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Abstracts of Communications

The Ultrastructure of the Cell Wall of Bacillus megaterium. M. V. NERMUT, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

Cell walls of *Bacillus megaterium* were isolated by grinding with "ballotini" and a part was then extracted with hot formamide. The mucopeptide membranes so obtained were processed in the same way as the walls, i.e. fixed with OsO_4, $KMnO_4$ and uranyl acetate and embedded in Vestopal or Epon. Ultrathin sections were then examined under the JEM 6c electronmicroscope. The walls are about 250—300 Å wide, the width of the mucopeptide membranes is about half of this, i.e. about 110 to 150 Å. On the basis of the study of sections and metal-shadowed preparations, a scheme was constructed of the structure of the cell walls. They are composed of two layers, an under layer which is formed of mucopeptides and is rigid and an upper layer composed of mucopolysaccharides (in *Bacilus megaterium* it is teichoic acid). The wall of *Bacillus megaterium* is much more porous than the wall of *Proteus*, for example, since it easily permits the penetration of phosphotungstic acid into the space between the wall and the cytoplasmic membrane. This permits the showing up of the mesosomes which had not previously been possible in gram-negative bacteria.

The Properties and Incidence of Mesosomes in the Cells of Bacillus megaterium. M. V. NERMUT, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

Mesosomes are the equivalent of mitochondria in bacterial cells. They arise as recesses of the cytoplasmic membrane in the cytoplasm. They can be suitably demonstrated by negative staining with phosphotungstic acid as well as in ultrathin sections. The first method is much quicker and easily permits the determination of the number of mesosomes in a cell. We have, therefore, used it to study the form, structure and incidence of mesosomes in cells at different stages of the growth curve. Their number was found to be relatively constant, but their form and size vary from simple fine canaliculi to complex "globules": Mesosomes are found even in two-day cultures, but their demonstration is not so easy as in young cells.

The Liberation of Alkaline Phosphatase in Cultures of Escherichia coli Inhibited in the Synthesis of Cell Walls. F. SMÉKAL, M. STEHLÍKOVÁ, Department of Microbiology, Faculty of Science, Charles University, Prague.

A study was made of the connection between the liberation of alkaline phosphatase from the cells of *Escherichia coli* K 12 and damage to the mucopolymere of cell walls by the action of lysozyme, penicillin and glycine. By conversion of intact cells to spheroplasts almost quantitative liberation of the enzyme into the medium occurred (more than 90% of activity), whereas the activity of the enzyme in the spheroplasts was small. The results showed that the liberation of enzyme from cells is not dependent on the degree of degradation of the mucopolymere of the cell wall. The liberation of the enzyme from cells by degradation of the wall mucopolymere or by inhibition of the synthesis of this rigid component of the cell wall, shows that in *Escherichia coli* K 12 alkaline phosphatase is located between the cell wall and the cytoplasmic membrane.

Metabolic Activity of Spheroplasts and Intact Escherichia coli B Cells and the Effect of Inhibitors. D, NEUWIRTHOVÁ, M. BABULÍKOVÁ, O. ONDREJIČKOVÁ. Ľ. DROBNICA, Department of Microbiology and Biochemistry, Faculty of Chemistry, Slovak Technical College, Bratislava.

A study of the kinetics of respiration, the kinetics of incorporation of ^{14}C-adenine, ^{14}C-valine and ^{14}C-glycine and reducing activity with the addition of further sources of energy, was made in parallel cultures of normal cells (further N) and penicillin spheroplasts (S) of *Escherichia coli* B in synthetic B medium and in mp broth. The spheroplasts showed a lower respiratory rate, a lower reducing activity (with the exception of the substrate glucose) and also lower incorporation activity. The rate of incorporation of ^{14}C-compounds which in S was within the range of 90—310 minutes (90 minutes from the addition of sucrose and penicillin; 95% conversion to S) was unaltered, which is evidence of the relative stability of S in the corresponding media. Centrifugation and resuspension led to marked damage to S. The effect of inhibitors, including chloramphenicol, trypaflavine, 4-bromobenzy- and 2-naphthylisothio-cyanate on N and S was characterized by values of ID_{50}%, i.e. the concentration of inhibitor in μg/ml necessary for 50% decrease of incorporation of ^{14}C-compounds. Chloramphenicol, as a known inhibitor of proteosynthesis, inhibited incorporation of ^{14}C-valine in N and S (3.8 and 3.4 mcg per ml. resp.) and glycine (7.25 and 4.08 μg/ml resp.) in almost equal concentrations, but it inhibited the incorporation of ^{14}C-adenine in markedly different concentrations (100 and 15.9 μg/ml resp.). In the case of isothiocyanates as inhibitors of energy metabolism, the ID_{50} values are almost equal both in relation to type of incorporated compound and to difference in objective. Trypaflavine had a similar effect. The importance of the demonstrated findings is discussed also in relation to results which will be given in more detail in a further paper.

The Synthesis of Ribosomal Proteins in Bacillus megaterium. P. KŘEČKOVÁ, J. CHALOUPKA, Department of General Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The synthesis of ribosomal proteins was investigated with the aid of ^{14}C-leucine in cultures of *Bacillus megaterium* subjected to sudden changes by the effect of shift up and shift down. Incorporation was carried out in pulses for 10—20 min. At the end of this time the synthesis of protein was stopped by chloramphenicol. The cells were converted to protoplasts by lysosyme. These were broken up by osmotic shock or detergent and the individual components isolated by ultracentrifugation. Shift down was immediately evident in decreased incorporation of ^{14}C-leucine into ribosomal proteins; shift up, on the other hand, led to the stimulation of incorporation. Some residual incorporation into ribosomal proteins persisted after prolonged starvation in a nitrogen-free medium. Cells preincubated with chloramphenicol behaved after the removal of the antibiotic like cells going through the physiological state of shift up. Control mechanisms of synthesis of ribosomal protein and the structural cell components of *Bacillus megaterium* are discussed.

The Effect of ATP on β-galactosidase Formation in Escherichia coli. E. STEJSKALOVÁ, M. BURGER, Department of Technical Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The specific inhibitory effect of ATP on β-galactosidase formation was determined in *Escherichia coli*. The inhibitory effect of ATP was investigated in spheroplasts, prepared by the lysosyme technique in cultures of bacteria in the logarithmic phase of growth. A considerable repression was found even at low molar concentrations of ATP (10^{-4}M). On the other hand, ATP was observed to exhibit a stimulatory effect in the same concentrations in cultures of whole intact *Escherichia coli* cells, in the logarithmic phase of growth.

A Study of Factors Participating in the Mechanism of Competence. M. KOHOUTOVÁ, H. KOPECKÁ, Department of Genetics and Variability of Microorganisms, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

A kinetics was studied of the effect of a competence factor on the development of competence in a culture of *Pneumococcus* in transformation system. The relation of nuclease activity in sterile filtrates of a receptor culture to competence was studied at the same time. The part played by some factors in competence was studied using transformable and non-transformable strains.

The Effect of Ethionine on the Synthesis of Inducible Enzymes in Escherichia coli. J. SPÍŽEK, J. JANEČEK, Department of General Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

A study was made of the effect of ethionine on the synthesis of β-galactosidase and tryptophanase in *Escherichia coli*. Ethionine considerably decreases the formation of inducible β-galactosidase in *Escherichia coli* ML-30 and of constitutive β-galactosidase in *Escherichia coli* ML-308. The formation of inducible tryptophanase in *Escherichia coli* is similarly inhibited by ethionine. The results obtained seem to support the possibility that enzymatically inactive proteins are synthesized in both cases.

The Influence of ITC on the Metabolism of Escherichia coli and the Distribution of Inhibitors in the Cell. J. AUGUSTIN, L. DROBNICA, P. NEMEC, Department of Microbiology and Biochemistry, Faculty of Chemistry, Slovak Technical College, Bratislava.

Of the 250 derivatives of natural and particularly of synthetic isothiocyanates (ITC) so far tested for antimicrobial activity, the most effective antibacterial ITC belong to the group of benzylisothiocyanate derivatives (group I) and some alicyclic ITC with a large aromatic radical (group II). Compounds from the latter group are specifically effective on bacteria. A further, less effective antibacterial group is formed by monosubstituted phenylisothiocyanate derivatives (group III). The mechanism of antibacterial action was studied in more detail particularly in representatives of group I derivatives. ITC of that group interfere directly with the essential processes of energy metabolism, resulting in the observed inhibition of proteosynthesis, the synthesis of nucleic acids and therefore of reproduction. Further observations on the effect of ITC of the group III on the metabolism of *Escherichia coli*, retention and distribution of 4-bromophenylisothiocyanate-^{35}S in *Escherichia coli* (chemical and structural fractionation) gave precision to the picture of the mechanism of action of derivatives of phenylisothiocyanates and pointed to differences in their action from that of derivatives of group I.

Interaction of Phosphates with the Inhibitory Effect of Penicillin in Relation to Cell Division. J. STÁRKA, J. MORAVOVÁ, Department of Microbiology, Faculty of Science, Charles University, Prague.

Penicillin in low concentration exhibits a specific inhibitory effect on cell division of *Escherichia coli* without influencing growth. The thus induced filamentous forms were selected as model for the study of cell division and processes associated with it. The effective concentration of penicillin (1—3 units/ml) changes in indirect relation to the concentration of phosphate (0—0.2 M) in the medium (tryptose). Thus phosphates potentiate the effect of penicillin. The possibility of the influence of pH is excluded for two reasons: In the given time interval (120 min.) the pH was unaltered, and the action of penicillin is not sensitive to pH changes within the physiological range values for cells. If adenosine triphosphate (ATP) is added simultaneously with the antibiotic (1 unit/0.1 M phosphate) partial reversal to division occurs in the course of 60 min. The application of ATP after 60 minutes action of penicillin (fibres already formed) results in a rapid increase in the number of living cells. The theory could perhaps be admitted that in addition to inhibition of the synthesis of the wall mucopeptides (mucopeptides or diaminopimelic acid and hexosamines in fibres were reduced from 25%), penicillin also acts on some processes in the cytoplasmic membrane taking part in the formation of septa. The formation from filaments of lysozyme spheroplasts which are several times larger than those formed from one cell, and also the course of killing after infection with UV inactivated T2 phage, rather suggest that filaments behave as a single cell with several nuclear equivalents. Final proof will be provided by electronoptical studies.

Some Morphological and Biochemical Changes, Characteristic for the Differentiation of a Germinating Bacterial Spore into a Primary Vegetative Cell. V. VINTER, Department of General Microbiology, Czechoslovak Academy of Sciences, Prague.

The postgerminative development of the spores of *Bacillus cereus* is accompanied by the degradation of some envelope structures and at this time the new cell is protected by a probably incomplete atypical cell wall. When investigating the synthesis of ribonucleic acid and proteins during postgerminative development of spores, using the pulse-labelling with radioactive precursors, we found that the extractable pool of the cell may be enriched with these radioactive compounds in the preelongation period. During the synthesis of RNA at this time there is an increased rate of utilization of ^{14}C-uracil and, though to a lesser degree, of ^{14}C-adenine. During the period of swelling, labelled amino acids (^{14}C-leucine, ^{35}S-methionine and ^{35}S-cysteine) are not utilized at an increased rate for the synthesis of proteins. The increased content of precursors in the "pool" of swelling spores is connected either with temporary reorganization of the peripheral cell layers and changes in permeability, or is an artifact produced by the labile binding of low-molecular substances in these structures. Differences in the utilizability of these substances for the synthesis of macromolecules are either conditioned by their availability or may be a reflection of certain deficiencies of the cell in the synthesis of some precursors. During the later stages of differentiation, the primary cells evidently renew normal permeability regulating mechanisms and/or the synthesis of precursors typical for the vegetative cell.

The Effect of Pre-irradiation Inhibition of Macromolecular Synthesis on the Sensitivity of Cells to Ultraviolet Radiation. M. SEDLIAKOVÁ, F. MAŠEK, J. BROZMANOVÁ, Department of Genetics, Institute of Biology, Slovak Academy of Sciences, Bratislava.

Thymine, uracil and glucose were temporarily omitted in separate samples of the strain *Escherichia coli* T^-, U^-, $hist^-$, in the pre-radiation stage. After irradiation the missing substance was added to the medium. In all cases a record was made of the proportion of surviving cells, the DNA content of

the cells at the time of irradiation and the curve of synthesis of DNA after irradiation. In samples where glucose was omitted in the presence of a low concentration of thymine (2 μg/ml) cell survival was 70 times greater than in samples with sufficient glucose and the same amount of thymine (2 μg/ml.). The marked increase in resistance was not accompanied by a greater cell DNA content at the time of irradiation or by delay in the postirradiation synthesis of DNA. On the other hand, a marked delay in the postirradiation synthesis of DNA was noted in samples starved of uracil, where the proportion of surviving cells was increased about three-fold.

Unstable Forms of "Mutations" Induced by Ultraviolet Radiation. M. SEDLIAKOVÁ, D. SLAMEŇOVÁ, Department of Genetics, Institute of Biology, Slovak Academy of Sciences, Bratislava.

Mutation to the prototrophic state was induced in the strain *Escherichia coli* WP_2 try^-. From these forms isolations were made of slowly growing clones forming small colonies and rapidly growing clones whose colonies did not differ phenotypically from those of the original wild type. Both types of clones were passaged in conditions permitting growth of both prototrophic and auxotrophic cells. It was found that in both cases a certain percentage of the induced changes were unstable, but that in the slowly growing forms reversal to the auxotrophic state occurred more frequently and after a smaller number of passages than in rapidly growing forms. In some cases the rate of reversal in the populations of prototrophic cells was so high that it could be explained by the mechanism of spontaneous reversal at genome level. Since unstable changes occurred more often in slowly growing clones, the possibility was considered that the fall in the proportion of prototrophic cells occurred as a result of selective proliferation of the more rapidly growing auxotrophic cells, on the assumption that mutation to auxotrophy was associated with shortening of the generation time. In some cases, however, a fall in the proportion of prototrophs was found already in the primary culture where auxotrophic cells could not reproduce since the essential substance was not present. It would thus appear that some nonlethal damage produced by ultraviolet irradiation is of such a character that it can be repaired not only after the end of the postirradiation sensitivity period, but also after phenotypic expression. It is improbable that "structural systems" are responsible for this labile damage, but more likely genes concerned with the transfer and expression of genetic information.

