SUMMARY

Effect of Substitution of Diaminopimelic Acid in the Wall by its 4-hydroxy Derivative on the Growth of Escherichia coli and Turnover of Murein. M. Sterndová, J. Chaloupka, J. Časlavská, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

3H-4-hydroxy-diaminopimelic acid (OH-dap) is incorporated into the wall of both dap-dependent mutant and prototrophic strain of Escherichia coli. The prototrophic strain grows normally in the presence of OH-dap; however, the dap- mutant forms swollen formations and gradually lyzes. An increased content of the analogue in the medium prevents morphological aberrations and postpones the onset of the lysis of cells. During growth with the analogue an autolytic system is activated and its activity outlasts for a certain time even after the addition of dap. The increased activity of the autolytic enzymes results in turnover of murein, the rate of which is indirectly proportional to the concentration of the analogue. The dap analogue can apparently replace dap in the synthesis of murein. However, as the affinity of enzymes incorporating dap is probably lower to the analogue as compared with that to the natural substrate, synthesis of murein is impaired during growth of the dap- mutant in the presence of low concentrations of OH-dap being manifested in a similar way as the effect of penicillin. Therefore the prototrophic strains grows normally in the presence of OH-dap, in spite of the fact that it incorporates the analogue.

Changes of Biosynthetic Activity of Escherichia coli B exposed to Different Conditions. D. Tóth, Limnological Institute, Slovak Academy of Sciences, Bratislava.

Cells of Escherichia coli B labeled during growth in a synthetic medium containing mineral compounds, glucose and ammonium sulphate (SyB) and 14C-valine and transferred after washing to a medium without a carbon source (SyBC-) or without carbon and nitrogen sources (SyBC-N-) increased the number of counts per 1 ml of the suspension during first 8 hours of the exposure. After this time interval counts per min decrease again at a practically identical rate in both media. A differentiated decrease can be observed after about 48 hours, when the decrease of the number of counts slows down in samples taken from the SyBC-. This difference becomes more substantial during further incubation up to 168 hours. After a transfer of cells of Escherichia coli exposed in the mineral medium at 37°C to the SyB medium with radioactive precursors the incorporation of both valine and adenine is decreased, depending on time of the starvation. A similar phenomenon was observed even when these cells were transferred to other cultivation media, e.g. meat-peptone broth. Values of radioactivity in individual components of the suspension were compared in cells of Escherichia coli B cultivated in the SyB medium and pulse-labeled with 14C-valine. The content of radioactive compounds in the supernatant increases depending on the time of starvation, whereas a decrease is observed in the sediment. The sum of both values at a given time is close to the state found in samples of the corresponding suspensions. Labelled compounds of the non-protein nature were found both in the medium and cells. Comparison of concentrations of labelled compounds found in TCA, precipitates and without precipitation makes it possible to evaluate the extent of degradation of cellular proteins as well as lysis of the studied cells during starvation.

The Relationship between Histone and Nucleic Acids in Escherichia coli. E. Masnerová, Ch. Zimmer, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague; Institute of Microbiology and Experimental Therapy, German Academy of Sciences (GDR), Jena.

Histone added to minimal medium during the exponential phase of growth of Escherichia coli influences of alkaline phosphatase shifting onset of derepression of its synthesis by about 60–80 min and, in addition, inhibiting synthesis of the enzyme. The inhibitory effect is not removed even after the removal of histone from the medium. According to literature data histone is bound to chromosomal DNA in eucaryotic organisms and influences regulation of protein synthesis. Similar relationships between histone and nucleic acids were investigated in bacteria. The effect of histone on nucleic acids was studied in a cell-free extract of Escherichia coli disintegrated ultrasonically using a method of determination of circular dichroism. The cells cultivated in the presence of histone exhibited in the range characteristic for nucleic acid different CD spectra than cells cultivated without histone. This fact justifies an assumption that histone forms a complex with nucleic acids in Escherichia coli. This situation is probably reflected...
also by the amount of the synthesized alkaline phosphatase.

**Effect of Externally Added Histone on Protein Synthesis in Bacillus cereus.** E. Pavlasová, E. Štěrskalová, J. Šťastná, V. Vinter, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

It follows from our previous studies that histone added externally to *Escherichia coli* influences protein synthesis probably at the level of transcription and the mechanism of its action depends on growth phase of the culture. In this study another model organism, *Bacillus cereus*, in which individual stages of differentiation are morphologically characterized, was used. The effect of histone on the early stage of development of the vegetative cell from the spore was studied so far. It was found that during this stage histone inhibits incorporation of 14C-leucine into cellular proteins. Its effect cannot be removed by adding higher concentrations of certain monovalent or divalent cations. The incorporation of 14C-uraesl into the total RNA fraction was substantially inhibited as well. The results obtained favour the idea that even in this case histone influences protein synthesis at the level of transcription. Thus, it can be assumed that the effect of histone in microorganisms is apparently of general validity.

**Morphological Changes in Escherichia coli and Bacillus cereus Induced by Externally Added Histone.** E. Štěrskalová, E. Pavlasová, J. Čáslavská, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

It has already been shown that histone influences protein synthesis in *Escherichia coli* and *Bacillus cereus*. In addition, it was found that histone can penetrate into the cells of *Escherichia coli*. Therefore it was of interest to investigate whether the addition of histone to these organisms results also in ultrastructural changes. Comparison of ultrathin sections of cells of *Escherichia coli* in the absence and presence of histone revealed the following differences: (1) In control cells both the cell wall and cytoplasmic membrane are well visible, ribosomes and nuclear structure can be seen in the cytoplasm. (2) Under the influence of histone the inner electron-dense layer of the cell wall is thickened, the cytoplasm is condensed and neither ribosomes nor the nuclear structure are clearly discriminated. Considerable electron-transparent zones can be observed in this cytoplasm. The following differences were found in *Bacillus cereus*: (1) After 30 min of incubation control cells germinate normally and also the beginning of the postgerminative development is normal (i.e., residues of spore envelopes outlast around the swelling protoplast, free exosporium can be clearly seen and individual structures are clearly differentiated in the cytoplasm. (2) In most spores, to which histone was added at the beginning of germination, even the initial phases of germination did not proceed during a 30 min incubation. An undamaged cortex can be observed on the surface of the spore protoplast and the protoplast is not differentiated. In a portion of spores the inhibition of germination by histone was not observed. In both types (non-germinated and partially germinated spores) a fine electron-dense layer at the surface of the exosporium was detected.

**Effect of the Germination Exudate on Synthetic Activities of Spores of Bacillus cereus Germinated in a Limited Medium.** J. Šťastná, V. Vinter, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Heat-activated spores (65°C/15 min) of *Bacillus cereus* were germinated in a minimal medium which does not facilitate a further development. A limited synthesis of proteins and (tested by incorporation of 14C-leucine) and RNA (tested by incorporation of 14C-uraesl) took place. Synthesis of the cell wall [measured by incorporation of 14C-diaminopimelic acid (dap)] continued for several hours. The above mentioned synthetic processes were studied in a medium containing the exudate released during germination of spores and in a fresh medium replacing after the germination the original one. Synthesis of the cell wall was accelerated in the presence of the exudate. In a limited medium the incorporation of 14C-dap was highly actininmycin D-sensitive and relatively chloramphenicol-resistant. The spore exudate accelerated also synthesis of proteins and RNA. After germination of radioactive spores, the cortical layer of which was pre-labeled with 14C-dap, a 14C-dap-containing material was released into the medium. The incorporation of 14C-dap from the exudate after germination of the labeled spores into germinated unlabeled spores was negligible. The presented results are discussed with respect to a possible reutilization of the exudate components by the germinated spores in a limited medium.

**On a Possible Role of Basic Proteins in Evolution of the Cell.** V. Liebl, L. Bržňovcová, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Pre-erythrocytic cells, bacterial cells in the first place, are suitable also for studying certain basic yet not fully understood developmental problems. It was found by analysis (mainly electrophoretic) of the bacterial cytoplasm and its components (ribosomes and membranes in particular) that the cytoplasm deprived of ribosomes practically does not contain basic proteins. Under physiological conditions cytoplasm thus cannot represent a typical dicomplex ascoevate with polyionic linkages. This coacervate would be unsuitable for complex biochemical processes due to its high viscosity, strong interactions among components, relatively simple structure etc. Cytoplasm is apparently of a more
Isolation of Nucleus-free Yeast Protoplasts and their Inability to Synthesise Glucan Fibris of the Cell Wall. M. Kopečka, M. Gabriel, O. Nečas, Institute of Biology, Medical School, J. E. Purkyně University, Brno.

A mixed population of nucleus-containing and nucleus-free protoplasts originates when preparing protoplasts from logarithmically growing cell of Saccharomyces cerevisiae by means of snail enzymes. The nucleus-free protoplasts did not synthesise glucan fibris, in spite of the fact that they contained all basic cytoplasmic structures except for the nucleus and that the structure of their cytoplasmic membrane remained unchanged. On the contrary, nucleus-containing yeast protoplasts synthesise glucan fibris even after the protein synthesis has been stopped by cycloheximide. The above-described behaviour of the nucleus-free protoplasts is not clear. The nucleus-free yeast protoplasts are the first case when a uniform fraction of nucleus-free fungi was isolated by means of a reproducible method.

Ultrastructure of the Yeast Trigonopsis variabilis. V. Šnedelar, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

During submerged cultivation in a Nickerson medium the yeast Trigonopsis variabilis grows as a mixture of triangular and oval cells. Electron microscopic studies of both forms of cells after fixation with KMnO₄ and fixation according to Sabatini and coworkers showed that their inner structure is comparable with that of other, particularly ascomycetal yeasts. This holds true also for ultrastructure of the cell wall of both forms, for budding and for character of scars on the mother cell, except for the fact that on ultrathin sections chitin tears could not be observed. From above the triangular cells have a shape of equilateral triangle with rounded peaks and with sides depressed inside. On a cross section they appear as flat discs. Budding takes place at the peaks of the triangular cells. In the oval cells budding occurs mostly on one of the poles of the cell, whereas in the triangular forms the termini are equivalent with respect to their ability to form buds. The cell wall is identical in the two types, it is three-layered and covered with a layer of mucigel. Cytochemical staining of polysaccharides showed that ultrastructure of the cell wall of the two forms is also identical and resembles that of Saccharomyces. A centripetal proliferation of the inner layer of the cell wall could be observed in the triangular forms in the middle part of their inside depressed sides. This proliferation is associated with an increased number of invaginations attached to the plasmalemma. These anomalies were not observed in the oval cells. On the basis of the above observations it can be assumed that the inner layer of the cell wall, together with the system of invaginations of the plasmalemma, play the most important role in the process of triangular morphogenesis of the yeast Trigonopsis variabilis.

Ultrastructure of a Dimorphic Yeast Trigonopsis variabilis Studied by Freeze-etching. A. Svoboda, F. Kevéi Institute of Biology, Medical School, J. E. Purkyně University, Brno.

A mixed culture of ellipsoid and triangular forms growing in a worth medium was frozen in Freon 22 and liquid nitrogen. The samples, either directly, or after incubation in 20% glycerol or after fixation with 5% glutaraldehyde and incubation in 20–30% glycerol. Studies of replicas in an electron microscope did not reveal any substantial differences in a submicroscopic structure of both vegetative forms except for the outer shape of the cells. The cell wall of the two forms is of the same thickness and its surface is smooth. A fine fibrillar material could be observed only after fixation with glutaraldehyde. On a fracture a granular structure discriminated to two layers can be observed. The cytoplasmic membrane is covered with granules 10–15 mm in size. These granules are missing at the site of invaginations. In both vegetative forms no considerable differences could be detected, both in arrangement of the granules and localization of the invaginations. The cytoplasm contains numerous mitochondria and rare cisternae of the endoplasmic reticulum. Clusters of short cisternae with small vesicles, perhaps analogues of the dictyosome, were observed on several fractures. The nucleus is relatively large and nuclear pores can...
to the wall and with production of hormonal compounds. The cell wall plays an important role in conjugation of yeasts. Modification of the shape of conjugating cells, their fusion facilitated by conjugative channels and lysis of the septum separating conjugating cells forming the zygote are the main stages of the wall differentiation. However, individual wall architectures of conjugating cells, their fusion and dividing yeasts do not influence this differentiation. The interaction with the competitive cell is the most important factor here. Control mechanisms of the formation of zygotes, associated with enzyme activities linked to the wall and with production of hormonal compounds are discussed.

Localization of Biosynthesis of Mannan in Yeasts. A. Košinová, S. Bauer, V. Parkaš, Chemical Institute, Slovak Academy of Sciences, Bratislava.

The autoradiographic method on ultrathin sections was used to localize enzymes involved in biosynthesis of mannan in yeasts Saccharomyces cerevisiae under conditions of selective incorporation of exogenous D-mannose-3H into mannan (1). After a 1 min incubation of cells in a medium containing radioactive mannose the predominating portion of the incorporated radioactivity was localized in the cytoplasm and in membranes of endoplasmic reticulum in the neighborhood of plasmalemma. In cells that were just in the stage of division the radioactivity was concentrated in the neighborhood of the septum to be formed. When after a 1 min incubation with radioactive mannose the cells were incubated for a further 10 min in a non-radioactive growth medium, the dominating portion of the radioactivity was detected in the cell wall. Structures resembling the Golgi apparatus could not be observed on ultrathin sections. Thus, it is assumed that enzymes synthesizing mannan are not exclusively bound to these structures, but are probably associated even with membranes of endoplasmic reticulum. It cannot be excluded that these enzymes are activated in these membranes occurring in the proximity of sites, in which an intensive construction of the cell wall just takes place. It was found by means of separation of the cell membranes in density gradients (2) that fractions of membranes of the endoplasmic reticulum contain roughly a 12-fold specific activity of mannan-synthetase as compared with fractions of the plasmalemma.

1) V. Parkaš, J. Kovařík, A. Košinová and S. Bauer, J. Bacteriol., January 1974 (in press).
2) P. Matile, H. Moor and K. Mühlethaler, Arch. Mikrobiol. 55, 201 (1967).

Changes in the Chemical Composition of Cell Walls of the Yeast Rhodotorula gracilis Related to Oxygen Sufficiency. Z. Holan, K. Berán, M. Novák, J. Baldrian, Institute of Microbiology, Czechoslovak Academy of Sciences, and Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague.

It has already been shown that under conditions of low aeration (KLa = 1.25 - 4.6 min⁻¹) the growth culture is influenced by the oxygen limit. In addition, when pH is decreased naturally, cytological changes occur on the inner side of the cell wall, mostly in the form of equatorial rings (Snider et al., 1973). Chemical analyses show that under good aeration conditions (KLa = 11.8 min⁻¹) and at constant pH 4.5 the content of glucosamine and protein in the cell walls equals to 1.88% and 8.5%, respectively (A type). When aeration is decreased the above values increase reaching up to 6.04 - 6.6%, glucosamine and 11.5 - 13.5% proteins at KLa = 3.3 min⁻¹, irrespective of whether pH of the cultivation was regulated or not and, thus, whether the cells form under the influence of pH the rings or not (B type). X-ray analysis of the cell walls of the B type revealed presence of chitin, whereas in the walls of the B type chitin can be detected only after partial hydrolyses being localized exclusively in mother scars. In the B type of the cell walls chitin is localized in the whole cell wall and not only in the ring. A relationship between KLa and content of protein and chitin in the cell walls exists. As shown by X-ray diffraction the content of nitrogen, glucosamine and sugars in the cell walls of the B type does not change considerably when the yeasts are cultivated within the range of KLa = 1.25 - 4.6 min⁻¹. However, under
the influence of pH they can differ only cytologically by the presence of the ring. Gas chromatography revealed the presence of glucose, mannose and arabinose and their quantitative proportions do not considerably differ, irrespective of whether the amount of the ring is 0, 40 and/or 80%. It follows from comparison of the chemical composition of sugars in the cell walls of the A and B types that the proportion of arabinose decreases with increasing $R_L$, whereas the amount of glucose increases. Partial hydrolysis of the cell walls containing the equatorial ring show that the ring is constructed from the same glycoprotein material as the rest of the cell wall; the cell walls of yeasts cultivated at the same $R_L$ and differing only by the presence of the ring have roughly identical chemical composition.

**Effect of Derivatives of Saccharides on Growth of Yeasts.** J. ŠANDUĽA, K. LINÉK, Institute of Chemistry, Slovak Academy of Sciences, Bratislava.

The effect of derivatives of saccharides prepared in this institute on growth of some species of yeasts and yeast-like microorganisms was studied. Out of the tested 30 derivatives of tetrooses, D-erythrose (p-nitrophenyl) hydrazone, D-threose (p-nitrophenyl) hydrazone, D-threose (2,5-dichlorophenyl) hydrazone and hexoses, D-glucose phenylhydrazone, D-galactose (p-nitrophenyl) hydrazone, L-ribofuranose (p-nitrophenyl) hydrazone have a strong inhibitory effect on growth of the cultures. Derivatives of trioses and pentoses do not substantially influence growth of the cells. Species of the genus *Saccharomyces* with a well developed system of glycosidic enzymes are less sensitive, whereas pathogenic species of the genera *Candida* and *Cryptococcus*, as well as species of the genus *Rhodotorula* are strongly inhibited by the above-mentioned compounds.

**Comparison of Cytomorphological Properties of Cells of Candida utilis at Different Growth Rates.** D. VRÁNA, J. LIBEĽOVÁ, K. BERAN, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Lengths and widths of mother and daughter cells of *Candida utilis* and *Saccharomyces cerevisiae* were compared during a single-step continuous cultivation at $D_1 = 0.05$, 0.1, 0.25 and 0.35 h⁻¹. Fluorescence microscopy following “staining” of scars by primulin was used to discriminate between mother and daughter cells. In a population of *Saccharomyces cerevisiae* the average size of mother cells containing one to two scars reaches about 150 μm³ and does not change substantially with increasing growth rate. However, the growth rate considerably influences size of the scar-less daughter cells. At $D_1 = 0.05$ and 0.1 h⁻¹ the daughter cells reach about 40% volume of mother cells. At $D_1 = 0.25$ h⁻¹ this value equals 75% and at $D_1 = 0.35$ h⁻¹ the daughter cells are practically of the same size as the mother cells (97%). Size of mother cells with four scars was indirectly proportional to the growth rate and was apparently a function of the time of delay. In a population of *Candida utilis* the growth rate is of considerable importance even for the average size of the mother cells ranging from 25 μm³ to 50 μm³ at the lowest and highest growth rate, respectively. The relative size of the daughter and mother cells was similar as in the population of *Saccharomyces cerevisiae*. The results are discussed with respect to a physiological state conditioning separation of the daughter and mother cells in the populations of yeasts. Generally, it can be postulated that the higher the growth rate of the population the more physiologically similar is the daughter cell to its mother cell and the shorter the time interval between its separation and origin of a new bud on its surface.

**Uptake of Deoxyglucoses by Baker’s Yeast.** D. MIČHALJANÍČOVÁ, A. KOTYK, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The specific carrier for monosaccharides in *Saccharomyces cerevisiae* does not transfer sugars with the axial hydroxyl group at C-4. The galactose induced carrier transports these sugars preferentially. 4-Deoxy-glucose (which is simultaneously 4-deoxy-galactose) is transferred by both carriers with an activation energy of 40–30 kJ/mol, thus indicating that neither of the carriers requires the presence of the hydroxy group at C-4. 4-Deoxy-D-glucose is apparently oxidized without phosphorylation in yeasts. 6-Deoxy-D-glucose is also transferred by both transport systems, but it is not further changed inside the cells. None of the sugars can serve as substrate for yeast hexokinase.

**Transport of disaccharides in the yeast Rhodotorula glutinis.** S. JANDA, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Transport of eight disaccharides and one trisaccharide was followed in the lipid-forming yeast *Rhodotorula glutinis*. Sucrose, trehalose, maltose, cellobiose and the trisaccharide raffinose (this sugar only partially) were taken up from the medium. Lactose, melibiose, isomaltose and gentiose were not utilized. Out of the utilized sugars sucrose and trehalose are degraded by periplasmic hydrolases on the cell surface, however, these enzymes are partially released into the medium. Glucose (and fructose) are then transported inside the cells. From raffinose only D-glucose is split off and transported. Maltose is transported in an unchanged form, probably by means of two transport mechanisms. Transport of cellobiose has not yet been clarified.

**Transport of Sorbitol in Saccharomyces cerevisiae.** L. ŘÍHOVÁ, D. S. CANÓ, J. HORÁK, A. KOTYK, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Similarly to monosaccharides, certain acyclic polyols are transported in *Saccharomyces cerevisiae*
without participation of metabolic energy. It even appears that uptake of these compounds proceeds without any specific carrier. Thus sorbitol is transported roughly to 75% of cell water within a broad concentration range (from $10^{-6}$ M to 1M), with a negligible temperature dependence, a very broad pH optimum (from 3 to 8) the $K_m$ and $V$ of this transport being infinitely high. No competition with other polyols or sugars was detected, the transport was not influenced by either metabolic (3,4-dinitrophenol, iodoacetamide) or transport (uranyl ions) inhibitors.

**Intracellular Level and Rate of Transport of Amino Acids in Saccharomyces cerevisiae.** A. Kotyk, A. Kotyk, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The intensity of transport of amino acids (glycine, methionine, lysine, glutamic acid, α-amino-isobutyric acid) is increased up to 25-fold after previous incubation with some metabolized sugars. It increases only 10-fold after incubation with substrates of the strictly oxidative metabolism (ethanol, acetate). The increased transport may be explained in two ways: (1) The previous incubation results in the accumulation of energy sources required for the transport of amino acids; (2) the levels of intracellular amino acids decrease during the preincubation due to the proceeding protein synthesis and the transport-inhibitory effect on the transport is thus removed. Determination of the total intracellular level of amino acids after different types of preincubation followed by deprivation show that the second explanation is not likely (after ethanol the total level of amino acids does not change at all decreasing to about 50% after glucose). The direct energy source for the transport of amino acids cannot be identified at present, in spite of the fact that a remarkable increase in the total intracellular level of amino acids after differentiation of transport systems for amino acids exists.

**Attempts at Solubilizing Transport Proteins from Saccharomyces cerevisiae.** J. Horák, M. Opěkárová, L. Řihová, A. Kotyk, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Solubilization of the plasma membrane of yeasts by means of detergents for purification of the binding protein for β-glucose and related sugars includes a series of difficulties, and the final preparation contains residues of detergents. The use of the osmotic shock described in bacteria avoids these shortcomings, but its application to yeasts requires certain modifications. By using a saturated mannitol solution it is possible to release the binding affinity for β-galactose from suitably induced cells in the stationary phase of growth. A peptide with specific binding affinity for L-arginine is released after a simple transfer of the growing cells to distilled water.

**Effect of Glucose on the Oxidation of Vanillic Acid in Bacteria.** F. Kunc, A. Kotyk, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The stimulatory effect of glucose and other energy sources on the oxidation of aromatic monomers and more complex compounds (e.g. fulvic acids) described in previous papers was investigated. The effect of glucose on the oxidation of 14COOH-vanillic acid by a washed suspension of cells of *Cellulomonas* sp. was studied by means of respiratory and radiometric methods, cell-free extracts, inhibitors and by adjustment of osmotic conditions. The obtained results showed that the lag phase was reduced in the presence of glucose and the oxidation of vanillic acid was thus accelerated. It was further found that the incorporation of the carboxyl carbon of vanillic acid into cellular structures was increased in the presence of glucose and that glucose decreases the inhibitory effect of higher concentrations of vanillic acid on the respiratory activity of bacteria. It followed from the results that the effect of glucose was due to an increase of biomass or enzymatic activity. Glucose rather served as an energy source for membrane transport mechanisms facilitating contact of the substrate with endoenzymes by the accelerated transport of vanillic acid into the cells. A similar effect was also observed when using succinic acid and acetate and also in cases when the content of accessible energy substrates in the soil was increased by sterilization by heat or radiation. A possible relationship between the above findings and the "priming effect" is discussed.

**Selection of Respiratory Mutants in the Basidiospore Schizophyllum commune.** J. Nisvěra, Department of Genetics, Faculty of Sciences, Charles University, Prague.

