nucleoli and round cytoplasmic inclusions of *Drosophila* fat bodies. In addition, the dense granules of clubs (one of the Y-chromosome loops in *D. hydei* spermatocytes) also appear stained by Schiff's reagent. Interestingly, all these stained structures are very compact and highly positive after staining techniques selective for proteins in Epon semithin sections[9,10].

Today it is widely accepted that ribosomal DNA probably embedded within the fibrillar region is a principal component of the nucleolar body[11]. In such a situation, the concentration of DNA seems to be simply too low to give a positive Feulgen reaction, as revealed in nucleoli from squashes and paraffin sections[4,12]. After acidic toluidine blue has been applied to Epon sections, the typical orthochromatic staining of DNA is also lacking from *Allium* and *Chironomus* nucleoli[13]. Other organisms in which nucleoli show conspicuous positive reactions for DNA are clearly different, since they are surrounded or permeated by large masses of condensated chromatin[14]. It is already known that some plant nuclei (particularly algae and bryophites) show very weak Feulgen staining[15]. Unmistakable Feulgen reactions are obviously expected to occur in cells with a great amount of condensed chromatin (e.g. polytene bands, chromocenters, mitotic chromosomes). However, the nonspecific staining on non-hydrolyzed sections could be caused by Schiff-reactive groups already present. As a consequence of incomplete cross-linking reactions with tissue elements, glutaraldehyde fixation may introduce free aldehyde groups[16–19], specially in protein-rich cell structures.

Since glutaraldehyde-fixed, Epon-embedded sections are capable of giving perceptible staining directly after the Schiff's reagent, careful control seems to be necessary to decide whether or not a given structure contains DNA. The present results suggest that by using the Feulgen reaction on semithin sections, not only false negatives but also false positives are possible.

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CONGRESSUS

Italy

3rd international symposium of cytopharmacology

in Venice, 10–14 July 1978

This symposium will be dedicated to 'Neurotoxins and their use as tools in neurobiology'. For further information write to B. Ceccarelli, C. N. R. Center of Cytopharmacology, Institute of Pharmacology of Milano, via Vanvitelli 32, I–20129 Milano, Italy.

9th international symposium on chromatography and electrophoresis

Riva del Garda (Italy), 15–17 May 1978

The symposium is organized by the Belgian Society for Pharmaceutical Sciences, the Italian Group for Mass Spectrometry in Biochemistry and Medicine and the Italian Society for Pharmaceutical Sciences. Communications should be submitted before 4 February 1978.

Further information by the chairman: Dr Alberto Frigerio, Istituto de Ricerche Farmacologiche 'Mario Negri', via Eritrea 62, I–20157 Milano, Italy.

5th international symposium on mass spectrometry in biochemistry and medicine

at Rimini, 19–21 June 1978

This symposium by the Italian Group for Mass Spectrometry in Biochemistry and Medicine will discuss all the latest aspects of mass spectrometry and their areas of application and consist of presentations of invited speakers and free communications. Deadline 28 February 1978.

Further information by the president, Dr Alberto Frigerio, Istituto di Ricerche Farmacologiche 'Mario Negri', via Eritrea 62, I–20157 Milano, Italy.

Belgium

Satellite symposium of the 7th international congress of pharmacology

in Brussels, 24 July 1978

Topic of the symposium: The inflammatory process. For information contact: Dr J. P. Famaey, Service de Rhumatologie et Physiothérapie, Hop. Univ. Saint-Pierre, 322, rue Haute, B–1000 Bruxelles.

France

Satellite symposium of the 7th international congress of pharmacology

in Paris, 22 July 1978

Topic of the symposium: Antiinflammatory and antirheumatic drugs. For information contact Prof. J. P. Giroud, Department of Pharmacology, 27, rue du Faubourg Saint-Jacques, C. H. U. Cochin, Paris XIV, France.

Switzerland

EUCHEM Conference on Stereochemistry

at the Bürgenstock, near Lucerne, 30 April–6 May 1978

The number of participants will be limited. Inquiries and applications (no special forms are required) should be addressed before January 15, 1978 to the Chairman: Prof. H. A. Staab, Max-Planck-Institut, Organ.-chem. Abteilung, Jahnstrasse 29, D–69 Heidelberg.

The diagnosis of anaerobic infections

by J. Wüst

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In recent years, there has been an enormous increase in reports concerning infections caused by anaerobic bacteria of endogenous origin. This new interest can be credited to several developments: clarification in the taxonomy of anaerobes, technical advances facilitating the culture of these bacteria and the availability of effective antimicrobial agents. These developments have produced a demand for accurate bacteriological diagnosis (for reviews[1–4]). There is no single definition of an anaerobe[5]. In general, anaerobes are bacteria that survive and grow only in an environment with a relative lack of oxygen. There are considerable differences in the ability of different anaerobes to survive and grow in different concentration of oxygen[6].

I. Anaerobes as normal flora

Critical to the proper diagnosis of anaerobic infections is the fact that anaerobic bacteria are the predominant part of the normal flora in the oral cavity, the intestinal tract and in the genito-urinary tract (especially in women). In the normal colon, anaerobic bacteria outnumber the aerobes by 1000 to 1, and in the skin, mouth and vagina by 10 to 1.

With few exceptions, such as tetanus, gas gangrene, crepitant cellulitis, botulism and septic abortion, most anaerobic infections are of endogenous origin, i.e. they stem from the patients own flora. Consequently, it is of utmost importance that clinical specimens represent the material from the infectious process. If a specimen is contaminated with normal flora, the results reported by the bacteriological laboratory may be very misleading.

II. Clinical clues suggesting anaerobic infection

There are a number of clues that suggest infection by anaerobic bacteria. These include[7]:

- foul-smelling discharge
- location of infection in proximity to a mucosal surface
- necrotic tissue, gangrene
- gas in tissues or discharges
- endocarditis with negative routine blood cultures
- infections associated with malignancy or other processes resulting in tissue destruction
- infections related to the use of aminoglycosides, to which most anaerobes are resistant
- infections following human or animal bites
- septic thrombophlebitis
- the presence of 'sulfur' granules in discharges (actinomycosis)
- clinical setting suggestive of anaerobic infection (e.g., septic abortion, infection following gastrointestinal surgery)

III. Specimen selection and collection

To avoid invalid and misleading results, certain specimens should not be cultured for anaerobes. For example, sputum is unsuitable as the oropharynx contains large number of anaerobic bacteria. Other clinical specimens which should not be cultured anaerobically are nasal and throat swabs, feces or rectal swabs, voided or catheterized urines, vaginal or cervical swabs (unless collected by visualization) and material from abdominal wounds obviously contaminated with intestinal contents.

Acceptable specimens include the following: body fluids that are normally sterile such as bile, pleural, sinusitis, joint or peritonitis fluid; surgical specimens obtained from normally sterile sites; abscess contents; blood cultures; deep aspirates of wounds; and specimens collected by specialized procedures such as suprapublic bladder, transtracheal and culdoscopy aspiration.

When culturing material from wounds and abscesses, it is necessary to obtain an aspiration sample with a sterile needle or a catheter attached to a syringe. Swabs are far less suitable, because such specimens are usually very small and difficult to protect from oxygen[8]. Bartlett et al.[9] have shown in 11 specimens containing at least 2 ml aspirate, that of the 26 anaerobes isolated with processing the specimen within 10 min after collection, 22 were still present after exposure to air for 24 h.

IV. Specimen transport

The specimen may be delivered in the syringe in which it was obtained, if the culture is set up within 30 min after collection. For that purpose the syringe should be closed by means of a sterile rubber stopper[4]. When

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longer periods are required for transport, gassed out tubes with a rubber stopper are convenient[10,11]. The tubes are filled with oxygen-free gas and contain an indicator so that one may be certain that the atmosphere is anaerobic. The indicator may be incorporated in agar or in a fluid diluent. Only a small amount of the diluent (which usually contains cysteine to help maintain a reduced atmosphere) should be used to minimize dilution of the specimen.

b) The roll-tube system[23] uses oxygen-free tubes with pre-reduced, anaerobically sterilized media. The oxygen-free atmosphere in the tubes is maintained by inoculation under a stream of oxygen-free gas. To obtain isolated colonies in these tubes, an agar film on the inner surface of the tube can be streaked. The major advantage of this system is that every culture tube is its own anaerobic container which can be inspected and handled at any time without exposing the

V. Laboratory processing

A. Direct examination. First, a gram stain should be made. The fact that many anaerobic bacteria have a characteristic pleomorphic morphology, can make it possible to provide the clinician with a presumptive diagnosis.

Fluorescent antibody staining appears promising[12–16]. L. R. Stauffer[17] could presumptively diagnose *Bacteroides fragilis* by immunofluorescence in 15 of 17 clinical specimens yielding *B. fragilis* on culture. No false positive reactions were encountered in 15 specimens yielding negative culture results.

Quantitative direct gas-liquid chromatography of clinical specimens has also been proposed as an aid in presumptive diagnosis. Gorbach et al.[18] have found that the presence of isobutyric, butyric and/or succinic acids in amounts $\geq$ 0.1 μmoles/ml are suggestive for anaerobic infections with gram negative anaerobic rods, i.e. *Bacteroidaceae*. In contrast to Gorbach et al.[18] I have found similar and higher amounts of succinic acids in purulent specimens from pure infections by *Staphylococcus aureus* (0.1–0.9 μmoles/ml), β-hemolytic streptococci group A (0.15 μmoles/ml), *Pseudomonas aeruginosa* (0.2 μmoles/ml), *Nocardia asteroides* (0.15 μmoles/ml) and in a case of multiple amebic liver abscesses (0.5 μmoles/ml; amebiasis diagnosed by serology)[19]. Although direct gas-liquid chromatography of clinical specimens is, in my opinion, a useful tool in establishing a presumptive and rapid diagnosis, the presence of succinic acid has to be interpreted with caution.

B. Anaerobic culture techniques. There are 3 major culture systems in use for the isolation of anaerobic bacteria:

a) The most commonly used method is the anaerobic jar. The availability of the simple GasPak jar[20] has facilitated anaerobic bacteriology in the clinical laboratory to a great extent. Studies by Killgore et al.[21] and Rosenblatt et al.[22] have shown that recovery of anaerobic bacteria is as good as with the more complex methods mentioned below, as long as the cultures are incubated for 48 h without interruption. The major drawback of the method is that cultures cannot be inspected at any time, and that all plates are exposed to oxygen as soon as the jar is opened.

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bacteria to oxygen. The drawbacks are the somewhat more complicated techniques and that colony morphology is less distinctive than on plates.
c) The third approach is the anaerobic 'glove box' or chamber into which specimens and media are introduced through an entry lock[24]. All procedures are performed within the chamber by means of rubber gloves. Thus, the techniques employed are basically the same as in aerobic bench bacteriology. Plates can be inspected at any time under anaerobic conditions. The disadvantages of the glove box are its large space requirement, its relatively high initial cost, and that all manipulations have to be done with gloves and observed through the plastic wall.