The Autodegradation of Nucleic Acids in Frozen and Lyophilized Bacterial Cells. J. ARPAI, Z. LEŠKOVÁ, D. LONGAUEROVÁ, Department of Microbiology, Central Research Institute of the Foodstuffs Industry, Bratislava.

An investigation was made of changes in the nucleic acid level in the cells of psychrophilic and mesophilic bacteria in the frozen and in the lyophilized state, and of factors conditioning the rate of autodegradation processes at low temperatures or humidity. The relations between the physiological state of the organism at the time of stabilization and during the decrease in nucleic acids in preserved cell material were determined with respect to the ability of organisms to survive thawing and rehydration. The results of the experiments suggested that the kinetics of autodegradation processes, such as the functional reaction rate of specific polynucleotidephosphatases or their activation energy, is significantly different in the psychrotolerant organism *Pseudomonas fluorescens* in its dependence on hypothermia, than in the mesophilic strain *Escherichia coli* B. Reversible inhibition of depolymerase degradation of nucleic acids in lyophilized cells follows the curve of sorption isotherms in relation to increased humidity, on the basis of which optimal conditions of stabilization can be determined.

Changes in the Redox-potential of Aerated Cultures of Escherichia coli and Staphylococcus aureus under the Infuence of Oxytetracycline. J. KELLEN, V. KRČMÉRY, J. MAYER, Institute of Hygiene, Bratislava.

The redox-potential of growing aerated cultures of *Escherichia coli* 514 and *Staphylococcus aureus* Oxford falls steadily in the logarithmic phase of growth. Oxytetracycline immediately arrests this fall, which indicates the immediate onset of bacteriostasis. Arrest of the fall in redox-potential did not occur in resistant strains. The measuring of rH by the method elaborated by the authors proved adequate for studying the mechanism of action of tetracyline and possibly of other antibiotics. The arrest of rH changes by antibiotics (in the course of one minute) is further proof that one of the primary sites of action of tetracyclines is in the transfer of electrons in the "respiratory chain" in sensitive microbial cells.

The Ecology of Antibiosis. P. NEMEC, V. BETINA, J. BALAN, Z. BARÁTH, J. EBRINGEROVÁ, L. EBRINGER, L. KRIŽKOVÁ, Department of Microbiology, Institute of Biology, Slovak Academy of Sciences, Bratislava.

A relation was found between the quality and quantity of antibiosis and environmental factors.

These relations were studied in the lower mycelial fungi, the Ascomycetes, fungi imperfecti and also in Phytomycetes. A definite relation was found between geographical conditions (altitude and latitude) and the quality of antibiosis or the width of the spectrum of the antibiotics produced. The results obtained suggest that there is also a relation between the biological effectiveness of the substance produced and the presence of biological objects in the environment which may attack or be resistant to the lower fungi.

The Question of the Architecture of Cell Walls in Schizosaccharomyces pombe. E. STREIBLOVÁ, K. BERAN, Department of Technical Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The various stages in the growth and division of the cells of *Schizosaccharomyces pombe* were studied by fluorescence microscopy, and electron-optically using metal-shadowed isolated walls, replicas and ultrathin sections. A description is given of the origin and structure of scars arising from cell division. Details of the growth of the arthrospores were discovered. The question of the origin of septum is discussed and on the basis of this the whole structure of the cell wall is interpreted. New observations were made on the structure of the cell wall — original wall ring, newly synthetized cell wall, secondary ring zone of scar plugs. The process of septal division is shown digramatically to the fourth generation.

The Structure and Properties of the Fibrillary Network Formed on the Surface of Yeast Protoplasts as the First Stage in the Biosynthesis of Cell Walls. M. KOPECKÁ, Institute of Biology, Faculty of Medicine, Purkyně University, Brno.

Yeast protoplasts, prepared by autolysis and with snail enzymes, do not contain any structural fragments of the original cell wall on their surface. On cultivation in gelatine the protoplasts form a complete cell wall, on culture on agar and in liquid media they form only a fibrillary network. A study was made of the first stages in the synthesis of the cell wall under different conditions of cultivation and of the properties of the foundation of the cell wall, the fibrillary network. The morphology of the fibrillary network was studied by electron microscopy partly on metal-shadowed preparations and partly on ultrathin sections. The following properties were also determined: solubility in acids and alkalies, how it is affected by snail enzymes, staining by PAS reaction according to Mundkure, conditions for the formation of the network; observations using fluorescence microscopy.

Comparative Study of the Ultramicroscopic Structure of Protoplasts of Different Yeast Cells. M. HAVELKOVÁ, Institute of Biology, Faculty of Medicine, Purkyně University, Brno.

The experiments were made on species of yeasts reproducing by budding — *Candida utilis, Saccharomyces lactis* and *Saccharomyces carlsbergensis* —, by dividing — *Schizosaccharomyces pombe* — and *Saccharomyces ludwigi* from the group of apiculate yeasts. The purpose of the work was to determine the character of the changes differentiating the ultrastructure of protoplasts from that of the mother cells. These are changes related to the cell membrane systems and to differences in the number of some cell organelles. It was important to distinguish changes conditioned by the fixation and prefixing method used in processing the osmotically very labile protoplasts, from those typical for the actual process of conversion of cells to protoplasts. The question of whether these changes are of such a character that they obliterate species specific ultramicroscopic structure of protoplasts, is resolved.

The Regenerating Ability of Protoplasts of Different Yeast Species. A. SVOBODA, Institute of Biology, Faculty of Medicine, Purkyně University, Brno.

Experiments were made with type strains of *Saccharomyces cerevisae, Saccharomyces ellipsoideus, Saccharomyces lactis, Saccharomyces chevalieri, Candida utilis, Schizosaccharomyces pombe, Nadsonia elongata, Saccharomycoides ludwigii* and *Rhodotolorula glutinis.* Protoplasts were prepared by the action of snail juices on yeast cultures in the logarithmic phase of growth. Protoplasts were cultivated on the surface of agar films, in a liquid medium and in various concentrations of gelatine. Protoplasts of *Schizosaccharomyces pombe* and *Nadsonia elongata* regenerate in all cultivation media, whereas the protoplasts of the other species regenerate exclusively in high per cent gelatine. The ability to regenerate in different media is given in connection with the composition of the cell walls of the initial yeasts. In the absence of gelatine, the cell walls of regenerating yeast protoplasts are deficient in mannans. It is suggested that a high per cent gelatine may have an influence on the synthesis of the mannan component of walls.

The Preparation of Protoplasts in Aspergillus niger M. MUSÍLKOVÁ, Z. FENCL, Department of Technical Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Much work has been published on the preparation of bacterial protoplasts and spheroplasts but far less

data exist on the protoplasts of yeasts and mycelial fungi. Protoplasts were prepared from the mycelium of *Aspergillus niger* by the action of lyophilized snail gastric juices and stabilized in a medium with a high osmotic pressure by the addition of $CaCl_2$. The amount of protoplasts liberated depended both on the conditions under which the hyphae were cultivated and on factors acting during the liberation of the protoplasts from the mycelium. When the activity of snail enzymes in the medium decreased, the protoplasts started growing and greatly increased in volume. In several hours they began to develop cell walls and to form normal straight mycelia.

Essential Nutrition of Species of the Second Fermentation Type of the Genus Candida. M. POKORNÁ, V. STUCHLÍK jun., M. VAJDOVÁ, A. KOCKOVÁ-KRATOCHVÍLOVÁ, Institute of Chemistry, Slovak Academy of Sciences, Bratislava.

An investigation was made of the essential conditions of nutrition of 26 strains of *Candida tropicalis*, 8 strains of *Candida pelliculosa*, 5 strains of *Candida intermedia*, 4 strains of *Candida robusta*, 2 strains of *Candida langeroni*, one strain of *Candida obtusa* and various intermediate forms. The following media were used: Minimal = A; medium A supplemented with amino acids = B, medium B supplemented with vitamins = C. It was found that these species of *Candida* have weaker growth in media supplemented with amino acids and vitamins than *Candida albicans*. In some cases the mixture of amino acids without vitamins even decreased growth. The mixture of vitamins increased growth, but the values reached were less than in *Candida albicans*. The experiments show that there are differences in growth requirements for substances among the species.

The Distribution of Sugars in Yeasts. A. KOTYK, Laboratory for Cell Metabolism, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Nonmetabolizable sugars can be divided into three groups according to their distribution in the *Saccharomyces cerevisiae* yeasts: (1) Sugars which, in the state of equilibrium, are dissolved in all cell water, e.g. D-xylose and D-arabinose; (2) Sugars appearing to be present in higher concentration than that corresponding to diffusion equilibrium, e.g. L-sorbose; this is due to adsorption in addition to distribution in the solution; (3) Sugars which apparently reach only 30—50% distribution, e. g. L-xylose, L-arabinose, D-ribose. As distinct from the preceding groups, the uptake and distribution of these sugars are affected by anaerobiosis and some metabolic inhibitors.

The Action of Inhibitors of Oxidative Phosphorylation on the Assimilation of Ammonia in Anaerobically Growing Yeasts. M. GREKSÁK, L. KOVÁČ, Department of Biochemistry, Faculty of Science, Comenius University, Bratislava.

Anaerobically growing yeasts assimilated NH_4^+ only in the presence of glucose. The important products of assimilation were glutamine and alanine. Assimilation was inhibited by dinitrophenol and azide but not by carbonyl cyanide phenylhydrazone. The results are discussed from the aspect of the existence of partial oxidative phosphorylation reactions in anaerobic cells.

The Study of Momentary Metabolic Changes Provoked by Contact of Cells with Substrates. K. KOLLÁR, L. KOVÁČ, Department of Biochemistry, Faculty of Science, Comenius University, Bratislava.

Momentary changes in cell respiration after the addition of substrate were studied by electrochemical methods. When glucose was added to aerobically growing yeasts in the stationary phase, the rate of oxygen consumption gradually increased and only after a few minutes did it reach a stationary value which could be measured manometrically. In aerobically growing cells in the exponential and stationary phases, the addition of glucose caused a decrease in endogenous oxidation. In mutant yeasts with the impaired oxidative phosphorylation the increased oxygen consumption after the addition of glucose occurred only after a latent period depending on the level of endogenous reserves. The results were discussed from the aspect of the mechanism of the Pasteur and Crabtree effect and from the point of view of the efficiency of oxidative phosphorylation.

The Effect of Surface Active Substances on the Electrophoretic Mobility of Rough and Smooth Mutants of Yeasts. L. ŠILHÁNKOVÁ, Department of Biological Sciences, College of Chemical Technology, Prague.

It was found that dimethyllaurylbenzylammonium chloride, as a representative of the cation-type surface active substances, produces a diverse effect on the electrophoretic mobility of cells of rough and smooth mutants of yeasts of the genus *Saccharomyces*. In smooth mutants it causes decreased mobility by decreasing the negative charge on the surface of the cell, whereas in rough mutants a change in the direction of movement of the cells occurs under these conditions, caused by the resultant positive charge of the cell surface. Laurylsulphate, as a representative of anion-type surface active substances, on the other hand, caused about the same increase in negative charge at the surface of cells of both types of yeasts. These results suggest

basic differences in the composition of surface lipids in the two types of yeasts, and are in accord with our previous findings of the increased sensitivity of rough mutants of *Saccharomyces* to the inhibitory effect of cation-active detergents.

The Fatty Acids in the Different Types of Lipids in Streptomyces aureofaciens. V. Běhal V. Procházková, Department of Biogenesis of Natural Substances, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

An investigation was made of the relation between the biosynthesis of fatty acids and tetracyclines in their producer *Streptomyces aureofaciens* to detect the conditions which would change their mutual relation. In subsequent experiments the lipids were separated and the fatty acids present in them determined. The amount of lipids varied between 1.1 and 1.4% dry weight of mycelium. The lipid content was not dependent on the production of tetracyclines or on fermentation conditions. The lipids were divided into fractions: triglycerides (34.2%), diglycerides (39.5%) and free fatty acids (26.3%). Sterols, steroid esters and phospholipids were not demonstrated. Using gas chromatography, in most types of lipids fatty acids were found with a chain containing 14—18 carbon atoms, with odd and even number of carbon atoms, straight and branched and mostly saturated.

The Comparison of the Antibacterial Activity of Neomycin with 7 Quaternary Ammonium Compounds on Pseudomonas aeruginosa. I. Táborský, J. Nezval, E. Smékal, R. Janisch, Department of Hygiene and Epidemiology, Department of Medical Physics and Department of Biology, Faculty of Medicine, Purkyně University, Brno.

The antibacterial activity of neomycin with three routinely used and four new synthetic quaternary ammonium compounds (QAC) was compared turbidimetrically on *Pseudomonas aeruginosa*. A comparison was also made in the presence of EDTA (ethylene diaminotetraacetic acid) and Ca^{2+} in the medium. Under the conditions of our experiment, the antibacterial activity of a mixture of neomycin + QAC or neomycin + EDTA was statistically significantly higher than the activity of the substances alone. The addition of Ca^{2+} on the other hand, led to a decrease in antibacterial activity in all cases, but least, however, in the mixture of neomycin + newly synthetized substance 3549 — VÚFB. Some electronoptic studies were made in addition to turbidimetric methods. Although electronoptic observations are greatly dependent on the variability of the microorganisms studied, they confirm the results obtained by the turbidimetric method.