When basidiospores of *Schizophyllum commune* are treated with ethyl methanesulfonate for 24 hours, up to 60% mutants with a decreased growth rate are detected among the surviving population. Out of a total of 75 mutants 15 were selected for further studies. These mutants grow slowly in a medium containing glucose and do not grow at all even after 7 days in the presence of acetate. It was assumed that the decreased growth rate was caused by a defect in the respiratory system and energetic metabolism. The inability to grow in the medium with acetate was recessive in all studied mutants. It was found on the basis of the analysis of segregation of basidiospores after crossing of these mutants with standard strains that in 11 mutants the inability to grow in the acetate medium is due to a mutation in the nuclear gene. An extra-nuclear mutation can be assumed in 1 mutant. Results of mutual complementation made it possible to estimate a minimum of 8 nuclear loci governing the utilization of acetate. Measurement
of the respiratory capacity by the oxygen electrode showed that the KCN-sensitive respiration was decreased in one nuclear mutant.

Antifungal Activity of Compounds Interfering with the Function and Biogenesis of Mitochondria. J. Súňík, M. Berčič, Food Research Institute, Bratislava.

The role of mitochondria in germination of spores of filamentous fungi was investigated. It was found that strict anaerobiosis, cyanide, azide, oligomycin, bongkrekic acid, acriflavine and ethidium bromide prevent germination of spores of Aspergillus niger and Penicillus italicum in a liquid germination medium. The effect of azide, oligomycin, bongkrekic acid and ethidium bromide was of fungicidal type. Cyanide and azide completely inhibited the incorporation of $^{14}$C-leucine and $^{14}$C-uracil by germinating conidia of Aspergillus niger. Oligomycin, bongkrekic acid and ethidium bromide reduced the incorporation of both precursors during first hours of the germination of conidia, inhibiting it completely during further hours of incubation. The inhibition of the germination of spores as well as the inhibition of synthesis of macromolecules during germination of the conidia of Aspergillus niger were related with the specific inhibitory effect of these compounds on the respiratory activity of dormant conidia and mycelial cells. The results indicate that the function of the mitochondrial genetic and protein synthesizing system, as well as the function of oxidative phosphorylation are essential for the normal germination of spores and growth of molds.

Effect of Phaltan on Certain Microorganisms Important from the Point of View of Food Industry. V. Orbačálek, V. Sicho, Department of Biochemistry and Microbiology, University of Chemical Technology, Prague.

The effect of phaltan (N-trichloromethyltetrahydrodiphthalimide), a pesticide, the use of which during storage of sugar beet and some types of fruit and vegetable is considered, was investigated. The following microorganisms were used throughout: Saccharomyces cerevisiae, Bacillus megaterium and several molds belonging to different genera. It was found that growth of Saccharomyces cerevisiae is fully inhibited by phaltan at a concentration of 0.03 mg%. It is difficult to determine accurately the inhibitory concentration of phaltan as this compound is highly unstable in aqueous solutions, at high pH in particular. Phaltan is rapidly decomposed in water and other complex media and concentrations higher than 0.1 mg% are required for the inhibition of growth. Growth of Bacillus megaterium in a mineral medium with glucose, followed by measuring absorbancy, was inhibited only partially by phaltan at a concentration of 1 mg%.

Antimicrobial Effect of Some Amine-N-oxides. D. Mlynárčík, D. Georch, M. Figurová, I. Lacko, Pharmaceutical Faculty, Komenský University, Bratislava.

The antimicrobial effect of 14 newly synthesized amine-N-oxides differing by substitutions on the nitrogen atom was investigated. The relationship between the chemical structure and effectiveness was studied by determining MIC in strains of Staphylococcus pyogenes, Bacillus subtilis, Escherichia coli, Salmonella minnesota, Candida albicans, Trichophyton terrestre and Microsporum gypseum. Length of the alkyl chain appears to be the most important factor here. Of the tested compounds 1-pentadecylpiperidine-N-oxide was most effective on Gram-positive bacteria and molds, whereas N,N-di-methylpiperidylamine-N-oxide was more effective on Gram-negative bacteria.

Screening of Insecticides Produced by Molds of the Class Fungi imperfecti. F. Nemec, J. Dobias, Kollárová, Institute of Biology, Slovak Academy of Sciences, Bratislava.

The contact insecticidal effect of extracts of 193 cultures of the class Fungi imperfecti was investigated. A method for screening of the contact insecticides using a model organism Drosophila melanogaster was described. According to the intensity of the insecticidal effect the studied molds were divided into groups with increasing effectiveness; each group consists of cultures with the effectiveness range within 10% mortality of the model organism. The results show that molds of the class Fungi imperfecti produce effective insecticides.

Wild Fungi Produce Attractors and Nematocides. J. Balan, L. Kuťková, P. Nemec, V. Volék*, Institute of Biology, Slovak Academy of Sciences, Bratislava; *Department of Technical Microbiology and Biochemistry, Faculty of Chemistry, Bratislava.

Wild fungi are little known microorganisms belonging mostly to the order Moniliales reacting to the presence of nematodes by the formation of passive or active trapping structures for catching microscopic worms. It was found that Arthrobotrys conoides, Arthrobotrys dactyloides, Arthrobotrys oligospora and Monacrosporum ruigeriense produce attractors highly effective for nematodes. The attracted worms are caught by the trapping structures and killed by the produced toxic compounds. The presence of worms induces formation of the trapping structures. In addition, production of attractors and even toxic compounds is induced or stimulated. The chemical nature of the produced attractors has not yet been elucidated; however, it has been found that one of the compounds is 3'5'-AMP, but this latter compound itself is much less effective than filtrates of media after the submerged cultivation. The observed facts indicate that the
worm may be attacked by the wild fungus as follows: The trapping structures are formed as a morphological reaction to the presence of nematodes, production of the attractors being induced or stimulated at the same time. The concentration gradient of the attractor attracts the worm to the trapping structures and formation of nematocides is induced or stimulated simultaneously. Hyphae of the fungus then enter the body of the nematode which serves as a source of nutrition. A semiquantitative method for determining the attraction will be briefly described.

Degradation of Pyrimidine Bases by Light of \( \lambda > 310 \text{ nm} \) in the Presence of \( \text{Fe}^{3+} \).

I. J. Černohorsky, G. M. Blackburn, Faculty of Sciences, Charles University, Prague and University of Sheffield, England.

Many organic dyes degrading photodynamically purine bases or aromatic amino acids during irradiation with light of a suitable wavelength in the presence of oxygen exist. On the other hand, data concerning a similar effect of inorganic ions are only limited. Studies of the interaction of metals with DNA showed that the quality of DNA changes with the time of contact with \( \text{Fe}^{3+} \) ions. The effect of light of a wavelength higher than 310 nm on purine and pyrimidine bases in the presence of \( \text{FeCl}_3 \) and at \( \text{pH} 5, 4, 3, 2, \) and 1 was hence investigated. Concentration of bases before and after the photoreactivation was studied spectrophotometrically. The highest decrease of bases was found at \( \text{pH} 3 \) in the case of pyrimidines. Purines are nonreactive under these conditions. Kinetic measurements of thymine showed that the reaction is first order and is decelerated slowly down by formaldehyde, indicating that \( \text{OH} \) ions are the active agent here. Paper chromatography revealed the presence of thymine-glycols (cis and trans), thymine-hydroperoxides (cis and trans), 5-hydroxy-methyluracil, 6-hydro-5-hydroxyorotic acid, 6-hydroxy-5-hydrothymine and a product that could not be identified by this technique. Mass spectrometry identified this product as thymine hydrate.

Photosensitization Effect of Transition Metals on Escherichia coli. P. Studená, A. Kubešková-Blahusková, I. J. Černohorský, Faculty of Sciences, Charles University, Prague.

It follows from the in vitro experiments that the irradiation of aqueous solutions of pyrimidine bases with light of wavelength higher than 310 nm in the presence of ferric ions results in degradation of bases, independently of the presence of oxygen in the medium. When studying in vitro survival of the bacterial strain Escherichia coli K12, SmR, \( \lambda^+ \) we used solutions of \( \text{Fe}^{3+} \), viz. \( \text{FeCl}_3 \)-sodium citrate, ferric ammonium citrate, potassium (hexa/cyanoferrate/III) and \( \text{FeCl}_3 \)-Tris at \( \text{pH} 5 \). The irradiation was performed with light of wavelength higher than 310 nm and intensity of 800 erg/mm²/s, temperature of samples at the end of the irradiation did not exceed 22°C. The highest effect was found with \( 10^{-2} \text{M} \) \( \text{FeCl}_3 \) in \( 10^{-2} \text{M} \) sodium citrate, when a dose of \( 96 \times 10^4 \text{ erg/mm}^2 \) yielded 5.8% survival, whereas in the control in the absence of \( \text{Fe}^{3+} \) a minimum of 90% bacteria survived. In the system containing \( 10^{-2} \text{M} \) ferric ammonium citrate 60% bacteria survived. The systems with \( 10^{-2} \text{M} \) potassium (hexa/cyanoferrate/III) and \( \text{FeCl}_3 \)-Tris did not differ from the controls. The photosensitization effect of \( \text{K}_4\text{Mo(CN)}_6, \text{MnCl}_2, \text{CoSO}_4, \text{UO}_2\text{(C}_2\text{H}_3\text{O}_2)_3 \) and \( \text{CuSO}_4 \) at concentrations from \( 10^{-4} \text{M} \) to \( 10^{-2} \text{M} \) was investigated in a similar way. It appears that the transition metals behave similarly to the well known organic dyes. However, these metals do not require the presence of oxygen, so that rather a clear photosensitization effect than photodynamics is involved here.

Thymine Starvation, UV Resistance and UV Mutability of Escherichia coli B/r Her+. P. Balgávý, R. Turček*, Institute of Experimental Oncology, Slovak Academy of Sciences, Bratislava; *Institute of Endocrinology, Slovak Academy of Sciences, Bratislava.

When the cells of Escherichia coli B/r Her+ thy trp- are transferred from a complete synthetic glucose medium to a medium without thymine they lose their colony forming ability after 45 min and frequency of trp+ revertants increases in the surviving fraction. Thymine starvation lasting up to 40 min (which does not substantially influence viability of cells and frequency of trp+ revertants in the non-irradiated culture) decreases resistance and increases mutability of the cells as compared with the control from the exponential phase of growth. As compared with the control the irradiation of the prestarved culture with a dose of 756 erg mm⁻² results in a delayed DNA synthesis and higher DNA degradation. However, excision of thymine dimers is not influenced. The lower survival of cells after the UV irradiation can be explained by the increased degradation of DNA after the irradiation, due to the inhibition of DNA ligase. As the mutations can be manifested only in the surviving population, the higher frequency of the UV-induced trp+ revertants in the prestarved culture must be brought about by a complete but a less accurate filling of gaps in DNA, originating by excision of thymine dimers and other damage caused by the UV radiation. Experimental details: M medium + 2 µg/ml thymine + 28 µg/ml DL-tryptophan served as a complete medium; viability was determined on the same medium solidified with 2%agar; the same plates with a decreased content of DL-tryptophan (1.5 µg ml⁻¹) served to determine the trp+ revertants; synthesis and degradation of DNA labelled with T-2A4C (IvVVR, Prague) was determined in a TCA-preeipitable fraction; DNA used for a radiochromatographic determination of thymine dimers, in the hydrolysate of high molecular DNA, was labelled in the same way.
Content of Guanine and Cytosine in Deoxyribonucleic Acids in the Genus Pasteurella. J. BOHAČEK, O. MRAZ, Biophysical Institute, Czechoslovak Academy of Sciences, Brno; Department of Epidemiology and Microbiology, Veterinary University, Brno.

Content of guanine and cytosine (GC) in DNA of two strains of Pasteurella multocida, eight strains of Pasteurella pneumotropica and fourteen strains of Pasteurella ureae, isolated from pathological changes or from upper respiratory tract of man and domestic or laboratory animals, was determined. The method of Marmur (1961) based on heat denaturation in 10⁻²M phosphate buffer with 10⁻⁸M EDTA, pH 7, was applied. Calculation of GC content from the ratio E₂₅₀/E₂₈₀ according to Frederig (1961) served as the control method. Average values of the GC content in Pasteurella multocida were 38.6±0.6%, the overall range was 38.6--38.7%. Corresponding values in Pasteurella pneumotropica and Pasteurella ureae were 39.6% (37.1--40.8%) and 41.6% (38.3--45.5%), respectively. The obtained results reveal a close relationship among all studied species and a genetic homogeneity of the whole genus. Further classification of the genus Pasteurella thus consists in existing differences in their biochemical properties.

Changes in Molecular Weight of DNA of Acholeplasma laidlawii after Irradiation with Gamma and UV Rays. J. HUTKOVÁ, V. DRÁŠTÍ, M. KLÍMEK, Biophysical Institute, Czechoslovak Academy of Sciences, Brno.

Enzymic degradation of DNA causes a relatively high radiosensitivity of Acholeplasma laidlawii (D₀ = 5000 rad). It follows from the hitherto experiments, in which changes in molecular weight of DNA of irradiated cells of Acholeplasma laidlawii were followed by means of centrifugation in neutral and alkaline sucrose gradients, that the induction of single and double breaks highly depends on the presence of oxygen during the irradiation. The absorption of 58 eV in the presence of O₂ at 0°C results in a single break in the DNA molecule, whereas the absorption of 469 eV gives origin to a double break. Removal of oxygen results in an increase of these values to 159 and 1863 eV, respectively. The relationship between the radiation dose and number of single or double breaks in the molecule of DNA cannot be accurately determined, when the enzymic degradation occurring after the irradiation is not excluded or minimized. Repair of breaks in DNA after the irradiation with gamma rays could not be observed in the studied strain. In the other group of experiments a question, whether the UV irradiation of Acholeplasma laidlawii results in degradation of DNA, similar to that after the irradiation with ionizing radiation, was investigated. It was found that the UV radiation does not bring about the enzymic degradation of DNA in Acholeplasma laidlawii. In addition to the enzymic degradation, the occurrence of DNA fragments of low molecular weight was also followed using doses from D₀ to doses several times higher. A possible repair of the originating damage was also followed.

A Two-step Induction of Auxotrophic Mutants in Mycobacterium phlei. M. KOSÍKOVÁ-RADOSHOVÁ, V. KOŠTA, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Double auxotrophic mutants were obtained in the prototrophic PA strain of Mycobacterium phlei by treating auxotrophic mutants, induced in the first step by UV radiation or N-methyl-N-nitroso-N'-nitroguanidine (nitrosoguanidine), with nitrosoguanidine under standard conditions (concentration 1 mg/ml, pH 6, survival of cells of the basic suspension 5--10%). Four auxotrophic mutants were used: PA met and PA his induced by nitrosoguanidine and mutants PA his and PA leu induced by UV radiation. The induction of double auxotrophic mutants was evaluated quantitatively in original single auxotrophic mutants and the results were compared with those obtained with the prototrophic PA strain. Only the PA leu mutant retained practically the same sensitivity to a repeated mutagenic treatment (detection of newly induced auxotrophic mutants 0.23%) as that observed in the original prototrophic PA strain (detection of auxotrophic mutants 0.11%). On the other hand, the remaining three single auxotrophic mutants exhibited a higher resistance to a repeated treatment with the mutagen (detection of newly induced auxotrophic mutants 0.02--0.03%). It cannot be concluded, whether this fact can be ascribed to changed structural conditions of DNA previously treated with the mutagen. The induction of newly induced phenotypes probably depends to a certain extent on character of an already existing mutational change. In the mutant PA met we obtained a wide range of phenotypes frequently induced in the prototrophic PA strain (e.g. arg, his, leu, ileu-val, pur, pyr etc.). Completely new phenotypes (z-amino-butyric acid, riboflavin) were also detected. In the PA his strain (originally induced by UV radiation) phenotypes leu, ileu, val predominated, whereas in the PA leu strain the phenotype ser was most common; the lys phenotype was also detected and the pur phenotype was rather rare. Other changes of the phenotype could not be detected.

Constitutive and Inducible Nucleoside Permease in Escherichia coli. J. DOSKOCIL, Institute of Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague.

Mutants deficient in nucleoside permease were obtained by selecting for a crossed resistance to 5-azacytidine and showdomycin. Out of 20 mutants of the zCyd⁻ type 12 were simultaneously resistant to showdomycin (zCyd⁻ Shm⁺), the remainder being of the zCyd⁻ Shm⁻ type. The incorporation of 5-aza-
cytidine was about eight times slower in both groups as compared with wild bacteria. In addition, mutants of the first group exhibited a decreased substrate affinity during metabolic conversion of a number of other nucleosides. However, all the above-mentioned properties were again comparable with the wild type when the cells were cultivated in the presence of thymine; they became sensitive to 5-azacytidine but not to show domycin. Resistance of the zCydr Shm mutants was not changed by the induction. The obtained results show that wild type bacteria have two different nucleoside-permeases determined by two different genes; the first is constitutive, whereas the other is a part of the deo-operon and is induced simultaneously with deo-enzymes. The mutants zCydr Shm are defective in the constitutive permease but retain the inducible enzyme. The inducible permease transports 5-aza-cytidine almost as effectively as the constitutive component, but it cannot transport showdomycin. Different character of the two permeases was verified by their different sensitivity to the inhibitory effect of some analogues of nucleosides (J. Daskočil and A. Holý, in press). The inhibitory studies showed at the same time that the permease induced in bacteria of the wild type differs from their constitutive permease and is identical with permease of the zCydr Shm mutants.

The Relationship between Synthesis of DNA and Division of Cells of Escherichia coli 15 TAU after Synchronization by Starvation for Arginine and Ura. M. Lhotská, V. Vondřejs, Department of Biophysics, Faculty of Sciences, Charles University, Prague.

Cells of Escherichia coli 15 TAU synchronized by starvation for arginine and uracil divide after the addition of arginine and uracil only on the condition that synthesis of DNA is not simultaneously inhibited, at least for 5 min, within 35–55 min after termination of the synchronization. DNA synthesis was inhibited by thymine starvation, by nalidixic acid and by oxoline acid. We assume that the DNA synthesis is necessary for completion of replication cycles that were not fully completed under the conditions of synchronization. The addition of thymine for 5 min beginning at any time interval within 35–55 min induces division of a fraction of cells beginning with the 60th min of incubation. It follows that completion of replication of the chromosome is necessary for the division, but it is not a signal of a time process determining the interval, when the subsequent division will take place. As chloramphenicol added after 45 min did not inhibit division induced by the addition of thymine 50 min after the synchronization, it may be assumed that after completion of the replication cycle protein synthesis associated with the division need not proceed. If thus synthesis of a regulatory protein were associated with completion of the replication (terminal protein — Jones and Donachie, 1973), the synthesis could not be sensitive even to very high concentrations of chloramphenicol (up to 400 μg/ml).

Inhibition of Replication and Division of Bacteria by Derivatives of 3-quinoline Carboxylic Acid. J. Havlova-Moravova, V. Sulcová, A. Capek, V. Vondřejs, Department of Biophysics, Faculty of Sciences, Charles University; Research Institute of Pharmacy and Biochemistry, Prague.

The antimicrobial effect of 11 derivatives of 3-quinoline carboxylic acid and nalidixic acid on several species of microorganisms was compared. In addition, the effect of these compounds on DNA synthesis, growth and colony forming ability was studied in more detail in Escherichia coli 15 TAU. The most effective was 1-ethyl-4-oxo-1,4-dihydro-6,7-methylenedioxy-3-quinoline carboxylic acid (oxolinic acid) completely inhibiting DNA synthesis at a 10 times lower concentration as compared with the commonly used inhibitor, nalidixic acid, or with any of the tested derivatives lacking the methylene bridge bound to other oxygens in positions 6 and 7 of the quinoline ring.

Mutagenesis of the Replication Point in Mycobacterium phlei by N-methyl-N-nitroso-N′-nitroguanidine. M. Koničková-Radochová, J. Koniček, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Mutagenesis of the replication point of auxotrophic mutants of the PA strain of Mycobacterium phlei was studied from the point of view of induction of resistance to antituberculosis agents. Mutagenic processes were induced by N-methyl-N-nitroso-N′-nitroguanidine, the mutagenic activity of which is maximal in the replicating region of DNA. The method of induction of revertants in a synchronized culture, consisting of the synchronization procedure and the mutational process proper, was applied. The culture was synchronized by a combination of the cold shock with a previous centrifugation. The procedure of the induction of revertants in the synchronized culture has already been worked out and verified with three auxotrophic mutants of Mycobacterium phlei. In this work we used double auxotrophic mutants PA leu met, PA met put, PA met arg, in which the induction of reversions was followed, and single mutants PA met and PA leu, in which the induction of resistance to streptomycin, ethionamide, cyclerine and resitomycin was investigated. By calculating maximal induction of the followed mutational changes we determined localization of eight genes on the chromosome of Mycobacterium phlei.
Cellular Receptors of the Competence Factor and Kinetics of Binding of the Factor to the Receptors. M. Konôrková. Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

It was found that D-glucosamine and D-galactosamine form a determinant part of receptors of the competence factor (CF) on the surface of the *Pneumococcus* (Kohoutová and Kocurek, Nature 247, 277 1974). Binding of CF to the receptors as a function of time was followed biologically in a transformation medium without bovine serum albumin. Under these conditions the control without the added CF was zero. After 3—5 min at 37°C the receptor is bound completely to the receptors. This is demonstrated by a loss of activity in the supernatant. After 10 min, when the induction of competence in non-competent cells reaches its maximum, CF is released from the receptors to the supernatant. Under conditions, when the spontaneous competence originates cyclically in the recipient culture, CF is cyclically bound to receptors of non-competent cells. However, after the induction only a single sharp peak of competence with a maximum after 10 min can be detected. The first step of the induction process, i.e., the ability to bind the added CF to the receptors, can be studied in the mutant blocked in the reversible and irreversible binding of DNA and not producing CF, by means of the described method.

Effect of NaN₃ on the Irreversible Binding of DNA in the Transformation Process in *Bacillus subtilis*. P. Tichý, Z. Fuchsovík, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The effect of NaN₃ on the irreversible binding of the donor DNA by the recipient cell and its transformation was studied in the strains *Bacillus subtilis* 168 *trp*₂ and *Bacillus subtilis* 168 *trp*₂ *thi*. The method of collection of cells on membrane filters was used to follow the amount of irreversibly bound ³H-labelled DNA; the number of transformants was followed by spraying on plates. The addition of NaN₃ at a concentration of 5 × 10⁻² M inhibited the transformation completely, but did not inhibit the irreversible binding of the ³H-labelled DNA by the recipient cell. However, a considerable degradation of the irreversibly bound DNA occurs in the presence of the metabolic inhibitor. A 40 min incubation of the cells (37°C) after the irreversible binding of DNA by the recipient cell showed that in the presence of NaN₃ 50% of the irreversibly bound DNA is degraded to TCA soluble products, as compared with the control. A new model of the irreversible binding of the transformation DNA by the recipient cell was postulated on the basis of the obtained results.

On Conjugation in *Mycobacterium smegmatis*. J. Konôrková, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Conjugation procedures usually used with other model microorganisms were unsuccessful with *Mycobacterium phlei*. Therefore, when working with auxotrophic mutants of two different strains of *Mycobacterium smegmatis* (CCM 2300 and My 23/64) we modified the conjugation method and performed it on surface of a solid complete medium used for *Nocardia* and *Streptomyces*. A high number of auxotrophic mutants requiring amino acids and to a lesser extent components of nucleic acids and vitamins were obtained by induction with N-methyl-N-nitroso-N'-nitroguanidine (1 mg/ml, pH 6 and survival of the basic suspension of 30% and 5%). Four auxotrophic mutants of *Mycobacterium smegmatis* CCM 2300 and eight auxotrophic mutants of *Mycobacterium smegmatis* My 23/64 that were mutually crossed were used in the experiments proper. Prototrophic colonies originating as a result of a simultaneous growth always of two auxotrophic mutants did not exceed substantially in their frequency the frequency of spontaneous reversions of individual auxotrophic mutants. Negative results are probably caused either by a limited number of used mutants or by unsuitability of the selected strains of *Mycobacterium smegmatis*.