C. Identification. It is beyond the scope of this review to give explicit details on the processing and examination of anaerobic bacteria. There are several manuals to which the reader is referred[23,25,26]. Besides 'enrichment broth' such as thioglycollate, enriched agar media are essential to the recovery of anaerobes. The use of selective media such as those containing kanamycin and vancomycin is frequently helpful in isolating *Bacteroides* species. The identification is based on morphology, biochemical reactions and the use of gas-liquid chromatography.

Biochemical identification usually requires a large battery of tests which makes an exact identification quite costly. The systems now commercially available (API, Minitek) do not give satisfactory results when the results obtained in these systems are evaluated by means of the standard tables published in the anaerobic manuals mentioned[27–29].

Gas-liquid chromatography of fermentation products produced by the anaerobic bacteria under investigation often allows a genus identification. Gas chromatography may be unfamiliar to many bacteriologists but it is a simple procedure providing much information. One may question whether definitive identification of anaerobes is necessary for the clinical laboratory. For most clinical purposes, the exact identification is not necessary for the management of an infection. However, laboratories should at least be able to rapidly recognize the bacteria in the *Bacteroides fragilis* group[30] and *Clostridium perfringens*, as these are the most frequently encountered anaerobes, possessing the greatest pathogenic potential. In addition, the *B. fragilis* group is resistant to the most often used antibiotics such as the aminoglycosides and the β-lactam antibiotics.
Nevertheless, there are arguments which favor a definitive identification: the portal of entry or source may be found in cases of unclear bacteremia. The strong association between *Clostridium septicum* infection and malignancy underlines the desirability of proper identification[31]. In certain cases, accurate identification permits differentiation between relapse of an old infection and a new one in the same patient. Finally, careful bacteriological and clinical studies will permit us to gain information regarding the importance of various anaerobes in different infections and the management and prognosis of these infectious processes.

D. Antimicrobial susceptibility testing. Controversy exists on whether or not anaerobic bacteria should be tested routinely as to their susceptibility to antimicrobial agents or if the treatment should rely upon published data obtained by minimum inhibitory concentration determination[32–35]. As I have encountered several strains with atypical resistance over a period of a few months (*Bacteroides fragilis* resistant to 100 µg clindamycin/ml and *Clostridium perfringens* resistant to 25 µg penicillin/ml or 50 µg chloramphenicol/ml), I am in favor of routinely testing at least those anaerobes which may have a clinical significance from sources not contaminated with normal flora[36]. This testing should include the agents known to be effective against anaerobes, i.e. chloramphenicol, clindamycin, erythromycin, penicillin, tetracycline and probably metronidazole or a related compound such as ornidazole or tinidazole.
Unfortunately, the methods used in aerobic bacteriology cannot be used in testing anaerobes. This especially applies to the Bauer-Kirby method[37,38]. Sutter et al.[26] have published a reliable disk diffusion method. However, it has several disadvantages such as the need for plates poured immediately prior to use. It also requires species identification because there are different standards for interpreting zone diameters. A simple and reliable method is the broth-disk test by Wilkins and Thiel[39,40]. It consists of adding regular antibiotic susceptibility disks to tubes of anaerobic broth in order to achieve an antibiotic concentration in the broth that is similar to that obtained in blood. The tubes are inoculated with 1 drop of the culture to be tested. The organism is considered resistant to an antimicrobial agent if, after incubation, the turbidity is greater than 50% of a control tube without any antimicrobial agent. For a review of methods, see Wilkins and Appleman[34].

VI. Serology

Serologic studies have been tried for the diagnosis and subsequent follow-up of patients with infections caused mainly by the *Bacteroides fragilis* group using agglutination, indirect fluorescent antibody and agar gel diffusion tests[41]. Antibodies could be found to the homologous bacterial strains isolated from the patients, whereas titers to heterologous strains of the same species were usually very low. Antibodies to various members of the *Bacteroidaceae* could be detected in the serum of normal individuals[42–45]. Due to the great many genera and species comprised of different sero-

types involved in anaerobic infections[46–49], it will be difficult to establish reliable and relatively simple serological methods. Nevertheless, the attempts ought to be continued for patients in whom anaerobic infection is suspected, but the etiologic agent cannot be cultured. The main problem will consist in developing more sensitive methods and in differentiation between titers found in healthy individuals and in infected patients when a rise in titer cannot be found.

VII. Conclusion

Over the last decade, important progress has been made in the knowledge of infections caused by anaerobic bacteria. There is still more effort needed to gain a better understanding, finally resulting for the benefit of the patient.

The diagnosis of anaerobic infection requires a good relationship between the physician and the bacteriological laboratory, otherwise much of the effort, and the resources, are spent in vain, e.g. in examining specimens contaminated by normal flora. Establishing a good contact between physician and laboratory will pose problems in countries such as Switzerland, with a centralized structure where bacteriological laboratories are usually not incorported into the hospitals. The laboratory has to inform physicians which specimens are suitable and offer them facilities and advice for transport. On the other hand, the physician ought to give as much information as possible to the laboratory. As current methods for anaerobic cultures require much time, reports will often be too late to benefit the patient. Therefore, rapid methods must be developed such as immunofluorescence, direct gas-liquid chromatography for metabolites and serology. Perhaps some totally different approach may be found; in some respects it is amazing that bacteriology still basically relies on the methods developed towards the end of the last century.

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Virus diagnostic procedures in clinical virology[1]

by P. S. Gardner

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In the 1950's, Sir James Spence, Professor of Child Health, initiated a study in Newcastle of a thousand families to observe all the illnesses which befall children. This led to descriptions of clinical conditions and brought to the forefront that infectious illness, especially respiratory, despite antibiotics, was still the major cause of childhood disease, but the agents responsible were unknown.

This study coincided with the period when new virus groups were being disccovered; the adenovirus 1954, the parainfluenza viruses 1957/58, respiratory syncytial virus in 1960 and the rhinoviruses 1961, to quote just a few examples. With this interest in acute infectious illness in childhood, the development of clinical virology in Newcastle in the late 1950's and early 1960's had not only a population conditioned to research, but clinicians who wished to know the answers.

By the mid 1960's it became clear that conventional diagnostic techniques, although serving a research and epidemiological need, gave little management aid to the clinicians. The developments needed were methods for the rapid diagnosis of virus infection which would bring a finite diagnosis to the clinician within a few h of the patient's admission.

There are many methods of rapid virus diagnosis but this communication is limited to the detection of virus antigen present in clinical material, by electron microscopy and immunofluorescence but mention will be made of immunoperoxidase technique which is still developmental.

Diagnosis by electron microscopy

Electron microscopy has become the method of choice for the diagnosis of smallpox. With rapid air travel and an increasing susceptible population, rapid means for smallpox diagnosis is still required until its final eradication. Electron microscopy will differentiate smallpox from chickenpox but not from other poxvirus; only egg culture will do this.

Another emergency for rapid diagnosis is suspected herpesvirus encephalitis; this can be achieved by examining brain biopsy material by electron microscopy.

There are many viruses which have not as yet been satisfactorily cultivated. Among these is orf virus, primary an infection of sheep man may acquire. The characteristic morphology can be recognized by electron microscopy.

Recently the importance of rotaviruses as a cause of

infant diarrhoea, has been determined by electron microscopy. The name 'rotavirus' has resulted from its wheel-like appearance.
Hepatitis A may be clumped in a stool specimen by a human convalescent serum, i.e. by immune electron microscopy. These particles could not be found in an untreated specimen.

Diagnosis by immunofluorescence

The limitation of the technique is in only making a morphological diagnosis, other techniques may have a more universal application. Immunofluorescence is one, another is immunoperoxidase. The principles of immunofluorescence are simple and have been fully described elsewhere[2].
The reason that many use the indirect in preference to direct, is that when many different viral antisera are handled their labelling would be tedious especially as adequate commercial antispecies conjugate are available. Moreover, conjugation of antisera, even in the best of hands, lowers the titre. The larger complexes one obtains in the indirect, is approximately 10 times brighter than that for the direct. Nevertheless, when one is only investigating a small number of antigens, the direct method may be of great use e.g. in the diagnosis of rabies.
The success of this relatively simple technique depends on adequate specimens and adequate numbers of infected cells in them. More areas of the body are being investigated by biopsy but the most frequent received is a brain biopsy. Biopsy material are investigated for rapid diagnosis by staining impression smears.
However, Swedish workers have used frozen sections of skin biopsy for rapid diagnosis of skin infections by the pox group, herpes group and measles. Answers are available in approximately 4 h[3].
The author uses skin and conjunctival scrapings for the investigation of superficial virus infections, the scrapings being placed in phosphate buffered saline and teased into smaller cell groups with needles[4].
Respiratory specimens are removed from nasopharyngeal secretions by suction using a fine catheter attached to a mucus extractor. Specimens are brought on ice rapidly to the laboratory; cells in specimens are separated by slow centrifugation, washed free of mucus, resuspended in phosphate buffered saline, placed in squares on prepared slides, air dried and fixed in acetone. These cell specimens are reading for staining.
One of the main reasons for the establishment of the European Group for Rapid Laboratory Viral Diagnosis was to ensure that reagents used by European virologists for rapid diagnosis, were of the highest quality and would give no nonspecific activity. Commercial reagents are becoming available and these too are subjected to quality control by laboratories of the European Group. Staff must be adequately trained and the reading of results must be in the hands of senior staff; all members of staff, even juniors, must be trained in the preparation and staining of the specimens, for these are part of routine laboratory work.

Diagnosis by immunoperoxidase

The immunoperoxidase technique is based on very similar principles. An enzyme, usually horseradish peroxidase, is conjugated with the antispecies globulin. The reaction is visualized by using the appropriate substrate and can be viewed by an ordinary light microscope. The technique has been shown to work in tissue culture and for clinical material such as brain in the diagnosis of rabies but specimens from the respiratory tract, contain endogenous peroxidase in the inflammatory cells causing difficulties in interpretation[5].
Immunofluorescence can be used for the diagnosis of herpesvirus hominis encephalitis in biopsy material; therapy with cytosine arabinoside can then be quickly started.

Comparison between immunofluorescence technique and virus isolation

Comparison between immunofluorescence and isolation techniques on eye scrapings showed good correlation; skin scrapings were a little less satisfactory but suitable if cells were taken from the base of vesicles – crusted lesions were difficult to interpret[4]. Investigations of nearly 6000 admissions showed that over 90% of viruses bringing children into hospital, are amenable to rapid virus diagnosis; that surveys can be extensive and therefore one can draw conclusions about virus infection in the community; that some viruses cause various syndromes and many viruses cause all respiratory syndromes; that R.S. virus and bronchiolitis is a common combinatipn, croup and parainfluenza viruses is frequent and that influenza viruses are important causes of childhood respiratory infection, an observation only recently made. Viruses which can be detected in respiratory cells are R.S. virus, influenza A and B, parainfluenza 1, 2, 3, 4a and 4b, adenovirus group and measles.
Unusual symptoms occur with virus infection; a frequent cause for admission to hospital with influenza A was a febrile convulsion, another in children with influenza B was abdominal pain, a finding helpful to the surgeon.