Comparative Study of the Action of Inhibitors on Microorganisms and HeLa Cells. M. Zemanová, K. Horáková, Department of Microbiology, Faculty of Science, Comenius University and Department of Technical Microbiology and Biochemistry, Faculty of Chemical Technology, Bratislava.

Selected metabolic inhibitors, cytostatics and other antimicrobial substances including isothiocyanates were characterized by their action against representatives of various groups of microorganisms (bacteria, yeasts, fungi, protozoa and algae) and HeLa cells. The ED_{50} and ED_{100} were calculated from the toxicity curves. It was found that the tested substances have different effects on the models used. Some substances exhibit a strong antibacterial action, in others marked antifungal action predominates. Some are much more effective against microorganisms than against animal cells. On the other hand, some tested substances were clearly cytotoxic and almost without effect on microorganisms. In addition to this divergent action of tested substances on different objects, it was found that many substances with antifungal action also had a cytotoxic effect, whereas they were inactive against bacteria. Most of these substances are poorly soluble in water.

The Antiprotozoal Activity of Some Chemically Similar Antibiotics. J. Balan, J. Ebringerová, L. Ebringer, Department of Technical Microbiology, Institute of Biology, Slovak Academy of Sciences and Department of Microbiology, Faculty of Science, Comenius University, Bratislava.

The antibiotic trypacidin was discovered on the basis of specific screening in a search for new antiprotozoal antibiotics. After working out the chemical structure of the substance it was shown that it is a substance of a structure similar to geodin. For this reason the antiprotozoal effect of several antibiotics of the geodin group were tested and it was found that some of them have a noteworthy action against some pathogenic protozoa.

The Influence of Cyanein on the Synthesis of Nucleic Acids and Proteins in Yeasts and Bacteria. Ľ. Drobnica, V. Betina, Department of Microbiology and Biochemistry, Faculty of Chemistry, Slovak Technical College, Bratislava.

The antibiotic cyanein, isolated from *Penicillium cyaneum* (Betina *et al.*, 1962) is particularly active against yeasts and yeast-like microorganisms and ineffective on bacteria and protozoa. It depresses the growth of Hea cells in tissue culture in strikingly low concentration (ED_{50} : 0.01 μg/m) and also shows a cancerostatic effect on some types of tumours in experimental animals. In the cells of Ehrlich's

ascitic carcinoma *in vitro*, proportional concentrations inhibit the incorporation of ^{14}C-compounds and inorg. ^{32}P into RNA and DNA. It markedly inhibits the incorporation of $^{32}P_a$, especially into ribosomal and nuclear RNA, at the same time incorporation into sRNA is not influenced (Drobnica & Klenow, 1964). Further investigation of the effect of cyanein on energy metabolism and the synthesis of macromolecules (incorporation of ^{14}C-adenine, ^{14}C-valine) in yeasts suggests that the influence on the reproduction of yeast cells is the result of interference with the synthesis of nucleic acids. In experiments with normal cells and penicillin spheroplasts of *Escherichia coli*, it was found that only in the case of spheroplasts does cyanein inhibit the incorporation of ^{14}C-adenine. Incorporation of ^{14}C-valine and ^{14}C-glycine under the same conditions and in the same time interval is not affected.

A Study on the Synergic Effect of a Combination of Natural and Synthetic Substances on Candida albicans Pn 10. K. Horáková, Faculty of Chemistry, Slovak Technical College, Bratislava.

In research on synergic effect an evaluation was made of some antifungal antibiotics and the group of synthetic isothiocyanates in all mutual combinations, on *Candida albicans* Pn 10. In determining this effect it was important first to establish the concentration of substances causing a 0—100% inhibition of reproduction of *Candida albicans*, .i.e. to determine the shape of the toxicity curves. Their determination permitted the rational programming of the concentrations of substances used in the combinations. Substances were combined in retarding concentrations, the values of which were calculated from the corresponding toxicity curves. When this concentration corresponded to the value ED_{10}, relatively exact criteria were already provided for the actual evaluation of the synergic effect. A marked synergic effect was observed on *Candida albicans* of the mutual combination of isothiocyanates and a combination of these substances with antifungal antibiotics, particularly the combinations: amphotericin B with 4-bromophenylisothiocyanate and with 4-bromobenzylisothiocyanate, trichomycin with 4-bromobenzylisothiocyanate, 4-bromophenylisothiocyanate with 4-diphenyl- and 4-bromobenzylisothiocyanate, 1-naphthylisothiocyanate with phenylisothiocyanate. The degree of synergic effect, i.e. the measure of the synergism of these combinations of substances is expressed mathematically. Since the above substances show anti-yeast properties, the results obtained on the synergic effect can probably serve as a basis for practical experiments.

A Special Form of Antagonism in Producers of Macrolide Antibiotics. L. Ebringer, Department of Microbiology, Faculty of Science, Comenius University, Bratislava.

All basic non-polyene macrolide antibiotics produce apochlorosis in *Euglena gracilis* and thus convert autotrophic cells to heterotrophic ones. After the addition of macrolide antibiotic to a culture of green Euglena, the chloroplasts gradually decrease in size and are destroyed; but with a decrease in the concentration of the antibiotic, plastids regenerate and increase to the original size, although, of course, only leucoplasts are present. This special form of antagonism is now known only in actinomycetes. It seems that under the influence of macrolide antibiotics, various colourless flagellates could develop from their pigmented partners under natural conditions. This would suggest that macrolide antibiotics also played an important role along these lines in phylogenesis. The differential sensitivity of chloroplasts or the relative insensitivity of the actual cells of *Euglena gracilis* to antibiotics at the same time supports the Mereshkovsky's hypothesis that plastids were originally independent autotrophic microorganisms which probably became adapted to their present form of existence by symbiosis.

The Metabolization of Cancerogenic Compounds by the Fungus Botrytis cinerea. L. Bahna, Oncological Research Institute, Bratislava.

A report is given of a suitable microbiological model for the study of the biological oxidation of aromatic amines, aminostilbenes, aminoazocompounds and aromatic carbohydrates. The biological synthesis of indamines, quinhydrones, azines and quinones was detected.

Nonsteady States in Continuous Cultures. B. Sikyta, J. Slezák, Institute of Antibiotics, Roztoky near Prague.

Nonsteady states in the continuous culture o microorganisms can be divided into three groups: (1) A nonsteady state in the culture produced by a change in part of the population (mutation); (2) Systems not attaining steady state (oscillation); (3) Nonsteady states produced by a change in operative parameters (contamination, a change in the rate of dilution). Nonsteady states produced by changes in operative parameters (changes in D), particularly by methods that can be described as "transient states and washing out" have not so far been used experimentally, although in some cases the use of such methods is useful. The principle of both methods is the study of changes in the cultures

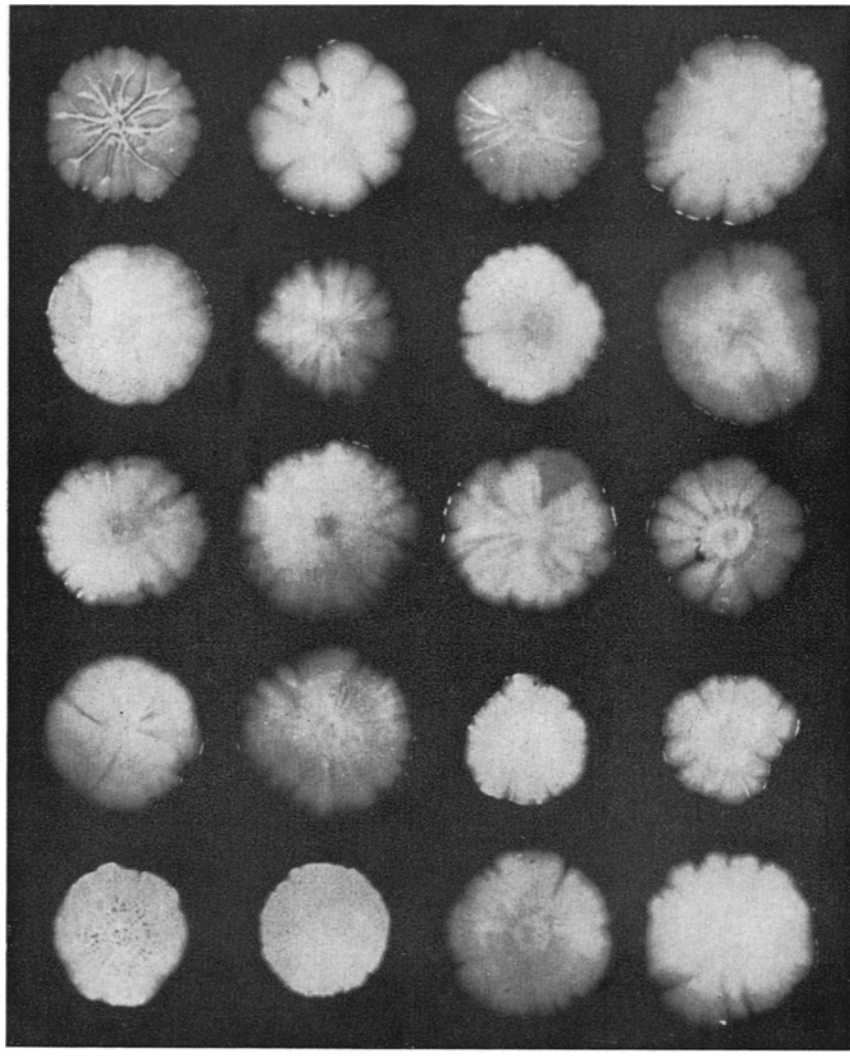


Fig. 1. Giant colonies.

48—11*	48—27*	48—28*	48—29*
48—40*	48—41*	48—42*	48—51*
48—56*	48—43*	48—24*	48—14*
48—62*	48—44*	21—4—17	22—3—13*
21—14—1	21—15—2	21—45—1	48—81

* Strains of *Saccharomyces cerevisiae*. All the others are comparative strains.

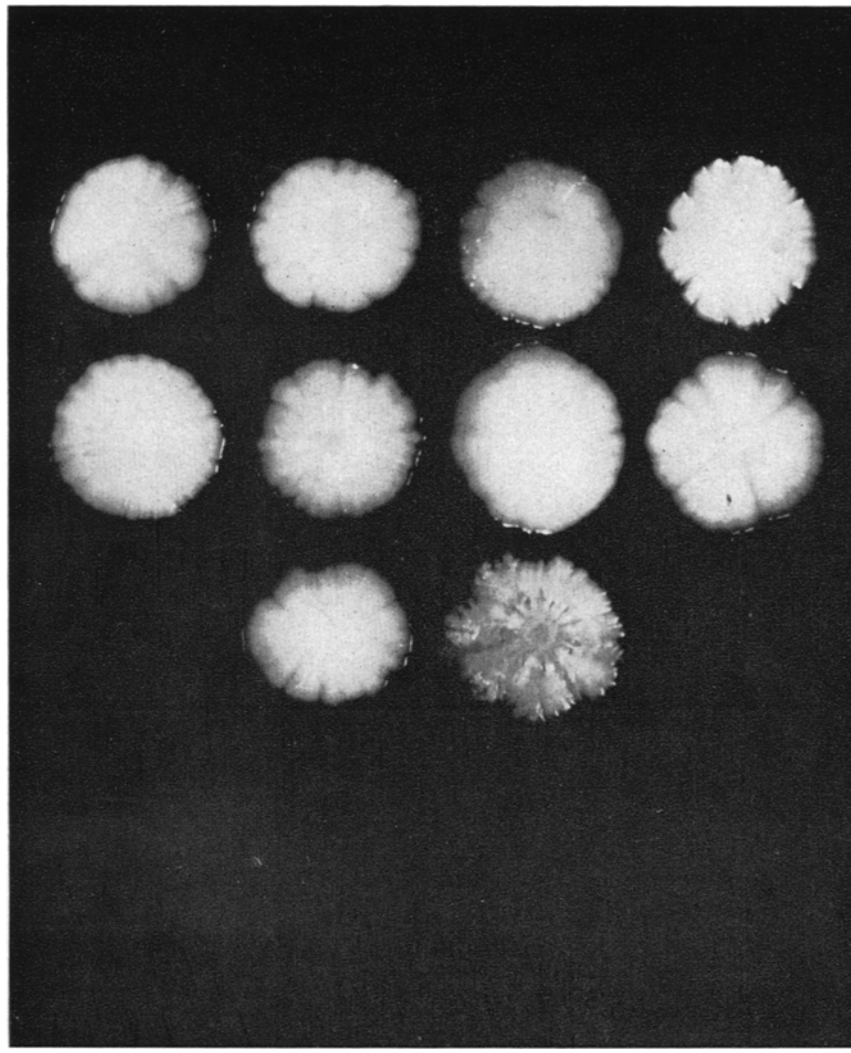


Fig. 2. Giant colonies.

48—12**	48—53**	48—52**	48—71**
48—82**	48—77**	48—63**	48—54**
	48—19**	29—3—12*	

* Strains of *Saccharomyces cerevisiae*; ** strains of *Saccharomyces carlsbergensis*.