Adsorption and Effect of Colicins on Sensitive Bacteria *Escherichia coli* with Altered Cell Surface. J. Šmařda, J. Kouvalík, I. Paval, Department of Biology, Medical School, J. E. Purkyně University, Brno.

Surface layers of the bacterial cell play an important and incompletely understood role in processes of adsorption and inhibitory effect of colicins. Quantitative ranges in these two processes were hence followed in sensitive bacteria exposed to: (1) plasmolysis and deplasmolysis, (2) urea, (3) surface active compounds. The experiments were performed with colicins E2 and K. Adsorption capacity of the plasmolyzed bacteria for colicins is decreased; their inhibitory effect is also proportionally decreased. However, colicin bound to bacteria cannot be released by transfer to a hypertonic solution after the adsorption. The decreased adsorption capacity, as well as the decreased effect of colicins, remain unchanged even after deplasmolysis (restoration of the physical contact of the cytoplasmic membrane with the wall does not restore its functional integrity). The hypertonic medium does not influence the adsorption capacity of the isolated fraction of the surface structures. Highest concentrations of urea (highest possible concentration with respect to activity of colicins) were used; urea did not have any effect either on the adsorption or the effect of both colicins. On the other hand, the adsorption capacity, as well as the effect on bacteria, were decreased when treating the cells with detergents attacking lipoproteins of the cell surfaces, i.e. dodecylsulphate and sodium deoxycholate. However, none of these agents could release colicins that had already been adsorbed on bacteria or reverse their inhibitory effect. Damage of surface structures of bacteria thus increases their resistance to colicins, simultaneously with a decrease of their adsorption capacity.
Transferable Resistance in Urinary Strains of Escherichia coli. V. Chaloupček, Institute of Hygiene and Epidemiology, Prague.

Of the strains of Escherichia coli isolated as etiological agents of diseases of the urinary tract 23.5% were able to transfer resistance to certain antibiotics by conjugation with Citrobacter freundii in vitro. Two R-factors and sometimes R-factor and col-factor were present in cells of some strains. However, the serotype of the strains or an occasional ability to haemolyze red blood cells were not associated with the presence of a col-factor or R-factor of a certain fi character. Degree of resistance determined by the R-factors differed only insignificantly in the Citrobacter and Escherichia coli K 13 Hfr. Resistance was induced to chloramphenicol (256 μg/ml and more), neomycin (126 to 256 μg/ml), streptomycin (8–512 μg/ml and even higher values found in the studied strains were not transferred) and tetracycline (64–512 μg/ml). Concentrations of chloramphenicol, streptomycin and tetracycline that can be reached in urine are 30 μg/ml, 1000 μg/ml and 300 μg/ml, respectively. It follows that the application of the antibiotic does not result in selection of the R strains of bacteria only in the case of streptomycin. From the comparison of sensitivity of the R and R+ bacteria concentrations of the antibodies suitable for the selection of the R+ exconjugants can be deduced: 22 μg/ml of chloramphenicol, neomycin and tetracycline and apparently only 5 μg/ml of streptomycin with a subsequent control of the presence of the R-factor in this latter case. As all strains resistant to streptomycin were also insensitive to spectinomycin, it may be assumed that the mechanism of resistance consists in adenylate of molecules of the above-mentioned antibiotics.

Regulation of Synthesis of Chloramphenicol-acetyl-transferase in Escherichia coli R+. H. Braná, J. Huseck, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

In strains Escherichia coli K12 X8011, HK26 and HK66, infected with the R1-dr 19 plasmid we tested activity of the enzyme chloramphenicol-acetyl-transferase, coded for by the above-mentioned plasmid. The enzyme was isolated during different phases of growth of cultures, the activity was measured in supernatants of sonicates by means of a standard spectrophotometric method. The enzyme is synthesized constitutively and its synthesis is regulated by catabolite repression. The specific activity is increased 5–10 times during the stationary phase in a complete medium (PNS); the number of enzyme units per cell increases also during this period. It was demonstrated that the increase of the specific activity of the enzyme is caused by synthesis of the enzyme de novo, being repressed by higher concentrations of glucose and blocked in the presence of chlorotetacycline. The specific activity of the enzyme did not increase in the synthetic medium (M9), on the contrary, the number of enzyme units per cell decreased. This fact is more pronounced in a medium with glycerol. The question of preferential synthesis of chloramphenicol-acetyl-transferase during the stationary phase in a rich medium is discussed.

Occurrence of Individual Categories of Plasmids in Haemolytic Strains of Escherichia coli Isolated from Freely Living Animals. A. Sokol, F. Federač, M. Špěwik, O. Hanván, Department of Microbiology and Zoohygiene, Veterinary University, Košice.

The presence of independently mobile determinants of resistance to tetracycline (T), Col factors and Hly factors was tested in 90 haemolytic strains of Escherichia coli. A simple conjugation test with Escherichia coli 185 N× was applied. Strains bearing independently immobile plasmids were subjected to tests of mobilization of plasmids using strains Escherichia coli 50 Col- Hly+, Escherichia coli CS 53 and Escherichia coli dispers P 14 as donors of the transfer factor. (a) Out of 30 strains isolated from the feathered utility animals cured with tetracycline 15 strains were T resistant, out of that 8 strains carried the independently mobile T determinant and in 2 strains the independently immobile T determinant of resistance was mobilized at least in one of the used tests of mobilization of plasmids, i.e. 18/8/2. None of 10 colicinogenic strains carried the independently transferable Col factor; Col factors of 2 strains could be mobilized, i.e. 10/0/2. Out of 30 haemolytic strains only one strain had the independently mobile Hly factor; in 4 strains the Hly factor could be mobilized, i.e. 30/1/4. (b) The following representation of categories of plasmids was found in 30 strains isolated from the feathered vermin animals (without a previous contact with antibiotics): resistance to T — 8/5/2, Col factors — 9/1/2 and haemolytic activity — 30/2/2. (c) The following representation of categories of plasmids was found in 30 strains isolated from deer and hairy utility small animals (without a previous contact with antibiotics): resistance to T — 10/6/2, Col factors — 5/1/2, haemolytic activity — 30/4/4. The results obtained extend the present view on ecology, occurrence and circulation of individual categories of plasmids in Escherichia coli, even without a selection pressure of antibiotics.

Effect of Peroral Application of a Colicinogenic Strain of Escherichia coli on the Incidence of Different Categories of Plasmids in Escherichia coli Isolated from Weaned Piglets Fed on Flavomycin. F. Federač, A. Sokol, Department of Microbiology and Zoohygiene, Veterinary University, Košice.

The effect of the peroral application of flavomycin on the occurrence of markers in Escherichia coli that can be determined by plasmids (resistance
to antibiotics, hemolytic activity and colicinogeny) was followed for 28 days. The following doses were applied: (a) flavomycin 10 mg/kg of diet; (b) flavomycin 10 mg/kg of diet together with the perorally applied colicinogenic strain Escherichia coli (a week dose of 5 x 10^6 of Escherichia coli In^+ per animal); (c) the colicinogenic strain alone (a week dose of 5 x 10^6 of Escherichia coli In^+ per animal). Both experimental and control groups consisted of 25 animals. (a) In the experimental group, in which only flavomycin was added the initial 41.0% incidence of strains of Escherichia coli with the mobile plasmid decreased to 11.2%. The incidence of hemolytic strains of Escherichia coli decreased considerably — from 19.2 to 4%. The initial occurrence of 34.4% of the colicinogenic strains of Escherichia coli decreased to 14.4%. (b) In the experimental group fed additional flavomycin together with the colicinogenic strain of Escherichia coli a considerable decrease of strains with the mobile R plasmid (from 36.8 to 6.4%), strains with the hemolytic activity (from 18.2 to 2.0%) but a less considerable decrease of the incidence of the colicinogenic strains (from 34.6 to 18.4%) occurred. (c) In the experimental group, in which only the colicinogenic strain of Escherichia coli was added, the occurrence of strains with the mobile R factor varied within a narrow range (from 38.4 to 32.0%), the occurrence of strains with the hemolytic activity decreased (from 20 to 14.4%) but the occurrence of the colicinogenic strains increased (from 23.0 to 49.8%). (d) In the control group the occurrence of the followed markers varied within a narrow range — the original incidence of the mobile R plasmid of 38.4% was decreased by at most 4%, the 17.6% incidence of the hemolytic activity by at most 4% and the 30.4% incidence of colicinogeny by at most 4.2%. The inhibitory effect of flavomycin on the occurrence of all three types of plasmids may be deduced on the basis of the results obtained. In addition, a possibility of substitution of the strains of Escherichia coli carrying R plasmids and the Hly factor by the newly accepted strain of Escherichia coli carrying the ColFa factor can be considered.

An Attempt to Transfer the Chromosomal and Plasmic Genes in E. coli by Means of Transformation. M. Šhogr, V. Rytíř, J. Hrubáček, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

In order to determine the relative quantity of plasmid and chromosomal genes and according to that the number of copies of the plasmids in the cell, we transformed the acceptor strains of Escherichia coli JC 7923 with DNA isolated from the strain 1100 R^+ containing the R^+ drd-19 plasmid. Synthesis of leucine and resistance to chloramphenicol were transferred as the chromosomal and plasmid markers, respectively. The acceptor cells were gradually washed with 10mM CaCl_2 in the cold (0-2°C), transferred to 30mM CaCl_2 and DNA at a final concentration of 10-20 µg/ml was added to the suspension of cells. The cells were sprayed on selective media and the frequency of both markers was followed separately. The frequency of transformants for the chromosomal marker varied from 1.0 x 10^{-7} to 4.0 x 10^{-7}. The frequency of transformants for the plasmid gene represents 2 to 5% of the frequency of transformants of the chromosomal gene. The frequency of the chromosomal gene. The frequency of the chromosomal and plasmid markers was roughly the same when using the acceptor strain Escherichia coli C6000.

**R-Factors in Strains of Shigella sonnei Isolated in the Central Bohemian Area.** E. Schön, Z. Mandlíková, V. Rytíř, Jr. Hrubáček, Central Bohemian Station of Hygiene Prague; Institute of Epidemiology, School of Medicine, J. E. Purkyně University, Brno; Institute of Hygiene and Epidemiology, Prague.

Sensitivity to antibiotics of 800 strains of Shigella sonnei was tested during 1971-1972. Out of the strains 463 were resistant to one or more antibiotics. Monoresistance (TC, CM or SU) was found roughly in one half of the strains, 13% of strains were biresistant, 23% of the strains were resistant to 3 antibiotics and 14% of the strains were resistant to 4 and even more antibiotics. Types of the resistance change: whereas in 1971 strains resistant to tetracycline predominated, in 1972 the relative frequency of chloramphenicol resistant strains increased. Changes of the type of resistance could be observed also during individual epidemics, namely in strains belonging to a single lysotype and colicin type. Conjugation experiments performed in 13.0 strains with a recipient strain of Citrobacter revealed the transferable character of the R-factors in 77%.

**Changes of R-Factors in Shigella sonnei during Dysentery Epidemics.** Z. Mandlíková, E. Schön, K. Červinka, Regional Hygienical Station of the Middle Bohemian Region, Prague; District Hygienical Station, Mladá Boleslav.

Changes of R factors were detected in strains of Shigella sonnei during dysentery epidemics. These changes can occur by accepting R factors from the resistant physiological flora, or by segregation of R factors in strains that were originally resistant, leading to a complete loss of resistance. Changes can be observed both during the followed epidemic and in repeatedly isolated Shigella strains in individuals. A possibility of a mutual interaction between pathogens and non-pathogens is in several cases supported by demonstration of transferability of the R factors in strains of Shigella and Escherichia coli isolated simultaneously from the same person.
Mass Balance during the Synthesis of Yeast Biomass from Ethanol. M. Ruz, F. Števos, Research Institute of Fodder Industry, Department of Microbial Production, Prague.

Junkalor industrial gas analysers, adjusted for laboratory use, were employed to determine the consumption of oxygen and the formation of carbon dioxide during the synthesis of yeast biomass from ethanol. In the first phase of batch cultivation the oxygen consumption attains 1.11 g O₂/g yeast dry wt., during the following phase it reaches a value of 1.76 g O₂/g yeast dry wt. The formation of CO₂ also increases but its increase is relatively more rapid; this is reflected in an increasing respiration coefficient during the cultivation. In the first phase the RQ is 0.28—0.32, rising in the second phase up to 0.4. Mass balance was established for both cultivation phases; it yielded stoichiometric relationships which were found to fit the experimental data in all respects. The characteristic features of the two cultivation phases are the accumulation of acetic acid during the first phase and its subsequent consumption during the second one. This second phase coincides with increased oxygen consumption, production of carbon dioxide and the RQ value; a calculation shows that the accumulated acetic acid is oxidized to CO₂ and H₂O.

Cultivation of Yeasts on Methanol. O. Volfová, P. Pilát, J. Panoš, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

A total of 10 yeast strains, utilizing methanol as the only source of carbon and energy, were isolated from soil. Their growth was studied at 30°C in a mineral medium with a small amount of yeast extract, NH₄+, and with 1% methanol. The strain yielding the highest percentage of biomass on cultivation in flasks (Y 33%) was further studied as to the growth parameters and biomass composition. The biomass was found to consist of 44% crude proteins, 2—3% esterified fatty acids, 1—2% free fatty acids, 5.7—6.0% RNA and 0.25% DNA. Amino acid analysis revealed a high content of aspartic acid, glutamic acid, lysine, leucine, valine and threonine. The composition of fatty acids in the cell lipids of this strain was similar to that found in lipids formed during the growth of the cells on glucose and ethanol (the strain grows better on these substrates than on methanol). The lipids contained 96% fatty acids with even number of carbon atoms and 4% of acids with odd number of C atoms in the molecule. Oleic acid was found to have the highest relative content. The suitability of application of this strain for the production of SCP from methanol is discussed.

Correlation of the Oxygen Demand with the Formation of Fatty Acids during the Cultivation on Hydrocarbons. M. Sobota, A. Prokop, J. Panoš, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The consumption of oxygen during fermentation may be measured by balance method as the product of the air flow and the difference in its partial pressure between the fermentor inlet and outlet air stream. The content of oxygen, carbon dioxide and nitrogen was measured by gas chromatography. Nitrogen served also as an internal standard for calculating the air flow rate at the fermentor outlet as this differed from the inlet value. The example of fermentation of n-alkanes by the yeast Candida lipolytica 4-1 served to demonstrate the calculation of the specific oxygen consumption rate in mmol/g.h and the oxygen demand per biomass unit (g O₂/g biomass) obtained by dividing the former value by productivity. At initial growth stages, the specific oxygen consumption rate and oxygen demand are in a correlation with an enhanced production of fatty acids present in both the oil and water phases. At this time the biomass yield is also lower. The results of this study may serve to the optimization of biomass production.

Substrate Specificity of Yeast Growing on Solid n-Alkanes. P. Pilát, A. Prokop, O. Volfová, J. Panoš, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The generally low growth rates (productivities) of microorganisms growing on solid n-alkanes hint at a different type of uptake of these substrates from that found with liquid hydrocarbons. To obtain higher productivities, the solid substrate may be dissolved either in liquid hydrocarbons (n-decane) or in highly branched inert hydrocarbons (pristane, squalane) or deparaffinated gas oil. The last technique was used for comparing the substrate specificity of two strains of Candida lipolytica; strain 4-1, already used for preparing biomass from gas oil, and strain K capable of removing paraffin from mineral oil. The substrate was a model mixture of C₁₀—C₃₆ n-alkanes. The consumption of individual n-alkanes, as determined by gas chromatography, was considerably different with each of the two strains. The substrate specificity of strain K was found to be shifted to higher n-alkanes, the relative utilization of individual alkanes being also different from the strain 4-1. The results were verified with crude oil (melting point 33°C, n-alkanes C₆—C₈). The results obtained with the utilization of n-alkanes from gas and mineral oils (previous data), from model mixture and from the crude oil point at an adaptation of the two strains to higher n-alkanes. The substrate specificity depends thus to a certain extent on the substrate used, its spectrum of n-alkanes and the fashion and form of its application.
Changes in drop size distribution are typical for the onset of hydrocarbon fermentation. Experimental data on the distribution of hydrocarbon droplets at different times during the fermentation, when plotted in non-dimensionalized plots, show that the distribution has a tendency towards self-preservation; the non-dimensionalized plots are obtained by normalizing the distribution according to the Sauter mean diameter. Also, the curves so obtained for different initial dispersed phase fractions and for two different kinds of substrates (gas oil and a model dispersed-phase system - n-hexadecane dissolved in biologically dewaxed gas oil) were found to have similar shapes. Using this self-preserving property, an empirical (single parameter) equation was set up for the drop size distributions. The proposed empirical distribution was found to hold for different reactor sizes (working volumes of 1.5—850 l). An attempt was made to correlate the parameter, the Sauter mean diameter, with the operating conditions. The usefulness of the empirical distribution for the prediction of the performance of fermentors was demonstrated on an example.

Effect of Oxygen Supply on the Behaviour of Cultivatively Anaerobic Bacteria during Batch and Continuous Cultivation. J. PÁCA, V. GRŠOR, Department of Fermentation Chemistry and Technology, Institute of Chemical Technology, Prague.

Oxygen limitation of the growth of a bacterial culture may be accomplished either by changing the partial oxygen pressure in the intake air or by altering the volumetric oxygen transfer coefficient (KLa) which is a function of agitator speed, aeration intensity and the physical properties of the liquid medium. Experiments were done with the organism Klebsiella aerogenes CCM 2318 grown on a minimal glucose medium at constant pH. Oxygen supply was controlled by changing KLa; this coefficient was measured during the cultivation by a dynamic method using a membrane electrode and also calculated from both the differential and the integral form of the oxygen transfer equation. The experimental data provided a basis for an analysis of the factors affecting the accuracy of the determination of KLa by the two methods. On studying the effect of KLa on specific cell growth rate in batch cultivations the value of μ was found to be in the range of 0.66—1.1 h⁻¹ throughout the region from anaerobic to aerobic conditions. Changes were found in biomass yield (23.4—47.3%) and in the production of acid products (difference of 26—51%). Continuous cultivations were carried out at dilution rates of 0.96 h⁻¹ and 0.178 h⁻¹ under otherwise identical conditions. During the transition from anaerobic to aerobic conditions, the steady-state changes in biomass yields were considerably higher than with batch cultivations (9—51%). At otherwise identical values of the physical parameters affecting the oxygen supply, a change in the dilution rate was found to bring about not only a change in the specific oxygen uptake rate, but also a change in the coefficient KLa.

Study of the Growth of Microorganisms on Fermentor Walls and of its Effect on the Dynamics Characteristics of the Chemostat. M. RYCHTER, V. GRŠOR, Department of Fermentation Chemistry and Technology, Institute of Chemical Technology, Prague.

To assess its maximum production of itaconic acid, Aspergillus terreus ATCC 10020 was cultivated on a glucose synthetic medium. Prolonged cultivation, especially at pH above 2.6, gives rise to a very intense growth of the microorganism on fermentor walls, above the surface of the agitated liquid. The adhesion of the microorganism depends both on its properties and on the properties of the fermentor walls. In contrast to bacteria or yeasts, the cultivation of mycelar forms gives rise not to a thin film, but to a thick layer (thickness 3—5 mm). The fermentor thus represents a continuous agitated biological reactor and, at the same time, a continuous film reactor with no regulation of the thickness of the biologically active layer. Under experimental conditions the system cannot be flushed out completely. The surface-growing microorganisms differ considerably from the actual submerged culture. The effect of dilution rate (0.2—0.4 h⁻¹), pH (2.3—3.6) and phosphorus concentration (0.002 to 0.05%; P) on the behaviour of the culture were studied. The results obtained with the wall growth differ considerably from those obtained when the wall growth was almost completely removed. A simplified mathematical model was proposed permitting the calculation of steady-state concentration of the limiting substrate in the system. The model was verified on a large number of experiments. The knowledge of quantitative relationships during the growth of microorganisms on the walls of biological reactors is of a great importance for the study of the behaviour and stability of continuous cultivations, especially for the application of small-scale results on a larger scale.

Study of the Propagation of Yeast Biomass for the Purposes of Wine Technology. F. MALÍK, J. HRONČEK, Faculty of Chemical Technology, Slovak Technical University, Bratislava.

Experiments were carried out with 3 wine yeast strains, Saccharomyces bayanus Saccharo 1805 (Bratislava 0 and 1), and Saccharomyces cerevisiae (Bínik 1), in an automatic-intake functional fermentor model. The nutrient medium was a concentrated grape must diluted prior to the fermentation. The experiments included both continuous and period-
ical fermentations with irregular dosage. On evaluating the interrelationships between the rate of substrate consumption and biomass concentration during the propagation from an inoculum (neglecting the effect of the alcohol formed) we found that at higher concentrations of yeast biomass the rate of multiplication declines. When taking into account the effect of alcohol (using an equation similar in form to that for noncompetitive inhibition) its inhibiting effect on the multiplication rate may be demonstrated. The appraisal of experimental data on the propagation of yeast biomass obtained by anaerobic fermentation shows that the yeast multiplication rate grows until a certain maximum concentration of the biomass is attained. A 20% replacement of the grape must by beet sugar is permissible for technological purposes. The propagation of yeast biomass on grape must is suitable only when the must is complemented by nutrients lost during the technological treatment of the concentrated must.

Production of Polysaccharides from Ethanol. J. Říčka, E. Masnerová, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

In various fields of technology, an attention has recently centered on the application of polysaccharides produced by microorganisms and accumulated in the cultivation medium. The substrates used are for the most part glucose or sucrose which are, however, relatively expensive. As synthetic ethanol has become accessible in large amounts, a special attention has been paid to microorganisms capable of producing polysaccharide from this substrate on a minimal medium. We carried out experiments of this type with unidentified bacteria isolated from compost soil. Cultivation conditions were tested in laboratory fermentors. Increased amount of oxygen promoted the decomposition of polysaccharides and the formation of acetic acid at later growth stages. Organic nitrogen, in the form of peptone and yeast extract, suppressed the formation of polysaccharide. In cultures without any limitation by inorganic nitrogen source (e.g. KNO₃) the polysaccharide yield was half that in limited cultures even though the limitation decreased the final concentration of cells. 2% glucose yielded 0.3—0.5% polysaccharide while 2% ethanol gave only 0.6—0.1% of the product. Increase in ethanol concentration to 5% did not result in any increase of polysaccharide production. The highest yield was observed with 1—2% ethanol.

Inhibition of Tetrahionate Reductase Synthesis and Activity by Nitrate Respiration. O. Akráttný, F. Kárpálek, Department of Genetics, Microbiology and Biophysics, Faculty of Sciences, Charles University, Prague.

Respiratory nitrate reductase (NR) and tetrahydroxys reductase (TTR) catalyse the reduction of terminal electron acceptors, nitrate or tetrahydroxys (anaerobic respiration). The presence of oxygen both inhibits the activities of the two enzymes and represses their synthesis. We attempted to establish if a similar regulation relationship exists mutually between the two reductases. Washed un-growing Citrobacter cells possessing NR synthesize TTR inductively in the presence of K₂S₄O₆; the NR activity being not affected by this synthesis. On the other hand, the induction of NR in cells possessing TTR causes a reduction in the TTR activity. The NR induction having been completed, the TTR activity is gradually restored to its original level. If the same experiment is carried out in the presence of KCN, which inhibits the NR activity but does not repress its synthesis and does not affect the TTR activity, the temporary decrease does not occur. If KCN is added it the moment of the most pronounced decrease, an instantaneous restoration of the NR activity to the original level follows. If chloramphenicol is added in the same way, no activity restoration is observed. Hence, the temporary decrease in the TTR activity is due to the action of the already existing NR and not to its synthesis; protein synthesis is thus required for the renewal of the activity. The two reductases were simultaneously induced in growing cells. While the TTR synthesis was repressed, the NR synthesis was not influenced. In the presence of KCN, which inhibited the NR activity, however, the repression did not occur and the two enzymes were synthesized simultaneously. Thus it may be concluded that the TTR synthesis is repressed by the activity and not by the synthesis of NR.