1 Abstract. Short version of the main lecture.
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The proof that a new technique is reliable needs careful comparison with conventional techniques. The few failures to make a diagnosis in the case of R.S. virus where virus was present in secretions, were due to poor specimens submitted. There were however a number of secretions which had positive cells present without virus being isolated. Their fluorescence was of a different quality to normal being dull and hazy and the patients had long clinical histories. Subsequent experiments proved conclusively that these dull infected cells are coated with antibody making virus cultivation more difficult. Comparison of the fluorescent antibody technique and isolation methods for influenza A, B and parainfluenza 1, 2 and 3 were satisfactory.

Respiratory virus deaths

Childhood deaths from respiratory infection still occur, the majority of these are probably viral. Immunofluorescence has helped to determine the cause of death as virus isolation may be difficult to achieve from autopsy specimens. There are 2 kinds of respiratory death, pneumonia and bronchiolitis, both with classical pathological features. Respiratory syncytial virus is the most frequent cause of respiratory death and may be found in abundance in the lungs of the deaths from pneumonia but in bronchiolitis virus is scanty and must be sought carefully[6]. About a quarter of 'cot deaths' are associated with a virus, usually R. S. virus; they have the histological features of bronchiolitis with only scanty virus present[7]. Most respiratory viruses can cause death; influenza A, the parainfluenza viruses and adenoviruses are not infrequent causes.

Application of rapid diagnostic techniques
a) Management of immunosuppressed patients

The applications of rapid virus diagnosis to many clinical problems has already been mentioned – immunosuppression is now common place in treatment – – it involves all grafting operations and also occurs during the treatment of leukaemia. Acute lymphatic leukaemia in children can in many instances be cured but the immunosuppression leads to death from infection, the great majority being viral. The viruses involved are rhinoviruses, measles, mumps from which normal children rapidly recover. Leukaemic children often show the phenomenon of long virus excretion; excretion of R. S. virus was noted for 64 days and giant cells in measles on the 24th day of rash.
Rapid diagnosis has provided a means of treatment of leukaemic children with virus infection. It is possible that interferon given early in an acute viral infection may effect the course of the illness and at the moment a double blind control trial is proceeding.

b) Prevention of hospital cross-infection

Rapid diagnostic techniques should prevent cross-infections from occurring in wards. Influenza A is a major culprit and occurs frequently when a child with an influenza A febrile convulsion on admission recovers and runs around the ward excreting influenza A over those with congenital heart disease, mucoviscidosis and other illnesses. R. S. virus too, especially in the young, causes bronchiolitis in cross-infections and all need oxygen and other resuscitative therapy. These rapid diagnostic techniques must be utilized for making hospitals safe places where children can be nursed and not acquire superadded infections.

c) Virus diagnosis at a distance

Immunofluorescent techniques lend themselves well for diagnosis of virus infections from distant places and a comprehensive diagnostic service for respiratory viruses is run for a hospital 100 miles away in the Lake District[8].
Besides the many aspects covered, the presence of a virus in clinical material means illness and infection. With few exceptions notably influenza A, in those with a previously damaged lung, the findings of a virus in a specimen means that antibiotics will not help and paediatricians are now able to be discriminatory in their use of antibiotics. Physicians now know that answers are available that day and curiosity does not need to be satisfied many weeks later as in the early days of virology.

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Nosocomial ecology of *Pseudomonas aeruginosa*. Analysis of 1545 strains isolated from 1970 to 1976

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The department of 'Hygiène Hospitalière de Strasbourg' has isolated, during 6 years, 1545 strains of *Pseudomonas aeruginosa* from various units in the hospital: surgery, intensive care, hemodialysis, etc. The samples were taken from patients (428 strains), personnel (72 strains) and environment (1045 strains). All these strains have been individualized by epidemiological markers: 1. Serogrouping with the help of 15 agglutinating sera of I. Habs system completed by M. Veron and distributed by Institut Pasteur. 2. Phage-typing on a set of 17 bacteriophages of Lindberg et al. This set was kindly made available by Dr J. F. Vieu of the Institut Pasteur.
Results. The study of these strains corroborates a highly heterogeneous distribution of the germ indicating the probability of an origin outside the hospital.
The study of distribution of strains by unit
1. of serotypes, shows that serogroup 6 is the most frequent (14.9%) followed by serogroup 11 (14.8%) and serogroup 1 (9%), and that 28% of strains of all units are auto-agglutinable or not agglutinable. The serogroups 12, 14 and 15 are almost absent.
2. Of phages, shows that for all the strains the phage 68, than the phage 44, 7, 21, 119 X are the most frequently encountered, whereas the distribution is significatively different for different units. The phage F 10 lyses only 0.8% of strains.
3. Of resistance to antibiotics: shows total resistance to all the tested antibiotics except carbenicilline (51% of resistant strains), streptomycine (85%), neomycine (65%), gentamycine (14%), colimycine (0.4%), polymyxine (27%), and nitro-5-hydroxy-8-quinoleine (27%). The study of resistance shows: a) a different percentage of resistance for different units, b) the resistance does not increase with time.
4. The computer study by the method of 'analyse factorielle des correspondances' does not permit a grouping of strains or of phages.

Standardized antibiotic assays with preserved, ready-made inocula

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To ensure that antibiotic assays will yield comparable results over periods of many years, the qualities of the laboratory strains and clinical isolates used must be kept as nearly constant as possible. This requirement can be met by using standardized, ready-made inocula from a deep-frozen stock, thus obviating the need for recultivation.
Our bacterial cultures are suspended at a defined cell density in broth or phosphate buffer supplemented with foetal calf serum and stored in portions of about 0.3 ml in plastic ampoules at a temperature of –140 °C. When needed for MIC or MBC determination, the ampoules are thawed and the contents diluted to give the desired inoculum and used immediately. For studies of bactericidal kinetics or morphology, in which proliferating cells are required, we first incubate the samples for 2–3 h, until the log phase of growth commences.
This method is less timeconsuming than others and considerably enhances the reproducibility of the assays, notably with prolific β-lactamase-producing strains and the fastiduous strains of *Neisseria and Haemophilus*, the characteristics of which are often altered by storage and passage.
This method of preservation makes it possible to perform antibiotic assays over periods of years under standardized conditions.

Sensitivity tests on strains of *Gonococcus* originating from the Basle district

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During the first 4 months of 1977 about 100 different strains of *Gonococcus* were isolated in the out-patient department of the Dermatology Clinic of the University of Basle. These were systematically tested for sensitivity to penicillin G, spectinomycin, thiamphenicol, doxycyclin and rifampicin. The MIC values were determined by the agar-dilution method on Müller-Hinton chocolate agar with 2 inocula, and the diameter of the zones of inhibition by the disc method according to Bauer-Kirby. Tests for β-lactamase production were made using the chromogenic cephalosporin Glaxo 87/312.
The MIC values found were: penicillin G 0.001–1 μg/ml; spectinomycin 2–16 μg/ml; thiamphenicol 0.12–4 μg/ml; doxycyclin 0.06–4 μg/ml; rifampicin 0.016–4 μg/ml. The size of the inoculum had little influence on the activity of the substances, except for that of penicillin G against the β-lactamase-positive reference strains. No β-lactamase-producing strains were detected amongst the clinical isolates. In the case of penicillin G, thiamphenicol and doxycyclin, there was a correlation between the MIC and the inhibition-zone diameter.

Standardization of the sero-immunological tests by their mechanization or automation by means of the Biodilutor and Bioreactor (Biotechnia)

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In most cases the demonstration of an antibody or an antigen has to be completed with a determination of their concentration in the sample, which has to be sufficiently accurate to allow a comparison to be made with the results obtained on foregoing or succeeding samples of the same origin, or on a known positive control sample. The lack of precision of the results obtained by hand-executed, or primitively mechanized tests, renders such a comparison rather hazardeous. Furthermore, the work itself being time-consuming and tedious, most often semi-quantitative, or even purely qualitative methods are employed when the number of samples to be examined is large in comparison to the time or the personnal available. In order to ensure quantitative results, which can always be reproduced at any time, in any work situation, we developed an accurate serial diluting and dispensing

system, based on a new peristaltic pump system, and realized the Biodilutor, a simple and cheap table-top device, as well as the Bioreactor, an automatic machine with insertable programme. Both devices are now available through Biotechnia, P.O.B. 562, Voorburg 2119, Holland. The Biodilutor allows the various operations to be performed mechanically with a high output, the Bioreactor executes any classical sero-immunological test in a fully automatic way, according to the instructions furnished by the operator, and excluding any human error. The accuracy of the basic system is such that after 10 2fold dilutions the diluting error remains limited to $\pm$ $^1/_2$ of the 2fold dilutional step. Complement fixation tests, antistreptolysin determinations, haemagglutination, direct agglutination and immuno-precipitation tests, radioimmuno assays, enzyme linked immuno-sorbent assays, and MIC determinations are now routinely performed by means of these devices.

A novel method for the production of *Salmonella* flagellar antigen, used in the preparation of H-antisera

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In the classical procedure for making *Salmonella* flagellar antigen, whole formalinized cultures of highly motile *Salmonella* are used to immunize rabbits. This method yields OH-sera, often with disturbingly high O-titers. It would be desirable to use pure flagellar antigens, but until now its preparation has proved laborious (differential centrifugation). Earlier we (Fey and Wetzstein, Med. Microbiol. Immun. *161*, 73, 1975) proposed the use of immunoabsorption for antigen preparation, and now describe a very simple new method which gives a high yield and sufficiently pure flagellar antigen for the production of antisera with high H-titers and very low O-titers.

When flagella are brougth to a pH of 1.5, they depolymerize to monomeric subunits of flagellin (mol. wt ~30,000 daltons). When the pH is adjusted back to neutrality in a high salt concentration they spontaneously reaggregate to polymeric flagellin. We make use of these qualities in the following method of preparation: A dense suspension of highly motile *Salmonellae* is brought to pH 1.5 and left overnight (at 4 °C). In some experiments the bacteria were deflagellated mechanically with a waring blendor, centrifugated at 4000 × g and the supernatant brought to pH 1.5. Both supernatants are centrifugated at 49,000 × g (20,000 rpm Sorvall SS34) for 60 min. Solid ammonium sulfate is added to the supernatant to a $^2/_3$ saturation (i.e., $^2/_3$ of 4 M = 2.66 M) and left overnight at 4 °C. The sediment is reprecipitated twice with $^2/_3$ saturated ammonium sulfate. The sediment is dialyzed against phosphate buffered saline (pH 7.2). The end product developed a strong precipitation line with the homologous anti-H-serum and a strong band with 2 faint minor bands in the polyacrylamide electrophoresis. Examination by transmission and scanning EM revealed a fairly uniform suspension of flagellar threads with no visible bacteria. 20 rabbits were immunized and produced the following titers (number of sera): *H*: 3200 (4), 6400 (6), 12,800 (6), 25,600 (2), 51,600 (2). *O*: < 50 (9), 50 (1), 100 (6), 200 (4).