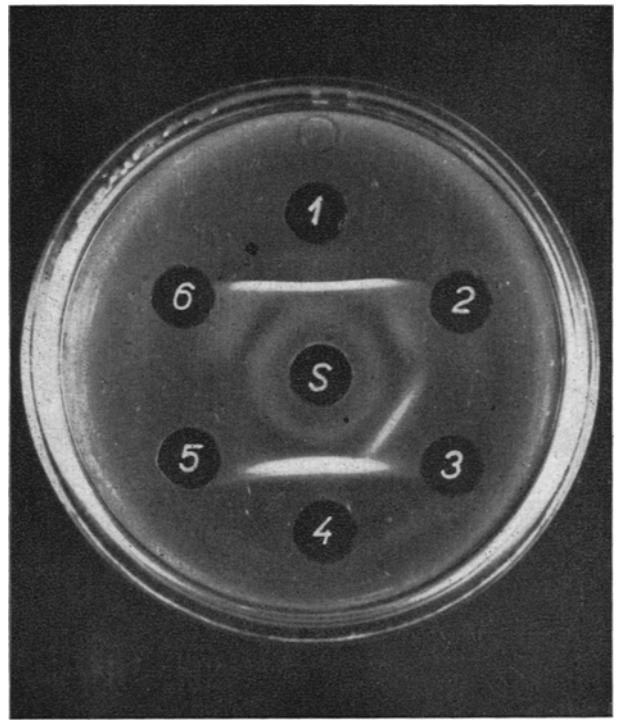


Fig. 3. Precipitation reaction of 48—44 antiserum. 1 — Autolysate of strain 48—44; 2 — autolysate of *Saccharomyces logos* 21 — 1 — 1; 3 — autolysate of *Saccharomyces carlsbergensis* (large morphological type, 48—54); 4 — autolysate of *Saccharomyces monacensis* 48—82; 5 — autolysate of *Saccharomyces mandshuricus* 48—81; S- 48—44 serum. The pronounced precipitation line in 1 and 4 denotes the presence of antigen "M". In 2, 5 and 6, common antigens give a faint precipitation line; these common antigens can be removed by adsorption. In 3, the common antigen appears in a high concentration.

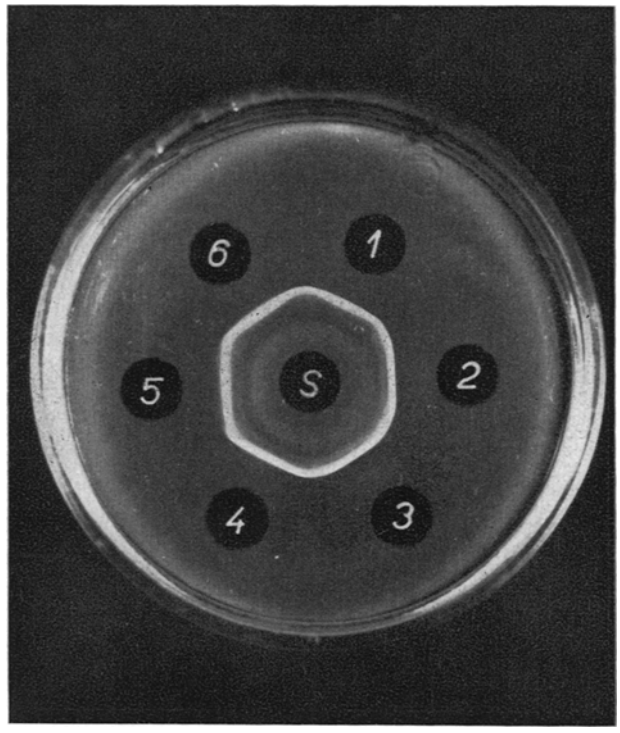


Fig. 4. Precipitation reaction of 48—44 antiserum. 1 — 48—14 autolysate; 2 — 48—24 autolysate 3 — 48—44 autolysate. The strains belong to the group which gives one third fermentation of raffinose. 4—48—71 autolysate, 5 — 48—77 autolysate; 6 — 48—82 autolysate. These strains belong to the group which completely ferments raffinose. All the strains possess antigen "M".

while reaching steady state. In "transient states", new media are added during batch cultivation at a rate which is less than the maximum specific growth rate of the microorganisms. The growth curve can be prolonged by this method. In "washing out" a higher flow-rate is used than that corresponding to the maximum specific growth rate, so that the cell concentration decreases with time. The cell concentration changes in both cases according to exactly definable relations. The kinetic relations between the formation of various components can by assessed from the change in concentration of other components in the culture. The use of the method is illustrated by the example of pyruvic acid formation in cultures of *Escherichia coli* B.

Kinetic Relations in Batch and Continuous Culture in Microorganisms with a Linear Growth Curve. J. Slezák, B. Sikyta, Institute of Antibiotics, Roztoky near Prague.

When predicting the behaviour of cultures of a continuous system it is usual to begin from an analysis of the form of the batch growth curve. Relations were thus deduced for the growth of cultures in continuous systems from growth curves which run exponentially to the exhaustion of the substrate (classical substrate limitation — Monod, Novick & Szilard). In some cases the growth curve or a part of it, has a linear course; however, these cases have not yet been searched from the viewpoint of the kinetics of continuous cultivation. When cultures grow on media with a low concentration of certain trace elements, vitamins etc., the exponential phase of growth is followed by a linear growth phase. Linear growth was also found when bacteria were grown in the presence of some amino acid analogues. The kinetic relations in a continuous system in one or more stages can be deduced on the assumption that the growth curve is biphasic, logarithmic and linear or only linear.

New Methods of Recording the Course of Fermentation Processes. J. Hronček, Department of Biotechnology, Faculty of Chemical Technology, Slovak Technical College, Bratislava.

It is usual to record the course of fermentation processes by means of growth and fermentation curves. The author suggests recording the amount of biomass and product (metabolite), not in relation to time, but in relation to the concentration of the limiting substrate. The curves obtained have no time scale. The author points to the advantages of these records and methods of evaluating them. The method is of value in bioengineering calculations.

Submerged Citric Fermentation on Beet Molasses in Fermentation Tanks. A. Čejková, J. Rybářová, M. Šestáková, Research Institute of the Distilling and Food-Preserving Industry, Prague.

Excess potassium ferrocyanide added to molasses mash for citric fermentation for the purpose of removing inhibitory substances (metals, betaine, etc.) remains in the molasses solution in the form of free ferrocyanide ions and has a positive effect on the fermentation process. The stimulating effect of these ions is only effective in cultures of *Aspergillus niger* R 3—56 in the presence of a sufficient amount of phosphorus. Under these conditions citric acid formation increases in proportion to increases in the concentration of free ferrocyanide ions, to the value of 25 mg Fe $(CN)_6^{4-}$ per 100 ml mash at the beginning of fermentation. Acid synthesis is not altered by further increase in the concentration of ions. During the fermentation process the concentration of free $Fe(CN)^{4-}$ gradually decreases to its almost complete exhaustion in the solution. The course of the fermentation process is connected with the decrease in the ions in the medium. The growth phase needs a higher concentration than the fermentation phase. By increasing $Fe(CN)_6^{4-}$ concentration in the growth phase to 50—200 mg/100 ml depending on the type of fermentation molasses, and its removal from the solution after 24—48 hours, the duration of fermentation was reduced to 96—120 hours and the yield of citric acid increased to 59.3—61.0%.

Methods of Separating Amylolytic Complexes of Aspergillus awamori. J. Kodešová, V. Munk, Department of Technical Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Amylase produced by the mould *Aspergillus awamori*, splits starch into maltose and glucose. The optimum activity for the production of the first and second sugar depends on pH and temperature which suggests the existence of at least two enzymes. There is a brief evaluation of the method of separating the complex of these enzymes in the molecular structure by the DEAE cellulose, electrophoretic and chemical methods.

Nutrition of Lactobaccilus delbrückii Related to Lactic Acid Production D. Halama, Department of Biotechnology, Faculty of Chemical Technology, Slovak Technical College, Bratislava.

In laboratory experiments, the course of fermentation of production cultures of *Lactobacillus delbrückii* was investigated in media with molasses, pure and raw sucrose and in media made up from a sucrose obtained by desugarization of molasses.

Peramin (feather hydrolysate) and malt extract prepared by different methods, were used as sources of growth factors, or amino acids were added. The stimulating effect of these sources of growth factors and the advantages of the different fermentation media were not dependent on the amino-nitrogen content. The rate of sucrose consumption and lactic acid formation decreased after valine and leucine had been exhausted; their addition again increased the fermentation rate.

The Biosynthesis of Lysine in Synthetic Media. J. PLACHÝ, A. BŘEČKA, Institute of Antibiotics, Roztoky near Prague.

Lysine is produced by auxotrophic mutants of strains producing glutamic acid and requiring biotin, and accumulates in the medium in industrially important amounts. The level of lysine production in synthetic medium depends on the concentration of biotin and the amino acids present in the medium. The aim of the work was to determine which amino acids and at what concentration are required for maximum production of lysine in synthetic media. Using synthetic media with different concentrations of homoserine and threonine + methionine, the optimum concentration of amino acids was determined for the production of lysine by the mutant producing glutamic acid and requiring homoserine which can be replaced by threonine and methionine. Apart from these three, other amino acids do not affect the production of lysine by the above mutant as determined by adding a solution of amino acids contained in corn-steep (corn-steep used as a source of amino acids in complex media). The effect of homoserine and threonine, homoserine and methionine and homoserine and threonine + methionine on the production of lysine was studied using synthetic media.

The Biotin Concentration in the Mycelium of Streptomyces aureofaciens during Fermentation. J. ZELINKA, V. SYHOROVÁ, Department for the Biochemistry of Microorganisms, Institute of Biology, Slovak Academy of Sciences, Bratislava, and Research Institute of the Distilling and Food-Preserving Industry, Prague.

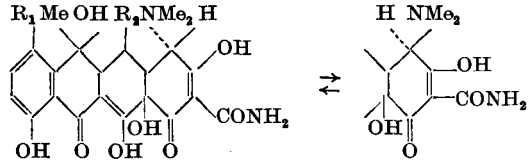
A study was made of the biotin concentration in the mycelium of *Streptomyces aureofaciens* during fermentation of chlorotetrycycline. The biotin level increases from the beginning of fermentation and reaches a maximum of 3.08 μg per g dried mycelium between the fifth and tenth hour of fermentation. Then a progressive decrease occurs which is proportional to the increase in chlorotetracycline. At the time of the greatest fall of the biotin content in the mycelium its level in the supernatant after centrifuging off the mycelia, can be considered to be unchanged.

Preparation of Glucamylase and Enzymatic Hydrolysis of Starch to Glucose. M. KULHÁNEK, M. TADRA, V. MANSFELD, Research Institute of Pharmacy and Biochemistry, Prague.

155 strains of *Aspergillus* and *Rhizopus delemar*, coming mainly from the Collection of the Institute of Microbiology of the Czechoslovak Academy of Sciences, were tested for glucamylase production. *Aspergillus awamori* NRRL 3112 was found to be the best, since it had the highest glucamylase production with a relatively low transglucosidase activity. For fermentation the surface cultivation on bran or submerged one in liquid media containing maize was used. Glucamylase was isolated by precipitating the enzyme with solvents or by the use of the method for decreasing the transglucosidase concentration at the same time. The glucamylase preparation thus obtained hydrolyses liquid starch mash almost completely to glucose. In this way solutions of glucose were prepared containing 0—3% isolmaltose, from which anhydrous crystalline glucose could be isolated with a yield of about 95% in relation to the dry weight of the starch used.

The Course of Epimerization of Tetracycline Derivatives in the Fermentation of Streptomyces aureofaciens. Z. JANGLOVÁ, J. SUCHÝ, Department of Biogenesis of Natural Substances, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

In the group of tetracycline antibiotics, isomerization at C_4 occurs in solution with the production of the biologically inactive epimer.



The equilibrium of the system depends on pH and the concentration of buffer. In the strain of *Streptomyces aureofaciens* producing tetracycline, the system should contain about 20% epimer at equilibrium under fermentation conditions (pH 6.5). But the concentration of the epimer in the course of fermentation process is much lower. From the beginning of production there is a distinct increase in the concentration of the epimer, approximately proportional to the increase in the concentration of the normer, to about 5% of the total tetracycline derivative. From 90 hours of fermentation, however, the concentration of epimer begins to fall to a fifth. A fall in the concentration of the epimer to about the same level also occurred if the epimer was added to the fermentation liquid in several-fold excess. The fall in the concentration of epimer is evidently associated with the completion of tetracycline production. The question of the mechanism of epimerization during fermentation is discussed.

A method was worked out for the determination of the epimer based on the polarographic determination of the chromatographically separated epimer.

A Study of the Rate of Utilization of Sugars in the Alcoholic Fermentation of Sulphite Liquors. Z. RADĚJ, Paper and Cellulose Research Institute, Bratislava.

A qualitative and quantitative study was made of the rate of consumption of the individual hexoses in the alcoholic fermentation of sulphite liquors, using both batch and continuous cultivation. It was found that sugars are utilized progressively by the yeast *Saccharomyces cerevisiae*, glucose being fermented first and the consumption of mannose only starting after its complete utilization. The galactose content decreased inappreciably during fermentation. A sulphite liquors adapted culture of *Saccharomyces fragilis* permitted the complete utilization of galactose during fermentation. Increased stirring of the fermentation wort accelerated fermentation and decreased the duration of the fermenting or retention time. The correctness of the laboratory results was confirmed under production conditions.

An Unusual Type of Fermenting Peptone Water. O. MRÁZ, J. CHURÝ, Veterinary Faculty, Agricultural College, Brno.

According to current data and experimental work, peptone water is the basis of a diverse series of changes by bacteria into neutral or various alkaline products. In addition, current methods of control check only the influence of incubation on the colour of the sterile medium and the identity of added carbohydrates according to inoculated test microorganisms. The indefinite results obtained when studying the fermentation properties of strains of *Actinobacillus lignieresii*, led both authors to inoculate only peptone water with a pH indicator. After 7 to 11 days of incubation, most of the 70 strains of *Actinobacillus lignieresii* changes the striking colour of bromothymol blue to yellow and more exact measurement showed a shift from the original pH of 7.4 to pH 6. The authors document the exceptional nature of this phenomenon by microbiological research with 20 families of significance in medicine, using seven standard peptones of the firm Difco. The cause of the acidity is demonstrated in more detail by chromatography.