Physiological State of a Culture in a Chemostat at the Same Growth Rate under Various Experimental Conditions. J. Doležal, F. Kaprálek, Department of Genetics, Microbiology and Biophysics, Faculty of Sciences, Charles University, Prague.

Citrobacter was cultivated aerobically in a chemostat at 30°C in a minimal mineral medium under the following conditions: with glucose at glucose limitation; with galactose at galactose limitation; with glucose at NH₄Cl limitation; with galactose at NH₄Cl limitation. The culture was analysed in the steady state at different dilution rates D (10—85% μₘₚₐₙ₉) and the following parameters were determined: growth yield K, specific metabolic rate q, intracellular concentration of pyruvate, ATP, ADP and AMP. A change in the physiology of the culture was found to occur at D = 70% μₘₚₐₙ₉ and near D₉; the value of K dropped, q increased, as also did the intracellular concentrations of pyruvate, ATP, ADP and AMP. The reason for this was taken to be a partial uncoupling of catabolism from anabolism or a decrease in the efficiency of oxidative phosphorylation. The above criteria served to compare 3a culture growing with the same growth rate but (a) with D lower than D₉ and D higher than D₉; (b) cultures growing on glucose or on galactose; (c) at carbon limitation and nitrogen source limitation;
The localization of EC-2 L-asparaginase was studied in E. coli ATCC 9637 growing on a synthetic medium with glycerol under slight aeration at 37°C. The medium contained sodium lactate ensuring the synthesis of the enzyme under aerobic conditions or, sometimes, also an inductor L-asparagine. The cells served for the preparation of spheroplasts that were then further fractionated. The enzyme was then assayed in individual cell fractions. The main fraction of the EC-2 L-asparaginase was found in the space between the cell wall and the cytoplasmic membrane, independently of the presence of the inductor. At the beginning of the induction, a portion of the activity was localized in the cytoplasm, in cells grown with L-asparagine this portion was four times larger than with control cells. With proceeding cultivation the content of enzyme in the cytoplasm declined gradually to zero. The membrane fraction of cells grown with the inducer contained, at the onset of induction, up to 6% of the total enzyme amount. With proceeding culture growth this fraction also disappeared gradually. Cytoplasmic membranes prepared from control cells possessed no asparaginase activity. On the other hand, a significant fraction of the enzyme was found in the cytoplasm and cytoplasmic membranes of cells grown with the inductor L-asparagine. This fact indicates that these cellular structures participate in the induction of the EC-2 L-asparaginase, especially in the preparatory stage.

Phenylacetylamidohydrolase (EC 3.5.1.11) in Escherichia coli. Substrate Specificity. V. Vortník, J. Slezák, Research Institute of Antibiotics and Biotransformations, Roztoky near Prague.

The substrate specificity of bacterial L-phenylacetylamidohydrolase was studied by measuring the initial rates of hydrolysis of various substrates in zero-order kinetics range. The substrates used included some L-phenylacetyl derivatives of L-amino acids, amides of phenylacetic and phenoxyacetic acids, various substituted derivatives of these amides or some other amides related structurally or chemically to the above two. When using a toluene-treated suspension of bacterial cells or crude enzyme preparation, significant differences were found in the ratios of initial rates of hydrolysis of various substrates, even though the enzyme is known to be localized in the space between the cell wall and the cytoplasmic membrane. The differences were probably due to a different rate of diffusion of individual substrates through the cell wall and, in some cases, probably also to steric hindrances. Alternately, the presence of two or more isoenzymes cannot be completely excluded. The most rapid hydrolysis by the enzyme was found with L-phenylacetylated L-amino acids. 2-Phenylbutyramide is a very poor substrate, 3-phenylpropionamide and 4-phenylbutyramide are not substrates at all. The substrate specificity of the enzyme is discussed with respect to the ap-
plication of some coloured substrates for a rapid and simple determination of the enzyme activity.

**Isolation of \( \alpha \)-Amylase by Means of DEAE-starch.**

J. HANUS, A. Pašek, H. ŠKACHOVÁ, Czech Agricultural Academy, Food Research Institute, Prague.

Experiments were done with pure enzyme from *Aspergillus oryzae* (100 000 Bern.U./g) and with cultivation liquid (mean activity 30–40 Bern.U./ml). Samples of DEAE-starch were prepared at the Institute of Chemistry, Slovak Academy of Sciences, Bratislava. It was proved that only \( \alpha \)-amylase is bound to DEAE-starch in the \( H^+ \)-form, glucoamylase remaining in the supernatant or the eluate. The optimum pH of the enzyme solution or cultivation liquid, ensuring a 100% sorption, was determined. The isolation efficiency was found to depend directly on the relationship activity — amount of DEAE-starch and a curve depicting this dependence was drawn. It was found to hold for both purified enzyme solutions and the cultivation liquid of *A. oryzae*. No effect of the medium composition on the isolation efficiency was found.

The elution of \( \alpha \)-amylase from a column or a stationary elution has a maximum yield (50–60%) with the solution of 0.5–0.6M NaCl, pH gradient was not found to be suitable. No complete elution of the enzyme was found. The technique may be used for the preparation of pure \( \alpha \)-amylase as well as for technical preparations.

**Application of Starch gel in Gel Chromatography.**

J. Kučera, J. Kodeť, Czech Agricultural Academy Food Research Institute, Prague; Starch Factories, Havlíčkův Brod.

The gel-filtration properties of a starch gel prepared by a cold treatment of starch smear were compared with individual types of dextran gel (Sephadex). The measurement of \( K_w \) of various proteins showed that the starch gel has properties similar to the Sephadex G-50. The slope of the plot of \( K_w \) versus log molecular weights for the two gels differs only negligibly. The dependence of the flow rate on hydrostatic pressure is linear throughout the region applicable for gel chromatography and the gel is thus in this region incompressible. Attempts to immobilize enzymes by inclusion into this gel revealed that under any conditions the enzymes are eluted from it, the presence of some high-molecular proteins (glucose oxidases) preventing even the transition of starch smear to utilizable starch gel. The starch gel prepared by the above technique may be used for desalting of high-molecular substances and for gel chromatography in the range of molecular weights up to approximately 25 000. Immobilization of enzymes by inclusion into the gel is not feasible.

**Mutagenic Effect of Ethionine on Candida lipolytica.**

R. Joseph*, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The inhibition of microorganisms, especially of many yeast species, by ethionine was demonstrated. Regulatory yeast mutants with respect to the metabolism of methionine and related amino acids were obtained by inducing the resistance to ethionine. Evidence was obtained to show that ethionine itself can act as a mutagen to the yeast *Candida lipolytica*. The frequency of ethionine-resistant strains after the application of growth-inhibiting concentrations of ethionine increased after a prolonged pretreatment with its lower concentrations. Morphological variants and auxotrophs could also be induced by treating the culture with ethionine.

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**Effect of Quantitative and Qualitative Amino Acid Composition of Nutrient Media on the Biosynthesis of L-Lysine.**

M. Bučko, A. Hanč, Biotika, Slovenská Lupča.

During the biosynthesis of L-lysine, the content of free amino acids in the nutrient media plays a significant role since the production strain lacks proteolytic enzymes. The concentration curves of individual amino acids in the course of biosynthesis of L-lysine were used to determine the optimum levels of the most important amino acids in the nutrient medium at time zero: aspartic acid 2.5 g/litre, leucine 1.0 g/litre, isoleucine 0.25 g/litre, threonine 0.38 g/litre, methionine 0.18 g/litre, and serine 0.7 g/litre. These data enabled us to replace the conventional nitrogen source (soybean flour) with waste products from other biosynthetic productions and protein wastes from other industries.

**Enrichment of Fermented Milk by Vitamin B12 by Means of a Precultivation with Propionibacterium shermanii.**

J. Černá, Z. Peck, H. Hrabová, Czech Agricultural Academy, Food Research Institute; Dairy Research Institute, Prague.

The study of the properties of *P. shermanii* in a special semi-synthetic medium at neutral and alkaline pH formed a basis for determining optimum conditions for the preparation of inoculum utilisable for enrichment of fermented milk by vitamin B12. The strain exhibited a high growth activity even at pH 10.3, probably owing to a protective cell coat and a mucous layer which, as determined by electron microscopy, is formed at alkaline pH above 8.3. The ability of the strain to synthesize the vitamin depended above all on the vegetative stage of the inoculum cells and on the type of microflora in the milk starter used. The highest enrichment was achieved with inoculum taken at the logarithmic growth stage and added to a kefir starter. The resulting kefir milk was left to ripen for 24 h and its content of vitamin B12 was then 70 times that in the original milk, its absolute value being approximately 14 µg/100 ml. In comparison with the non-enriched product, the
milk contained also some other vitamins from the B group; the level of these substances was assayed by microbiological tests. Fermented milk beverages enriched in this way are faultless as to their sensory properties and may be used mainly in children's nutrition and in various diets.

Technical Problems of Determining the Biodeterioration of Asphalts. D. Halama, Z. Ulčiná, Faculty of Chemical Technology, Slovak Technical University Research Institute of Oil and Hydrocarbon Gases, Bratislava

Asphalts are not completely chemically defined, the most detailed information being usually their type composition. The conditions of their application are extremely variable and the spectrum of the biodeterioration agents is very broad. This hinders the development of generally applicable methods for the determination of the biological deterioration of asphalts. The methods mentioned in the literature include mostly the determination of the rate of degradation of thin films or emulsions or the assessment of the degree of corrosion of metal surfaces protected by asphalt layers. The testing is done with either pure or mixed (accumulation) cultures. The results obtained with various methods on different asphalts are compared. The parameters studied are the rate of degradation of asphalts by mixed and pure cultures, effect of nutrients and other cultivation conditions, the size of the inoculum and the optimum pH. The applicability of individual methods and the possibility of their unification and standardization is discussed.

Biodeterioration of Plastics Subjected to Artificially Accelerated Ageing. R. Waserbauer, EZU, Prague-Troja.

The ageing of plastics includes mainly oxidative processes which lead to alterations in the physicochemical properties of the materials and to changes in the degree of resistance towards biodeterioration. Laboratory photo- and thermooxidative acceleration of ageing resulted, in all cellulose-containing phenol-formaldehyde moulding materials studied, in up to 90 times higher rate of colonization of the surfaces of the materials by fungi. Analogous results were obtained with oil varnishes, nitrocellulose lacquers and polyamide. With plasticized PVC, cellulose triacetate and acrybutyrate, on the other hand, an increased resistance towards the biodeterioration was found after the treatment. The measurement of conductance characteristics, permeability, water vapour sorption and insulation resistance of the aged materials revealed that the decreased resistance of the phenolformaldehyde plastics is due to an enhanced rate of transport of water vapour into the materials whereas with the nitrocellulose lacquers, oil varnishes and polyamide the rate of colonization is affected by oxidation products. The increased resistance of plasticized PVC, cellulose acetobutyrate and triacetate is caused by the release of volatile plasticizing agents.

Structures of Some Novel Biologically Active Microbial Metabolites. M. Podojízl, Z. Vaněk, P. Sedmera, J. Vokoun, J. Taxa, I. Kuhar, J. Fusska, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague; Research Institute of Veterinary Medicine, Brno; Slovak Technical University, Bratislava.

Analyses performed at the laboratory of physical chemistry, department of biogenesis of natural products, led to the elucidation of the structure of substances with antiprotozoal activity (vermikulin), antifungal (MBU-18), antibacterial (61-pyromycine) and cancerostable activity (duelauxin). Extremely useful tools in the analyses were the proton magnetic resonance, mass spectrometry, and the use of a computer.

Microbial Glucosidation of Hydroxyanthraquinones. N. Steinerková, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The aureovocin-producing Streptomyces aureofaciens, strain B-96, was found to be able to glucosidize isomeric dihydroxyanthraquinones, added to the cultivation medium, to corresponding mono- and di-glucosides. With alizarine (1,2-dihydroxyanthraquinone) the conversion attains 23%, with anthraflavine (2,6-dihydroxyanthraquinone) it reaches 45%. Also, both isomeric monohydroxyanthraquinones were subjected to microbial glucosidation under analogous conditions. Though the glucosidation takes place also in these cases, the conversion is much less than with dihydroxyanthraquinones. The identity of all the products was confirmed by comparing them with authentic samples prepared chemically. The IR-, UV- and NMR spectra of all derivatives under study were measured.

Isolation and Some Properties of Glucosyl Transferase from Streptomyces aureofaciens. J. Matějů, K. Mikušik, M. Blumaerová, Z. Vaněk, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Enzyme preparation with transglucosidase activity was isolated from the mutant strain Streptomyces aureofaciens B-96. Partially purified enzyme was prepared by differential centrifugation, salting-out with ammonium sulphate to 50% saturation, and by chromatography on DEAE-cellulose. It catalyses the transfer of glucose from the glucosyluridylylphosphatase to 1,2-dihydroxyanthraquinone, which gives rise to 1-hydroxy-2-(6-glucopyranosyloxy)anthraquinone. The substrate specificity and physico-chemical properties of the enzyme were studied.
During the production of tetracycline antibiotics, the fermentation media are supplemented with calcium carbonate which is to maintain an optimum pH of the submerged medium and to bind the antibiotics formed into insoluble Ca-complexes. We tested 7 different kinds of CaCO₃, both purely mineral and chemically precipitated, in order to determine the relations between their physical properties (microscopic particle size, particle shape, their conglomeration tendency, weight volume and sedimentation rate), chemical properties (purity, buffering properties, change in pH of fermentation medium with CaCO₃ before and after sterilization), and the production activity of chlorotetracycline during the cultivation with the tested strain S. aureofaciens. With respect to the production of antibiotics, the most suitable carbonate was the microparticle ground one (VJM) which had the production activity higher by 20% than precipitated CaCO₃. Also, a buffering capacity test showed that this type of carbonate has the lowest inflection pH point among all samples tested. The above criteria may assist in finding the most suitable type of carbonate, or a combination of carbonates, for other kinds of biosyntheses.

The Problems of Chlorination of Perhydronaphthacene in Streptomyces aureofaciens. F. SměkáL, K. Mikulík, Research Institute of Antibiotics and Bio-transformations, Roztoky near Prague; Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The biosynthesis of chlorotetracycline (CTC) by Streptomyces aureofaciens is controlled by a certain concentration of chloride ions in the medium, e.g. by 0.02 M NaCl which is fed into the medium between the 40th and 50th h of cultivation. The production of the antibiotic attains 10—12 mg CTC/ml medium (ČS PV 337-73). Alkali metal chlorides (Na, K, Ca) do not affect the chlorination of the perhydronaphthacene ring and the amount of CTC corresponds to 85—95%. Another group of chlorides (Mg, Co, Mn, Fe(III)) suppresses considerably the activity of the chlorination process and the level of the antibiotic is reduced to 40 to 60%, with a concomitant high proportion of tetracycline and its epimers. Application of chlorides [Co, Mn, Fe(III)] at the onset of cultivation results in an inhibition of the synthesis of the enzyme catalyzing the chlorination of perhydronaphthacene. The amount of CTC corresponds then to 20—50%.

Some chlorides (NH₄Cl, KCl, LiCl) abolish the inhibitory effect of the Co, Mn and Fe(III) salts on the synthesis of the enzyme. The results indicate the regulatory function of chloride ions in the synthesis of the enzyme participating in the chlorination of perhydronaphthacene.

Energy Charge and the ATPase Activity in Streptomyces aureofaciens. E. Čurbová, Z. Hoštálek, Z. Vaněk, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The relationship was studied between the energy metabolism of S. aureofaciens and the biosynthesis of tetracyclines. The metabolic activity of a low-production strain of the wild type was compared with the activity of a production variant. The energy charge values for the two strains were almost identical throughout the cultivation but the low-production strain displayed an appreciably higher level of adenylates than the production variant. The total ATP-ase activity of S. aureofaciens increased strongly after 24 h of cultivation, i.e. in the phase when the anabolic phase terminated and ATP ceased to be utilized in biosynthetic processes. The ATP-ase activity had an identical course in both strains. The ATP-degrading enzymes were divided into two fractions: (i) a typical membrane (Mg²⁺)ATPase of the bacterial type, and (ii) a soluble cytoplasmic ATP-ase activity whose synthesis was repressed by inorganic phosphate. This enzyme is likely to control the level of accessible inorganic phosphate in the cell. The role of the membrane ATP-ase may be the regulation of the level of ATP in the cell and, hence, the control of its allosteric action.

Developmental Changes of Ribosomes from Spores and Vegetative Cells of Streptomyces graminicolor. K. Mikulík, I. Janda, A. Říčková, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The structure of ribosomes from the spores and vegetative cells of S. graminicolor was studied. The spore ribosomes bind very strongly a dark pigment which is then not removable by dialysis, washing with 1 M NaCl, sedimentation in 30% sucrose or by detergents. In the presence of 10⁻² M Mg²⁺ the spore ribosomes form aggregates while the majority of ribosomes from vegetative cells remain in a 70 S form. When the Mg²⁺ concentration is decreased to 10⁻⁴ M, both types of ribosomes dissociate into 30 and 50 S subunits. On lowering the Mg²⁺ concentration as far as 10⁻⁵ M an irreversible disintegration of the subunits occurs. This phenomenon is more pronounced with ribosomes from vegetative cells. Qualitative differences between the protein composition of the two ribosome types were detected. Since the ribosomal proteins include a proteolytic enzyme and a ribonuclease which are activated during the isolation procedure, the isolation of ribosomal proteins had to be carried out in the presence of 10⁻³ M phenylmethylsulphonylfluoride.

During the isolation of spore rRNA the ribonuclease activity was inhibited by diethylpyrocarbonate and bentonite in all extraction media. Two types of rRNA, 16 S and 23 S, were isolated from the ribosomes of vegetative cells and spores. The nucleotide composition of the rRNA in both de-
Passive Selection of Antibiotic Producers with High Production Variability. J. Pásková, Research Institute of Antibiotics and Biotransformations, Roztoky near Prague.

After a transfer into a further spore generation, some strains of actinomycetes used in the fermentation production of antibiotics disintegrate into low-production and high-production variants so that they have to be subjected to a continuous passive selection. The main problem is the acquisition of a sufficient amount of spores for inoculation of large fermentation volumes, since each transfer into another spore generation is accompanied by a decrease in production activity. Selected variants of the erythromycin producer S. erythreus had a considerably lower production activity in 3rd spore generation whilst during a vegetative transfer no attenuation of antibiotic production was observed even after eight-fold transfer. Monosporal colonies of the strain grown on an agar sporulation medium after inoculation by spore suspension were transferred into flasks with liquid medium and cultivated for 24 h on a rotary shaker. Submerged mycelium in the logarithmic growth phase was lyophilized and kept under an inert gas. After evaluation of the selectates by production test portions of the lyophilized young submerged mycelium were used to inoculate "mother" agar slopes of the 1st spore generation which, in turn, served for preparation of inoculum for large fermentation volumes.

Bacterial Dehydrogenation of Aldoses and Aldonic Acids. M. Kulhánek, M. Tadra, Research Institute for Pharmacy and Biochemistry, Prague.

The relationship between the configuration and bacterial dehydrogenation of aldonic acids by active strains of bacteria of the genera Acetomonas and Pseudomonas has, some time ago, been found to obey the so-called gluconic rule: Aldonic acid is dehydrogenated only at the second or the last-but-one C atom of the sugar chain, only when the configuration at this and the neighbouring asymmetric C atom is identical with the configuration of the D-gluconic acid at the same site. The bacterial strains active in this respect are only those dehydrogenating D-gluconic at the corresponding C atom. Some other ketoaldonic acids were now prepared in fermentation experiments from aldonic acids or aldoses. The strains used were selected because of their high activity to D-gluconic; the C-2 dehydrogenation was carried out using Pseudomonas aeruginosa, that on the last-but-one C atom with Acetomonas oxydans. As the sugars used were mostly non-physiological for the bacteria in question, the fermentation liquid was supplemented by a small amount of well assimilated sugar as growth inductor. The aldonic acids were neutralized by insoluble carbonate. D-mannose and D-mannuron yielded thus D-lyxo-5-hexulosonic acid (5-keto-D-mannonoic, 5-keto-L-gulonic acid), D-ribonan gave as yet undescribed D-erythro-4-pentulosonic acid (4-keto-D-ribonic acid), L-arabinose or L-arabonan provided L-erythro-2-pentulosonic acid (2-keto-L-arabonic acid) prepared so far only chemically, and D-xylene or D-xylonan gave D-threo-2-pentulosonic acid (2-keto-D-xylonic acid) isolated so far only a dehydrogenation of D-xyllose by Pseudomonas milderbergii. All these dehydrogenation reactions are in agreement with the gluconic rule.

The Relationship of Conidiation of a Submerged Culture of Claviceps purpurea to the Physiology of the Strain and to Alkaloid Formation. J. Kožová, P. Saudl, Z. Řeháček, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The formation of conidia in a submerged culture of C. purpurea (Fr. Tul) was accompanied by an increased consumption of ATP, almost constant protein synthesis, constant level of orthophosphate in the mycelium, increasing activity of both intracellular and extracellular proteases and the accumulation of lipids in the mycelium. Conidiation was suppressed by ergotamine (5.10^{-4}M), ergometrin (5.10^{-4}M) and glycerol (5.10^{-3}M). Ergotamine also inhibited the activity of leucine aminopeptidase. Inhibitors of conidiation favourably affected the formation of alkaloids, the effect of ergotamine and glycerol in a mixture being synergistic.

Effect of Different Water Environment on Physiological and Metabolical Properties of Escherichia coli. I. Daunener, E. Ježová, D. Töth, Institute of Limnobiology, Slovak Academy of Sciences, Bratislava.

E. coli was cultivated on meat-peptone broth. In the logarithmic growth phase it was washed and transferred to sterile distilled water or sterile surface water from Danube. The cells were kept in these media for 3 weeks at 20°C under constant agitation. A conspicuous decrease was found to occur during this period in the number of cells capable of reproduction on meat-peptone agar and in the respiration and dehydrogenase activity. The decrease was deeper in cells exposed to surface water; the number of colonies determined by plating dropped to 50% during approximately 140 h while in distilled water an equal drop required 210 h. The specific respiration activity, referred to 1 mg cell proteins, was attenuated much more rapidly; in surface water cells it was reduced to 50% after cca 50 h, in distilled water after 60 h. Corresponding data for the decrease in specific dehydrogenase activity are 40 and 50 h, respectively. These results imply that (a) the sterile surface water has a more inhibitory effect on the microorganisms tested than has the distilled water, and...
(b) the attenuation of the metabolic functions of the cells in both media is more rapid than the decrease in reproducing ability.

Some Microbiological Aspects of Water. J. Pokorny, R. Adamek, Institute of Hygiene and Epidemiology, and Centre of Hygiene, Prague.

Microbiological considerations form one aspect of appraisal of water as life environment. The consumption of water by man, whether for personal use or in technological processes, increases steadily, with increased demands on its quality. The increased consumption is satisfied by treatment of the ever more contaminated surface waters; this water may mean a health hazard from the microbiological viewpoint. Microbiology is thus of a prime importance for control and evaluation as well as for diagnostic methods and quality tests. The main task with both drinking and surface water, is the detection of total microbial colonization and, in particular, of faecal contamination including intestinal pathogens, esp. those recently recognized as harmful. The attendant problems are the indicator value, survival of the microbes and their ecology. Microbiological testing is important also with recreation-used water, e.g. in swimming pools, where the questions of occurrence and significance of some microbes (streptococci, staphylococci, candida) and their resistance to chlorine have not yet been unambiguously solved. Microbiology has its say also in the problems of both streaming and accumulated surface waters and of the degradation of some foreign substances in water, e.g. pesticide residues. These aspects delineate both the present state and the perspectives in microbiology of water.

Physiological and Biochemical Activity of the Species Sporocytophaga cauliformis (Myxococcaceae) Isolated from Water. B. Trcilov, L. Mikošovičová, Institute of Limnology, Slovak Academy of Sciences, Bratislava.