In the Limulus test our flagellar antigen is strongly positive, thus still containing endotoxin.

Detection of staphylococcal enterotoxin B with a new modification of the enzyme linked immuno assay (ELISA)

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The demonstration of staphylococcal enterotoxin in cultures and food is still not widely done because of the laborious methods of extraction and concentrating and, after all, because of the exorbitant prices of antisera and test toxins. Internationally the microslide technique is mainly used which needs only 10 µl of antiserum. After preliminary experiments with a radio immunosorbent test and an enterotoxin-coupled bacteriophage inhibition test we decided to apply the ELISA which exhibits a comparable sensitivity. We deviced a modification which allows 20 ml of toxin-extract instead of 10 µl to react with antibody. This makes tedious manipulations of concentration, which always cause a heavy loss of protein superfluous.

Polystyrol petri dishes are coated with anti enterotoxin B immunoglobulin (ammonium sulfate precipitated). 20 ml of enterotoxin is added and 6 h later a phosphatase-coupled enterotoxin B conjugate as well. Both antigens compete for the antibody sites. After incubation over night at room-temperature the plates are washed and substrate NPP (p-nitro phenyl phosphate) is added. The enzymatic reaction is stopped after 30 min by addition of 2 N NaOH (Engvall and Perlman, J. Immun. *109*, 129, 1972). If enterotoxin is present the substrate remains colorless, if not, enterotoxin-phosphatase is bound and breaks the NPP down to the yellow NP (p-nitrophenol) the extinction of which is measured at 403 nm. With this simple method we detected 1 ng/ml enterotoxin B in 9 experiments, 0.5 ng/ml in 8, 0.25 ng/ml in 6 and even 0.1 ng/ml in 3 experiments.

Since most sensible persons react clinically if the incriminated food contains 1–10 µg of enterotoxin/100 g (i.e. 10–100 ng/ml), our method is sufficiently sensitive for the detection of clinically dangerous specimens.

We obtained positive results with extracts from milk and meat which had been contaminated with 0.5–1 µg/100 g enterotoxin B before the extraction procedure (i.e. 5–10 ng/ml).

A rapid chromatographic separation of IgM from 50 µl of whole serum: its application to diagnostic tests

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The presence of specific IgM in serum is normally considered to signify a recent infection with a particular disease agent. To ascertain the specific IgM titer it has proved in many instances useful, if not necessary, to remove the IgG fraction from the material to be tested. We have adapted a preparative gel chromatography procedure to a rapid, reproducible small column technique. Diagnostic results on 10–15 separated IgM fractions can be obtained per day.

A sample (50–100 µl) of whole serum is applied to a 1.1 × 3 cm column of Biogel A-5m resin in 0.05 Tris-HCl, pH 7.2, 0.15 M NaCl and 0.02% NaN_3. The 1.1 ml void volume is discarded and the following 0.3 ml is collected as the IgM fraction. This fraction contains about 50% of the total IgM applied and is about 8fold diluted, but

contains no detectable IgG or IgA. The effluent is used directly in complement-fixation, hemagglutination inhibition, or immunofluorescence (IF) tests.
Using this technique we have a) eliminated false negative results due to IgG interference in IF for Toxoplasma-specific IgM;
b) replaced the cumbersome sucrose density gradient technique for Rubella-specific IgM, and
c) simplified the EB virus IF method (triple sandwich) by using the column fractionated IgM.
The small column technique is highly reproducible and is much faster, cheaper and simpler than other currently employed procedures for the separation of IgM from serum.

Microcalorimetry, a new procedure for registering the antibacterial activity of chemotherapeutic substances

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Microcalorimetry: a new procedure for registering the antibacterial activity of chemotherapeutic substances. Microcalorimetry makes it possible to show the heat production of a growing bacterial culture graphically, a culture during the logarithmic phase giving as a result a characteristic thermogram. By addition of chemotherapeutic substances this result will be changed. These alterations are the expression of the chemotherapeutic activity by the substance. In our research the tested substances showed a dose dependent effect on the microcalorimetric activator (i.e. the tested bacterial strains). The addition of tetracycline compounds to the growing bacterial culture altered the thermogram to an immediate fall of the calorimetric curve. Gentamycin and Ampicillin changed the microcalorimetric curve in another specific way according to the used substance. Furthermore it was possible to demonstrate synergistic effects of chemotherapeutics with the microcalorimeter. This microcalorimetrical researches have been carried out using *E. coli* strain H 3579 and *Staphylococcus aur. haem.* strain A 13665.

Tobramycin-nucleotidyltransferase, a unique enzyme of staphylococci inactivating aminoglycoside antibiotics

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Tobramycin resistant *Staphylococcus epidermidis* strains produce an enzyme, which efficiently adenylylates and inactivates Tob and many of the other aminoglycosides, but not gentamycin and sisomycin. Experiments with a crude enzymatic extract suggested that the hydroxyl-group 4 of the amino sugar I might be the target site of this enzyme (Santanam and Kayser, J. Infect. Dis. *1345*, 33, 1976). Our results with a partially purified enzyme preparation confirm this suggestion. In addition, our results suggest that the 4″–OH group of aminoglycosides also can be modified, thus enabling the enzyme to have a broad substrate-spectrum that surpasses in range, all the aminoglycoside modifying enzymes that have been characterized hitherto.
The enzyme was prepared by the osmotic shock procedure from *Staph. epidermidis* strain FK109. Purification was done by affinity chromatography either on dibekacin or tobramycin bound CNBr-activated sepharose-4B followed by chromatography on DEAE-sepharose CL-6B. The results support the inference that adenylylation of 4′–OH as well as 4″–OH is mediated by one and the same enzyme. The enzyme requires divalent cations and dithiotreitol for activity. The purine nucleotide triphosphates are more efficient as cosubstrates than the pyrimidine triphosphates. All the aminoglycoside non-substrates, notably gentamycin C_{1a} and streptomycin, inhibit adenylylation of most of the substrates to varying degrees. Although all the drugs have a specific optimal pH for adenylylation, those that are highly efficient as substrates are modified over a broad pH range, between 4.5 and 9.5. There is, in general, a close correlation between the MIC of the drugs and their relative efficiency as substrates. Adenylylation results in inactivation of all the substrates.

Characterization of the R factor P111-ACS and of its derivatives

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Preliminary studies showed that the transferable R factor P111-ACS (A: ampicillin, C: chloramphenicol, S: streptomycine) has an aggregate structure whereas its infectious derivative P111-A is a co-integrate (Experientia *33*, 131, 1976). Genetic experiments lead to the isolation of the P111-AS, P111-AC and P111-S transferable derivatives. C and SC segregants were not found. Transfer assays to different *Salmonella* showed that for P111-ACS and P111-AS, the resistance determinants are transferred at different frequencies, whereas for P111-AC, the 2 determinants are always transferred at the same frequency. These results suggest that the determinants of P111-ACS and P111-AS are transferred independently and that those of P111-AC are transferred together. Agarose gel electrophoresis and electron microscopy studies are in agreement with these data: in *E. coli* K12 F⁻, P111-ACS shows the structure of both the plasmid co-integrate (molecule length: 29.6 μm) and of the plasmid aggregate (19.2 μm molecules inferred to be the transfer factor and 3.2 μm molecules inferred to be the resistance determinants A, C or S). P111-AS and P111-S demonstrate similar behaviour (for P111-AS: 25.3 μm molecules inferred to be the co-integrate structure, and 19.0 μm and 3.2 μm molecules representing the aggregate form; for P111-S: 22.5 μm, 19.5 μm and 3.2 μm molecules). P111-A shows only a plasmid co-integrate structure of 22.4 μm in length. For P111-AC, a co-integrate structure of 29.4 μm and a population of 18.9 μm molecules were found. No molecules of 3.2 μm (resistance determinants) were detected. Further studies showed that P111-AC actually has an S determinant which is not expressed. This explains the co-integrate length similar to that of P111-ACS. Similar dissociation patterns are found in *E. coli* Rec A⁻ harbouring these plasmids. Furthermore, the presence of these different R factors in Rec A⁺ or Rec A⁻ hosts does not interfere with the UV survival of the cell. These results suggest a possible role of insertion sequences (IS) in the dissociation phenomenon, which could be controlled, at least in part, by a specific region on the plasmid itself. Further experiments are in progress to confirm these hypotheses.

The use of ether-treated cells to analyze the biochemistry of rifampicin resistance in clinical isolates

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The antibiotic rifampicin inhibits the growth of bacteria by forming a tight complex with the bacterial RNA polymerase and thus inhibiting the enzyme. Rifampicin resistant mutants obtained in the laboratory have been shown to contain an RNA polymerase which is not affected by the drug.
The question arose whether the rifampicin resistance of clinical isolates is also based mainly on a change of enzyme sensitivity, or whether other mechanisms of resistance can be found. To analyze such resistance, a number of gram-negative clinical isolates were treated with ether, rendering them permeable to nucleoside triphosphates. The RNA polymerase in these cells could then be directly analyzed for sensitivity towards rifampicin. Several highly resistant strains (minimal inhibitory concentration (MIC) $>$ 250 µg/ml) were found which contained an RNA polymerase with a drug sensitivity very similar to normal cells (MIC 4–16 µg/ml). Since only a limited number of strains has been tested so far, nothing can be said about the frequency of such mutants. However, it is clear that the change in sensitivity of RNA polymerase to rifampicin is not the only mechanism of resistance. In preliminary experiments, no rifampicin degrading enzymes have been found, leaving a change in cell permeability as a possible resistance mechanism.

Use of a cell-free protein synthesizing system in the evaluation and characterization of antibiotics

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Cell-free protein synthesizing systems with transducing phage DNA containing the lac operon as template and crude extract from an *E. coli* strain containing a chromosomal lac deletion are capable of synthesizing enzymatically active β-galactosidase. We used such a system to study the influence of several antibiotics on transcription and translation under conditions where the permeability barrier normally present in gram-negative organisms is completely lacking.
In a first approach the specificity and sensitivity of this system were studied using several antibiotics with known mode of action. β-Galactosidase synthesis was inhibited by all known inhibitors of transcription and translation studied, irrespective of wether they were active or inactive when tested against whole *E. coli* cells. On the other hand, no influence by antibiotics not interfering with protein synthesis could be detected at concentrations up to 100 µg/ml. We therefore conclude that this system might be a useful tool in a crude elucidation of the mode of action of new antibiotics and in the differentiation between resistance due to a permeability barrier or due to lack of a target.
In a further series of experiments the usefulness of the system as a possible screening for the detection of new antibiotics in fermentation broths was evaluated. When tested at a final concentration of 1% (v/v), such broths did normally not inhibit the system. On the other hand, there was a good correlation between antibacterial activity detected in fermentation broths by conventional methods and inhibition of β-galactosidase synthesis, indicating similar sensitivity of both systems.