The Effect of Fatty Acids and their Derivatives on the Utilization of Phosphate. Z. HUŇKOVÁ, Z. FENCL, Department of Technical Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The use of hydrocarbons as a source of carbon for the growth of microorganismus has again increased interest in the action of their oxidation products in the cell. A study was made of the effect of the lower fatty acids and their derivatives on the transport of phosphate by the cells and on the degradation of glucose. This included a study of the role of the aliphatic chain and carboxyl groups in the process and the question of the mechanism of action of fatty acids is discussed on the basis of the results.

On the Role of Phosphorus in the Metabolism of Claviceps purpurea (Fr./TUL). J. KYBAL, J. MAJER, I. KOMERSOVÁ, Research Institute for Natural Drugs, and Department of Biogenesis of Natural Substances, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Cultivation experiments on synthetic media showed that conidia formation only occurred in media containing very little phosphate and only after nearly all the phosphorus has been exhausted. Resorption of phosphate is very rapid and occurs already during the germination of the conidia. These findings permitted the elaboration of the essential requirements for the production of conidia, used in the artificial, field production of ergot for infecting rye, in submerged cultures, on a technical scale. During the production phase of the parasitic culture on rye, the phosphorus, nitrogen, fat and alkaloid content was determined in maturing sclerotia. It was found that in the production phase the character of nutrition is governed not only by the predominant sugar component, but also by a relative deficiency of phosphorus against nitrogen. The relations between the investigated parameters were also confirmed by the analysis of sclerotia from various hosts (wild grass ergot). The importance of phosphorus for the biosynthesis of alkaloids is discussed.

Phosphate Utilization in the Submerged Cultivation of Ergot. J. MAJER, J. KYBAL, I. KOMERSOVÁ, D.D. WANI, Department of Biogenesis of Natural Substances, Institute of Microbiology, Czechoslovak Academy of Sciences, and Research Institute for Natural Drugs, Prague.

The submerged cultivation of ergot and the production of alkaloids are sensitive in certain phases to the concentration of nutritional elements and to their mutual concentrations. These relations

were studied by investigating the demands of the organism for phosphate, if this was the limiting component in the medium. The determinations were made during the growth of the vegetative inoculum at the beginning, middle and at the end of this stage of cultivation. It was found that primary saturation of the mycelium with phosphate occurs about 27 hours after inoculation with a spore suspension. This was verified by the use of $^{32}PO_4^{3-}$. The question of the possible resorption of phosphate by resting spores of this organism was also investigated and also demonstrated by means of $^{32}PO_4^{3-}$.

The Growth and Oxidation-Reduction Activity of the Basidiomycetes Oudemansiella mucida. J. ČERNÁ, Department of Biogenesis of Natural Substances, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The occurrence of cytochromes and the activity of enzymes of the flavine type, polyphenoloxidase, peroxidase and catalase were studied in the course of submerged growth of the basidiomycetes *Oudemansiella mucida*. The sequence of these enzymatic systems in the growth cycle of the fungus was demonstrated. The ability of the mycelium to oxidize DPNH was also determined and an experiment made to fractionate the enzymatically active subcellular structures. A protein structure having diaphorase activity was found in the sediment of washed and disintegrated mycelium after fractionation by centrifuging at 100,000 *g*.

Growth and the Antibiotic Activity of Mycorrhizal Fungi. V. ŠAŠEK, Department of Biogenesis of Natural Substances, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

In order to test the hypothesis on the role of antibiotics from mycorrhizal fungi in increasing the resistance of the host plant to parasitic microorganisms, a study was made of the antibiotic activity of 17 species (35 strains) of fungi in mycorrhizal relationship to the pine. The fungi were cultivated statically and in submerged cultures in several nutrient media under various conditions and their activity against standard sensitive test microorganisms and against pine fungal parasites, was determined. To test the activity of mycorrhizal fungi against parasites attacking young pines, experiments were set up in vitro with the simultaneous culture of pine seedlings, parasites and mycorrhizal fungi. A more marked antibiotic activity was found only in two species (*Tricholoma saponaceum* and *Rhizopogon roseolus*). The results obtained do not permit the conclusion to be drawn that antibiotic production plays an essential role in the relation between the two partners. The results extend our previous knowledge about the surface and submerged growth of pure cultures of mycorrhizal fungi under laboratory conditions. Species of the family *Suillus* can be placed among the well growing fungi. Representatives of the *Amanita* and *Lactarius* families showed the worst growth. Concentrates of an antibiotic were obtained from cultures of the two antibiotically active species and its essential biological and physico-chemical characteristics determined. The results suggest that it is a new antibiotic.

The Enzymatic Degradation of Cholesterol. A. ČAPEK, O. HANČ, M. TADRA, Research Institute of Pharmacy and Biochemistry, Prague.

Cholesterol commands attention from two main aspects, firstly from the the chemical point of view as a cheap home-produced raw material for the production of steroid compounds and secondly from the physiological point of view as a compound occurring in the healthy and diseased organism (the precursor of the biosynthesis of steroid hormones; atherosclerosis). The first step in the microbial transformation of cholesterol is oxidation into $\triangle^4$-cholestene-3-one. The end product of the microbial degradation of cholesterol is CO_2. C_4 of the steroid A ring is oxidized about four times more quickly to CO_2 than C_{26} of the lateral chain. Bacterial strains able to dehydrogenate the steroid molecule in position 1—2 of the A ring, transform cholesterol in a suitable manner in relation to the quality of the metabolites. The formation of phenolic substances (secoderivatives) and keto acids with splitting of the A ring were demonstrated as intermediary-products of degradation. The strain *Proactinomyces ruber* No. 932 was used for studying transformation processes in the cholesterol molecule.

The Transformation of Steroids by Means of Ustilago violacaea. B. ŠKÁRKA, Department of Microbiology and Biochemistry, Faculty of Chemistry, Slovak Technical College, Bratislava.

A study was made of the ability of *Ustilago violacaea* to transform the steroid molecule. The steroid was added dissolved in acetone (20 mg/ml) to different cultures of the microorganism and left for 6—144 hours. At the end of this time samples were extracted with chloroform (1 : 1), evaporated in vacuo to dryness and the residue dissolved in 0.5 ml chloroform. The steroids were determined in the extracts prepared in this way by means of chromatography in different systems, the separate substances eluted from the paper were evaluated in the UV and IR regions of the spectrum and their melting points determined. Quantitative paper chromatograms were also prepared. It was found that *Ustilago violacaea* reduces the steroid molecule by the transformation of 11α-hydroxyprogesterone to progesterone, $\Delta^{1,4}$-androstadiene-3,17-dione to

Δe-androstene-3,17-dione to Δ^4-androstene-3,17--dione to testosterone.

Some Microbiological Parameters Characterizing the Course of Maturation of Meat after Treatment with Peracetic Acid. O. PAWEL, J. VRANOVSKÁ, Veterinary Research Station, Prague-Motol.

Peracetic acid appears to be one of the substances which can be used for the surface disinfection of foodstuffs and possibly for the improvement of their keeping qualities. Fresh beef was submerged in a 0.1% solution of peracetic acid immediately after slaughter. Meat from the same animal taken from the same site of the other side of the body, served as control. The meat was kept at 20° C and 70% relative humidity, and 4° C and 85% relative humidity. Before treatment and during maturation, up to the onset of decay, determinations were made of the numbers of the following microorganisms in the meat: psychro- and mesophilic aerobes (blood and meat peptone agar), anaerobes (meat peptone agar with added thioglycolate), coliform bacilli (Klimmer agar), yeasts and moulds (Sabouraud agar). The numbers of microorganisms on the surface of meat were markedly decreased after treatment with peracetic acid. The number in the depth of the meat was lower throughout the experiment than in the control meat. The keeping qualities of the treated meat were better as demonstrated by smell and by the chemical determination of pH and ammonia. Peracetic acid acted bactericidally on the meat and later acted as a bacteriostatic.

Proliferation of the Tick-borne Encephalitis Virus in Parenterally Infected Ticks. J. ŘEHÁČEK, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

When the tick-borne encephalitis virus is injected into the body cavity of half-saturated females of the ticks *Ixodes ricinus*, *Dermacentor pictus*, *Haemaphysalis inermis* and *Hyalomma dromedarii* it proliferates in all organs irrespective of pathological changes in the tissues of the ticks and changes in behaviour. The highest virus titres were found in the alimentary tract, hypodermis, muscles, in the Geneo organ and haemolymph, the lowest in the Malpighian and salivary glands. The tick organism can retain and proliferate the virus to high titres up to 1 LD_{50} (mouse)/0.03 ml. We consider this method of infecting ticks, i.e. inoculating the virus directly into their body cavity, could be used with advantage in virus isolation experiments in field work and for the preparation of the virus for transport over long distances.

The Distribution of Virus Neutralizing Antibodies against the Tick-borne Encephalitis Virus in Domestic Animals in the Tribeč Area. E. ERNEK, O. KOŽUCH, M. GREŠÍKOVÁ, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

An investigation was made of the sera of cattle and goats from six villages lying on the southern and south-western slopes of the Tribeč mountains, a well-known focus of tick-borne encephalitis in southern Slovakia, for the presence of tick-borne encephalitis antibodies, using the virus-neutralization test on monkey myocardial cells. The investigation showed that in the area investigated these domestic animals often come into contact with *Ixodes ricinus* ticks and the tick-borne encephalitis virus. The percentage of contact with tick-borne encephalitis virus in the cattles was 6—28% and of goats 0—34.7%, which varies with the locality. The percentage is related not only to the number of animals but also to their age. In all areas there was a direct relationship between the percentage and the age of the animals.

The Properties and Origin of Serum Interferon Inducted by Bacillus pertussis or by the Newcastle Disease Virus. L. BORECKÝ, V. LACKOVIČ, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

It was found that *Bacillus pertussis* is another non-virus agent which inducts the formation of interferon *in vivo*. The well-known effect of *Bacillus pertussis* infection on the white cell component of the blood was used in analysing changes in the blood picture accompanying the production or release of interferon in virus infection. The results suggest that the release of interferon in infection with NDV is in correlation with changes in the mononuclear blood count and that interferon formation after *Bacillus pertussis* inoculation is reflected by changes in the polymorphonuclear count. It was found that interferon formation is the same in splenectomized mice as in controls.

Optimum Conditions for Interferon Formation Inducted in Cultures of Chick Embryonic Cells Infected with Tick-borne Encephalitis Virus. E. HENSLOVÁ, H. LIBÍKOVÁ, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

An investigation was made of the influence of different factors on the production of interferon in chick embryonic cells infected with tick-borne encephalitis virus. The temperature of cultivation was 39° C. In the system used the higher degree of multiplication of the infection had no effect on the interferon content in preparations harvested three days after infection. A definite increase in interferon formation was obtained if already infected cultures

were incubated for more than three days and if older than 6-day cultures of chick embryonic cells were infected. These "old" cultures produced seven-fold more interferon per cell than cultures infected after 24 hours of cultivation.

The Mutual Action of Interferon and Some Other Defence Mechanisms of Cells Infected with Virus. D. STANČEK, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

The production and action of interferon, as a natural antivirus factor of infected cells and the organism, is dependent on a number of factors which determine the character of metabolism of the infected cells or result from it. These are primarily changes in the metabolic rate of cells and associated changes in the pH of the cells and media, reproductive activity of the cells, etc. The character of the virus infection of the cells and of the organism seems to be dependent on the mutual relations of these and other factors with interferon.

The Rapid Diagnosis of Some Virus Diseases using Fluorescent Antibodies. J. LEŠŠO, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

The viruses of epidemic parotitis, herpes simplex and *Coxiella burneti* were demonstrated by the direct fluorescent method with the aim of speeding up the diagnosis of mumps, parotitic meningitis, herpetic disease and Q fever. The epidemic parotitis virus was demonstrated in material from patients, in amniotic fluid and in the amniotic membrane of chick embryos. The herpes simplex virus was also demonstrated in human material and on tissue cultures of HeLa cells and Detroit 6. *Coxiella burneti* was demonstrated in mouse and guinea pig spleen. It was shown that the demonstration of the pathogenic agents by the fluorescent antibody method is more rapid and simpler than by the routinely used virological methods.

The Demonstration of Coxiella burneti in Phase I using Fluorescent Antibodies. R. BREZINA, E. KOVÁČOVÁ, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

Ten strains of *Coxiella burneti* were used on preparations of conjugates. The conjugates were prepared from rabbit sera twelve, twenty and forty days after infection. Preparations of mouse spleen infected with the virulent strain Florian in phase I were used for the demonstration of antigen. The results can be summed up as follows: (1) No differences were found in the sensitivity of different sera which could be ascribed to antigenic differences among the strains. (2) The sera of virulent strains showed a much higher sensitivity. This fact can be explained by the formation of antibodies against antigens which are decisive for the visualization of *Coxiella burneti* in phase I. In sensitive sera there is no actual correlation between the degree of fluorescence of the antigen and the time of collection of the serum. (3) The Nine Mile strain which has been greatly adapted to yolk sac and the avirulent strain Ž 57 gave sera with which *Coxiella burneti* in mouse spleen could not be detected at all. (4) The strains *Henzerling* and *Constanta* showed a slight increase in sensitivity in correlation with the time of collection. It cannot at present be decided whether it is due to the appearance of antibody against phase I antigen not detectable serologically, or by the alternation of "light" and "heavy" antibodies.

Comparative Study of the Reproduction of Three Variants of the Ťahyňa Virus in Tissue Cultures. Z. WALLNEROVÁ, Research Institute of Epidemiology and Microbiology, Bratislava.