In 1972–1973, a total of 135 strains of the species Sporocytophaga cauliformis were isolated from surface (Danube) and drinking (town water main I and II) water on the basis of their morphological and physiological properties. Out of 5 media tested, the best myxobacteria-retaining ability was exhibited by modified Moutonagar and the B/10 agar medium modified by us. Electron and light microscopy were used to study the developmental cycle of this species, beginning with the stage of vegetative cells without microcysts until the formation of the new generation of vegetative cells; the cycle was found to last 8 days. According to biochemical tests (Graf and Sturzenhofer, 1965) the species S. cauliformis was divided into 2 types. Type I ferment dextrose, with attendant acid formation of catalase: type II forms catalase but does not ferment dextrose (MTQ < 0.33  oligotrophic waters, MTQ > 0.33  eutrophic waters). The MTQ value of Danube water is 0.4–2.3, placing it among eutrophic waters. Although myxobacteria were originally taken to be cellulolytic bacteria, only 50% of the isolated strains were capable of utilizing cellulose as the only carbon source. Proteolytic activity was displayed by 45.4% of the strains, indicating their significance in the mineralization of organic water contaminants. In surface water, myxobacteria form 0.36–3.0% of total microbial count in 1 ml, in drinking water 0.05–0.2%. Out of the count of heterotrophic bacteria, they comprise 2.2–22.5% in surface water and 2.5–10.0% in drinking water.

Measurement of Various Characteristics of Bacterial Isolates from Surface Waters. P. Puncchak, Laboratory for Hydrobiology, Institute of Botany, Czechoslovak Academy of Sciences, Prague.

Bacterial strains isolated from the Slapy reservoir were transferred to a defined medium with a single energy substrate (Na citrate or glucose) and inorganic sources of N and P. The ability of the isolates to grow on minimal media and to maintain the steady state were tested in continuous cultures. Strains growing for a long time period, viz. 50 or more doubling times, were used to measure growth parameters $\mu_{max}$, $K_0$, Y. The experiments were carried out at constant temperature of 20°C. The cell biomass was determined by measuring organic nitrogen, $\mu_{max}$ was calculated from the changes in cell count of individual cultures. Also, in the strain 8-71-PU (Alcaligene faecalis, det. M. Kocour, CCM Brno) the measured parameters included its growth rate in the fermentor and the time interval necessary for attaining steady state after altering the dilution rate in the fermentor. The characterized strains will be used in laboratory experiments with multicomponent systems (in the presence of predators).

A Method for Detecting the LPP-1 Virus in Drinking Water. E. Stuchlíková, Research Office of Water Management, Prague.

The contemporary knowledge of antivirus disinfection processes is meagre because of the inadequacy of the methods for virus detection (as compared with conventional microbiological methods). The pathogenic nature of viruses prevents their routine determination in water works. The algal viruses LPP-1, LPP-2, SM-1, discovered by Safermann (1963), attack only some species of blue-green algae but are not pathogenic to man. Neither the algae nor the viruses are exacting as to cultivation conditions, we tested the virus as a model in water-works technology. The detection method used was that of Schneeweis and Stifter (1971), modified for our conditions. The water sample was converted into a saline and filtered through a membrane filter Sympor 8 with a medium pore size (0.23 μm). The viruses were thereby absorbed on the filter matrix and then retrieved by
Patient's treatment and counting (in PFU/1 ml) were done according to Jackson and Sládeček (1969): a nutrient agar was inoculated by 0.5 ml of the tested sample mixed with top agar and with the alga Plectonema boryanum. After solidification, the Petri dishes were turned upside down and cultivation was carried out at constant artificial light for 4 days.

Changes in Bacterial Populations and the Occurrence of Salmonellas in a Contaminated River Section. L. Mašinová, V. Bernatová, Institute of Hygiene and Epidemiology, Prague.

A significant part of the appraisal of hygienic water quality is the determination of its microbial colonization. Microorganisms take part in natural processes occurring in water and some of them may be potentially pathogenic for man. The populations of both saprophytic and some pathogenic bacteria were studied in a 15-km section of the river Ohře, contaminated with waste water from Cheb; the river is the source of drinking and service water and attracts increasing numbers of people for recreation. The direct drainage of untreated waste waters into the river degrades considerably the quality of its water and may, from the microbiological viewpoint, mean a source of infections. The results showed that the longitudinal profile of the curve of decreasing bacterial count, especially in coliform bacteria, has the same character as the deoxygenation line. The unfavourable state of the waste waters is also indicated by the presence of Salmonellas. During the two-year observations, the percentage of Salmonella-positive findings at the outlet of the waste waters and at the end of the experimental river section was 19% and 11%, respectively. The results were supplemented by a study of the occurrence of alimentary infections in the corresponding district.

The Diagnostics of Iron Bacteria. L. Švorcová, Balneological Research Institute, Mariánské Lázně.

Iron bacteria may be divided, according to the type of nutrition, to autotrophic, mixotrophic and heterotrophic, and according to morphological features into filamentous, rod-like and cocci. The diagnostics of the filamentous species, embodied in the order Chlamydiobacteriales, is less complicated owing to the considerable shape variability. On the other hand, the classification of individual genera and species of the family Siderovascales of the order Pseudomonadales is difficult because of the ambiguity of diagnoses of individual genera and, especially, species. A classification is therefore proposed, based on cell morphology, which would divide the genera into I. coccal forms, including genera (1) Siderovasca, (2) Sideroepheara, (3) Siderococcus; II. rodlike forms with a ring-shaped capsule, including genera (1) Naumanniella, (2) Ochrobium; III. rodlike forms with a capsule surrounding the whole cell, with genera (1) Sideronema, (2) Ferribacterium, (3) Sideromonas, (4) Siderobacter (Ferrobacillus).

Bacteriological Contamination of the Working Environment in City Sewage Plants. E. Valterová, Municipal Water Management, Brno.

In the process of waste water treatment bacteria are transferred into the atmosphere with water droplets and the sedimentation of the bacterial aerosol results in a contamination of the working environment. Quantitative determination of psychrophilic, mesophilic and coliform bacteria in the air of the plants was carried out using Aerosol. Replica microbiotests served to take samples for the determination of coli — aerogenes bacteria, fungi and enterococci. Samples were taken from the rack house, pumping station, aeration tanks, biofilters, workshops, laboratories and offices. To have a standard of the degree of air contamination, samples were also taken in a place with a heavy traffic in the centre, in laboratories of a drinking water processing plant and in a wood. The effect of UV-radiation on the bacterial air contamination was studied by a Microlux; the working place was irradiated for 15 min and samples were then taken from a distance of 1 m from the radiation source. The most sensitive towards the radiation were coliform bacteria and enterococci which were almost annihilated, more resistant are psychrophilic bacteria, mesophilic bacteria and fungi. The effect of the radiation was rapidly abolished due to proceeding transfer of bacteria from waste water into the atmosphere and stirring up of the sedimented bacterial dust. One of the best methods for decreasing the concentration of microorganisms in
the air is an effective ventilation which may reduce the number of bacteria by 50%. The experimental values of the bacterial contamination in individual places formed a basis for a proposed system of evaluation of the life environment with respect to bacteriology.

SECTION OF ENVIRONMENTAL MICROBIOLOGY

Nitrification by Heterotrophs. E. BERGEROVÁ, Department of Microbiology, Faculty of Sciences, Comenius University, Bratislava.

The existence of heterotrophic nitrifiers has long been denied, the results concerning heterotrophic nitrification being taken to be erroneous. Though the existence of heterotrophic nitrifiers — bacteria, actinomycetes and fungi — is no longer doubted, the biochemistry of the process is not yet clear. Heterotrophic microorganisms do not belong to a single taxonomic group; apart from fungi, oxidizing avidly NH₄⁺ to NO₂⁻ or NO₃⁻, bacteria and actinomycetes form also these substances, though to a lesser extent. The capability to oxidize NH₄⁺ seems to be possessed also by other, not yet identified bacteria and fungi. The ability of heterotrophic nitrification was tested in micromyceetes Aspergillus flavus Wehmer, Aspergillus flavus Link, Chaetomium globosum Kunze and Penicillium solium Westling. All of them oxidized NH₄⁺ if grown on a medium with reduced nitrogen forms. Various amino acids and ammonium sulphate served as nitrogen sources for the formation of hydroxylamine, nitrite and nitrate. The intensity of NO₂⁻ and NO₃⁻ formation depends on the species, substrate, and the C : N ratio, which is one of the limiting factors in the release of the NH₄⁺ ions and, consequently, one of the decisive factors in heterotrophic nitrification. The course of the process is also strongly dependent on the pH of the medium, the optimum being at pH 7—9. The intensity of NH₄⁺ oxidation is not directly proportional to growth intensity. Further experiments aimed at determining the effect of some metals on heterotrophic nitrification in the above micromyceetes and in the cell extract from Chaetomium globosum Kunze. The problem of the utilization of energy formed by the process under natural conditions has not yet been elucidated.

Mechanisms were studied which control the synthesis and activity of enzymes participating in the degradation and utilization of amino acids by soil microflora. Enzyme synthesis and activity were assessed from the rate of disappearance of glutamic acid present in a nitrogen-free mineral solution inoculated by a medium preincubated with glutamic acid or with glucose. Enzymes catabolizing glutamic acid were synthetized in the medium enriched by this acid, and the glutamic acid present in the mineral medium disappeared at a constant rate. In glucose-enriched medium, on the other hand, the activity of these enzymes was low; the glutamic acid present in the mineral medium was initially utilized at a low rate, which increased appreciably after 2—3 h. The results indicated that the glucose-enriched medium allowed only very low constitutive synthesis of appropriate enzymes or that these enzymes were repressed or inhibited by glucose. The increase in the glutamic acid degradation would then be due either to inductive enzyme synthesis or to derepression. Glucose was found to repress the synthesis of the enzymes catabolizing glutamic acid and to inhibit their activity. The activity of the enzymes was also inhibited in the presence of ammonium ions; this inhibitory effect of ammonium ions decreased in the presence of glucose, and vice versa. The results confirmed the existence of regulatory mechanisms and pointed at the significance of nutritional conditions in the synthesis and activity control of enzymes in mixed soil populations.

Biochemical Evaluation of the Effect of Fertilization on the Utilization of Nitrogen in Soil. B. NovÁK, Research Institutes for Crop Production, Prague-Ruzyně.

Two-culture crop rotating system (sugar beet, spring wheat) was systematically fertilized by both organic and mineral fertilizers. All fertilizing variants included also plots of black-soil fallow. The physiological utilisability of nitrogen was evaluated from the values of respirometric test, N : B, NG : G, and those obtained by cellulolytic test, CN : Cₓ. The value N : B indicates the actual shortage of utilisable nitrogen in the soil while the quotients NG : G and CN : Cₓ indicate its potential shortage. The physiological utilisability of nitrogen is enhanced by fertilizers, especially mineral. It decreases partially spontaneously with time, and partially due to the crop growth. The compost used prevented, to a considerable extent, the losses in nitrogen utilisability, particularly in fallow soil. The use of manure brought about conspicuous changes in the nitrogen utilization, especially in soil with growing crops.

Regulation of Enzymes Participating in Utilization of Glutamic Acid by Soil Microflora. J. MACURA, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Determination of Fluorescent Pseudomonas Strains in the Rhizosphere of Inoculated Plants. V. VÁSÍČKA, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The survival and multiplication was studied of various strains of Pseudomonas putida and P. phascolcola on the roots and in the rhizosphere of inoculated plants. Two methods were used. The first method is based on the ability of fluorescing Pseudomonas cells to utilize pipelicolic acid. Diluted suspensions of rhizosphere soil or root washes were
inoculated in to a medium containing mineral salts, glucose and 0.1% piceolic acid. After 14 days of cultivation at 27°C the level of piceolic acid in the medium was assayed. Its disappearance indicated the growth of microorganisms utilizing it. The results of the test were quantified by means of probability tables. The second method makes use of the antibiotic-resistance of Pseudomonas cells. A medium according to Simon, Kovács and Sands was used in which novobiocin was replaced by erythromycin. Both methods were used simultaneously in a number of pot experiments. The results, completely compatible, showed the changes in the number of the bacteria in the rhizosphere of inoculated plants. Strains of Pseudomonas putida are capable of surviving and multiplying in the rhizosphere of bean plants, wheat and cucumber. These strains affect favourably the growth, development and health condition of the plants. Supernatants of pure cultures of Pseudomonas putida cells were found to contain a number of auxins and gibberellins.

**Polysaccharides Exuded by Plant Roots. L. Kalačová, V. Vančura, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.**

Epidermal root surface is covered by a layer of viscous material, the so-called mucigel. It is formed even on the surface of sterile roots. We studied the formation of this polymer by wheat roots during the first four weeks of plant growth in Robinson medium under sterile and controlled conditions. The dynamics of the formation of polysaccharides exuded by plant roots is similar to that of excretion of other exudates. When referred to 1 plant/1 day, the values show a decrease in the second week of growth, followed by rise in the third week which continues probably till the flowering phase. During the first week the germinating plant has sufficient energy from seed storage substances and the seeds themselves exude also a certain amount of polysaccharides. In the second week the nutrition of the germinating plant is switched mainly to photosynthesis and the amount of exuded polysaccharides declines as compared with the first week. In the following weeks the amount of exuded polysaccharides increases, amounting to 30 mg/day/1 g dry root weight, or 7.5 mg/day/1 g dry wt. of whole wheat plants. Similar values were obtained also with maize. If the nutrient solution is not replaced, the formation of polysaccharides decreases. The composition of acid hydrolysates of polysaccharides was determined by paper and gas chromatography; wheat root polysaccharides contained uronic acid, galactose, glucose, arabinose, mannose and xylose. Maize root polysaccharides contained, in addition, fucose.

**Changes in Microbial Population of Bean Plant Rhizosphere after Colonization of the Root Surface by the Bacterium Xanthomonas fuscans. J. Lastix,** M. Staněk, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

In a preceding communication, the possibility was outlined to study the interaction of root plants with bacteria colonizing the rhizosphere and the factors limiting the occurrence of individual species on root surface, by means of the bacterium Xanthomonas fuscans. When the bacterium was artificially inoculated on bean seeds, and thus colonized the roots of germinating plants, the species P. fluorescens and some other species originally inhabiting the germinating seeds disappeared within the first month of vegetation. The concomitant decrease in the total bacterial count on the root surface was due to altered nutritional conditions in this phase and the transition from cotyledon nutrition (heterotrophic type) to leaf-and-root nutrition (autotrophic type). In the period of leaf formation the root-surface count of X. fuscans decreased but the total bacterial count was considerably higher. Physiological properties (e.g. nutritional requirements) of bacteria colonizing the roots after the disappearance of X. fuscans differed from those of the bacteria inhabiting the roots of control plants. The bacteria also decomposed polysaccharide produced in the rhizosphere by Xanthomonas cells. The rhizosphere microorganisms were thus shown to be able to utilize, apart from root exudates, also the metabolites of bacteria previously colonizing the surface of the roots.

**Effect of Foliar Application of Urea in Combination with Pesticides on Oxygen Consumption in Rhizosphere Soil. J. Vraný, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.**

Winter wheat was grown in soil in pots. In the phase of 2–3 proper leaves the above-ground parts were treated 4–5 times with 2% solution of urea, 0.4% Benlate (methyl-1-carbamoyl)-2-benzimidazole carbamate), 0.6% Aminex (4-chloro-2-methylphenoxycetic acid), or a combination of these compounds. Oxygen consumption was then measured in rhizosphere soils (RS) of control and treated plants 1–8 weeks after the last application. Approximately 0.2 g RS was suspended in 0.9% buffered NaCl and measured by conventional Warburg technique. The oxygen consumption in RS was 3–7 times higher than that in control soil without plants, taken from parallel pots. The respiration rate in RS of urea-treated plants was enhanced, the oxygen consumption during a 3-h period being higher by 50%. In RS of plants treated with Aminex or Benlate the oxygen consumption fell below that found in controls. When the two agents were applied together with urea, the O2 consumption was also lower than with untreated plants. With combination urea-Benlate, however, the O2 consumption was higher than with Benlate alone. The bacterial counts in the RS of both treated and untreated plants were in correlation with the experimentally assessed oxygen consumption by the
soil. An attempt was made to relate these results to the data on the effect of urea and pesticides on the microbial counts in the RS, with the aim to obtain a suitable criterion for evaluating the effect of pesticides on the microflora and the treated plant.

**Effect of Mineral Nitrogen and Some Rhizobium trifolii Strains on Nodulation and Symbiotic Fixation in Red Clover.** H. MAREČKOVÁ, Research Institutes of Crop Production, Prague-Ruzyně.

The effect of 7 strains of *R. trifolii* was tested at 3 levels of mineral nitrogen (14, 70, 140 p.p.m. N) in plants of red clover by greenhouse agar tests on a Jensen medium. The parameters tested were nodule formation, rate of nodule setting and plant dry weight. With the last parameter, a significant difference existed between the 70 and 140 p.p.m. N variants. Individual strains responded differently to the addition of KNO₃; the highest plant mass yield was in a symbiosis with the strain S 190. The dose of 14 p.p.m. N increased the yield as compared to the nitrogen-less variant, further increase in nitrogen level did not affect the yield. Concerning nodule formation, a significant difference was found between the nitrogen-less variant and those with up to 70 p.p.m. N. The dose 14 p.p.m. had almost no effect, doses exceeding 70 p.p.m. N inhibited the nodulation.

**Effect of Prolonged Herbicide Application on the Decomposition of Cellulose in the Rhizosphere of Continuous Maize Crop.** M. HUSÁROVÁ, Maize Research Institute, Trnava.

The decomposition of cellulose was studied in three variants of eleven-year-old culture after nine years of herbicide application: control without herbicides (B₁), classical treatment (B₂) and yearly treatment with a mixture of Zeazine and Selectine (2.5 kg/ha Oleogespamine. Chemically treated variant (B₃) was given 8 kg/ha Zeazine, lower since 1964 to 4 kg/ha. In 1969 the dose given to B₂ and B₃ was supplemented by 7 kg/ha Ramrod against *Echinochloa crus-galli* (L.) P. Beauv., and in 1970 by 7 kg/ha of the preparation Lasso. Fertilizing was identical in all variants. Cellulose decomposition was determined by the method of Pokorná and Kozová (1965). Prolonged application of herbicides suppressed the relative occurrence of cellulolytic microorganisms and thus also the basic cellulose decomposition. The potential decomposition of cellulose with the addition of nitrogen increased significantly during the prolonged herbicide application. The control without herbicides showed a higher basic decomposition and a lower potential decomposition of cellulose as compared with chemically treated variant. Under natural conditions, the cellulose decomposition is in a correlation with the amount of rainfall and with soil humidity.

**Effect of Allylisothiocyanate on Soil Microflora.**

V. DROBNÍKOVÁ, Z. PRÍHKRY, Agricultural Soil Research Institute and Faculty of Sciences, Charles University, Prague.

The effect of allylisothiocyanate (AITC), in the form of aqueous solution or vapour, on soil bacteria, actinomycetes and micromycetes was studied by Warburg method and by plating technique. The solution of AITC exhibited an inhibitory effect on soil microflora only at high concentrations (400 p.p.m.); this inhibition was short and was followed by a stimulation. The fungicide effects of the solution, which were observed also with lower concentrations (40 p.p.m.) were also transient. The inhibitory effects of AITC vapours, on the other hand, are more prominent, the length of inhibition being directly proportional to the length of exposure. After a certain exposure both the physiological activity and the count of bacteria increase, this stimulation being again directly proportional to the length of action of AITC vapours. 24-h exposure to AITC-saturated atmosphere resulted in a complete abolishment of the growth of micromycetes in soil sample. This inhibitory action was permanent. Even at lower partial pressures of the AITC vapour the fungicide effect was high. At a required degree of fungicide effect, the length of necessary exposure to AITC vapours increases with increasing soil layer. Fungicide properties of AITC vapour were found also with phytopathogenic micromycetes isolated from infected plants and soil.

**Effect of Fertilization by Straw and Pig Manure on Biochemical Processes in Soil.** J. POKORNÁ, Research Institute of Crop Production, Prague-Ruzyně.

The effect was studied of straw and pig manure on soil processes in a two-crop rotation system and in black-soil fallow. In comparison with both control variant and with variant fertilized by mineral NPK fertilizers, both the organic fertilizers enhance the respiration activity of soil and the counts of soil microorganisms. Specific mineralization intensity (referred to the number of microorganisms) decreases in the presence of easily utilisable organic substances (pig manure root exudates) and the assimilation and synthetic ability of the microbes increases. The straw brings about an immobilization of soil nitrogen and a drop in its actual and potential utilizability, especially in cultivated plots. It prevents, to a great extent, the losses of nitrogen from the fallow soil. Pig manure accelerates the dynamics of soil nitrogen turnover; its nitrogen is physiologically more utilisable than the nitrogen of mineral fertilizers.
In two plots in highland meadows and one in a lowland meadow in southern Slovakia, the intensity of ammonization and nitrification was studied. The soils of the meadow phytocoenoses in question were characterized by a low level of mineral nitrogen forms. The proportion of nitrate nitrogen was lower than that of ammonium nitrogen. On applying a maximum dose of nitrogen (480 kg N/ha) it was accumulated predominantly as NO$_3^-$, especially in the period of decreased biological sorption, which indicated low utilization of this form by the herbage and soil microflora, and a better utilization of NH$_4^+$. After the application of nitrogen fertilizers considerable proportion of N became immobilized by soil microflora. The mineralization of organic nitrogenous substances was significantly affected by soil and climatic factors, developmental stage of the herbage and the fertilization with maximum doses. In plots with acid soil reaction the proportion of nitrified nitrogen was low and generally directly proportional to the productivity of the plots. At high levels of superficially applied nitrogen the soil reaction changed, which resulted in heavy losses by denitrification and volatilization. The results show that the dose 480 kg N/ha and, in arid years, even 240 kg N/ha are unsuitable. In view of the heavy losses of nitrogen by way of microorganisms, the fertilizers used for meadow coenoses should be supplemented by compounds inhibiting the nitrification and retarding the ammonization of urea.

Changes in Optical Density of Humus Acids during Microbial Decomposition. A. Dubovská, M. Mačor, Department of Microbiology, Faculty of Sciences, Comenius University, Bratislava.

The utilization of carbon and nitrogen from the fulvoacid (FK$_2$) and humine acid (HK$_2$), isolated from podzolic soil according to Roebus, by soil micromycetes was studied. The cultures included Aspergillus versicolor, A. glauea, Penicillium citrinum and P. lilacinum, all of them being previously found to utilize carbon and nitrogen uniformly from all fractions of fulvoacid. C and N utilization was determined from increased mycelium dry weight; the level of carbon and the optical density were measured also in repreeipitated fractions of humus acids. The comparison of these data with the original ones gives a fairly accurate measure of the degree of decomposition of the acids and of carbon utilization. The decomposition of the above fractions of humus acids was measured after 1 and 2 months of cultivation. The increment of mycelium dry weight was found to vary with the variant used (humus acid, C or N source) and species of micromycetes, no correlation being revealed between the increment and the loss of carbon from the humic acid fractions used. Total amount of FK$_2$ decreased, during the cultivation, by 54% at most, the maximum loss of HK$_2$ being 64%. The loss of C from FK$_2$ after 30 and 60 days of cultivation was 10–45%, similar data for HK$_2$ were 12–38% depending on micromycetes species. The utilization of humus acids by soil micromycetes may also be characterized by decrease in the optical density of appropriate fractions.

Microflora of Various Systems. Release and Incorporation of Mineral Nutrients during the Decomposition of Plant Material in the Reedy Pond Nesyt. B. Ulehlova, B. Vašúková, Institute of Botany, Czechoslovak Academy of Sciences, Brno.

The levels of mineral nutrients and microbial colonization of a number of materials were studied at 1-month intervals in a cross-section of typical coastal vegetation zones of the pond Nesyt in southern Moravia in 1972. In the terrestrial ecophase standing dead parts of main macrophytes, plant material strewn on the ground and semi-decomposed material mixed with soil were analysed. The hydrophase gave data on standing dead parts of main macrophytes, floating decomposing material, material lying on the bottom, and sapropel. All materials studied differed considerably in both chemical composition and microbial colonization. The terrestrial ecophase was characterized by mineralization of plant material, enrichment by mineral substances, especially Ca; K and P were readily released during the decomposition. The decomposing plant material exhibited a gradually increasing level of N, providing apparently conditions favourable to its fixation. In the hydrophase, the predominant process was humification, the level of C remaining high even in sapropel. Some accumulation of mineral materials was also observed. The level of N and P of floating material increased.

