells from backcross populations. We conclude that a genetically determined difference, which is virus specific, seems to exist between strains of mice in their sensitivity to IF.

Differential sensitivities of cellular RNA and protein species to enterovirus-induced shut-off

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Infection of cells with enteroviruses results in inhibition of cellular RNA and protein synthesis. The extent and kinetics of enterovirus shut-off, however, varies in different cell lines. In addition, the synthesis of different cellular RNA and protein species exhibit differential sensitivities against enteroviral shut-off.
RNA-synthesis: Infection of L-cells with mengovirus results in a rapid and complete shut-off of cellular RNA synthesis. In polio- and mengovirus-infected HEp-2 cells, inhibition of cellular RNA-synthesis is slower and incomplete. The remaining RNA synthesis persists throughout the infection cycle, occurs in the nucleolus and can be blocked with actinomycin D. The extracted RNA cosediments with rRNA precursors.
Protein synthesis: Poliovirus infection of HEp-2 cells rapidly decreases the overall rate of protein synthesis. Some of the protein products disappear soon after infection, whereas others persist or even increase their amount over the time of maximal viral protein synthesis. These alterations in the relative ratios of cellular proteins result more clearly from experiments using guanidine-HCl where poliovirus replication is blocked and virus-induced shut-off needs longer times to get established. The shut-off resistant cellular proteins are even synthesized after hypertonic treatment of the cells, which can be used to unmask viral proteins early in poliovirus infection.
The results implicate differential sensitivities of the synthesis of cellular proteins against poliovirus-induced shut-off mechanism. Differential inhibition of cellular proteins may result from different binding affinities of the corresponding mRNAs, reflecting their dependence from factors which initiate protein synthesis and their ability to compete for these factors.

Kinetics and localization of poliovirus replication related to host cell metabolism and CPE

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In the time course of poliovirus specific macromolecular synthesis, maximal viral protein synthesis preceeds peak viral RNA synthesis by 30–60 min. ^{3}H-choline incorporation, a measure for virus-induced cytopathology, starts to increase above control levels at 3 h p.i., shortly before maximal viral RNA synthesis. Ultrastructural localization of the viral synthetic activities revealed viral protein synthesis to be randomly distributed in an unaltered cytoplasm. In contrast, viral RNA synthesis is exclusively found associated with small, newly formed vacuoles. Such clusters of vacuoles arise multifocally in the cytoplasm and coalesce at or shortly after peak viral RNA synthesis into a single vacuolated field. Thus, viral RNA synthesis (the 'replication complex') is found in structures considered to be the morphological equivalent of CPE.
In order to study further the events responsible for CPE induction, poliovirus-infected cells were treated with guanidine-HCl. Addition of guanidine early after infection suppresses virus replication completely, but virus-induced shut-off of host cell macromolecular synthesis takes place. Such cells, with their synthetic activities suppress d to the same extend as in nontreated, infected cultures, show no CPE. Therefore, viral macromolecular synthesis seems to be required for CPE. This was substantiated in experiments where guanidine was added later in infection, so that viral proteins (detected by PAGE) were made: such cells invariably became cytopathic.
It is concluded, therefore, that viral protein synthesis (not shut-off of host cell functions) is a prerequisite for CPE and that CPE, in turn, is necessary for, or at least tightly connected with, viral RNA synthesis.

A fungicidal and bactericidal gas from the mycelium of a *Paecilomyces* strain

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After submerged cultivation of a fungus belonging to the genus *Paecilomyces* (the strain combines characters of *P. marquandii* Hughes and *P. lilacinus* Samson) in a 25% sucrose medium, the collected mycelium exhibited a high fungicidal and bactericidal activity against a wide range of yeasts, hyphomycetes and gram-positive and gram-negative bacteria. On conventional agar test plates clear inhibition zones are formed even when the active mycelium has no direct contact with the test plate surfaces. Moreover, when a slow aircurrent is passed over active mycelium and directed onto a test plate surface for a period of time, it

induces a clear inhibition zone following incubation. On a *Bacillus megaterium* or *Sarcina lutea* test plate, the aircurrent will induce clear zone of ca. 20 mm after a treatment period of 3 min, ca. 30 mm after 10 min and 50–60 mm after 30 min. The activity therefore results from a volatile metabolite.