A study was made of the reproduction of two strains of Ťahyňa virus in chick embryonic cell tissue cultures by titration of the infective virus, the immunofluorescence technique and morphological methods. The neuroadapted variants of strain 236 and the line of the neuroadapted variant of strain 92 propagated in chick embryonic cells, proliferated well in chick embryonic cell tissue cultures with complete cytopathic effect and characteristic cytoplasmic localization of the fluorescence of virus antigen. There was early disintegration of the nucleoli with typical morphological changes in the infected cells. On the other hand, the extraneural variant of strain 236 proliferated in the above tissue cultures, was without cytopathic effect and the titres of infecting virus were low. Infected cells showed no morphological changes and no specific virus antigen could be detected by the direct fluorescence antibody method. Since this variant has properties closest to those of the virus in natural circulation, it can be concluded from the results obtained that tissue cultures of chick embryonic cells are not a suitable substrate for the immunofluorescence detection of freshly isolated strains of Ťahyňa virus.

Comparison of the Susceptibility of Some Strains of Inbred and Randomly Crossed Mice to the Ťahyňa Virus. V. SCHWANZER, Research Institute of Epidemiology and Microbiology, Bratislava.

On the basis of the death curve of mice after intracerebral, intranasal and subcutaneous inoculation of the Ťahyňa virus (with determination of the LD_{50} and ED_{50}) the susceptibility of 10 strains of inbred mice and two groups of noninbred mice was

determined (calculating the "SPR" of the animals) to two variants of the Ťahyňa virus, strain 236. The above-mentioned indices confirmed: (a) That there is no marked difference in the susceptibility of the tested groups, (b) That in inbred strains, unlike noninbred strains, there is no parallelism of the dependence of incubation period on the amount of virus inoculated. The results are also given of comparing the course of the viraemic phase of infection in mice with two variants of the Ťahyňa virus on the basis of the determination of virus in the blood of young mice of three inbred strains and two groups of randomly crossed mice. The course of the viraemic phase of the disease in mice after intranasal inoculation with strain 236 Ťahyňa virus showed that viraemia is greater after the extraneural inoculation of the passaged variant than after that of the neuroadapted variant.

Contribution on the Part Played by Complement and its Components in Inactivating Viruses. V. RATHOVÁ, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

A comparison was made of processes affecting the virus-neutralizing activity of guinea pig serum, leading either to the inactivation of complement or to the removal of its beta or gamma inhibitory activity. The work showed that after thermal inactivation of complement, beta-inhibitory neutralizing activity fell almost to zero. The serum continued, even in an enhanced manner, to neutralize the avid A2 virus (the gamma-inhibitor is activated). After removing beta-inhibitor with trypsin, complement fell to unmeasurable values, and the neutralization of A1 virus also. Neutralization activity to A2 virus was preserved. After treating the serum with potassium periodidate to remove gamma-inhibitor, complement fell about five-fold, the neutralization activity of sera to the A1 virus two-fold, and to A2 virus many-fold, although a definite neutralization activity to A2 virus was preserved.

The Plaque Method in Rickettsias. N. KORDOVÁ, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava.

When inoculated on to monolayer primocultures of chick embryonic cells treated and cultivated *in vitro* according to Dulbecco and Vogt, rickettsias (yolk sac suspensions infected with *Rickettsia prowazeki, R. mooseri, R. acari* and *R. conori*) form plaques whose size and shape are species specific. The number of plaques is directly related to the dilution of the inoculum. Antibiotics (penicillin and streptomycin) inhibited plaque formation.

Lysogeny and Polylysogeny in Staphylococcus aureus. J. PILLICH, Z. HRADEČNÁ, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

A study was made of the incidence of lysogeny and polylysogeny in bacterial strains of *Staphylococcus aureus*. Most of the lysogenic strains of the above species could be identified by the use of suitable indicators. It was found that some of these strains were polylysogenic and liberated several bacteriophages. The strains of *Staphylococcus aureus* 55 and 812 belonging to different serological groups were the main objects of study. 2—3 different phage strains were liberated from these strains by induction with ultraviolet light and were successfully distinguished by suitable bacterial strain indicators.

Lytic Manifestations in Irradiated and Nonirradiated Bacterial Strains of Pseudomonas aeruginosa. J. PILLICH, Z. HRADEČNÁ, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

While studying lysogeny in a number of strains of *Pseudomonas aeruginosa* isolated from pathogenic material, the authors succeeded in isolating suitable indicator bacterial strains both for the isolation of new bacteriophages and for the identification of the production of bacteriocins. Using these strains, several highly active bacteriophages were isolated, i.g. the PL phage which lysed more than 70% of all tested bacterial strains.

The Radiosensitivity of Staphylococcus aureus Bacteriophages. B. LIŠKA, J. PILLICH, M. VÍZDALOVÁ, V. DRAHOŠ, B. KLHŮFKOVÁ, Institute of Biophysics of the Czechoslovak Academy of Sciences, and the Institute for Technical Instruments, Brno.

Ten different *Staphylococcus aureus* bacteriophages (phages 55, 81, Twort, ϕ 200, PA, 812, 42E, 75, 6, D) were irradiated with gamma radiation from a cobalt (Co^{60}) source. The bacteriophages differed in the morphology of their colonies and in the spectrum of their *Staphylococcus aureus* host strains. Bacteriophages with large colonies (phage 55 and Twort) were among the most sensitive to radiation; the most resistant were phages with small colonies (D and 6). The probable differences in target volume were calculated from the survival curves in accord with the target theory and the sizes of the bacteriophages and their structure were determined using the electron microscope.

The Sensitivity of some Bacillus subtilis Bacteriophages to Irradiation with UV Light. D. KALÁB, Research Laboratories, Bioveta, Ivanovice in Haná.

A study was made of the action of UV radiation (2,537 Å) on the bacteriophages SP 3 and SP 8

which are virulent for *Bacillus subtilis*. The DNA of the bacteriophage SP 3 contains only normal constituents, whereas all the thymine in the DNA of bacteriophage SP 8 is replaced by 5-hydroxymethyluracil. The photochemical reactivity of thymine and 5-hydroxymethyluracil is discussed.

Photodynamic Effect and Mutagenesis in Phages. E. JANOVSKÁ, J. KOUDELKA, V. KLEINWÄCHTER, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

A high percentage of mutations was found in phages inactivated by visible light in the presence of acridine orange (AO). A study was made of the incidence of morphologically different mutants at a survival rate of about 1% of the original particles. r II mutants of T4 phage with a known type of DNA damage (in the A—T or G—C pair) were used to classify the mutants arising, and a study was made of their reversal to the original type on irradiation with visible light in the presence of AO. Only some mutants of the G—C type were successfully reverted. The results show that AO is probably bound specifically to some pair of bases and that the resulting mutations are the result of photooxidation of the corresponding pair of bases.

Comparison of the Action of Hydroxylamine and UV Radiation on Escherichia coli Bacteriophages. M. VÍZDALOVÁ, J. PILLICH, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

A study was made of the inactivating action of hydroxylamine (a radiomimetic substance) in relation to the inactivating effect of UV radiation, in *Escherichia coli* bacteriophages of the T series. These phages differ from one another not only morphologically and serologically, but also in DNA content and in the ratio between the individual bases in the DNA. Three different molarities of hydroxylamine (1 M, 0.5 M and 0.05 M) were used for inactivation and UV radiation of wave-length 2,537 Å. It was shown that the mechanism of the inactivating effect of the tested radiomimetic and UV radiation are not the same. The sensitivity of phages to hydroxylamine was proportional to the amount of cytosine (or 5-hydroxymethylcytosine); sensitivity to UV radiation was proportional to the amount of thymine in the phage DNA.

Influencing the Reactivity of the Organism to the Biological Action of Scarlatina Toxin by Adrenocorticoidal Hormones. V. HŘÍBALOVÁ, V. SCHUH, Institute of Epidemiology and Microbiology, Prague.

A study was made of the power of glucocorticoids to modify the reactivity of rabbits to the pyrogenic, lethal and dermal action of scarlatina (erythrogenic) toxin (ET). Glucocorticoids (hydrocortisone is a little more effective than cortisone) in daily doses of 10 mg/kg subcutaneously, greatly decreased the pyrogenic and lethal effect of ET. 90% of the animals which had been under cortisone for two days survived 2.88 LD_{50} of the toxin (as against 33% of the control animals). When they had been given steroid for 7 days, more than 50% of the animals were protected against a dose of 2×10^6 STD, which was almost equal to LD_{100}. Under the given experimental conditions, protection afforded by steroids against ET is greater than against the reference typhoid endotoxin, in which, after being given cortisone for two days, the antilethal effect was just under the boundaries of significant difference from the control and only after seven days on cortisone was there a statistically significant decrease in lethality in all three doses of endotoxin used (0.24; 0.96; 3.84 LD_{50}). The positive Dick reaction is weakened or completely suppressed after two day's systemic administration of steroids. The pyrogenic and lethal activity of ET is modified by the antitoxic action of glucocorticoids in the same way as the corresponding activity of the endotoxins of gram-negative bacteria. According to the present conception, allergizing mechanisms come into play at least partly in the action of these toxins. Dermal activity of ET is inhibited in a similar manner to that described in local manifestations of delayed hypersensitivity. These results together with theories on the explanation of the antitoxic action of adrenocorticoids are discussed in connection with views on the sensitizing effect of ET in the organism.

The Reaction of Mice to the Injection of Phytohaemagglutinin. R. DVOŘÁK, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Numerous data in the literature on the action of extracts from the seeds of *Phaseolus vulgaris* in *in vitro* experiments (haemagglutination, mitogenic effect on lymphocytes, etc.) is in favour of the lectins having special properties and that when given by injection they may affect the total immunological reactivity of animals. In this work a study was made of the effect of giving a preparation of phytohaemagglutinin (PHA), of our own production, in experiments *in vivo*. Inbred mice were given PHA intravenously, intraperitoneally, subcutaneously and intralabially and a comparison was made of the macroscopic and histological, local and general changes after the first and repeated injection. Shock and the increase in titre of serum inhibitors in mice after repeated administration of PHA suggests a certain degree of acquired sensitization and resistance. In mice irradiated with gamma rays, PHA stimulates the repopulation of the spleen, particularly when accompanied by the

intravenous administration of cell suspension from the spleen of nonirradiated syngenic donors.

Changes in Proteolytic Systems and in the Level of Complement of Rabbit Serum after Bacterial Contamination in vitro. J. Štefanovič, Z. Staršia, J. Buranský, Department of Medical Microbiology and Immunology, Faculty of Medicine, Comenius University, Bratislava.

A study was made of changes in the proteolytic and antitryptic activity of rabbit serum at various time intervals after its contamination *in vitro* with the microorganisms *Escherichia coli, Proteus vulgaris, Bacillus anthracoides, Staphylococcus aureus* and *Streptococcus pyogenes haemolyticus*. Statistically significant changes in proteolytic activity were recorded in sera contaminated with *Staphylococcus aureus, Proteus vulgaris* and *Bacillus anthracoides*. Significant changes in antitryptic activity were recorded after contamination of sera with *Staphylococcus aureus* and *Streptococcus pyogenes haemolyticus*. Changes in the level of complement were studied in parallel with studying the changes in proteolytic systems of the serum. Complement was inactivated by the microorganisms in the following order: *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Streptococcus pyogenes haemolyticus* and *Bacillus anthracoides*.

The Results of an Investigation for Listeriosis in Rural Inhabitants in Slovakia. K. Elischerová, Research Institute of Epidemiology and Microbiology, Bratislava.

In view of the repeated incidence of listeriosis in animals in Slovakia, a serological and bacteriological investigation was made of various groups of rural inhabitants. Attention was particularly concentrated on detecting infection in people working in animal production, exposed to the risk of infection from occupational contact with listeriosis in domestic animals. In the group of the exposed, 14.6% showed an O-agglutinin titre of 1 : 160—1280 and 11% an H-agglutinin titre of 1 : 160—320. Of the rest of the people in these villages, 4,5% had a raised O-agglutinin titre and 3% a raised H-agglutinin titre, in workers on farms with no evident animal listeriosis, the figures were 3% and 2,4% respectively. The complement-fixation reaction (CFR) was positive in titres of 1 : 4—128 in 56.8% of the exposed subjects and in 9.6% and 19% respectively of subjects in the other groups. A CF-antibody titre of 1 : 32—128 was found in five exposed subjects who, like the rest of the workers from a focus of animal listeriosis, had not had any suspected clinical illness at the time in question. Antibodies at the higher titres recorded could also be detected in sera after absorption with strains of *Staphylococcus pyogenes* and *Enterococcus*. On the basis of the statistically significant differences in the incidence of raised titres of antibodies in various groups of the agricultural population it can be assumed that infection occurs in exposed persons without clinical manifestation. Listeria were not demonstrated in the throat swabs, nose swabs, blood or stools of exposed persons despite prolonged incubation in the cold, even from patients suspected clinically of listeriosis from villages with listeriosis among the animals. A titre of 1 : 256 was found in one woman after the perinatal death of her infant. The serological findings in exposed subjects and confirmation of listeriosis by cultivation in three persons from other villages, point to the need to follow-up this condition in diagnostic bacteriological laboratories in Slovakia.

The Effectiveness of Salmonella typhimurium Enterovaccines in Model Experiments. D. Georch, Pharmaceutical Faculty, Comenius University, Bratislava.

Formaldehyde, phenol, alcohol and acetone enterovaccines were prepared from 18-hour agar cultures of a virulent strain of *Salmonella typhimurium*, and were dried to constant weight. The dosing of the enterovaccine was measured in units of weight, 1 mg of the dry microbial mass containing about 3 milliard killed bacterial cells. Active protection tests were made using enterovaccine suspended in saline solution and given orally by metal catheter to white mice of the H strain. The best proved to be the acetone enterovaccine, given three times at 48-hour intervals, which protected 62% of mice against 1 MLD of a virulent strain of *Salmonella typhimurium*, 44 % against 2 MLD and 28% against 3 MLD. The results of the group of active protection tests were statistically evaluated by variance analysis and according to the Duncan test. This also confirmed the best immunizing effect of the acetone enterovaccine, not only against the control group but also as compared with the phenol and alcohol enterovaccines.