Decomposition of Cellulose in Soil and the Type of Phytocoenosis. M. Tesářová, Institute of Botany, Czechoslovak Academy of Sciences, Brno.

The rate of cellulose decomposition (RCD) was studied for several years in three different phytocoenoses; a tight relation was found between the RCD and the type of phytocoenosis. This may be derived from the following findings: (1) The study of the dependence of RCD on time and phytocoenosis type as the sources of its variability in soil showed that the primary source of variability is the type of phytocoenosis; (2) A tight correlation was found between the RCD in soil and the increasing production of plant biomass; (3) From the three soil profiles tested, 0–5 cm, 5–15 cm, and 15–25 cm the highest RCD was found in the depth of 5–15 cm, i.e. in the place of accumulation of plant root mass. The differences between RCD values obtained at corresponding soil profile depths with individual phytocoenoses were statistically evaluated; the coenoses were found to differ in the values corresponding to the depth of 5–15 cm; (4) The soil of phytocoenoses with broader spectrum of plant species gave a more heterogeneous cellulolytic microflora.
strikingly, probably owing to colonization by per-
iphyton. The microbial populations in standing,
decomposing and finally degraded materials dif-
fered both qualitatively and quantitatively. The
most abundant ones were in decomposing materials,
the poorest in the standing ones, with higher pro-
portion of micromycetes.

Comparison of the Effect of Bentonite and Kaolinite
on the Mineralization of Various Concentrations of
Glucose. J. Nováková, College of Agriculture,
Prague.

Sand containing a mineral nutrient solution, 3% bentonite or kaolinite and inoculated by soil micro-
flora was supplemented with 0; 0.1; 0.2; 0.5 or
1.0% glucose. The production of CO₂ during the
incubation was determined. Both clays suppressed
the mineralization of glucose at all concentrations,
kaolinite being more effective. The oxidation at the
beginning of incubation was enhanced by bentonite
and suppressed by kaolinite. With rising glucose
concentration the number of secondary respiration
peaks increased; these peaks were entirely elimin-
ated by the addition of bentonite. The smoothing-
out of the respiration curves by kaolinite was less
pronounced than with bentonite. These phenomena
are explained on the basis of a different adsorption
of the primary substrate and its metabolites by
bentonite and by kaolinite.

Effect of Clay Minerals on the Germination of Spores
of Some Microscopic Soil Fungi. Z. Filip, Depart-
ment of Microbiology, College of Agriculture,
Prague.

The droplet method was used to determine the
effect of clay minerals on spore germination and
sprout length of some microscopic soil fungi of the
class Deuteromycetes. Both germination percentage
and sprout length were affected particularly by
minerals of the montmorillonite type, the effect being
positive or negative depending on the fungal spe-
cies. For instance, the presence of montmorillonite
(Wyoming) caused an increase in the length of
sprouts of the fungus Stachybotrys atra by 25% and
increased the germination capacity by 6% as
compared to control while with Mucor hiemalis
and Fusarium oxysporum the sprout germination
capacity dropped by 20%, the length of sprouts
being only 0.2 that of the control. The results in-
dicate that the lower occurrence of some microscopic
fungi, e.g. Fusarium sp., known from the literature,
may be due not only to the effect of clays on soil
environment, but also to a direct fungistatic effect
of some clay minerals. The mechanism of this
effect deserved a further study.

SECTION OF IMMUNOLOGY

The Preparation and Characterization of Anti-
cathepsin Serum. J. Štefanović, M. Ferencík,
J. Belaš, Institute of Medical Microbiology and
Immunology, Medical Faculty, Comenius Univer-
sity, Bratislava.

Intracellular proteases (cathepsins) participate in
the destruction of phagocytosed material, and their
relationship to immunogenicity of antigens is
thought. As besides pepstatin, no other suitable
inhibitors of these enzymes are known, we prepared
antisemum against chicken and rabbit cathepsin D.
Antisemum to chicken cathepsin D was prepared by
a repeated immunization of rabbits with purified
cathepsin D isolated from chicken spleens, whereas
serum to rabbit cathepsin was prepared by im-
munization of sheep with highly purified prep-
paration of cathepsin D from rabbit spleens. The
activity of antisemum was determined on the basis
of inhibition of cathepsin degradation of bovine haem-
oglobin at pH 5.0 using immunoelectrophoresis
with subsequent detection of cathepsin D activity
using bovine haemoglobin (pH 3.0) as a substrate.
All antisemum possessed a significant inhibitory effect
on the activity of cathepsin D. The most active
serum inhibited completely the activity of 1 unit
of chicken cathepsin in the amount of 8 μl. One
unit of rabbit cathepsin D was fully inhibited with
approximately 15 μl of specific antisemum. The
fractionation of antisemum on a DEAE cellulose
column revealed that the anti-cathepsin D activity
was localised in the IgG fraction of the serum.

The Distribution of Bactericidal Substances and
Lysozomal Enzymes within Subcellular Fractions of
Rabbit and Chicken Phagocytes. M. Ferencík,
O. Absolonová, J. Štefanović, Institute of Medi-
cal Microbiology and Immunology, Medical Faculty,
Comenius University, Bratislava.

Rabbit and chicken PMN leucocytes were hom-
gonized in 0.34M saccharose. The homogenate
was submitted to differential centrifugation at 150,
800, 10,000 and 50,000 g. In the fractions, both
the overall bactericidal activity and activity of
myeloperoxidase (EC 1.11.17.), catalase (EC
1.11.1.6.), lysozyme (EC 3.2.1.17.), cathepsin D
(EC 3.4.4.23.) and E, beta glucuronidase (EC
3.2.1.31.) and acid phosphatase (EC 3.1.3.2.) were
determined. Antibacterial activity was found in
all fractions from rabbit leucocytes with the ex-
ception of the last one (above 50,000 g), whereas
in chicken leucocytes, the activity was localised in
the first fraction (150 g) only. The content of above
mentioned enzymes in individual fractions of rabbit
and chicken leucocytes was also different. Contrary
to rabbit leucocytes, in chicken peritoneal leuko-
cytes, myeloperoxidase, catalase and cathepsin E
could not be detected. However, despite the ab-
sence of the myeloperoxidase system, which is one
of main intracellular microbicidal mechanisms in
mammals, the overall antibacterial activity of chicken
leucocytes was comparable to that found in rabbit
leucocytes. These findings seem to suggest the
presence of more antibacterial mechanisms in
phagocytes and differences among animal species,
the variances being not necessarily accompanied by different final antibacterial activity.

The Effect of Hydrocortisone on the Plaque-forming Activity of Peripheral AT-Lymphocytes. A. Sokol, M. Kunay, M. Kandrác, M. Novák, School of Microbiology and Zoohygiene, Veterinary Faculty, Department of Microbiology, Faculty Hospital and the 1nd Clinic of Internal Diseases, Medical Faculty, Safrák University, Košice.

Administration of hydrocortisone elicited in experimental animals decrease of metabolic activity or even degeneration and lysis of thymocytes and proportion of lymphocytes. This phenomenon is caused by the inhibitory effect of glucocorticoids on synthesis of RNA, DNA and proteins, particularly on the RNA- and DNA-polymerase, as well as on membrane transport proteins. Lymphocytes, withdrawn from the organism in activated state (ATL), after incubation in the microchamber attached to glass and released a soluble factor which pushed off erythrocytes; thus a cell-free space—a plaque—was generated. The number of activated, plaque-forming lymphocytes was studied in guinea pigs (a) after a single injection; (b) after five intraperitoneal injections of hydrocortisone for four and/or five days. The blood differential count and cortisol level were determined quantitatively. Thymus and peripheral lymph nodes of control and experimental animals were examined histologically. After a single hydrocortisone administration, no change in the number of plaque-forming active lymphocytes was recorded in the blood with high level of cortisol; however, the number temporarily slightly increased following the decrease of the cortisol level. After the five-fold hydrocortisone administration, the number of plaque-forming active cells did not change again during the period of high level of cortisol, however, it increased abruptly following the decrease of the cortisol level. Based on the contemporary knowledge on the biphasic response of the organism to glucocorticoids, as well as on the results of our present study we may assume that the adherence, release of soluble factors (activators) into the microenvironment and the pushing off of erythrocytes, i.e. the plaque-forming capacity, do require not only the activated state of the given AT lymphocyte, but also appropriate conditions for realization of directed metabolic events (synthesis of RNA, DNA and membrane transport proteins). The function and cooperation of A-, T-, and B-cells is discussed.

The Influence of DNase, RNase and Trypsin on the Plaque-forming Capacity of Peripheral Lymphocytes. M. Kunay, A. Sokol, Department of Microbiology, Faculty Hospital, School of Microbiology and Zoohygiene, Veterinary Faculty, Košice.

An increased number of peripheral blood lymphocytes, withdrawn from patients 24 h after surgery, possessed the plaque-forming capacity in micro-chamber in vitro. The plaque-forming activity of lymphocytes was influenced by various concentrations of DN-ase, RN-ase and trypsin, added to buffered physiological saline used for dilutions of blood samples. DN-ase at concentration of 0.1 mg (18 U. Kunitz) and higher per 1 ml of diluted blood, inhibited completely the formation of plaques, whereas at concentrations of 0.1—0.02 mg/ml yielded a partial inhibition of the plaque-forming mass. On the other hand, RN-ase could not inhibit the plaque-formation at concentrations 0.5 mg (20 U. Kunitz) and higher per 1 ml of diluted blood. Trypsin (1:150) at concentrations above 15 mg/ml of diluted blood inhibited completely the plaque formation, whereas at concentrations 0.5—10 mg/ml inhibited partially not only the formation of the plaque-forming mass but decreased the number of plaque-forming active cells at the same time. We therefore assume that only DN-ase exerts a specific effect on the production of the plaque-forming mass in activated lymphocytes.

The Development and Degradation of Rough Endoplasmic Reticulum (rer) System in Maturing Antibody-forming Plasma Cells. L. Hájdu, J. Honigman, M. Holub, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague and Institute of Clinical Experimental Medicine, Prague.

In the course of the local primary immune response to intraperitoneally administered horse radish peroxidase together with endotoxin, several types of arrangement of rer, containing specific antiperoxidase antibodies in the channels, were found in omentum of mice: on the 3rd day after immunizations (1) short, narrow irregular tubules; (2) irregular convolute of narrow tubules; (3) "whirls" of concentric narrow tubules. On the 5th and 7th day after immunization, majority of productive plasma cells contained already rer system, which was arranged typically in dense parallel lameral tubules, filling up almost entirely the protoplasm. In addition, certain mature plasma cells contained anti-peroxidase antibodies in spherical dilated cysternae of rer, in which condensation or even crystallization of antibodies took place. On the basis of our findings we assume that the arrangement of the rer system within the plasma cells develops from short disarranged narrow tubules found in blast elements up to dense parallel lamellae of rer in mature plasma cells or even up to the stadium of large lacunar cysternae. The occurrence of sometimes even bizarre forms of the rer system could be attributed to the specific action of antigens used, and we consider them transitional and fully functional proteosynthetic structures which could be "reorganized" into an arrangement, typical for mature plasma cells. The discontinuation of the limiting membrane unit of rer during gradual crystallization of antibodies within the
cysternae can be considered as one of possible modes of degradation of the rer system.

The Morphology and Enzymatic Activity of Peritoneal Exudate Cells of Non-specifically Stimulated Mice. L. Jarosková, I. Hajdý, M. Holter, I. Terechavský, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague and Institute of Clinical Experimental Medicine, Prague.

The effect of intraperitoneal administration of protease peptone on the composition of peritoneal exudate of CBA mice was studied using light and electron microscopy. The enzymatic activity of cells was tested by histochemical and biochemical assays on acid phosphatase. As compared with control animals, an increase in the number of mono-histiocytic forms from 50% to 80% was found three days after peptone injection. Besides quantitative changes, an activation of cells of the macrophage type was detected: faster and firmer adherence to glass and ultrastructural changes that are characteristic for activated macrophages, particularly the enhanced content of lysosomes in correlation to increased level of acid phosphatase. The increased activity of peritoneal cells—adherence and enzymatic activity—was also found in mice following an intraperitoneal administration of Bordetella pertussis vaccine. The morphological heterogeneity of cells of the peritoneal exudate after activation with substances which influence the cells of the macrophage series is discussed as regards their origin, differentiation and functions performed by these cells.

The in vivo Opsonizing Activity of Fab µ, and F(ε)5 µ fragments of Pig Immunoglobulin M to Escherichia coli O 55 in Newborn, Precolostral Piglets. J. Zíkán, I. Míler, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague and Research Institute for the Care of Mother and Child, Prague.

Opsonizing activity detected by intravascular clearance of bacteria in newborn precolostral piglets, and bactericidal effect to Escherichia coli O 55 of immune pig IgM, its peptic Fab µ, and F(ε)5 µ fragments and tryptic Fab µ fragment were studied. Total reduction and carboxymethylation of disulfide bonds of the immune IgM molecule completely abolished its in vivo opsonizing activity, suggesting thus the importance of a tertiary structure stabilized by disulfide bonds. Tryptic and peptic Fab µ fragments differed significantly in biological activities. Unlike the tryptic Fab µ fragment, the 4,200 dalton larger peptic Fab µ fragment possessed both opsonizing and bactericidal activity. On the other hand, the peptic F(ε)5 µ fragment opsonized bacteria without any bactericidal effect. The possible participation of complement as well as the nature of nonspecific binding of the peptic F(ε)5 µ fragment to bacteria is discussed.

The Antibody Response and Immunoglobulin Synthesis in Germfree Piglets after Monocontamination and Peroral Vaccination. V. Dišabač, J. Hofman, J. Černá, J. Klepaloňová, Department of Immunology, Laboratory of Gnotobiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The antibody response and immunoglobulin synthesis was studied in piglets, reared under germfree conditions after monocontamination with gram-negative intestinal bacteria and after a long-term peroral administration of massive doses of killed bacteria. The antibody formation and immunoglobulin synthesis was significantly higher in monocontaminated piglets in comparison with the vaccinated group. These results are compatible with the finding that non-pathogenic gramnegative bacteria penetrate in high numbers into the regional lymph nodes which results in much higher antigenic stimulation.

The Protective Effect of Antibodies to Escherichia coli in Newborn Germfree piglets. I. Míler, J. Černá, J. Trávníček, J. Reinček, Research Institute for the Care of Mother and Child, Prague and Department of Immunology, Laboratory of Gnotobiology, Czechoslovak Academy of Sciences, Prague.

Using the germfree piglet model, the effect of the immune colostrum, serum and immunoglobulins IgA, IgM and IgG on the antibacterial and antienterotoxigenic local resistance of the small intestine to enteropathogenic Escherichia coli O 55 was studied. The in vivo assay, ligated intestinal loop of piglets, was employed for determination of enterotoxigenic activity and penetration of bacteria through the intestinal wall. The results demonstrated the protective effect of all three immunoglobulin classes used, the IgA being the most effective.

Comparison of the Adjuvant Effect of Lipopolysaccharide with its Toxicity and Chemical Structure. J. Hofman, B. Ríhová, V. Dišabač, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

Lipopolysaccharide of Salmonella typhimurium LT2 increases significantly both the primary and secondary antibody response if administered together with a low molecular hapten bound to a protein carrier ARS-BGG into inbred mice which do not respond to the antigen alone. The enhancement involves both 7S and 19S antibodies. Mild alkaline hydrolysis, which splits esterically and amicodically bound fatty acids in the lipidic portion, lowers the toxicity and adjuvant capacity of lipopolysaccharide. Therefore it seems that there is certain relationship between the adjuvant effect and certain other biological activities of the lipopolysaccharide, which are bound to the lipid portion of the molecule.
Purification of Interferon. N. Fuchsberger, V. Hajnica, L. Borecký, Institute of Virology, Slovak Academy of Sciences, Bratislava.

The disadvantage of purification procedures of interferon published so far is that in the process of purification great losses of interferon activity take place. We therefore modified the purification procedure in order to obtain a sufficient amount of highly purified material for its further testing. We concentrated on a fast and effective concentration of the preparation obtained from mouse L-cells, and, furthermore, on the possible shortening of a time period, at which the material is exposed to possible inactivation. The procedure includes formation of interferon in a minimal amount of serum-free medium, precipitation with zinc acetate, dialysis, centrifugation in a ionexchanger, pressure dialysis and electrophoresis in polyacrylamide gel. Using this procedure, no addition of stabilizing protein to the interferon preparation was needed. Thus we obtained in one purification 2 x 10^8 units of highly purified mouse interferon with a specific activity of > 10^8 units/mg of protein, which permits further testing of its properties.

Studies on Hyporeactivity of Mouse Peritoneal Cells to the Interferon-inducing Capacity of Lipopolysaccharide from Escherichia coli 0111:B4. V. Lackovic, L. Borecký, Institute of Virology, Slovak Academy of Sciences, Bratislava.

Using the model of peritoneal cells, the authors studied the onset of hyporeactivity after intravenous, subcutaneous and oral administration of lipopolysaccharide. We took advantage of the fact that cells of the peritoneal cavity play a central role in the production of interferon in the organism after the endotoxin stimulus, and that the stimulation of interferon formation occurs shortly after the intravenous, subcutaneous or oral administration. Liberation of interferon or an attempt to restimulate peritoneal cells was performed after their explanation in vitro. We found that: (1) Peritoneal cells became hyporeactive already within 30-60 min after intravenous administration of lipopolysaccharide, i.e. at time, when the serum interferon level has not reached the maximum; (2) after the subcutaneous administration of lipopolysaccharides, the hyporeactivity stadium was preceded by a phase of enhanced reactivity (30 to 60 min after administration); (3) oral administration of 1,000 μg of lipopolysaccharide stimulated the formation of interferon in peritoneal cells, however, did not induce the hyporeactivity state. These results support the author's view, that interferon released in the organism of mice by endotoxin, is originated from presensitised peritoneal leukocytes.

The Character of Mitogenic Activity of Scarlet Fever Toxin. V. Hářkalová, M. Pospišil, Institute of Hygiene and Epidemiology, Prague and Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The scarlet fever (erythrogenic toxin – ET) elicits blast transformation of lymphocytes in vitro. This reaction was found in lymphocytes of all animal species tested, i.e. rabbit, mouse, man and it possessed characteristics of non-specific stimulation, comparable to the response of lymphocytes to PHA. The mitogenic effect of ET, depending on the dose used, can be inhibited specifically in vivo by an induced pyrogenic tolerance to a given ET type; cross tolerance was used as an evidence that the carrier of mitogenic activity as virtually the pyrogenic exotoxin. The significance of mitogenic activity of ET for the pathogenicity of streptococcal infections is discussed.

Typhoid γG, γM and γA Antibodies in Men With Various History of Typhoid Fever Injection. G. Fabryková, M. Draškovičová, I. Čičář, J. Karolček, Research Institute of Epidemiology and Microbiology, Bratislava.

Antibodies were detected on the basis of decrease in immunoglobulin IgG, IgM and IgA levels after absorption with purified O, Vi and H antigens. The method according to Nash and Heremans was employed. The immunoglobulin levels were determined using the IDF Sevac kit. The results showed a significant decrease of IgA in sera of typhoid carriers and of IgM in sera of typhoid patients and reconvalescents. The amount of Absorbed IgA antibodies from sera of carriers was 1.5 times higher as compared with individuals with positive typhoid anamnesis but without a carrier state. In sera of typhoid patients and reconvalescents, the IgM antibodies level was 1.5 times higher than in sera of carriers. No major changes were observed in the IgG levels. These results were confronted with the specific bactericidal activity of corresponding sera.

Modification of Antigenic Determinants of Polysaccharides of Non-Cladhera (NAG) Vibrios. I. Čičář, G. Fabryková, J. Karolček, Research Institute of Epidemiology and Microbiology, Bratislava.

Lipopolysaccharide antigens of four strains of NAG vibrios isolated from patients suffering from diarrhoea, and of two strains from surface water were analysed. The purified antigens formed with homologous rabbit antisera 1-3 precipitin lines. The immunoelectrophoretic patterns of these antigens differed mutually and also differed from patterns which are typical for lipopolysaccharides of S-forms of Escherichia coli and Salmonella. The mild alcali hydrolysis of purified lipopolysaccharides altered their antigenic properties. According to altered reactions in gel, the lipopolysaccharides could be divided into three types: (a) antigens, in which determinant groups were inactivated by the mild alcali hydrolysis and therefore did not react with homologous antisera; (b) antigens, in which
only certain determinant groups were inactivated and therefore yielded semidentical reactions with homologous antisera or formed new precipitin lines; (c) antigens, gaining new determinants after mild alcali hydrolysis which react with homologous antisera. The mild alcali hydrolysis modified such antigenic determinants, in which the structure was based on O-acetyl group and monosaccharides, bound by a alcali-labile bond.

**Endotoxic Lipopolysaccharide of Coxiella burnetii.** S. Schramek, R. Březina, Institute of Virology, Slovak Academy of Sciences, Bratislava.

The causative agent of Q-fever, Coxiella burnetii, is a gram-labile rickettsia. Using the methods for isolation of lipopolysaccharide of gram-negative bacteria, we isolated from purified suspensions of phase I a soluble antigen, possessing the serologic activity of antigen of phase I and immunogenic activity of antigens of phase II and III. The chemical analysis of isolated antigens showed that these are lipopolysaccharide-protein complexes with various proportion of these components, depending on the isolation method used. The method of isolation (extraction with trichloroacetic acid or phenol), purification, chemical composition, physico-chemical properties (thermostability, sedimentation) and biological properties (toxicity, pyrogenicity) showed that isolated soluble antigen of Coxiella burnetii of phase I has typical properties of endotoxic lipopolysaccharide from a highly purified Virology, Slovak Academy of Sciences, Bratislava.

**Chemovaccine Against Q-Fever.** R. Březina, S. Schramek, J. Kazár, J. Urvölgyi, Institute of Virology, Slovak Academy of Sciences, Bratislava.

The protective antigen, obtained by extraction with trichloroacetic acid from a highly purified suspension of phase I Coxiella burnetii, represents a new type of vaccine against Q-fever in men. The immunogenicity of the vaccine was tested in laboratory animals and in group of 46 laboratory workers, potentially exposed to infection during laboratory work. 23 individuals were vaccinated with a single dose, another 23 people received 2 doses of the vaccine. The serological conversion was found in 23 persons with a single vaccination and in 21 with double vaccination. Antibodies against phase I (alone or together with antibodies to phase II) were formed in 16 persons belonging to the first group; antibodies to phase II only were found in 11 individuals. After two doses of the vaccine, phase I antibodies (alone or together with phase II antibodies) were found in 19 vaccinated and in 2 persons antibodies only to phase II occurred. No one of vaccinated persons, exposed daily to infection during work, contracted Q-fever.

**Determination of Cellular Hypersensitivity to Listeria monocytogenes.** I. Spratová, C. John, M. Mára, F. Patočka, Institute of Medical Microbiology and Immunology, Faculty of Medicine, Charles University, Prague.

Listeria complex Ei was used for sensitization of experimental animals and for liberation of mediators of cell-mediated hypersensitivity. For evaluation of the cell-mediated hypersensitivity and immunity, method of a direct inhibition of cells of the macrophage type was employed. Groups of rabbits were sensitised with factor Ei in incomplete Freund' adjuvant (20 mg in divided doses into fore and hind foot pads). Spleens were removed 4–5 weeks after immunization. Complex Ei was added to the tissue culture medium in various amounts. The lymphocytes, present in the spleen fragments, release under the influence of antigen a number of activities; the factor inhibiting macrophage migration was chosen for our study. The degree of inhibition of migration was determined by the migration index. After addition of 100 μg of Ei/1 ml of medium, the values of inhibition index were found to be 0.67, 0.64, 0.67 and after 1 μg of Ei/1 ml of medium, the values were: 0.80, 0.83, and 0.85. The statistical evaluation of the results revealed that values under 0.9 represent inhibition of migration. Similar results were obtained in experiments with rabbits, sensitised with Ei complex in complete Freund's adjuvant. The effect of the Listeria factor Ei can be demonstrated even in the indirect migration test. At present, the activity of the medium is being tested, which contains MIF produced by peripheral blood lymphocytes, influenced by Ei.