Probabilistic identification of nonfermentative gram-negative bacteria

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The results given by 943 fresh clinical isolates of nonfermenters were processed through a program developed to be run on a 4K PDP8/e computer. The method of identification is derived from that described by Dybowski and Franklin (J. gen. Microbiol. *54*, 215, 1968); the data base is taken from Gilardi (Antonie van Leeuwenhoek *39*, 229, 1973) in its 1976 revision. It consists of a matrix of probabilities of positive results for 45 tests given by 37 taxa. Identification was obtained with 686 strains (72.7%) at a relative likelihood level (RLL) of 99.9% or above, with 776 strains (82.3%) at a RLL of 99% or above, and with 897 strains (95.1%) at a RLL of 90% or above. Failure to obtain identification at a high level was due either to a low relative likelihood for all taxa stored in the matrix or to an approximately equal relative likelihood for 2 closely related taxa. A new matrix of conditional probabilities was derived from the strains identified at 99.9% RLL. χ^2 comparisons were made, test by test, for all taxa represented in our isolates, between Gilardi's figures and ours. Significant differences were noted for many tests. These differences may be due to geographical variability among strains or to multiple isolates from patients harbouring an atypical strain. The presence, in the matrix, of taxa having a low maximum possible absolute likelihood is one of the reasons for poor discriminating power between some species. Despite these few restrictions, the program has the advantage of reproducible interpretation of test results; moreover it gives a list of atypical results, if any, and a list of further tests required for a more precise differentiation.

Comparative study with the scanning electron microscope of cysts of *Naegleria* and *Acanthamoeba*

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The free living protozoa belonging to the *Limax* group are found in soft water (swimmingpools) and soil. Many of them were found to be pathogens. They cause a primary amoebic meningo-encephalitis, a lethal disease which killed hundreds of individuals throughout the world. Vegetative cells or cysts are normally non flagellated. In unfavorable conditions, the vegetative cells may be flagellated.
In a scanning electron microscopic study of cystic forms of strains isolated from soft water as well as from pathologic materials (CSF) of patients with meningo-encephalitis, we have confirmed the phase contrast microscopy observation that *Acanthamoeba* (Hartmannella) has a folded surface. Folds can be deep and numerous (*A. castellani* Neff and *A. polyphage*) or shallow and few (*A. rhysodes* 1534 and *A. culberstoni*). *Naegleria*, however, are characterized by cysts with regularly round shapes.

Cysts of *N. fowleri* 0361 exhibit a smooth surface whereas those of *N. gruberi* EG_S are rough.
All *Naegleriae* cysts exhibited pores, with exception for *N. jadini* 0400. *N. fowleri* has an average 1.7 pores per cyst. Their rims are smooth. In *N. gruberi NB-1* and NE_G, the rims are evaginated, with a diameter of 0.6 nm and a thickness of 0.4–1 nm. No pore was observed in *Acanthamoeba*.
The surface of cysts as well as the number and structure of pores can be of importance in taxonomic determinations.

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Immunological responsiveness of calves fatally affected by bovine virusdiarrhea - mucosal disease (MD)

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Fatal mucosal disease (MD) in cattle is characterized by a persistent viremia and the absence of detectable neutralizing antibodies even in subacute and chronic cases. In order to study the possibility of a preexisting immune deficiency, several aspects of the immune system were studied in cattle, 5-month- to 2-year-old, affected by fatal MD. In all animals, with the exception of 1 with a low titer of serum-neutralizing antibodies, a generalized infection and viremia and the absence of neutralizing antibodies against the Oregon strain of BVD-virus and the isolate from the patient itself was proven.
Immunoglobulin levels of the IgG, IgM and IgA classes were within the normal range (H. Fey, personal communication). Several animals surviving at least 9–10 days were producing precipitating antibodies to ferritin at a similar rate as the controls. The stimulation of lymphocytes by phythemagglutinin and Concanavalin A was also similar to the controls. However, the serum of some animals at late stages of the disease had a suppressing effect on lymphocyte stimulation.
In conclusion we consider, that the immunodeficiency observed in fatal MD is specific and is induced by BVD-virus most likely in the acute stage of infection.

Further evidence for spontaneous Bornavirus-infections in rabbits

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Borna disease, a sporadically occurring virus-infection of the central nervous system in horses and sheep, has been diagnosed exclusively in central Europe. Until now, only 2 reports on spontaneous outbreaks of the disease in rabbits have been published. These observations were confirmed by the diagnosis of Borna disease in 2 rabbits from a seemingly enzootic area, in which the first virologically established outbreak among sheep and horses in Switzerland was recently reported (Metzler et al., Schweiz. Arch. Tierheilk. *118*, 483, 1976). The affected animals were females, showing clinical symptoms a few days after parturition. In both cases, virus antigen could be demonstrated in neurons of the central nervous system by the fluorescent antibody technique. The virus, which is noncytocidal, was isolated in a cell line of rabbit kidney origin (RK-13) and further characterized as Bornavirus by immunofluorescence. Histology of the brain revealed a nonpurulent meningo-encephalo-myelitis. Only in 1 case, some pathognomonic intranuclear inclusion-bodies were found. The diagnosis was further confirmed by the demonstration of virus-specific antibodies in the sera and brains of the affected animals, using indirect immunofluorescence in a persistently Bornavirus-infected cell line.

Infectious bursal disease of chickens in Switzerland: A serological survey for precipitating antibodies

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The infectious bursal agent (IBA) is a RNA virus, which causes necrotizing inflammation of the bursa Fabricii in chicken. Mortality lies between 1 and 15% and secondary losses due to a functional impairment of the immune system induced by the virus are considerable.
1430 sera from 110 poultry herds in Switzerland were tested for precipitating antibodies to IBA using agargel-precipitation (AGP) and counterimmunoelectrophoresis (CEP). In 47 sera from 10 herds antibodies against IBA were detected in CEP as compared to 45 in AGP. The positive sera were titrated, again using CEP and AGP and, additionally, by a neutralization test in embryonated eggs from a susceptible flock. The titers ranged from 1 to 1:8 in CEP, 1 to 1:64 in AGP, 1:18 to > 1:243 in the neutralization test. CEP seems to be suitable for detecting precipitating antibodies to IBA. Results are obtained within hours and less antigen and serum is needed as compared to AGP.
We conclude from our results, that infectious bursal disease is not a serious problem in Switzerland. Serological evidence for its existence was obtained only in 9.1% of the herds investigated. A vaccination program is therefore not justified at the moment.

Determination of influenza and mumps viral antibodies by single radial hemolysis in agarose gels

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The single radial hemolysis test (SRH) is based on the lysis of erythrocytes coated with antigen by specific antibodies in the presence of complement. The diametre of the hemolysis zone is proportional to the antibody concentration in the serum. In contrast to the hemagglutination inhibition test (HI), the time consuming and laborious removal of nonspecific inhibitors is unnecessary since they do not interfere with antibody determination by the SRH method. Furthermore, the serum can be tested undiluted.
In order to find the best method suitable for routine laboratory use we tested several different SRH procedures (Russell et al., J. gen. Virol. *27*, 1, 1975; Schild et al., Bull. WHO *52*, 43 1975; Väänänen et al., Arch. Virol. *52*, 91, 1976) for the assay of influenza antibodies. Factors like incubation temperature, time of antibody diffusion, concentration of antigen (crude virus in allantoic fluid) were of great importance for the sensitivity of the assay. A

modification of the method of Väänänen et. al., using $CrCl_3$ as a coupling reagent and an incubation time of 20 h at 37 °C, was found to give the best results. Sera from 412 patients and blood donors were screened by SRH, and independently by HI for antibodies to influenza A/Victoria/3/75 virus. The measurement of mumps antibodies in 420 sera from patients was made by SRH and the results were compared with the HI and CF titres. There was a good correlation in sensitivity between the SRH assay and the HI test. Moreover, it was possible to detect rises in antibody levels by SRH. The SRH technique appears particularly suited to the screening of large numbers of sera like in vaccine trials or herd immunity studies since it is simple, accurate and unaffected by nonspecific inhibitors.

Functional alterations of cellular membranes following infection of HeLa cells with rabbitpox virus

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Monolayer cultures of HeLa cells were infected with rabbitpox virus (strains Utrecht) at a multiplicity of 5 PFU/cell and functional parameters of their plasma and mitochondrial membranes were measured at different times after infection. Cytoplasmic (lactate dehydrogenase, EC 1.1.1.27) and lysosomal (acid phosphatase, EC 3.1.3.2; β-glucuronidase, EC 3.2.1.31) enzymes were released from the cells into the supernatant medium as early as 7.5–10 h post infection (hpi). Ca^{2+}/O quotients, acceptor control ratios and oxygen consumption/mg protein as well as $^{45}Ca^{2+}$ uptake were measured under conditions permitting the assessment af the functional state of mitochondria in intact cells using succinate as a substrate (E. Peterhans, Biochem. biophys. Res. Commun. *75*, 1078, 1977). Preliminary results strongly indicate that respiratory rates, acceptor control ratios and $^{45}Ca^{2+}$ uptake decay from the 10th hpi. These data suggest that as early as 10 hpi the mitochondria lose the ability both of sustaining the Ca^{2+} gradient and of energy production and that the plasma membrane is then permeable for soluble proteins. Thus, the cell can be considered as metabolically dead or dying, even though neutral red uptake does not fall below control values before 12.5 hpi.

Rabbitpox virus and vaccinia virus DNA: Comparison of the *Hind*III and *Sst*I restriction maps

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The DNAs of the closely related rabbitpox virus (strain Utrecht) and vaccinia virus (strain Elstree) were cleaved with the restriction endonucleases *Hind*III and *Sst*I. The cleavage sites of the enzymes were mapped on both genomes using double digestion and cross-hybridization techniques. The 2 cross-links present at or near both ends of the DNA permitted the identification of the terminal restriction fragments due to their rapid renaturation after alkaline denaturation.
Vaccinia virus DNA was found to be some 6×10^6 daltons larger than rabbitpox virus DNA. The 2 DNAs display identical restriction patterns in an internal region comprising of approximately 60% of the genome whereas the differences both in the restriction patterns as well as in the mol. wt are confined to both ends of the 2 genomes.
The 2 opposite terminal restriction fragments of rabbitpox virus DNA hybridize to each other and thus contain some similar sequences.

The significance of viral antibody titers in systemic lupus erythematosis (SLE)

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SLE is characterized serologically by high antibody titers against nuclear components, especially native DNA. In addition, high titers of antibodies against viral antigens such as measles, cytomegalovirus (CMV), adenoviruses and picornaviruses have been observed in SLE sera. The presence of such antibodies suggested that viral antigens might be of significance in the pathogenesis of SLE, especially since virus-like particles have been occasionally observed by EM in skin biopsies. Thus we have studied the antigenic specificities of the high viral antibody titers (CF titers against CMV and measles simultaneously) in 3 patients with a well established diagnosis of SLE.
Serum from SLE patients and from patients with measles or CMV (indicated by a 4fold antibody titer increase) were examined by indirect immunofluorescence (IF) on cell cultures infected with measles or CMV. In addition, antinuclear antibodies were absorbed from the SLE sera with a nuclear preparation from uninfected cells.
The pictures obtained show that a) the IF image of measles virus-infected vero cells (or CMV-infected human fibroblasts) treated with SLE or specific anti-measles (or anti CMV) sera is significantly different and neither show characteristic viral antigen fluorescence, and b) absorption of antinuclear antibodies from SLE sera does not uncover fluorescence of typical CMV inclusion bodies. Specific viral antibodies were not absorbed in control experiments.
These preliminary findings suggest that the presence in SLE sera of high CF titers against measles or CMV antigens may not be evidence for a recent or persistent infection. It is possible that the CF viral antibodies in SLE sera react with other, not viral specific, antigens in the CF antigen preparation. The nature of such antigens remains to be elucidated.