Manifestations of Tissue Damage in the Histological Picture of Tuberculin Tests in Guinea Pigs Passively Sensitized by the Transfer of Lymphoid Cells. A. Kolín, J. Johanovský, J. Pekárek, Institute for Sera and Vaccines, Prague.

The authors studied the histological picture of skin tests in guinea pigs passively sensitized by the transfer of spleen cells and lymphatic node cells from BCG sensitized guinea pigs. As early as 90 minutes after the intradermal injection of PPD, they found numerous areas of necrosis in the subcutaneous muscle fibres and fairly frequent foci of lipophagia. No similar findings were detected in the reaction to the same dose of ovalbumin in the same animals and in tuberculin tests in the recipients

of nonsensitized cells. Muscle fibre necrosis arose without any obvious inflammatory infiltration, which, together with paresis of the subcutaneous venules developed more slowly. The authors consider that the histological changes described support the hypothesis that the reaction of hypersensitive cells with antigen leads to this type of tissue damage and thus forms the main cause of the inflammatory changes developing in the second nonspecific phase of the reaction.

The Influence of Certain Pharmacologically Active Substances on the Course of the Delayed Hypersensitivity Reaction in Tissue Cultures. J. Švejcar, J. Johanovský, Institute for Sera and Vaccines, Prague.

Specific antigen generally acts unfavourably on cultures of hypersensitive cells. Under certain conditions, i.e. either in the first stage of incubation or on using minimal doses of antigen, a reverse effect can be demonstrated displayed functionally by stimulation of the sensitive cells. A number of well-known pharmacological mediators (histamine, serotonin, adrenaline) were tested and adrenaline was found to have a similar effect on sensitized and control cells, and also the Schild hypothetical mediator of delayed hypersensitivity, known as LPF (lymph node permeability factor). The possible relation and part played by these substances in the mechanism of the delayed hypersensitivity reaction is discussed. The effect of nonspecific toxic antigen (endotoxin of *Salmonella paratyphi* B) was different from that of specific antigen.

The Effect of the Integrity of the Antigen on the Intensity of the Anaphylactic Reaction in Guinea Pigs. J. Štefanovič, L. Bergendi, I. Blašková, Department of Medical Microbiology and Immunology and Department of Pharmacology, Faculty of Medicine, Comenius University, Bratislava.

In guinea pigs passively sensitized by rabbit antibodies to human serum albumin, an anaphylactic reaction is evoked by the complete antigen and by the antigen degraded to various degrees by catepsin D prepared from rabbit spleen. The intensity of the anaphylactic reaction was studied *in vivo* and *in vitro* using the Schultz-Dale technique.

Inhibition of Neutralizing Antibody Formation by Actinomycin D. C. John, B. Dušková, Department of Medical Microbiology and Immunology, Charles University, Prague.

Inhibition of antibody formation by actinomycin D was studied by a method permitting the *in vitro* demonstration of antibodies released from lymphatic tissue fragments. Inhibition of the primary response: Groups of mice were immunized into the two hind pads with bacteriophage T2. 24 hours after immunization various doses of actinomycin D were given by the same route (5 and 10 μg/pad). At various time intervals after the primary stimulus, the lymph nodes draining the area were removed and cut into small fragments (1 mm). The fragments were fixed to the bottom of Petri dishes (6 cm in diameter) by nutrient medium, made up of Earle's solution containing 0.5% LAH and 1% agar. After incubation for 48 hours at 37° C, the agar layer was covered with a second layer of 0.7% agar in peptone water containing bacteriophage T2 and a sensitive bacterial strain. After further incubation for 6—24 hours marked zones of inhibition of plaque formation could be seen round the tissue fragments, caused by the neutralization of phage by the released diffusible antibodies. In the groups of mice which had been given actinomycin D 24 hours after the primary stimulus, antibody formation was inhibited; phage was not neutralized in the vicinity of lymphatic fragments. Inhibition of the secondary response: a short period of inhibition of the secondary response was attained in mice given actinomycin D (5 μg into the pad) 24 hours after secondary immunization, carried out five weeks after the first stimulus.

The Influencing of Serum Protease Inhibitors by Complexes of Antigen with Antibodies. J. Štefanovič, L. Bergendi, Department for Medical Microbiology and Immunology, Faculty of Medicine, Comenius University, Bratislava.

Different concentrations of antigens (*Salmonella paratyphi* B and human serum albumin) were added to hyperimmune rabbit serum and the level of trypsin and papain inhibitors and the level of complement were studied in this mixture. The work showed that immune aggregates do not influence the level of serum inhibitors of protease but that the level of complement changes in dependence on the quality and concentration of antigen at a constant concentration of antibodies.

The Determination of the Number of Cells forming Antibodies: Comparison of the Immunofluorescence and Plaque Method. V. Kamarýtová, M. Holub, J. Šterzl, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Spleen cells were isolated from normal mice and from mice immunized with sheep erythrocytes 4—5 days previously. Antibodies from them were determined by the immunofluorescence (sandwich) and plaque methods in parallel. The number of positive cells in the plaque method ranged from

0.0001% (in nonimmunized) to 0.1%, whereas the values established in the same samples by immunofluorescence were from 0.02% to 0.1% and were always higher. In both methods large and small lymphoid cells were the producers of antibodies which at the time of testing were type 19 S. They were identified in the immunofluorescent samples as blasts, large and small lymphocytes and quite exceptionally as immature plasmatic cells. Differences in the number of positive cells between the two methods can be ascribed to the following circumstances: (1) We are dealing with immunization by complex erythrocyte antigen, and critical amounts of antigen producing haemolytic antibodies determined by the plaque technique probably differ from the quantity of the antigen producing a response detectable by immunofluorescence. (2) Immunofluorescence detects antibodies situated in the cell, the plaque technique antibodies released actively or passively into the medium; it would be necessary to determine their amount quantitatively and thus compare the sensitivity of the two methods. (3) The results compared were obtained from a total number of 10^8 cells (in the plaque technique) and 10^3 cells (in immunofluorescence) — quantitative evaluation is so far difficult. To arrive closer to an explanation of the difference in the two methods a comparison was made of the number of cells forming plaques and the number of cells forming gamma globulin (technique of direct immunofluorescence with pig anti-rabbit serum).

Experimental Verification of Model for the Differentiation of Immunologically Competent Cells. J. Šterzl, M. Jílek, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

A model was developed for the differentiation of immunologically competent cells on the basis of which it is possible to explain the induction of antibody formation, primary response of the 19 S antibody type, the preparation of the organism for the secondary response (formation of 7 S antibodies) and the origin of immunological inhibition — tolerance — after giving large doses of antigen to young or adult individuals. Experimental data were obtained by determining the number of cells forming antibodies by the plaque technique. The following results were obtained which supported the suggested model of differentiation: A number of immunologically competent cells capable of reacting with the corresponding antigen remain constant in the organism without antigenic stimulus or under the influence of nonspecific stimulus. Under the influence of antigenic stimulus, competent cells pass into the active state. If the antigen supply is sufficient, most of the activated cells become differentiated into producing cells. The producing cells survive in the organism with a half-life of 2.3 days and after their death the organism reacts to a subsequent dose of antigen with only an insignificant antibody response — tolerance. On the basis of these results, tolerance is considered the consequence of exhaustive terminal differentiation in excess of antigen, which prevents the proliferation of activated cells. If small amount of antigen is introduced into the organism, only some of the activated cells differentiate terminally into producing cells; the rest of the activated cells proliferate and prepare for the secondary reaction. On the basis of the results it is assumed that during the proliferation of activated cells changes could take place in the genetic regulation — a shift from 19 S into 7 S antibody formation.

The Effect of Endotoxin on the Complement Level in the Serum of Newborn Precolostral Piglets. I. Miler, H. Tlaskalová, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Science, Prague.

Most authors working on the act on of endotoxin on the organism, describe a fall in the complement level several hours after its administration. The mechanism of this fall has so far not been explained. Some authors have tried to exclude immunological reactions. These authors, however, carried out their experiments on adult animals of various species with antibodies present in their serum so that a reaction of antigen, endotoxin, with antibody occurs. The present experiments were made with newborn precolostral piglets whose serum contained complement (2—7 CH_{50}) and did not contain antibodies. After giving large doses of *Salmonella paratyphi* B endotoxin, no fall in complement level occurred in the third and sixth hour. 24 hours after the injection of endotoxin a significant increase in the complement level occurred as against that of the control piglets which were given saline solution. Another group of piglets received an injection of antibody to *Salmonella paratyphi* B before the injection of endotoxin, and in the serum samples from these piglets a fall in complement was found in the third and sixth hour which point to the importance of the reaction of antigen with antibody for the inactivation of complement by endotoxin.

The Sensitivity of M-mutants of Salmonella typhimurium to Precolostral Piglet Serum. V. Dlabač, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague,

Mutants of the M type lack the enzyme UDP-gal-4-epimerase, which catalyses the synthesis of cell wall lipopolysaccharids. In media without galactose these mutants synthetize defective lipopolysaccharides, whereas in the presence of galactose, the composition of the lipopolysaccharides is similar to that of the wild strain. A study was made of the influence of the composition of the cultivation

medium on the sensitivity or resistance of various M-mutants to the bactericidal action of precolostral piglet serum. It was shown that M-mutants growing in media without galactose were sensitive to precolostral piglet serum like other R strains of *Enterobacteriaceae*. On the other hand, M-mutants which had utilized galactose added to the cultivation medium, were not killed by precolostral piglet serum. The role of cell wall lipopolysaccharides in the bactericidal reaction is discussed.

The Effect of the Cultivation Medium on the Form and Physiological Characteristics of Erysipelothrix insidiosa in Relation to Immunogenicity. K. GRÍGELOVÁ, O. ŽÁK, Research and Development Department, Bioveta, Nitra.

Cultures of the attenuated WR 2 strain of *Erysipelothrix insidiosa*, cultivated in media with limited and maximum source of nitrogen on a basis of protein hydrolysates, differed in the morphology of the microorganisms, rate of proliferation, density of population reached and survival time. These properties were correlated with the immunological response in mice. It was shown that the differences mentioned were a function of the protein hydrolysate, its type and degree of hydrolysis and its concentration in the nutrient medium.

The Effect of Lyophilization on the Antigenic Properties of Salmonella. E. KITTNAR, H. SRBOVÁ, A. PETERA, Institute for Sera and Vaccines, Prague.

A comparison was made of the percentage of surviving Salmonella and of changes in their antigenic capacity after lyophilization carried out in various media and under various physical conditions. It was found that the antigenic properties are better in the presence of worse survival; in further experiments the authors sought an explanation for this finding and reached the conclusion that a selective mechanism is involved.

The Intracellular Activation of Clostridium tetani protoxin. A. LETTL, K. NEKVASILOVÁ, K. MORAVEC, A. STEJSKAL, Institute for Sera and Vaccines, Prague.

A study was made of the course of activation of the precursor of tetanospasmine. Since it proved impossible to increase the toxicity of the supernatants of young cultures by digestion with trypsin and no increase whatsoever in the toxicity of this material was observed after maintenance for several days at +4° C, it can be affirmed that the protoxin is not present in the cultivation medium, but only the final tetanospasmine. During cultivation intracellular toxicity increases rapidly up to advanced autolysis. The addition of chloramphenicol in the exponential stage of growth leads to the definite differentiation of the synthesis of antigen from its later activation. On the basis of the experimental material, the precursor can be regarded as part of a little soluble cell structure. During activation, tetanospasmine is split off from this structure and only after this can it be released from the cell material into the medium.

New Antigenic Types of the Citrobacter genus. J. SEDLÁK, M. ŠLAJSOVÁ, Department of Microbiology, Medical Faculty of Hygiene, Charles University, Prague.

The drawing up of the first Citrobacter antigenic scheme by West and Edwards in 1954 made it possible to classify about 200 serotypes into 32 serological O groups. The present authors have used this scheme for 15 years for the serological examination and study of almost 5,000 Citrobacter cultures. This work has shown that the original West and Edwards scheme, although indubitably presenting a relatively very wide antigenic spectrum, does not detect about 20% of the serotypes current in Czechoslovakia. On the basis of detailed serological studies of these nontypable strains, 10 further serological O groups have been constituted. By means of this supplemented and extended West and Edwards scheme, more than 90% of the strains can now be classified into one of the 42 serological O groups. An account is also given of some new, related somatic antigens which have been found to exist among individual serotypes of *Citrobacter*, *Salmonella* and *Escherichia*.

The Content of Bases in the DNA of Species of the Micrococcus and Staphylococcus Families. S. ROSYPAL, A. ROSYPALOVÁ, J. BOHÁČEK, Department of Microbiology, Faculty of Science, Purkyně University, Brno.

Species of the *Staphylococcus* family have a GC content in the DNA within the limits of 30.7 — 36.4%. All gram-positive cocci up to now designated as micrococci and sarcinae, forming acid from glucose under anaerobic or aerobic conditions and having a GC content in their DNA within these limits, should be included in the *Staphyloccoccus* family. Species of the *Micrococcus* family have a GC content in their DNA of 66.3—73.3%. All gram-positive cocci, up to now designated as sarcinae and staphylococci and not forming acid from glucose either under aerobic or anaerobic conditions and having a GC content in the DNA within these limits, should be included in the *Micrococcus* family. The above limits of GC content are only approximate and may be wider when further data

on the DNA GC content in the *Micrococcus* and *Staphylococcus* families become available. Species belonging to the *Micrococcus* family can be divided into three groups according to the DNA base content: Group 1 (70.8—73.3% GC) group 2 (67.5—69.5% GC) and group 3 (66.3—67.0% GC). Strains of the *Staphylococcus* family were similarly divided into three groups: Group 5 (36.4% GC), group 6 (33.3—34.2% GC) and group 7 (30.7—32.7% GC). On the basis of Adamson's analysis, every group was then divided into subgroups made up of strains, not only with the same DNA base content, but also showing a high degree of similarity in their physiological and biochemical properties. Organisms belonging to a given subgroup can be considered to be very close genetically. The following subgroups were formed in the *Micrococcus* family: 1a, 1b, 2a, 2b, 3a, 3b, and in the *Staphylococcus* family 5a, 6a and 7a. 1b corresponds to the present species *Micrococcus roseus* and 7a to *Staphylococcus aureus*. *Micrococcus denitrificans* does not belong to the *Micrococcus* family.