**Haemolytic Streptococci of a Newly Serological Group.** J. Jelínková, V. Kubín, School of Medical Microbiology Postgraduate Medical and Pharmaceutical Institute and The Reference Laboratory for Streptococi, Institute of Hygiene and Epidemiology, Prague, Laboratory of Otorhinolaryngology, Czechoslovak Academy of Sciences, Prague.

During bacteriological examination of material from pigs from slaughter houses, we isolated from swollen and highly oedematous submandibular lymphnodes strains of beta haemolytic streptococi of serologic groups C, L, S, U, and further haemolytic strains, which could not be classified to any of so far known serologic groups A – U. Antisera against isolated strains contained antibodies which were active in the homologous system, different from groups A – U. As regards certain biological properties of these strains (particularly the CAM P test), and with respect to their origin, it is necessary to differentiate them from groups B, E, U, E and Str. uberis. Alltogether we examined 41 biological and fermentative properties. For differential diagnosis, following combination of properties is important: splitting of Ne hippurate (–), splitting of ascuclin (–), growth in 4% NaCl (+). On the basis of immunological antigenic studies we assume that the isolated strains belong into a new,
so far not described serological group of haemolytic streptococci. We therefore propose to designate this group with the letter "T".

Some Properties of Enterococci of Human Origin. Z. MATYÁŠ, District Hygiene Centre, Lušenec.

The results of studies of 256 strains of enterococci isolated within a period of one year from clinical material in the District hygiene Centre-Lušenec, permit to draw following conclusions: For isolation and identification of enterococci from clinical material, the use of blood agar (pH 9.6 with 6.5% NaCl, supplemented with 40% bile), appears to be the most advantageous. For detection of varieties of Str. zymogenes and Str. durans, human or rabbit blood is required (not sheep), temperature 37°C. In the stools of healthy individuals, Str. bovis was found most frequently (40%); in patients suffering from dyspepsia, Str. faecium (52%), and in 19% of cases Str. zymogenes was found, the majority of strains being non-proteolytic. Str. faecalis was found in both groups in about 20%. From infectious foci, localised outside the digestive tract, Str. faecalis was isolated most frequently (48%), then Str. liquefaciens (23%) and Str. zymogenes (19%). A significantly higher proportion of proteolytic and haemolytic enterococci in this group suggests their important role in the pathogenesis of the infectious process. The determination of sensitivity of enterococci to five basic antibiotics revealed two types of dependence: according to the species and according to the origin of the strain. The most sensitive strain was Str. bovis; in Str. zymogenes and Str. liquefaciens, high differences in sensitivity to individual antibiotics were observed. On the contrary, lowest differences were found in Str. faecium; most of all multiresistant strains belonged to this strain. Most sensitive strains were found in the stools of healthy individuals, most resistant strains were isolated from infectious processes outside the digestive tract.

Contribution to the Type Determination of Germs of Genus Bacillus. M. Bašták, Central State Veterinary Institute, Prague.

More accurate classification of germs of genus Bacillus can be done using agglutination (droplet method or in test tubes) or precipitation; sparse antigens and antisera to these antigens are used in the first place. Sera can be relatively easily produced and absorbed. For serological determinations of strains, mostly the methods according to Norris and Wolf (1961) were employed in our experiments. In addition, the gross appearance of colonies (morphology), microscopy of sporangium and spores and certain physiological and biochemical properties were evaluated. The determination key of Smith et al. (1952), modified by Wolf and Barker (1968) was used for evaluation of these properties. The number of false determinations which were done on the basis of biochemical, physiological and morphological criteria as compared to serological examination, comprised one fourth of all complex determinations of strains (altogether 84).

Quantitative Studies on the Influence of the Banana Pulp on the Bacterial Associations in vivo. H. Puzová, L. Dubay, E. Košíková, V. Janíková, D. Kahaneck, School of Microbiology, Faculty of Medicine, Šafář University, Košice and County Children's Hospital, Košice.

In studies on the effect of banana in meat-peptone agar on Escherichia coli and Proteus, various effects on monocultures and on association were observed in the correlation to the concentration used. A 5% dried banana mass in MPA suppressed the growth of bacteria in monocultures. In association, the growth of Proteus was significantly inhibited, whereas Escherichia coli exhibited normal growth. The described effect of banana was also studied quantitatively. If bacteria were transferred from a 8 h broth culture containing 5% of dry banana mass on MPA agar plates, no typical Proteus colonies were observed: on the other hand, the number of colonies of Escherichia coli corresponded to that obtained in broth culture without banana. After a 24 h cultivation in broth, no growth of Proteus was observed, whereas the number of Escherichia coli colonies exceeded significantly the number of colonies cultivated in medium without banana. In studies on the effect of banana diet in infants with diarrhoeal disease, similar results were recorded, i.e. disappearance of Proteus, increase in the number of Escherichia coli colonies and normalization of the stools.

Acute Respiratory Morbidity in Apprentices. I. Hána, P. Šervadák, Postgraduate Medical and Pharmaceutical Institute, Prague.

Morbidity rates for acute respiratory diseases (ARD) were analysed in seven different Railway Apprentice Centers in Czechoslovakia. The study lasted for 3 school years and covered 9,400 apprentices mostly aged 15 to 17 being based on doctors' diagnoses. The apprentices were divided into boarders and nonboarders. It was shown that ARD is the main cause of absenteeism from work and school in adolescents: it accounted for 42.5% of the lost days, whereas injuries contributed 17.1% and all the remaining diagnoses together 40.4%.

The attack rate in apprentices was more than double of that recorded in the Apprentice Centres' personnel. The ratio between acute tonsillitis and/or pharyngitis (ATPH) on the one hand and any other form of ARD on the other was approximately 1 : 2. Each year boarders displayed a higher ATPH attack rate than expected; life in dormitories seems to favour the incidence of ATPH as compared with other forms of ARD. It is highly probable that under these conditions the spread of beta-haemolytic streptococci is enhanced more than that of viral agents.
Types of Shigella sonnei Detected in Five Districts of East Bohemia and their Changes During Epidemics. V. Horaček, E. Aldová, I. Dvořáček.

Within a period of approximately one and half year, we determined in 115 strains of Shigella sonnei 12 lysotypes and 11 colicinogenotypes. Using the combination of both methods, the number of types increased to 23. Out of these 23 strains, 39% were sensitive to drugs, 52% possessed the R-factor, 9% had a non-transferable resistance and 13% were non-colicinogenic. Furthermore, we found that the lysotype 23 (XII-) colicinogenotype 4 (col Ib) keeps constantly in the Broumov region, probably as a carrier strain. During that time the change of the colicinogenotype to type 14 (col Ib + col E2) to type 2 (col Ia) and to further undefined type took place. In further case, one colicinogenotype 8 (col E2) changed in one epidemic to lysotype N.C. (III-IV-VIII) probably by acquiring the R-factor to lysotype N.C. (I-III-IV-VIII) and finally by acquisition of a col V factor to lysotype N.C. (I-III-IV-VII-VIII). Our lysotypes 65 were probably connected with the production of esculine E1 in colicinogenotypes 11 (col E1), 6 (col E1) and 13 (col E1 + col Ia). Sometimes identical types could be differentiated by a different colicinogenotype and vice versa. We recommend combination of both methods.

Arachnia propionica — a Causative Agent of Actinomycosis faciei. J. Scharen, Reference Laboratory for Pathogenic Actinomycetaceae, Department of Microbiology, District Hygiene Centre, Trutnov.

The genus Arachnia, represented by a single species Arachnia propionica (A.p.) is classified together with genera Actinomyces, Rothia, Bacteri- nema and Bifidobacterium into the family Actino- mycetaceae. A. propionica is defined as a micro- acrophil, catalase-negative G + filamentous organism that is able to form mycelium of a transitional type. A. propionica does not split urea but ferments glucose and propionic acid and contains in its peptidoglycane LL-diaminopimelic acid. A. pro- pionica is pathogenic for man — the disease is manifested as actinomycosis — and experimentally for mice. A. propionica is sensitive to penicillin. A well-documented isolation of A. propionica from such localisation which is typical for actinomycosis, is unique in our country. In this respect, A. pro- pionica belongs together with Actinomyces naeslundii and Actinomyces viscosus to microacrophil actino- mycotic organisms, which were reported to be pathogenic only recently and exceptionally. The proteus-like nature of actinomycosis almost ex- cludes its clinical diagnosis. However, the aimed laboratory diagnostics shows that microacrophile actinomycota participate in morbidity of our population in an unexpected large scale. Relative difficulties which are connected with isolation and identification of the causative agent of actino- mycotic diseases could be solved provided this problem will be submitted to careful studies and the diagnostic will be available for all laboratories.

Analysis of Peptido-glucan as a Diagnostic Tool in Genus Actinomyces. M. Žavadová, J. Chaloupka, P. Krčková, J. Adenská, V. Pokorny, Department of Microbiology, Thomayer Hospital, Prague and Department of General Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

The analysis of the mucopeptide component of the cell wall was used for differentiation of anaerobic corynebacteria and strictly anaerobic actino- mycetes. Mucopeptide of the genus Actinomyces (Actinobacterium) meyeri belongs to the lysine type with a following proportion of components in its mucopeptide: lys : glu : ala : N-ac-gln : N-ac-mur = 1.19 : 1.00 : 1.26 : 0.93 : 0.75. Mucopeptide of Corynebacterium granulosum contains diaminopimelic acid. The proportion of components of its cell wall is characterised by following numbers: dap : glu : ala : N-ac-gln : N-ac-mur : (gly : ser) = 0.59 : 1.00 : 1.38 : 0.77 : 0.85 : (0.92 : 0.51). In the diagnostic praxis, it is sufficient to use the method according to Park and Hancock (1960) which is based on gradual extraction of cells with hot TCA, ethanol, ethanolether and acetone and digestion of solid residue by trypsin. In case the analysed sample of extracted mucopeptide contains both diamino- pimelic acid and lysine, one can presume that the presence of lysine indicates contamination of the cell wall by traces of protein.

Electronmicroscopic Studies on Mycobacteria Cultivated Under Nutrition Deficiency Conditions. T. Strýčková, H. Mohelská, Research Institute of Epidemiology, Bratislava and Institute of Hygiene and Epidemiology, Prague.

This work presents the results of electronmicro- scopic studies of Mycobacterium smegmatis during three periods of cultivation: (1) on Youmanns me- dium; (2) subsequent cultivation in physiological solution or (3) cultivation in physiological solution supplemented with 1% glycerol. During the long- term cultivation, samples were withdrawn, fixed with OsO₄, embedded in agar and Durecupan and ultrathin sections were prepared. The electron- microscopic pictures showed heavy degranulation of cytoplasm and widening of the space between the cytoplasmatic membrane and outer coat in mycobacteria that were cultivated for 30 days in a nutritionally deficient medium. On the other hand, the characteristic appearance was maintained in the membrane structure of the cell wall, cytoplasmatic membrane, as well as in the mem- brane which envelops the lipid granules. In each of three mentioned alternatives, regardless from the length of cultivation, a sporadic occurrence of indi- viduals with an unusual rough lamellar structure and dense polymorph granula in the cytoplasm could be observed. The scanning microscope showed
differences in the regularity of surfaces and roughness of bacteria from individual cultivations. The pictures from the scanning microscope and the ultrathin sections revealed that the irregular roughness was in connection with the formation of giant vacuoles. It was also of interest that great irregularities of the surface were characteristic for cultures grown in physiological solution with 1% glycerol. Cultures grown in physiological solution without glycerol had a relatively regular surface similarly as the control culture grown in complete nutrient medium.

Effect of Antituberculotics on Biosynthetic Processes in Mycobacterium smegmatis. V. MAJTA, I. DROBNICKOVA, Research Institute of Epidemiology and Microbiology, Bratislava and School of Microbiology, Comenius University, Bratislava.

The effect of 12 antituberculous drugs on the growth and biosynthesis of macromolecules in Mycobacterium smegmatis was studied in time and concentration dependence. The alteration of multiplication of cells of Mycobacterium smegmatis was studied using the Merill's medium. The most effective agent appeared to be etambutol, hydrazide of the isonicotinic acid, etionamide and rifampicin. The influence of these substances on growth of Mycobacterium smegmatis was studied in the aim to investigate their effect in biosynthetic processes. These involve proteosynthesis indicated by the rate of incorporation of leucine-14C into the TCA-insoluble fraction of cells and synthesis of nucleic acids using adenine-14C. The rate of incorporation was studied using the Merill's synthetic medium with glucose which proved to be the most advantageous for this purpose. The effectiveness of tested substances was considered mainly on the basis of changes in the rate if incorporation of precursors used, and therefore it was followed in a relatively short time interval. As the concentration dependence of tested substances was used, we could determine graphically the ID50 value, i.e., such concentration of the substance, which was required for a 50% decrease of the incorporation rate into corresponding macromolecules of the cell. In addition, by expressing the ratio of radioactivity of incorporated adenine to radioactivity of incorporated leucine, different data were obtained which were suitable for evaluation of the effect of the substances under study on the metabolism of the cell. From the data obtained it follows that streptomycin, gentamycin, kanamycin and rifampicin influenced significantly incorporation of precursors-14C into corresponding macromolecules in Mycobacterium smegmatis cells.

The endocellular parasitism is characteristic for pathogenic mycobacteria. Atypical mycobacteria like Mycobacterium avium, elicit the Yersin-type of a lethal disease without typical symptoms of tuberculosis. We studied the course of infection in situ in the rabbit liver. Mycobacteria were found only within phagosomes of Kupffer cells, or eventually at later stages of infection, within phagosomes of giant multinuclear cells, which can be detected approximately in the half of the process. In the course of the process of infection, the enlargement of the volume of cytoplasm and an increase in the number of cellular organelia in the Kupffer cells could be observed. The cells could be seen in vast agglomerations around sinusoids. Mycobacteria maintained unchanged morphology and the structure of cell coats was undamaged as compared with mycobacteria cultivated in vitro. Only exceptionally lysed cells could be found. However, quite frequently the polymorph structures occurred in the plasma of mycobacteria and phagosomes. These structures were characterised by a high density and their morphology, size and frequency of occurrence did not correspond to volutine granules and, furthermore, they were never found in avian mycobacteria cultivated in vitro under standard laboratory conditions. Within certain mycobacteria, and further within mycobacteria that were released into the sinusoid lumen probably by a secondary disruption of cells, vacuolisation of plasma was observed probably as a consequence of inadequate cultivation conditions. Dense structures occurred even within these cells. Presumably, we were also dealing with a regressive change which might reflect the lack of oxygen or lack of some essential nutrient in the infected tissue. Macrophages obviously lack the capacity to degrade mycobacteria.

Some Chemical and Immunochemical Aspects of S- and R-Endotoxins of Shigella dysenteriae. J. ŠOVIK, T. TRNKA, Institute of Hygiene and Epidemiology, Prague and School of Organic Chemistry, Charles University, Prague.

The basic chemical composition of S- and R-endotoxins (lipopolysaccharide-protein complexes) of Shigella dysenteriae 1 (Shiga), partially purified by a two-step column chromatography on Sephadex G 200 and Sepharose 4B, was studied. As an original raw extract, we used the preparation obtained by treatment of cells with hypertonic solution of NaCl (1M) and Na nitrate (0.1M) according to Reynaud. The complexes consisted of 23—35% of lipids, 40—50% of sugars and 14—24% of amino-acids. Main part of the total lipid content formed the behenic acid 22:0 (about 50% for both growth forms). The value above 10% was recorded in fatty acid 16:0; within the range of 5—10% were detected 14:0 and OH 14:0. None of the odd fatty acids possessed a significant per cent proportion in the total lipid. The carbohydrate component contained following sugars: galactose (for S-30%/for R-30%), glucose (5/35), glucosamin (10/3), rham-
nose (40/0) and aldoheptose (0/25). Sixteen aminoacids were found in both forms in an identical composition; only in S-endotoxin less aminoacids were found quantitatively. The ratio of individual aminoacids between R/S endotoxins was maintained within the range 1.7 ± 0.2. The preparation of a given purification step contained 3.8-4.8% of N, 2-3% of P and traces of S. The keto-deoxyoctulonic acid (KDO) was found under 0.5%, 0-phosphoryl-ethanolamine could not be detected. The presence of several trace elements (inorganic cations) was demonstrated in both S- and R-forms (Cu, Zn, Ca, Mg, Fe, Mn). The results of the analysis of chemical composition should be taken as preliminary mean values which should serve as a guide for further steps of purification and isolation of active components of bacterial endotoxins. It turns out that the sugar ratio, which represents a different structure both quantitatively and qualitatively, is the main cause of different immunological behaviour of both types of endotoxin and therefore its immunodominant role is fully acceptable.

The Base Composition of Rickettsiae Deoxyribonucleic Acids. S. Schramek, Institute of Virology, Slovak Academy of Sciences, Bratislava.

Rickettsiae possess relatively little phenotypic characteristics suitable for classification. Until now, the classification of rickettsiae was based mainly on antigenic differences, epidemiological and ecological criteria and on comparatively few biological properties. For determination of an objective index of genetic relationships among rickettsiae, we established the molar content of bases from various strains of rickettsiae using the thermal denaturation method. The average molar content of guanin and cytosin was within the genus *Rickettsia* within the range of 29-39%. In subgenera following values were found: *Rickettsia* 29%, *Dermacentrotriton* 33%, *Rochalimaea* 39%. In the genus *Coxiella*, the average base composition was 43% for guanin and cytosin. The differences among species and strains within one subgenus were not significant. On the basis of the analysis of base composition of deoxyribonucleic acid in newly isolated rickettsiae in Slovakia, they belong genus *Rickettsia*, subgenus *Dermacentrotriton*.

Phase Variability of *Coxiella burnetii* during Transfers in Normal and Immunosuppressed Mice. J. Kazár, R. Brezina, E. Kovačová, Institute of Virology, Slovak Academy of Sciences, Bratislava.

We studied antigen-binding and antigen-inducing properties, virulence for mice and guinea pigs of strains of *Coxiella burnetii* 48 and Nine Mile, serologically in phase II during transfers in normal and immunosuppressed mice. The strain 48 (50 transfers on yolk sac of chicken embryos) could be transferred in normal mice; in immunosuppressed mice it changed to phase I during 4 passages. The Nine Mile strain (an undefined number of several hundreds of transfers on yolk sac of chicken embryos) could not be transferred in normal mice, however, it could be changed to phase I during 8 passages in immunosuppressed mice. These results invalidate the hypothesis on the significance of antibodies for conversion of phase II to phase I of *Coxiella burnetii*.

Structural Proteins of the Fowl Plague Virus. M. Sangret, O. J. Vertlak, V. Hrypko, Department of Infectious Diseases, Public and Forensic Veterinary Medicine, Veterinary School, Košice.

The virus of classical plague of fowl, strain Dobson, was grown in 10-11 day-old chicken embryos. The virus was then concentrated using the 2×10^5-10^6 zinc acetate and further purified on a DEAE cellulose. The pooled eluates were centrifuged for 1 h at 45,000 g. The sediment was dissolved in a minimal volume of 0.14M NaCl. The purified virus (concentration 10^6-10^7 HU/ml) was dissociated in phosphate buffer, pH 7.2, using a 2% sodium dodecylsulphate and 1% 2-mercaptoethanol. Electrophoresis was performed in 8% polyacrylamide gel. The polymerization took place in glass tubes 10×0.6 cm. Seven polypeptides could be clearly demonstrated electrophoretically in the virus studied. In some experiments, the SH groups were found to be carboxymethylated. Differences in the number of polypeptide chains were not observed. In four polypeptides the presence of glycoproteins could be demonstrated. The antigenic characteristic of the localization of virus polypeptides was in agreement with data of Klenk et al. (1972). Polypeptides designated HA, HA1, NA and HA2 are glycoproteins, originating in the capsule of the virion. If virus was treated with bromelain, all glycoproteins were split off the virus. Polypeptides P, NP and MP were detected by polyaerylamide electrophoresis even in this case. Using the protein markers, the molecular weight of individual virion polypeptides was established; the values vary within the range 26,000 to 91,000 daltons. The origin of HA polypeptide in the virus under study and in viruses in general, is discussed.

L-forms of Listeria monocytogenes. D. Kalvodová, F. Paročka, Institute of Medical Microbiology and Immunology, Faculty of Medicine, Charles University, Prague.

L-forms were induced by penicillin in three strains of Listeria monocytogenes out of 20 strains. These were stabilised after 5 passages on solid medium and did not revert into the bacterial form during subsequent 50 passages on media without an antibiotic. The colonies possessed characteristic morphology of L-forms. They were osmotically labile, did not grow at NaCl concentration below 1.5%. They required at least 1% of native serum, the optimum concentration being 10%. All strains produced weak haemolysis in a blood-containing me-
Morphological, Biochemical and Developmental Relations Between Globular Mycoplasmatales Viruses and Myxoviruses. B. Liška, Biophysical Institute, Czechoslovak Academy of Sciences, Brno.

A number of properties of mycoplasmatales viruses belonging to types MV-L2 and MV-Lg-L 172 were found to be identical to those of myxoviruses and kindred animal viruses which indicates the relation or identical origin of these groups of viruses. The properties are: (1) structure of particles, containing nucleic acid enclosed in the three layer lipoprotein, biological unit-membrane; (2) heterogeneity in the size of virus particles; the particles are not rigid but rather of pliant consistency; (3) electron microscopic studies show that mature particles are not present inside the cells but they can mature either at the moment of lysis of the host cell or by budding and become enveloped by the membrane at this final stage. Since mycoplasmas themselves are close to mammalian cells as to surface structure and they are elementary self-reproducing microorganisms, parasiting in plant and animal tissues where they can live together with their viruses, in many cases inside the cells, it is possible that these viruses could get adapted to the host cells of higher organisms or the viruses of these organisms could be taken over by mycoplasmas. The former of the two possibilities is very probable in view of the relation of the morphogenesis of these viruses to the characteristic features of mycoplasmal structure and development. The difference in the nucleic acid type of globular viruses of mycoplasmas hitherto described and of myxoviruses cannot be explained on the basis of present knowledge of mycoplasmatales viruses, but a number of processes are known which could take part in this conversion and the similarity of other properties of these viruses shows their obvious relationship.

SECTION OF VIROLOGY

Steric Effects in Reaction of Neuraminidase Virus with Antibodies. G. Russ, E. Makovniková, B. Styk, Institute of Virology, Slovak Academy of Sciences, Bratislava.

Antibody inhibition of neuraminidase activity was studied using influenza A virus recombinants containing haemagglutinin and different neuraminidase subtypes. Neuraminidase was inhibited in every case tested, even when hyperimmune serum containing antibodies only to the relevant haemagglutinin was used. This steric inhibition of virus neuraminidase was not observed if the inhibition test was carried out in the presence of a non-ionic detergent (Triton-X-100), however. Inhibition of neuraminidase by serum containing antibodies to the neuraminidase in question was not influenced by the presence of Triton-X-100. The use of Triton-X-100 allows very simple and quick detection of steric inhibition of virus neuraminidase by antihaemagglutinin antibodies.

Characterization of Influenza Virus ts Mutants. E. Tučková, E. Antisimová, V. Vonka, Institute of Sera and Vaccines, Prague.

After 5-fluorouracil induction, 2 ts mutants were isolated from a recombinant obtained in our laboratory by crossing the viruses A2/Singapore and A9/NWS. The mutants were characterized by growth tests at a permissive (35°C) and a non-permissive (39°C) temperature. Haemadsorptive, haemagglutinating and neuraminidase activity, RNP antigen formation and ultrastructural changes in the cells were studied and infectious virus production at both temperatures was determined. The results, including those of the temperature tests, show that ts defects of the given mutants involve various virus functions.