Inhibition of protein biosynthesis in Semliki Forest Virus infected cell cultures

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In chick embryo fibroblast cell cultures infected with Semliki Forest Virus (SFV) the rate of over-all protein synthesis is increasingly inhibited. The inhibition is late after infection and the onset slow (measurable 3 h after infection, leading to a nearly complete stop after 7 h). The inhibition parallels the exponential phase of viral RNA- and virion formation. Several explanations have been offered. 1. A decrease of the elongation- and/or release-rate of polypeptides. This cannot be shown, as equal masses of polysomes of infected or control cells yield in vitro the same amount of incorporation. 2. Out-

competing of host messenger RNA (mRNA) by viral mRNA. This is unprobable, because polysomes decay with a kinetic similar to that of the reduction of protein synthesis. We found, that polysomes are chased into single ribosomes; the latter are stable during infection. Part of these 'infection generated' free ribosomes contains viral RNA, which is heterogenous and sediments between 15 and 26 S. The association is stable in 0.5 M KCl, in 20 mM EDTA and in CsCl density gradients; it survives therefore the dissociation to ribosomal subunits, RNA being bound to large or small subunits. The 26 S-RNA is also found in large and small polysomes, indicating a different spacing of ribosomes on this RNA. Therefore, the following sequence of events, leading to inhibition of protein synthesis, can be visualized: Functional ribosomes and viral mRNA form polysomes, of which some are degraded by endonucleases because of large spacing. Ribosomes with attached viral mRNA fragments cannot be reused because of steric hindrance and are taken out of traffic.

Interaction of tetanus toxin and toxoid with cultured neuroblastoma cells: ^{125}I-toxin and toxoid binding studies

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Our previous investigations using immunofluorescent techniques have shown that tetanus toxin binds to mouse neuroblastoma C1300 cells in tissue culture under both growth and morphologically differentiated cell conditions. Using ^{125}I-labelled tetanus toxin and toxoid, we have confirmed these results. The toxoid does not bind at all. For the toxin, however, in growth culture cells, we now demonstrate 2 affinity levels of sialic-acid residue dependent binding: one binding displaceable by native toxin, and another which is not. There is not only no binding in presence of neuraminidase or β-galactosidase, but also after pre-incubation of the growth culture cells with ^{125}I-toxin, the action of either enzyme removes it entirely.

On the other hand, using morphologically differentiated cells there are 3 distinct classes of binding receptors: sialic-acid residue dependent, β-galactoside linkage dependent, and that which is independent. Class 1, neuraminidase sensitive, can be divided into 2 groups: displaceable and non-displaceable. Class 2 bound toxin can be removed only by β-galactosidase application. Class 3 sites are independent and defined by the proportion which is bound in presence of β-galactosidase. Our results demonstrate the kinetics of the interaction of ^{125}I-tetanus toxin under varying conditions. The binding is saturable within 1 h for either cell type, they support our hypothesis that ineffective and effective binding of the toxin are due to the different character of the separate receptors.

Genetic properties of two *E. coli* strains sensitive to the standard colicins

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During the last few years about 2000 strains of enterobacteria were examined in our laboratory for sensitivity to colicins and colicin production. Among them only 2 strains of *E. coli*, named Athens I and Athens II were found to be sensitive to the standard colicins A, B, C, D, E1+I, E2, E3+I, G, H, Iα, Iβ, K, L, S4, S5, X, V and Va. These strains can be very useful as colicin production indicators. Athens I was isolated from drinking water and Athens II from the faeces of a hospitalized patient. Both strains are not colicinogenic (sensitive indicator *E. coli*/Row) (Fredericq, A. Rev. Microbiol. *11*, 7, 1957). Athens I grows well on minimal medium containing agar, salts and glucose after 24 h of incubation at 37 °C. Athens II is an auxotrophic strain which requires minimal medium supplemented with methionin (DL-Methionin, 4000 μg/ml). The resistance of the 2 strains to penicillin G, chloramphenicol, kanamycin, streptomycin and tetracyclin was tested with antibiotic strips and their MIC's were estimated in liquid and solid (for the sulphonamides) media (Anderson et al., J. Hyg., Camb. *72*, 471, 1974). Athens I is slightly resistant to chloramphenicol (MIC = 3 μg/ml) and Athens II resistant to streptomycin (MIC = 600 μg/ml). No transfer of their resistances to *E. coli* K12 Nal^r was observed in overnight crosses. Athens I can mobilize the non-autotransferring plasmid NTP2 (resistance to streptomycin and sulfonamides) from *E. coli* K12 and transfer it to *E. coli* K12 Nal^r. Athens II does not mobilize NTP2 (Anderson et al., Nature *208*, 843, 1965). Athens I harbors an F-like RTF; it is not lysed in spot tests by the female specific phage φ2, it propagates phages μ2 and fd but not phage If1. Athens II is lysed by φ2 but it does not propagate phages μ2, fd or If1 (Pitton et al., Genet. Res. *16*, 215, 1970, and Grindley et al., Genet. Res. *17*, 267, 1971). Athens II is lysogenic; it produces phage lysing a λ^-*E. coli* indicator strain but not the parental strain, a λ^+*E. coli* and Athens I. The phage produced by Athens II can lysogenise the λ^- strain. (Bernstein et al., J. gen. Microbiol. *32*, 349, 1963). These findings and the fact that λ does not lyse Athens II suggest that this strain may be lysogenic with λ phage. Athens I does produce phage lysing the λ-strain or a number of wild type *E. coli* strains. The serotyping of our strains is under investigation.

Coliphage P1 DNA contains an insertion element IS1

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Bacteriophage P1 DNA contains 1 IS1 insertion element at unit 20 of the physical genome map. The P1 derivative P1Cm89 has a 2.6 kb (kilobasepairs) chloramphenicol resistance transposon inserted at map unit 4, which gives P1Cm89 2 additional IS1 elements. These are inserted in the same orientation but antipolar to the IS1 element at map unit 20. The evidence comes from electron microscope studies with *Bgl*II restriction fragments containing these elements and with an IS1 probe. Each of these IS1 elements is cleaved once by restriction endonuclease *Pst*I at 0.51 kb from one end and at 0.29 kb from the other.

The relationship between protein cleavage and head maturation in bacteriophage T4

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Protein cleavage has been shown to be important in the maturation of many bacteriophages and animal viruses. In T4 phage this cleavage has been suggested to be

necessary to convert a fragile prehead to the stable mature head. Other presumed steps in prehead maturation are lattice expansion, incorporation of the outer capsid proteins and DNA incorporation, all of which have been suggested to be dependent upon previous protein cleavage. We have now isolated 2 distinct types of particles from mutants in various T4 head genes. The characteristics of these mutant preheads suggest that the specific sequence of these maturation steps may not be obligatory.

Preheads produced by mutants in genes 21 and 24 are basically the same. They produce fragile particles which are membrane bound, have a protein core and a shell of uncleaved protein 23 (P23) in the unexpanded lattice configuration. The 24$^-$ mutants can be matured to normal capsids by adding P24 and then activating the cleavage enzyme coded for by gene 21.

The second class of particles is derived from a temperature sensitive mutant in gene 23 (tsA78). This strain makes aberrant particles which are also composed of uncleaved P23. However, they have many of the characteristics of mature capsids: they are resistant to 8 M urea to 1% SDS up to a temperature of 50 °C. Optical diffraction studies show that these particles have a lattice constant of 125 Å which is characteristic of the mature capsid. SDS gels show that they contain the outer capsid proteins hoc and soc never previously shown to be associated with structures made of uncleaved P23. The existence of these particles shows it is possible for lattice expansion and other maturation processes to precede rather than follow cleavage during bacteriophage T4 maturation.

A new class of bacteriophage T4 head related particles: Small and large capsoids in mutants in gene 17

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Under restrictive growth conditions, T4 mutants in gene 17 accumulate 2 different types of head related particles. Electron microscopy of thin sections showed that 1 type (esp) has a thick shell and the dimensions of T4 preheads. The other (elp) look like normal empty heads both in dimensions and shell thickness (H. Wunderli et al., J. supramol. Struct., in press). The isolated particles appear in negative staining as flat disks with a rough surface (esp's) and like normal empty capsids (elp's). The isolation procedure involves antibody precipitation of elp's and sucrose gradient centrifugation. Gel electrophoresis analysis has shown that bot particles contain the major head protein in the cleaved form (gp23*). Also, elp's have Hoc and Soc proteins while esp's do not. The study of the surface structure of giant variants of these particles, have revealed that the elp's have an expanded lattice (130 Å) while the esp's have an unexpanded lattice (112Å) in spite of being made of gp23*. They represent a new class of head related particles with different lattice constants and mass distribution from those previously described for other T4 head related particles (A. Steven et al., J. molec. Biol. *106*, 1976). In negative stained micrographs esp's look very similar to maturable particles (ε-particles) made of gp23* which accumulate when wtT4 infected cells are treated with acridine (C. Schärli et al., following abstract). We have found that esp's can be transformed in vitro (under low salt conditions) to particles similar to elp's, supporting the idea of the similarity of these particles with actual intermediates in T4 maturation.

A new class of bacteriophage T4 head related particles: The maturable ε-particles

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Acridines cause a delay in the phage head maturation (Piechowski and Susman, Virology *28*, 396, 1966). Electron microscopy of thin sections of infected cells shows: At low doses of 9 amino acridine (10–25% survivors at 60 min) normally sized with a reduced DNA content accumulate, while at higher doses ε-particles are predominant. These, as well as their breakdown products, become visible on sections of cells which are partly extracted during fixation; they are of the same size as the prehead, contain a core, but are morphologically and chemically distinct. Electron micrographs of negatively stained ε-particles show them as flat discs with a rough surface texture, while partially filled particles become empty and look like empty capsids. The amount of chemically determined gp23* (cleaved main capsid protein) corresponds to the number of particles counted on sections suggesting ε-particles to be made of gp23*.

Partially filled and ε-particles were tested for maturability by incubating the culture in the absence of new protein synthesis (25 µg/ml of chloramphenicol). Both types of particles become completely filled with DNA (black particles on sections). We propose tentatively: The gp23 of the prehead (τ-particle) becomes cleaved to gp23* without expansion forming the ε-particle. It then becomes expanded by 15% in each dimension, filled with DNA and stabilised. The step at which the core material is processed into the internal proteins and internal peptides of the mature head is not yet known.