Thoroughly washed *Paecilomyces* mycelium becomes practically inactive and by adding glucose or sucrose the activity is restored completely. Heat-killed mycelium (70–80 °C, 30 min) is inactive.

The active gas is absorbed by activated charcoal and by water, the latter becoming active itself when saturated.

The physical identification of this active gas is now in progress.

Debaryomyces hansenii, a yeast showing an unusual resistance to polyene antibiotics

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Polyene antibiotics which are frequently discovered during screening programmes may be identified by their characteristic UV-spectrum or a microbiological test using strains of oomycetes. These fungi do not synthesize sterols and therefore show cross-resistance to all polyenes.

For further differentiation attempts were made to isolate polyene resistant strains applying the enrichment technique reported by Flickinger and Perlman (J. Antibiot. *28*, 307, 1975)., On a minimal medium containing yeast extract and amphotericin B as the carbon source a yeast was isolated from horse manure. Agar diffusion and MIC assays showed it to be resistant to amphotericin B but not to other polyene antibiotics tested. This selective response is consistent with an enzymatic degradation of amphotericin B. However growth experiments using the isolation agar demonstrated the ability of the isolated yeast to utilize yeast extract but not amphotericin B as a carbon source. Other mechanisms beside an enzymatic degradation of amphotericin B have therefore to be considered to explain the selective resistance to this yeast.

Carbon utilization experiments showed the strain to grow on a variety of sugars under aerobic but not under anaerobic conditions. Nitrate could not be used as a nitrogen source. Applying this pattern to the taxonomic identification scheme by Barnett and Pankhurst (A New Key To The Yeasts, North Holland Publishing Company, 1974) the strain was identified as *Debaryomyces hansenii*. Spore formation on a yeast extract/malt extract agar showed the spore form to be in agreement with the previous classification.

CONGRESSUS

Switzerland

16th EUCHEM conference on stereochemistry

Bürgenstock, near Lucerne, 27 April–3 May 1980

Information by the chairman: Prof. J.D. Dunitz, Laboratorium für Organische Chemie, ETHZ, Universitätsstrasse 16, CH-8092 Zürich.

International symposium on inborn errors of metabolism in humans

Interlaken, near Berne, 2–5 September 1980

Note: change of dates. For information write to: Mrs Sonja R. Wyss, Medizinisch-chemisches Institut der Universität, Bühlstrasse 28, CH-3000 Bern 9, Switzerland.

Federal Republic of Germany

International symposium of melatonin

Bremen, 28–30 September 1980

For information write to the scientific secretariat: Dr. N. Birau, Institute for Preventive Endocrinology, Schwachhauser Heerstr. 52, D-2800 Bremen 1/BRD.

Poland

VI international congress of protozoology

Warsaw, 5–11 July 1981

General information by: Dr Stanislaw L. Kazubski, Secretary general, Departement of Cell Biology, M. Nencki Institute, 3, Pasteur St., 02-093 Warsaw/Poland.

Czechoslovakia

International congress of polarography, in memoriam Jaroslav Heyrovsky

Prague, 25–29 August 1980

Information by Dr J. Kûta, secretary of the congress, Vlašska 9, 118 40 Praha 1, Malá Strana, Czechoslovakia.

Great Britain

4th meeting of European neuroscience association

Brighton, 13–20 September 1980

Plenary lectures will be presented by H. Barlow, M. Burger, A. Burgen, E. Zaidel, S. Thesleff, P. Andersen, D. Ploog and L. Heimer.

For further information, please write to the secretary of the meeting, 142 Oxford Road, Cowley, Oxford 0X4 2DZ, England.