Pathogenic Effect of Strain A9/NWS ts Mutants on Monkeys. B. Doležalová, A. Jirásek, M. Stárek, E. Tučková, Institute of Sera and Vaccines, Prague; First Department of Morbid Anatomy, Faculty of Medicine, Charles University, Prague.

Neurotropism of strain A9/NWS thermoresistant mutant obtained by successive adaptation to high temperatures was tested in monkeys (Macaca rhesus). The monkeys were infected intranasally, intracerebrally and by a combination of the two methods. They were killed at different intervals. Histological changes in their brain, lungs and spleen were studied and the presence of virus in the organs was determined by cultivating organ suspensions. Specific antibodies were determined in a haemagglutination inhibition test. CNS lesions were found only in i.e. infected monkeys. Most of the findings support the hypothesis of a single, defective influenza virus replication cycle, but the fact that a brain suspension from i.e. infected monkeys produces typical influenza encephalitis in mice still remains unexplained. The question of the elongate structures released from the surface of monkey ovoidymal cells after i.e. infection is likewise still unresolved.

Removal of Nonspecific Serum Inhibitors of Recent Influenza Virus Strains by means of RDE (Receptor Destroying Enzyme). B. Styk, B. Tímová, J. Jakubková, V. Veber, I. Pečenková, R. Štandová, B. Šikorová, Z. Frištacká, G. Russ, E. Makovniková, P. Veber, Institute of Virology, Slovak Academy of Sciences, Bratislava; Institute of Hy-
giene and Epidemiology, Prague; HEO, Bratislava; Faculty of Pharmaceutics, Comenius University, Bratislava.

For removing nonspecific inhibitors of myxoviruses, WHO experts recommend preparing the sera with RDE. We tested this method, using RDE cholera filtrate preparations (Czechoslovak and foreign produced) and recent and prototype strains of influenza, parainfluenza and parotitis virus. We found that crude cholera filtrate preparations did not adequately remove nonspecific inhibitors of recent, epidemiologically important, influenza A virus strains and that semipurified RDE preparations (RBC eluates) were needed. Czechoslovak preparations were more potent than those produced by Messrs Duphar (Holland); they reliably removed nonspecific inhibitors without significantly impairing specific antibodies. The RDE preparations were produced, in principle, according to the method of Burnet and Stone, using developing chick embryos for the propagation of Y. choloreae and all-glass G5 filters for filtering the cleared allantoic fluid. The enzymatic activity (neuraminidase content) and RDE titre of the preparations and their ability to destroy inhibitors in standard sera were tested. The RDE preparations were supplied to Czechoslovak virology laboratories, usually in the lyophilized form. Another final way of preparing RDE was also tested. In this, the preparation was dried by atomization and the resultant powder was compressed into dry tablets. This is an international innovation and it has several advantages over the lyophilized form.

Study of the Biophysical Properties of Strain OC 43 of the Coronavirus Group. J. Pokorny, M. Brbecova, M. Ryv, Institute of Hygiene and Epidemiology, Prague.

The distribution of the virus particle and of its antigenic components was studied in virus preparations from embryonic mouse brain tissue by zonal centrifugation in a sucrose density gradient, by electron microscopy identification of the resultant fractions and by serological tests. Strain OC 43 virions were found in the 1.16 density zone. Haemagglutinating activity was associated mainly with complete virus, whereas complement-fixing activity was found in the ribosomal fraction (sp. density 1.14). Conditions for storing the isolated fractions so as to preserve their biological activity were determined.

Demonstration of Influenza A Virus in Organs of Subjects Dying during an Acute Influenza Infection. V. Fraškova, A. Jirasek, Military Institute of Hygiene, Epidemiology and Microbiology, Prague; First Department of Morbid Anatomy, Faculty of Medicine, Charles University, Prague.

During an epidemic caused by type A2/Hongkong and A2/England influenza virus, the authors systematically examined postmortem material from subjects who died during an acute influenza infection. In five, they demonstrated influenza virus in organs other than those of the respiratory tract (heart, liver, spleen). These were all subjects with lowered resistance: three were over the age of 70, one was a young woman who had given birth five days previously and one was an infant aged four months. The virus was demonstrated by isolation and by examining cryotome sections of the organs by the immunofluorescence method.

Study of the Prevalence of Adenoviruses and Herpes Virus in an Infant Community. J. Holý, K. Vaseček, Z. Pavlík, Department of Child Development Research, Faculty of Paediatrics, Prague; Department of Economic and Regional Geography, Faculty of Science, Charles University, Prague.

Virus infections of the respiratory system present clinically similar signs despite antigenic diversity of the virus pathogens. When evaluating the incidence of serum conversions against adenoviruses and four human herpes viruses among the infants in an infants' home, the authors used the statistical method employed in demography. This method is suitable for studying the progressive prevalence of different viruses in a child community, with or without clinical signs.

Antigenic Relationship between the Viruses A/Hong-kong/68 and A/England/42/72. Z. Závadová, V. Vonka, E. Domorázková, J. Bruij, Institute of Virology, Slovak Academy of Sciences, Bratislava.

The cross reactivity of the viruses A/England/42/72 and A/Hongkong/68 was studied by means of hyperimmune rabbit sera, the sera of subjects who had influenza in 1969 or 1972 and the sera of subjects of different ages vaccinated with inactivated vaccine prepared from the strain A/England/42/72. The results show that, according to its haemagglutinin, the virus A/England/42/72 is the "prime" strain in relation to the virus A/Hong-kong/68. Some evidence was likewise obtained indicating that the reverse applies to the neuraminidase antigens of the two viruses.

Electron Microscopy Study of the Structure of the Envelopes of Infectious Bovine Rhinotracheitis (IBR) Virus in Ultrathin Sections. L. Valšek, B. Šmíd, Institute of Veterinary Medicine, Brno, and Czechoslovak Collection of Microorganisms, Purkyně University, Brno.

The permanent cell line MDBK and a primary calf kidney cell culture were infected with two strains of IBR virus. It was found that: (1) The outer envelope of virus particles in the cytoplasm canals and the extracellular space has a distinct membrane unit structure and processes or globular formations on its surface, while the envelope of intranuclear particles and particles in the perinuclear cisterna consists of only a single dark layer.
(2) The outer envelope of cytoplasmic and extracellular virus particles is often pleomorphic compared with that of intranuclear particles and the space between the capsid and the outer envelope is filled with varying amounts of a dark substance. The substance is contiguous to the inner aspect of the outer envelope, but is separated from the capsid by a light zone. The dark substance, which is linked to the membranes of the cytoplasmic canals, enters the virion, together with a nucleocapsid acquiring an outer envelope, by being forced into these canals. The origin, chemical composition and function of the substance are still obscure. (3) A light zone can clearly be seen round some intranuclear nucleocapsids. The same zone separates the capsids of cytoplasmic and extracellular virions from the dark substance lying on the inner wall of the outer envelope. It is probably the inner envelope of the virion.

Action of Some Inhibitors on Influenza Virus.

S. Havířová, G. Rusek, Institute of Virology, Slovak Academy of Sciences, Bratislava.

The authors studied inhibition of the replication of influenza virus and neuraminidase synthesis by N-tosyl-L-phenylalanyl chloromethane, an inhibitor of proteolytic enzymes, in influenza virus-infected cells. Influenza virus replication was found to be inhibited; neuraminidase synthesis in the infected cells was inhibited at the same time. These results are in agreement with the concept that large protein molecule precursors are synthesized in influenza virus-infected cells and that they are then broken down to virus structural proteins by proteolysis. The influenza virus envelope proteins contain a large quantity of cysteine residues and the effect of S-methyl-L-cysteine on the replication of influenza virus was therefore tested. Influenza virus replication was found to be inhibited. The influence of temperature on the stability of the neuraminidase of influenza virus replicating in the presence of S-methyl-L-cysteine and on control virus neuraminidase was also compared. The cysteine analogue was not found to have any effect on the thermolability of neuraminidase.

Effect of Polyvalent Phage 812 on Oxidative Activity of Cells of Strains of Staphylococcus aureus.

K. Hošák, D. Horáková, M. Němec, Department of Microbiology, Genetics and Biophysics, Purkyně University, Brno.

The glucose oxidase activity of cells of S. aureus SA 812, NCTC 8511, S 26 and SA 66 in the presence of the polyvalent and virulent staphylococcal phage 812 is reduced to 85.3—66.6% of the activity of cells not exposed to the action of the phage. Oxidase activity was determined by the standard manometric Warburg method in 0.05M Tris medium at an input ratio of 10⁻¹. The smallest drop in glucose oxidase activity was recorded in SA 66 cells (by 14.7%), to which a large proportion (92.3%) of phage 812 is adsorbed, but does not replicate. In the other organisms studied, the decrease in oxidase activity was roughly the same (29.3—33.4%) and was uncorrelated to the number of phage virions adsorbed and to their ability to replicate. The presence of phage 812 likewise has a negative effect on oxidation of the endogenous substrates of strains SA 812, NCTC 8511 and S 26, whereas O₂ consumption by strain SA 66 cells for the oxidation of endogenous substrate does not alter significantly in the presence of phage 812. The drop in the glucose oxidase activity of the cells and in the oxidation of endogenous substrate is uncorrelated to the input ratio (IR), which is specific for the individual strains. Strain SA 66 again forms an exception, since its oxidative activity is influenced irrespective of the number of virions present in the phage-bacteria mixture.

Isolation of Phages from Coagulase-negative Staphylocoocci Strains Isolated from Clinical Material.

J. Pillich, J. Hájková, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

Today there is an increase in the number of infections caused by coagulase-negative staphylococci, which were once all regarded as saprophytes and contaminants of a primary pathogen. This group of bacteria now frequently causes chronic inflammations of the urinary passages, endocarditis, osteomyelitis and a number of other diseases. We have concentrated, in our laboratory, over 150 of these strains from different laboratories in other countries, where they were isolated from a wide variety of infections. Using mitomycin C and UV radiation, we succeeded in isolating phage strains with more or less specific effects in relation to the staphylococcal host. So far we have observed definite relationships between coagulase-positive and coagulase-negative staphylococci isolated from clinical material, which can be expressed in terms of their sensitivity to individual phages.

Autolytic Phenomenon in Pseudomonas aeruginosa.

A. Kázdová, J. Pillich, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

One of the properties of Pseudomonas aeruginosa are the spontaneous lytic manifestations which can be observed on solid media. These are often plaque-like clearings ("autoplaques", AP), which closely resemble phage plaques. About two thirds of Ps. aeruginosa strains are capable of forming AP. An analysis of 150 colonies of strain whose AP production can be influenced by passaging showed that it is composed of colonies both producing and not producing AP, whereas AP production by strains not influenced by passaging is uniform. Ultracentrifugation of a culture of the autolytic strain Ps 179003 unh in a CsCl density gradient showed the presence of phage in 10⁸ concentration in the zone corresponding to a density of 1,500 g/cc. The phage displayed normal morphology in the EM. Direct
evidence that it induced AP formation was not obtained. If AP are of phagic origin, the relationship between bacteriophage and host is evidently a specific, atypical one.

Utilization of the Agar Diffusion Method for Testing Radiomimetics. Z. Malinka, D. Toufarová, Z. Hradečná, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

The authors' aim was to verify the suitability of using the agar diffusion method to test radiomimetics and substances whose action has not, so far, been explained. Hydroxylamine, the monofunctional alkylating agents ethylmethane sulphonate, 2-chloroethylyamine and N,N-dimethyl-2-chloroethylamine and the bifunctional agents ypepsyl and mitomycin C were used for testing. Several nitrofurans, sugar analogues and microbial metabolites were also employed. The lysogenic strain E. coli C 600 lambda was used to study the inductive effect of the substances. Phage inactivation was studied in phage lambda eb2. In both cases the indicator organism was E. coli C 600. An inductive effect was manifested in an increase in the number of plaques, an antiviral effect in a distinct decrease in the number of plaques in the diffusion zone of the substance. None of the three monofunctional alkylating agents was found to influence prophage induction. With the two bifunctional alkylating agents, induction occurred in both cases. Some of the other test substances also had an inductive effect, but it was significantly weaker. 2-chloroethylyamine and some sugar analogues, nitrofurans and microbial metabolites displayed an antiviral effect.

Intracellular Transformations of the Replicating Form of RNA Phage f2. J. Dobskoch, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague.

The electrophoretic profiles of the double-stranded RNA formed in large amounts, also by sublethal doses of UV radiation, to which the host cell is exposed prior to infection with phage f2 su11 were determined. Short-term labelling at different intervals of the defective replication cycle shows that the molecular weight of the newly formed RNA is not progressively reduced, but that the double-stranded RNA formed after infection is progressively degraded to low molecular weight fragments. On using a strain deficient in RNAase III, which specifically breaks down double-stranded RNA, degradation does not occur and an intact replicating form is obtained. The results show that the greater part of the hyperpropced complementary RNA already forms a helical structure in the cells, i.e. prior to isolation, so that it loses its template function and undergoes gradual degradation by RNAse III. Only a small portion of the RNA keeps its monohelical-stranded structure and template function. This part of the RNA does not undergo intracellular degradation.

Virulent Mutants of Salmonella typhimurium Phage "L". J. Soška, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

A series of "clear" mutants of Salmonella typhimurium phage "L", different from the typical type cI, cII and cIII mutants, was isolated. The properties of the mutant designated as B 14 were studied in detail. The mutant was localized by means of crosses in the zone between the genes cII (the gene for the repressor) and cI (the gene for the activator of the repressor). This mutant, like other, similar, independently isolated mutants, is remarkable in that it produces phenotypically virulent mutants capable of vegetative growth on lysogen LT2 (L) with relatively high frequency. The nature of the other mutation leading to virulence has not yet been determined. According to the frequency of back-recombinations, it is likewise in the "c" zone of the phage chromosome, however. "Virulent" mutants are complementary with cI and cIII mutants. The initial B 14 mutant is capable of lysogenizing an infected cell. Some of the resultant lysogens are defective.

Mutagenic Effect of O-methylhydroxylamine on Phage T4. J. Koudejka, E. I. Budovský, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno; Institute of the Chemistry of Natural Substances, Academy of Sciences of the U.S.S.R., Moscow.

The biological effect of O-methylhydroxylamine (OMHA)-modified adenine on phage T4 was studied by means of a non-sense phage T4 mutant. The rate of OMHA modification of adenine was 50 to 100 times lower than the rate for cytosine. Modified adenine derivatives display very low activity in the RNA-polymerase system and their biological activity is therefore presumably also low. In the case of phage T4, however, study of reversions of amber and ochre mutants shows that OMHA also caused type A-G transitions. Unlike other systems, this transition accounts for a relatively large proportion of the total mutagenic effect of OMHA on phage T4.

Effect of O-methylhydroxylamine on Lambda Phage. L. Dobiáš, Z. Hradečná, I. Korčáková, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

Raised non-exponential inactivation of lambda phage was demonstrated during short-term exposure to 1M O-methylhydroxylamine. Under these conditions, the lethal effect is probably caused by bonds between the phage DNA and protein. Phage DNA damage can be repaired by the host cell's enzymatic system, as in UV inactivation. Phage DNA repair is stimulated either by the host cell alone, or by sublethal doses of UV radiation, to which the host cell is exposed prior to infection with phage.
Repair of Lambda Bacteriophage and E. coli Host Cells Exposed to the Action of UV Radiation. J. HAKLOVÁ, Z. HRADĚČNÁ, D. TOUFAROVÁ, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

The effect of UV radiation on the repair capacity of the host cell and bacteriophage was studied. The sensitivity of E. coli cells to the effects of UV radiation depends on the repair and recombination capacity of the cell and is influenced by UVr and rec genes. Bacteriophage repair capacity is closely associated with the genetic properties of the host cell. The significance of UVr and rec genes in the repair of bacteriophage after exposure to UV radiation was studied. Repair of bacteriophage by the host cell depends on the host gene UVr B; the rec A gene is of no significance for the course of repair. The results indicate that mechanism of lambda bacteriophage repair by the host cell consists in the breakdown of phage DNA dimers. UV reactivation of bacteriophage requires the host rec A gene. Genes for excision repair are probably not needed for this repair.

Host Specificity of Phages P22 and L. L. Sošková, Department of Biology, Faculty of Medicine, Purkyně University, Brno.

The Salmonella typhimurium phages P22 and L differ (among other things) in respect of their host specificity; strain LT2 is the host of phage P22, while the host strains of phage L are LT2 and 1559. The difference is not determined by modification of phage DNA by the host (the host conversion mechanism). Phage L mutants incapable of vegetative growth on a P22 host were found. These were divided, by complementation tests, into 3-4 groups. The mutants in the first group belong to the first of the series of genes controlling synthesis of the phage head, but do not possess phage P22 specificity. The second group was allocated to one of the genes for synthesis of the tail controlling adsorption to the host. These mutants likewise do not possess phage P22 specificity. Only the last group of mutants displayed phage P22 specificity in complementation. These mutants were localized by means of two- and three-factor crosses in the immediate vicinity of gene cII. In addition, it was found that some "clear" type cII L genes had similar limited capacity for vegetative growth on a strain 1559 host and that the adjacent regulatory gene for DNA synthesis in phage P22 and L was non-homologous.

Promoters and Two-way Transcription of the immC Zone of Phage P22. M. Bezdek, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

Lysogenization by phage P22 is controlled by the genes c1, c2 and c3 (the immC zone) and the cly gene. C1 and c2 function are essential for the institution of lysogeny, c2 function for its permanent maintenance. Cly function acts counter to the establishment of lysogeny. The interplay of immC and cly helps to decide between lysis and lysogenization. The elimination of cly mutations leads unequivocally to lysogenization. We found that mutations leading to suppression of a defect in cly function exist. They were characterized complementationally, physiologically and genetically as promoter mutations. In this way we functionally defined a promoter for left-sided transcription of genes c1 and c2 en bloc. (We presume that transcription on the left continues from a further promoter, etc.) Another promoter, prn, defined by K5 mutation, promotes only c2 transcription, in the same direction. We submitted a transcription regulation model for the immC zone, in which we assume that the prn promoter also determines transcription in the opposite direction (transcription of early phage functions). We further use the same model to explain the development of virulence in phage P22 as constitutive bilateral transcription from εZ and K5.

Non-mutational Hereditary Changes in Prophage Lambda Gene Activity in Non-defective Lysogens. J. ČRAÝN, E. KOUTECKÁ, Z. NEUBAUER, Faculty of Sciences, Charles University, Prague.

Clones able to grow normally at the inducing temperature were isolated from a heat-inducible E. coli recA- lysogen (intλE11557). The inability of the derepressed prophage to express its lethal functions is due neither to a genetic defect in the prophage, nor to mutation of its host. Two types of such heat-resistant clones, corresponding to different phage regulation patterns, have so far been defined (E1 and E2). E1 type bacteria do not support the growth of homoimmune phages, including λvir, and their capacity for reproducing λ17 and λ434 is diminished, but they are completely sensitive to λ21. E2 type bacteria are also insensitive to superinfection with homoimmune phages fully susceptible to λ-specific negative regulation (cl, tof), but the constitutive mutants λvir, λ2, λ1v3 and λ17 and the heteroimmune phages λ434 and λ21 grow normally in E2 type cells. E1 and E2 types also differ in respect of their prophage gene activity; in E2, expression of the left operon early gene N takes place, whereas N gene activity is absent in E1. On the other hand, in both E1 and E2 cells, the right operon early genes of their prophage, cI, O and P are expressed, while the genes of the prophage immunity region, cI and reX, are not. This contrasts with the normal lysogenic state (corresponding to the parental type PA), when only the prophage immunity zone is active. The characteristics of E1 and E2 clones are unstable hereditary features: reciprocal transitions occur in aging cultures or in bacteria starved and kept in buffer. The frequency and the direction of these transitions depend upon the history of each clone. It is also influenced by the temperature at which the non-growing populations are kept. At
30°C E1 shifts to E2 and PA and E2 to both E1 and Pa; at 45°C E1 populations are fairly stable, while in E2 populations transitions to E1 occur. The parent-like type PA behaves like the original heat-inducible lysogen: at 30°C it is stable and at temperatures over 37°C it is inactivated.

Differentiation in UV-irradiated Bacteria Lysogenic for a Defective Prophage and the Role of the CII Gene in this Process. D. Vihanová, Z. Neubauer, Faculty of Science, Charles University, Prague.

Lysogenic bacteria containing a doubly defective prophage ($\lambda N^{-} P^{-}$) non-lethal for its host were tested for their ability to promote growth of a superinfecting phage at various intervals after UV irradiation. Two successive processes ensued. During the first 20 minutes after irradiation, immunity to superinfection was lost; afterwards, 70% of the cells regained it, while the rest remained non-immune. The decision of each cell was transmitted to its progeny and became a hereditary character. Superinfection of the irradiated cells with a homimmune phage prevented postirradiation loss of immunity. This was due to the CI product (immune substance) added by the superinfecting genome, since a similar effect was observed when the superinfecting phage was $\lambda ^{-}$ (cII sus 10). If the defective phage was $\lambda I$- ($\lambda N^{-} cII^{-} P^{-}$), immunity was lost more slowly (it took 30 min for the whole population to become non-immune), but immunity was not regained. The cII gene thus seems to play an ambivalent role in the regulation of immunity: immediately after irradiation it promotes loss of immunity, but at later intervals it promotes its reestablishment.

An Attempt to Describe the Regulation of Lambda Bacteriophage by means of Situation Calculus. Z. Neubauer, K. Bendová, V. Bendova, Faculty of Science, Charles University, Prague; Mathematical Institute, Czechoslovak Academy of Sciences, Prague; Faculty of Mathematics and Physics, Charles University, Prague.

The situation calculus language L we use consists of three types of variables, unitary and binary predicates, binary relations, quantifiers and the usual logical connectives. In general, any system of regulation at transcription level can be interpreted as a structure of L. Compared with "weaker" calculi, e.g. Boolean algebra, etc., the situation calculus is less operable. On the other hand, it has the advantage of being suitable for an easy intuitive interpretation of formal results. It further allows any arbitrary initial conditions to be set. It is thus a uniform method for describing and studying both normal and defective genetic systems at each step of the regulation process and even permits analysis of the properties and behavior of theoretically constructed regulation states. The heuristic value of the situation calculus can be seen particularly in the possibility of studying and formulating the most general features of gene regulation at purely

Abstract Automatic Products and Logical Grids as Models of Gene Regulation. J. Soška, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

Genes and their products are considered as elements of a logical grid system in which the grid represents inter-element relationships. These relationships can be expressed by mathematical logic symbols, using logical functions of negation, conjunction, disjunction, etc. The allosteric inhibition system and the repression system are simple cases to which the above relationships can be applied. In biological material in which gene functions and products and the regulatory relationships between them are relatively well known, modelling by means of a logical grid can be used instead of the usual modelling of the topological relationships between the elements of the system, and the relationships within the functional subsystem can be expressed in terms of Boolean algebra. The interactions of genes and their products within the logical grid are of a dynamic character and they can be modelled as an abstract automatic system progressively acquiring a given number of defined states. Intermediate states are either of a cyclical character, or, after a given number of steps, they lead to the absorbing, final state. Part of the regulatory system of lambda phage is given as an example.

Repressor-antirepressor System Model Effected with an Electronic Computer. V. Drašil, J. Soška, Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

A system of four Boolean equations representing idealized relationships between repressor gene, repressor and antirepressor forms an abstract automatic model acquiring 16 possible states. Of these, only two alternative states, representing preponderance of either the repressor or the antirepressor, are final and stable. The other states are equilibrium and form dynamic cycles. The introduction of probability functions into the four-equation system showed that equilibrium states are labile and are transformed to modified final alternative states after a given number of cycles. On using further probability functions, alternative states occasionally change from the one to the other and back again, thereby modelling, for example, the phasic transitions of $\text{imm}^{+}$ to $\text{imm}^{-}$ lysogens described by Neubauer. The process of cell differentiation can be modelled on the same principle.
syntactic level, without encumbrance by the sem-
antical aspects of the terms (i.e. their biological, biophysical or mechanical meaning or spatial inter-
pretation). In this way, general axioms were for-
mulated, such as the axiom of Vitality, the Monot-
ony and the Production axiom and so on, which reflect the formal principles on which the con-
temporary concept of genetic regulation is based.