We propose that the empty small particles (esp's) which are found in cells infected with the T4 mutants 17$^-$, are breakdown products of this ε-particle (Carrascosa et al., preceding abstract).

Are *E. coli pel*$^-$ mutants, which block phage lambda DNA injection, isogenic with *ptsM*, a PTS enzyme II?

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Escherichia coli pel$^-$ mutations block the penetration of bacteriophage lambda DNA into the cell. Using P1 mediated cotransduction, we have mapped pel$^-$ mutations at min 40.5 on the *E. coli* map, between markers *fad* and *eda*. *PtsM*, the gene coding for the phosphoenolpyruvate-dependent phosphotransferase system (PTS) enzyme II specific for mannose, glucosamine, glucose and fructose, also maps at this position.

The following evidence suggests that *pel* and *ptsM* are isogenic or coordinately regulated: 1. All *pel*$^-$ mutants, including those isolated by resistance to a large deletion phage, are *ptsM*$^-$; although parental *pel*$^+$ strains are *ptsM*$^+$.

2. Of *ptsM*$^-$ mutants selected by glucosamine resistance at high temperature in a *fda*^{ts} mutant (Jones-Mortimer and Kornberg Proc. R. Soc. Lond. *B 193*, 313, 1976), 20% are *ptsM*$^-$ *pel*$^-$, and 80% are *ptsM*$^-$*pel*$^+$.

3. *Pel* and *ptsM*$^-$ are not separated py P1 cotransduction (500 transductants tested).

These results suggest that the injection of lambda DNA into the cell may require a cytoplasmic membrane protein involved in the transport of some PTS sugars.

Ribosomal DNA in spores in *Physarum polycephalum*

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Using RNA-DNA hybridization techniques spores of the true slime mold *Physarum polycephalum* were found to contain 320 genes, each coding for 19 S and 26 S rRNA. Hybridization of rRNA to spore DNA fractionated on CsCl density gradients shows that the sequences coding for 19 S and 26 S RNA are located at a satellite position (1.714 g/cm³) of greater density than the main band DNA (1.702 g/cm³). The data demonstrate that in spores ribosomal DNA is not degraded and that no amplification of these genes takes place in hatching amoebae. The DNA content of spores (0.6 pg/spore) and the number of extrachromosomal rRNA genes present suggest that spores are in G2 phase.

The organization of genes for transfer RNA and ribosomal RNA in amoebae and plasmodia of *Physarum polycephalum*

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Using RNA-DNA hybridization techniques nuclei from both amoebae and plasmodia of the true slime mould *Physarum polycephalum* were found to contain 275 genes each coding for 5.8 S, 19 S and 26 S rRNA, 685 genes for 5 S rRNA and 1050 genes for tRNA. Hybridization of these RNA species to both amoebal and plasmodial DNA fractionated on isopycnic CsCl gradients reveal that the 5.8 S and 26 S rRNA genes are located at a satellite position (ϱ = 1.714 g/cm³) with respect to the main band of DNA, whereas 4 S and 5 S RNA genes are located exclusively in the main band peak of DNA (ϱ = 1.702 g/cm³). This result was confirmed by demonstrating that only the 5.8 S, 19 S and 26 S rRNA species hybridize to purified plasmodial ribosomal DNA. The 19 S and 26 S rRNA genes are localized on extrachromosomal DNA molecules of a discrete size (38 million daltons) in amoebae as well as in plasmodia.

Subunit structure of chromatin from *Physarum polycephalum*

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Limited digestion of isolated nuclei from *Physarum* with micrococcal nuclease reveals DNA fragments which are multimers of a repeating subunit. The size of the subunit was calculated from acrylamide-agarose gels calibrated with rat liver DNA fragments. The subunit structure differs from that of higher eukaryotes: each subunit contains only 170–175 base pairs as compared to 190–200 base pairs in higher eukaryotes. However, a quasi limit digest of 140 base pairs is obtained with *Physarum* chromatin as with higher eukaryotes. The basic repetitive structure of chromatin from diploid plasmodia and haploid amoebae is the same. The extrachromosomal ribosomal DNA located in the nucleoli, is arranged in a similar chromatin structure. Ribosomal chromatin is somewhat more slowly digested by micrococcal nuclease than bulk chromatin as determined by acid soluble products after different digestion times. Isolated DNA fragments after chromatin digestion hybridize equally well with 19+26 S rRNA as does unfragmented DNA.

Alkalimetric determination of cephalosporins

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An alkalimetric assay of cephalosporins utilizing an immobilized acetyl esterase to split off the acetyl side chain has been described (Konecny, Experientia *33*, 141, 1977; Enzyme Engineering 3. Ed. E. K. Pye, Plenum Press, 1977). Analogous titrations have now been carried out with lactamase P-99 (Ross, Meth. Enzym. *43*, 678, 1975).
Immobilization by standard methods (Weetall and Filbert, Meth. Enzym. *34*, 59, 1974) at room temperature of 580 units of pure lactamase on 1 g of the glutaraldehyde derivarive of zirconia glad controlled-pore glass gave a very stable catalyst with 30% retention of activity. Titrations were carried out by the pH-stat method in a recirculation reactor with 1 g catalyst, 40 ml buffer (0.5 M KCl/10 mM phosphate) and 1 N KOH (2.5 ml Radiometer autoburette) at a high recirculation rate at pH 7.50 or 8.00. After preequilibrating the catalyst with buffer, substrate was added, pH readjusted to exactly 7.50 or 8.00, and the reaction started by turning on the pump. Reproducibility of the titrations was better than 1%. Cleavage of the β-lactam ring of deacetyl cephalosporin C generated exactly 1 equivalent of acid/mole. Exactly 2 equivalents acid were formed from cephalosporin C because fast expulsion of the acetyl side chain follows cleavage of the ring. Use of the esterase and lactamase separately or in sequence permits the assay of mixtures of the 2 compounds. Less reactive cephalosporins, requiring higher activities, were assayed with the free lactamase.

The role of the laboratory and pilot plant in the development of an antibiotic fermentation process

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In this communication the advantages and disadvantages of batch fermentation versus controlled feed fermentation and its impact on mutation work in the laboratory are considered and a generalised plan for development is suggested. Optimation methods for batch media are described by Auden et al. (Pathologia Microbiol. *30*, 858, 1967) and for a controlled fermentation procedure by Pan et al. and Squires (Dev. ind. Microbiol. *13*, 103 and 128, 1971).
Reasons for introducing a controlled fermentation procedure: a) When it is necessary to introduce a cheaper medium (e.g. elimination of lactose from the penicillin batch medium). b) When dissolved O_2 becomes limiting due to high carbohydrate content of a batch medium. c) When enzymatic destruction of end-product occurs and the enzyme can be repressed by a glucose feed (cephalosporin C fermentation).
Advantages and disadvantages of a controlled fermentation procedure. *Advantages*: 1. In general produces higher yields than a batch procedure. 2. Does not suffer from

the necessary restrictions of a batch process. 3. By developing such a process considerable knowledge of its kinetics and biochemistry is acquired.
Disadvantages: 1. The increase in production cannot compare with that obtained from regular production of new mutants.
2. Requires considerable investment in the productivity plant as well as operator know-how. 3. Only a relatively small number of pilot fermentations can be run at the same time: development proceeds slowly. 4. As the media differ considerably from the batch media used in flasks scale-up from flasks becomes more and more difficult. The decision to develop a controlled fermentation process should not be taken lightly as it is virtually irreversible. Initially laboratory mutant screening, which is much cheaper, should be given priority.

Glucose metabolism in *Acetobacter aceti*

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Acetobacter aceti NCIB 8554 is able to grow on ethanol, acetate, or pyruvate in a mineral medium but not on glucose or any other carbohydrate. Of glucose added to an ethanol medium, about 60% is oxidized to extracellular gluconic and oxogluconic acid. The rest metabolized in the cells. Enzymatic, radiorespirometric, and electrophoretic determinations have been performed to determine the fate of intracellular glucose.
Activity of the following enzymes has been demonstrated: gluconokinase, glucose-6-phosphate isomerase, glucose-6-phosphate dehydrogenase (G-6-P-DH), 6-phosphogluconate dehydrogenase (6-P-G-DH), ribosephosphate isomerase, and transaldalase. 6-phosphogluconate dehydrogenase could be determined only after elimination of a potent NADH oxidoreductase by ultracentrifugation. The 2 dehydrogenases of the pentosephosphate pathway show a peculiar coenzyme specificity: G-6-P-DH is NADP specific and 6-P-G-DH is NAD specific. The dehydrogenases of other acetic acid bacteria exhibit the same coenzyme preference but not such a high specificity as those of *A. aceti*. That 6-P-G-DH is of the decarboxylating type was demonstrated by radiorespirometry and by identification of the product, ribulose-phosphate, with high voltage electrophoresis. N-acetylglucosamine could be identified as an important product of intracellular glucose metabolism.

Induction of tyrosinase in *Streptomyces glaucescens*

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Streptomyces glaucescens produces intracellular and extracellular tyrosinase (EC 1.14.18.1 monophenol monooxygenase). The intracellular tyrosinase is inducible by the following amino acids which do not serve as substrates of tyrosinase: methionine, leucine, phenylalanine and norleucine. Tyrosine, the substrate, is not an inducer (H. P. Kocher 1976, Diss. ETH 5668).
In a batch culture growing on synthetic medium the enzyme may be induced only during a relatively short phase of maximal specific growth rate. After induction and a lag of about 1 h, there is a steep rise of intracellular tyrosinase activity during 4–6 h, followed by a decrease. Without induction, intracellular tyrosinase activity cannot be detected. Extracellular tyrosinase activity accumulates independently from the intracellular one, both in the presence and absence of inducer. In the presence of inducer it is excreted earlier.
The short duration of competence for induction may be related to factors in the mycelium or in the culture medium. In order to test the presence of such factors, aseptic filtration of cultures of different age was carried out simultaneously and the mycelia and filtrates were recombined in new combinations for further incubation and induction. It was shown that neither age nor concentration of the mycelium had a significant effect on inducibility. Induction occurred in 'young' but not in 'old' culture filtrate. As addition of fresh medium components to 'old' culture filtrate had no effect, it seems that a substance produced by the growing mycelium inhibits induction.