The Morphology of L-colonies of Haemolytic Streptococci. J. HAVLÍČEK, Institute of Epidemiology and Microbiology, Prague.

L-colonies of haemolytic streptococci do not differ morphologically from the L-colonies of other microbes. When growing on media with an NaCl concentration lower than the optimum (0.75% NaCl more than the physiological concentration) the colonies are gigantic, mucoid and composed mainly of a translucent mucoid substance corresponding to the classical L-colonies. The amount of this substance can be affected by hyaluronidase. Types 3A and 3B could not be distinguished because their main characteristics, power of reversal and growth on media without serum, cannot be used to make this distinction. Although there were considered to be colonies, corresponding morphologically to these types, reversal was very difficult and the growth of L-colonies was not observed at all in the absence of serum.

The Action of Cyanein on Pathogenic Fungi in vitro. V. BETINA, E. DROUHET, C. SEGRETAIN, Department of Microbiology and Biochemistry, Faculty of Chemistry, Slovak Technical College, Bratislava, and Institut Pasteur, Service de Mycologie, Paris.

The inhibitory action of the antibiotic cyanein was studied on 28 species of fungi from the following families: *Candida, Cryptococcus, Trichosporon, Rhizopus, Mucor, Aspergillus, Penicillium, Paecilomyces, Phialophora, Madurella, Geotrichum, Coccidioides, Sporotrichum, Histoplasma, Blastomyces, Trichophyton, Microsporon* and *Epidermophyton*. The following species were the most sensitive: *Candida albicans, Aspergillus fumigatus, Coccidioides immitis* and *Trichosporon capitatum*. Cyanein was also observed to have a morphological effect on *Trichosporon cutaneum, Aspergillus fumigatus* and *Paecilomyces viridis*. It prevented fructification in *Aspergillus fumigatus* and *Paecilomyces viridis*. In the dimorphic fungus *Paecilomyces viridis*, transformation of the type of mycelium to a yeast-like form was observed in addition, in the presence of subinhibitory concentrations of the antibiotic.

The Incidence of Pathogenic Mycelial Fungi from the Dematiaceae Family. Y. SVOBODOVÁ, Mycological Research Laboratory, Faculty of Medicine, Comenius University, Bratislava.

A report is given of the study of a number of pathogenic fungi belonging to the *Dematiaceae* family (from the genera *Madurella* Brumpt, *Cladosporium* Link, *Aureobasidium* Arnaud, *Phialophora* Emmons, *Torula* (Pers) Link and *Chmelia* Svobodová). The saprophytic and parasitic properties of these strains are described from experiments conducted *in vitro* and *in vivo*.

Antiglobulin Test in the Serology of Swine Erysipelas. O. ŽÁK, J. KOVÁČ, Research and Development Department, Bioveta, Nitra.

Specific antibodies can be detected in the serum of pigs hyperimmunized with a culture of *Erysipelothrix insidiosa* by means of the antiglobulin test, sooner and in the first phases of hyperimmunization and in much higher titres than by means of the ESCA-test and test tube agglutination in a solution of NaCl or polyvinylpyrolidon. The antibodies detected by the antiglobulin test are fairly thermostable; they are detected even in sera inactivated for 10 minutes at temperatures of up to 72° C. The use of this method of detection for determining the protective effect of serum *in vitro* is discussed.

Changes in the Coliform Flora in Connection with Colienterotoxaemia in Piglets after Weaning. E. SALAJKA, Research Institute for Veterinary Medicine, Brno-Medlánky.

One of the essential conditions for the development of colienterotoxaemia in piglets after weaning is the predominance of one of the species of pathogenic serotypes of *Escherichia coli* (08, 0138, 0139, 0141) in the intestines of the piglets of this age group. A number of authors considered that the predominance of pathogenic, usually haemolytic strains of *Escherichia coli* occurs as a result of changes connected with weaning, such as changing the stye, change of food, change of personnel, transport, vaccination and castration. In experiments with several litters of pigs the authors found that

even if the above-mentioned environmental factors are excluded, pathogenic *Escherichia coli* become the predominant organisms in the intestines of piglets during the second week after weaning. Where this disease occurs the serum of sows shows high titres of agglutinins against K- and O- antigens of *Escherichia coli* serotypes typical for this condition, mainly against 0139 : K 82 (B). 10—14 days after the brief predominance of stye specific serotypes of *Escherichia coli* in the alimentary tract of weanlings, specific agglutinins appear in the serum of all surviving animals, including those which were not ill. On the basis of these results, it is assumed that the brief preponderance of one of the pathogenic *Escherichia coli* at the time of weaning is connected with coproantibodies. While the piglets were being suckled, antibodies from the maternal milk fulfilled the function of coproantibodies. On weaning, this factor was excluded and the conditions thus created for massive proliferation of the stye specific pathogenic *Escherichia coli* serotype. This disappears in surviving piglets as a consequence of the local production of coproantibodies. The development of the immune response in the serum of piglets immediately after the preponderance of the specific serotypes of *Escherichia coli* in the intestines, is considered to be the consequence of the penetration of coproantibodies into the blood stream.

The Suitability of some Biochemical Tests for the Identification of Escherichia coli 0124. V. PAUČKOVÁ, J. BOHUŠ, H. LÁSKOVÁ, Department of Microbiology, District Hygiene and Epidemiological Station, Frýdek-Místek and Michalovce.

The strains of *Escherichia coli* 0124 which have been isolated by the authors since 1963 all have almost concordant biochemical properties. On the basis of absorption tests, too, they are considered to form an antigenically homogenous group, identical with *Shigella dysenteriae* 3. Many of these strains were isolated from patients with enteritis. 80 biochemical tests within the possibilities of a routine laboratory were tried out and on the basis of the results the authors recommend the use of the following supplementary tests, in addition to experimental keratoconjunctivitis, for the identification of this serotype of *Escherichia coli* in diagnostic practice: the ninhydrine test according to Carlquist, and growth on Christensen citrate medium as a means of distinguishing lactose defective strains of Escherichia and Alkalescens-Dispar on the one hand, and Shigellae on the other, and when using Na-malonate also from most Hafnii.

Verification of the Disinfectant Action of Hexachlorophen in Gynaecological Practice. J. KELETI, A. HUDCOVIČ, M. LUBOJACKÝ, E. SIROTNÝ, Pharmaceutical Faculty, Comenius University, Bratislava.

A qualitative and quantitative investigation was made of the microflora of the mouth cavity, nose, nipples and perineum in 100 parturient women at the Second Women's Department, Bratislava University, with special reference to *Staphylococcus pyogenes* and *Streptococcus pyogenes*. By the use of a 3% spray of Hexochlorophen of Czechoslovak production, prepared by the authors in sunflower seed oil, for daily disinfection of the nipples and perineum in 100 women in the puerperium, the number of bacterial organisms isolated was significantly decreased and all bacteria present of the *Staphylococcus pyogenes* and *Streptococcus pyogenes* species destroyed. Thus, 3% hexochlorophen in spray form behaves as an effective preventive of staphylococcal and streptococcal infection in obstetric and gynaecological practice.

The Incidence of Resistant Staphylococci in Various Population Groups in Slovakia. M. BETINOVÁ, P. NEMEC, Research Institute for Epidemiology and Microbiology, Bratislava and Department of Microbiology, Institute of Biology, Slovak Academy of Sciences, Bratislava.

Quantitative sensitivity to 8 antibiotics was investigated in coagulase positive strains of *Staphylococcus aureus*. The following antibiotics were used: benzyl penicillin (PNC), streptomycin (STM), chlorotetracycline (CTC), chloramphenicol (CHL), erythromycin (ERY), bacitracin (BAC), kanamycin (KAN) and vankomycin (VAN). Of the staphylococcal strains isolated from the town and village population 17% were resistant to PNC, 6.2% to STM and 0.7% to CHL. No difference in the incidence of resistance was found between the town and village population. Of the strains obtained from the attendants of domestic animals fed with additions of chlorotetracycline, 6.4%were resistant to PNC, 2.1% to STM and 19.2% to CTC. Of the strains obtained from people employed in the production of antibiotics (penicillin and tetracycline) 39% were resistant to PNC, 34.4% to STM, 31.3% to CTC, 1.5% to ERY and 1.5% to CHL. The highest incidence of staphylococcal strains resistant to CTC (63.1%) and to STM (71%) was in departments for the isolation and preparation of animal feed chlorotetracycline. Of the strains from patients, personnel and the environment of the surgical department, 57.7% were resistant to PNC, 42.8% to STM, 31,9% to CTC, 15.8% to ERY and 8.4% to CHL. Polyresistant strains were often isolated from patients and the surroundings (58%). On the other hand, among the personnel the incidence of polyresistant strains was rare in nurses and the incidence of monoresistant strains more frequent

(53.8%). Of the strains from the patients, personnel and surroundings of the Children's Department, 78.6% were resistant to PNC, 63.8% to STM, 56.5% to CTC, 55.2% to ERY and 47.7% to CHL. The incidence of polyresistant strains was high in patients (65.1%), from the surroundings (75%) and from the personnel (53.6%).

The Typization of Various Strains of Pasteurella multocida Isolated in Czechoslovakia. Z. MALÍK, Research and Development Department, Bioveta, Nitra.

A total of 113 strains of *Pasteurella multocida*, isolated from cases of chicken cholera and coming from different parts of Czechoslovakia were typed. Typing was carried out by means of the indirect haemagglutination test according to Carter and the passive mouse protection test according to Roberts. Modifications were made in both tests which proved useful. It was found that 82 strains belonged to type A (II), 8 strains to type D (IV) and 23 strains could not be typed even by one of the tests. The negative results were caused by the low virulence of the strains or by mucoidal forms as confirmed by the hyaluronidase tests with strain of *Staphylococcus aureus*. On the basis of the results, it can be concluded that the cause of acute chicken cholera in Czechoslovakia is most often type A (I) and that type D (IV) occurs much more rarely. Type C (III) was not found in connection with chicken cholera.

New Means of Rapid Differentiation of Acapsulated Vaccine Variants from Virulent Strains of Bacillus anthracis. Z. KOPPEL, A. SOKOL, F. HRUŠOVSKÝ, Veterinary Faculty, College of Agriculture, Košice.

The introduction of the Sterne vaccine into routine veterinary practice permits the differentiation of the two types of postvaccinial complications that occur, i.e. post-vaccinial complications caused by superinfection with fully virulent *Bacillus anthracis* and those caused by the long survival of the acapsular vaccine strain in the organism. The distinction of these two types is necessary from the hygienic, epizootological and economic aspects. A complex of five methods can be suitably used for rapid differentiation between acapsular vaccinial strains and fully virulent *Bacillus anthracis*. These proved fully satisfactory in four cases of post-vaccinial complications occurring in practice. These tests are: (a) the Čokolenko-Černý test; (b) the Smith test; (c) the Boik test; (d) the combined Smith-Jensen-Kleemayer test; (e) the combined Boik-Jensen-Kleemayer test. The discussion deals with the question of how far the two above types of *Bacillus anthracis* can be rapidly identified and differentiated and raises the demand for investigating the case within 3—6 hours after receiving the strain.

The Dynamics of Incidence of Resistant Escherichia coli and the Types of Polyresistant Escherichia coli in Pigs in Relation to Feeding with "Aureovit"$_{12}C_{20}$. A. SOKOL, Z. KOPPEL, I. MIKULA, Veterinary Faculty, College of Agriculture, Košice.

The dynamics of the incidence of resistant *Escherichia coli* in the rectal swab of pigs in relation to feeding with Aureovit$_{12}C_{20}$ was investigated under experimental and practical conditions and the incidence of the various types of polyresistant *Escherichia coli* determined by the disc method. A direct relation was found between increase in the ratio of resistant to sensitive *Escherichia coli*, and the duration of feeding with Aureovit$_{12}C_{20}$, containing subinhibitory doses of active CTC. When feeding with Aureovit$_{12}C_{20}$ is discontinued there is a gradual decrease in resistant strains of *Escherichia coli* and an increase of sensitive strains. This confirmed the validity of the Welsch phenomenon in both directions. A dependence was also demonstrated between the incidence of polyresistant types, the spectrum of polyresistance and the polyvalence of individual polyresistant types of *Escherichia coli* and the duration of feeding with Aureovit$_{12}C_{20}$. The mechanism of polyresistance is discussed and its medical significance, the danger of transferring polyresistance in *Escherichia coli* to other intestinal microorganisms, and measures that could be taken to speed up the loss of polyresistance both in *Escherichia coli* and other intestinal bacteria.

Precipitation Reactions of Polysaccharides Isolated from Yeasts. J. ŠANDULA, Institute of Chemistry, Slovak Academy of Sciences, Bratislava.

Polysaccharides isolated from the nutrient medium and cell walls of various species of *Candida* react not only with homologous sera, but also give cross reactions with antisera of some other species of the *Candida* family, with sera of *Saccharomyces* and *Torulopsis*. A comparison is given of the immunochemical properties of polysaccharides and glycoproteins obtained by different isolation processes.