Mutants of *Streptomyces glaucescens* with altered regulation of tyrosinase synthesis

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Tyrosinase is the enzyme for the first 2 steps of the conversion of tyrosine to melanin (tyrosine → dihydroxyphenylalanine → phenylalanine-3,4-quinone). In *Streptomyces glaucescens* tyrosinase is found intracellularly and extracellularly. Based on genetic evidence both forms of the enzyme are encoded by the same gene. Production of tyrosinase is dependent on the presence of certain α-amino acids in the growth medium. Among the best inducers of tyrosinase synthesis are methionine, leucine and phenylalanine. Tyrosine itself has no inducing effect. For elucidating the genetics and physiology of melanin production we have isolated mutants with altered regulation of tyrosinase synthesis.
Among mutants resistant to the methionine analogues selenomethionine (2×10^{-5} M) or ethionine (10^{-3} M) strains were found which produce tyrosinase in medium without inducer. Possibly these strains overproduce methionine. 2 types of such constitutive tyrosinase producers can be distinguished. One type shows high levels of both intra- and extracellular tyrosinase activity, the other shows only extracellular activity.
Furthermore colonies which, in contrast to the wild type strain, form melanin in medium containing only tyrosine but no other amino acid, arise spontaneously with a frequency up to 10^{-2} in freshly cloned cultures. Some of these mutants form tyrosinase constitutively. Others need tyrosine, methionine, or leucine for induction, but are not induced by phenylalanine anymore.
The results demonstrate that tyrosinase, the only enzyme known to be involved in melanin formation, is subject to an intricate pattern of regulation involving several genes.

The general control of amino acid biosynthesis in *Saccharomyces cerevisiae*

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Limitation of growth by single amino acids leads in *Saccharomyces cerevisiae* to higher specific activity of enzymes of the tryptophan-, histidine-, arginine- and possibly other amino acid-biosynthetic pathways (Schürch

et al., J. Bacteriol. *117*, 1131, 1974; Delforge et al., Eur. J. Biochem. *57*, 231, 1975; Niederberger, Dissertation ETH Nr. 5882, 1977). In our case the limitation has been accomplished with the amino acid analogue 5-methyltryptophan or by the use of bradytrophic mutants in the leucine-, lysine-, arginie-, serine- and histidine-biosynthetic pathways. The kinetics of the 'derepression' process was followed and a continuous increase of the activity over 3–7 h by factors of 1.5–5 was observed. In order to get some insight into this regulatory system the charging of tRNA and the behaviour of some aminoacyl-tRNA synthases was studied. Neither the charging nor the level of the leucine-tRNA were changed significantly in a leucine bradytrophic strain grown under leucine limitation. However the leucyl-tRNA synthetase was derepressed by a factor of 80 under that condition, whereas other aminoacyl-tRNA synthetases were either weakly or not at all derepressed.
In 3 mutant strains which are defective in this general regulatory system (Schürch et al., J. Bacteriol. *117*, 1131, 1974) not only the amino acid biosynthtic enzymes but also the aminoacyl-tRNA synthetases are unable to derepress under amino acid limitation. Nevertheless no influence on the charging and the level of leucine-tRNA was observed. It seems possible therefore that either only minor isospecies of tRNA's are involved in this regulatory system or the aminoacyl-tRNA synthetases play an active regulatory role.

Localization of arginine biosynthetic enzymes in yeast

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In eucaryotic microorganisms the compartmentation of enzymes and substrates is an important aspect of the regulation of amino acid biosynthesis. Previously, we have demonstrated the mitochondrial localization of N-acetylglutamate synthase, the first enzyme of arginine biosynthesis, which is feedback-inhibited by arginine. We now have analyzed the intracellular localization of the subsequent enzymes, leading from N-acetylglutamate to citrulline: N-acetylglutamate-5-phosphotransferase, N-acetyl-glutamic-γ-semialdehyde dehydrogenase, N-acetylornithine-δ-aminotransferase, N-acetylornithine transacetylase and ornithine transcarbamylase. For all studies, *Saccharomyces cerevisiae*, either the wild type H 1323 or a derivative with derepressed enzyme levels was used. The organism was grown on a minimal medium. Cells were lysed by osmotic shock after treatment with snail gut enzymes. The cell-free extract thus obtained was layered on top of a buffersolution containing 35% sucrose and centrifuged at 30,000 $\times$ g for 60 min. Enzymes were measured in the dialyzed sediment, which contained mainly mitochondria, and in the dialyzed supernatant (cytosol). Ornithine transcarbamylase was detected only in the cytosol fraction whereas 80% of N-acetylglutamate synthase activity was present in the sediment. 20–25% of the total activity of the other enzymes of citrulline biosynthesis were found in the sediment. The 75–80% of the total activities detected in the cytosol fraction may be due to breakage of organelles during cell lysis. However the specific activities of these enzymes were approximately 2fold higher in the sediment than in the cytosol. We therefore postulate that in *Saccharomyces cerevisiae* all 5 enzymatic steps leading from glutamate to ornithine occur in the mitochondria.

N-Acetylglutamate deacetylase in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa PAO 1 grows on N-acetyl-L-glutamate, the first intermediate of arginine-biosynthesis, as a sole carbon and nitrogen source. *ArgA* mutants, which are blocked in the first step of arginine-biosynthesis and thus are unable to synthesize N-acetyl-L-glutamate, have lost this ability. The rate of uptake of N-acetyl-L-glutamate is similar in the wild type and in *argA* mutants. Spontaneous mutants derived from *argA* strains have been found, which have regained the ability to grow on N-acetyl-L-glutamate.
These observations led us to investigate the metabolism of N-acetyl-L-glutamate in the cell. In addition to N-acetylglutamate synthase, N-acetylglutamate-5-phosphotransferase and ornithin-acetyltransferase, which produce or process N-acetyl-L-glutamate as an intermediate of arginine-biosynthesis, a N-acetyl-L-glutamate deacetylating activity has been found. In the wild type this enzyme is induced 5fold during growth on N-acetyl-L-glutamate. The enzyme has been purified 400fold and separated from other deacetylating activities, like N-acetylornithinase and formylaspartate deformylase.
Among 16 N-acetyl-L-amino acids tested as substrates, the partially purified preparation deacetylated N-acetyl-L-glutamine, N-acetyl-L-glycine and N-acetyl-L-methionine with 20% of the rate observed for N-acetyl-L-glutamate, whereas the other N-acetyl-amino acids were deacetylated with a rate of less than 10%. The enzyme was also specific with respect to the N-acyl-group and showed no peptidase activity.
The physiological role of N-acetylglutamate deacetylase and its possible involvement in the control of the intracellular pool of N-acetyl-L-glutamate are under investigation.

Microbial degradation of triazine herbicides

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Certain s-triazines and their derivatives occurring in the waste-water of production plants are degraded very slowly or not at all and can only be eliminated from the wastewater by complicated chemical and physical methods. We started a program to search for microorganisms, which can degrade those triazine derivatives, especially the hydroxy derivatives. We would later like to modify these organisms genetically in order to enhance the metabolic or degradative capabilities (for review on triazines see Esser et al., in: Herbicides: Chemistry, Degradation and Mode of Action, chapt. 2, p. 129. Ed. P.C. Kearney and D. D. Kaufman. Marcel Dekker Inc., New York 1976).
Microbial strains were isolated from soil samples, treated with triazine herbicides over many years, and from sewage plants from triazine production. The metabolic capabilities were investigated with ^{14}C-ring labelled s-triazines which were added to the growth medium at a concentration of 5 ppm. After fixed times of incubation samples were taken from the supernatant and were analyzed by thin layer chromatography, followed by autoradiography. In the case of cyanuric acid, the amount of $^{14}C\,O_2$ formed was measured.

Microorganisms were found which can perform the following degradation steps: N-ethylammeline to N-ethylammelide, N-isopropylammeline to N-isopropylammelide, ammeline to ammelide, ammelide to cyanuric acid, cyanuric acid to CO_2 and presumably NH_3.
At present we cannot perform deakylations of OH-atrazine, N-isopropylammeline, N-isopropylammelide, N-ethylammeline or N-ethylammelide. But dealkylations can be observed with atrazine. With the dealkylated OH-forms we can perform deaminations, and we can efficiently degrade cyanuric acid.

Enzymes of the sulfur metabolism of *Cephalosporium acremonium* assayed after disc electrophoresis

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For the biosynthesis of the antibiotic cephalosporin C by *Cephalosporium acremonium* the amino acid cysteine is an important precursor (Lemke et al., in: Cephalosporins and Penicillins, p. 370. Ed. E. H. Flynn. Academic Press, New York and London 1972).
Up to now 3 enzymes synthesizing cysteine could be detected in the fungus:

L-Serine + H_2S → L-cysteine + H_2O (1)
O-Acetyl-L-serine + H_2S → L-cysteine + acetate (2)
L-Cystathionine + H_2O → L-cysteine + 2-oxobutyrate + NH_3 (3)
(1) = Cystathionine β-synthase (EC 4.2.1.22)
(2) = Cysteine synthase (EC 4.2.99.8)
(3) = Cystathionine γ-lyase (EC 4.4.1.1)

For studying the influence of various sulfur sources (e.g. L-Met, L-Cys, $SO_4^=$ etc. in the culture media) on these enzymes, the electrophoresis of cell free extracts on polyacrylamide gels turned out to be a quite quick and effective method. 0.5–1 mg of protein of a $(NH_4)_2SO_4$-fraction (40–65% saturation) of a cell free extract was applied to a polyacrylamide gel (5 × 0.5 cm, pH 7.5, 7.5%, electrode buffer diethylbarbituric acid, Tris pH 7 [H. R. Maurer, in: Disc Electrophoresis. Ed W. de Gruyter, Berlin and New York 1971]). The gel was run for about 1 h at 5 mA. Afterwards the gel was slized into 1 mm discs, each disc was divided into 4 pieces, which were assayed for cysteine formation according to the equations 1–3.
Results with different mutant strains and the effect of various sulfur sources are presented and discussed.

A new approach to the biosynthesis of the rifamycin-chromophore in *Nocardia mediterranei*

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It is known that the ansa chain of the rifamycins is derived from acetate- and propionate-subunits. The origin of a sevencarbon amino unit in the naphthoquinone part of the rifamycins is still unknown. Carbon-13 incorporation studies have shown that this subunit could be derived from an intermediate of the shikimate pathway. Further studies with the incorporation method cannot be done, because precursors like shikimic acid are unable to penetrate into the cells of *N. mediterranei* and are therefore not incorporated into rifamycins.
By UV mutation we have isolated 2 mutants of *Nocardia mediterranei* N813 blocked in the biosynthesis of aromatic amino acids. Mutant A10 grows only in media supplemented with all the 3 aromatic amino acids Phe, Tyr and Try but not in media supplemented with shikimic acid or quinic acid. This mutant produces rifamycin in the same amount as the original strain. It accumulates shikimic acid and 3-dehydro shikimic acid in the medium. Enzymatic tests showed that the block in this mutant lies behind shikimic acid. Mutant A8 grows in media supplemented with either Phe and Tyr or Phe, Tyr and Try. It also grows slowly when supplemented with quinic acid but not with shikimic acid. The production of rifamycin by this mutant is very low. Mutant A8 accumulates a product in the medium which is not yet clearly identified. The product shows the properties of a ketopentose. The mutant must therefore be blocked somewhere in the pentose-shunt, in the biosynthesis of erythrose-4-phosphate.
The present results show clearly that the biosynthesis of rifamycin is correlated with the biosynthesis of aromatic amino acids. The seven-carbon amino unit of the rifamycin-chromophore must therefore be derived from an intermediate of the shikimate pathway not behind shikimic acid.

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