THE SOCIETY FOR GENERAL MICROBIOLOGY

The Society for General Microbiology held its forty-ninth General Meeting at Imperial College, London, on Monday, Tuesday and Wednesday, 3, 4 and 5 April 1967.
The following communications were made:

ORIGINAL PAPERS

Chlorohydrin Formation during Epoxide Sterilization of Culture Media. by T. J. Weston
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Propylene oxide and ethylene oxide can be used for the cold sterilization of instruments, culture media and tissue slices (Hansen, H. & Snyder, W. C. (1947), Phytopathology, 37, 369; Goss, R. C. & Marr, J. L. (1963), Proc. Iowa Acad. Sci. 70, 125). These epoxides react with chlorides to form persistent toxic chlorohydrins in dried foodstuffs, such as flour and pepper (Wesley, F., Rourke, B. & Darbishire, O. (1965), J. Food Sci. 30, 1037). Using a gas chromatograph, incorporating a halogen detector, chlorohydrins were found in agar media and potato tuber slices after fumigation with propylene oxide. The amount of chlorohydrins formed in the agar was dependent upon the amount of Cl⁻ present, fumigation time, and propylene oxide concentration.

The growth and morphology of fungi subsequently grown on the agar were affected for as long as the chlorohydrins remained. The chlorohydrins were not persistent in agar and potato slices for more than 1-2 weeks, being eliminated by hydrolysis during storage.

Haemadsorption and Haemagglutination by Mycoplasmas. By R. J. Manchee and D. Taylor-Robinson

Del Guidice & Pavia ((1964), Bact. Proc. p. 70) reported that erythrocytes from several species adsorbed to colonies of Mycoplasma pneumoniae. In the present study, the factors concerned in demonstrating haemadsorption and its occurrence among different mycoplasmas were investigated. Haemadsorption occurred best to colonies which had recently developed on agar at pH 6.5. Crowding of colonies could completely abolish haemadsorption. The phenomenon usually occurred with erythrocytes from a wide range of species. Mycoplasmas isolated from various bird and animal sources haemadsorbed, e.g. M. gallisepticum, M. agalactiae, M. bovigenitalium, M. pulmonis, but not all strains within a serotype did so. Thus the ‘Negroni’ strain of M. pulmonis did not haemadsorb. Generally antiserum titres obtained by haemadsorption inhibition (HAdI) were low in comparison with those obtained by metabolic inhibition, and HAdI was not useful as a routine serological technique.

Mycoplasma haemagglutination was first reported in 1945 (van Herick, W. & Eaton, M. D. (1945), J. Bact. 50, 47). In the present study haemagglutination occurred best at pH 6.5-7.0 in U-shaped cups at 37°C. Mycoplasmas isolated from birds, cattle, goats, man, mice, and pigs, and grown in liquid medium haemagglutinated, generally at low titre. Inhibition of haemagglutination by specific antisera was demonstrated. There was lack of correlation between haemadsorption and haemagglutination: both these phenomena were exhibited by some mycoplasmas while others haemadsorbed only, and others haemagglutinated only.
Effects of R Factors on UV-Susceptibility of Escherichia coli K12. By A. G. Siccardi
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R factors are plasmids (transmissible extra-chromosomal DNA structures) which determine resistance to various anti-bacterial drugs, and sometimes further properties such as phage restriction and modification. Some R factors, like some colicine factors, confer partial protection against the bactericidal effect of ultraviolet irradiation. Of 28 R factors (a few of them associated with colicine factors) tested in E. coli, 13 gave protection (either causing a 'shoulder' on the dose/log-survival curve or decreasing its slope), 10 had little or no effect and 5 caused increased UV susceptibility, in both lysogenic and non-lysogenic K12 hosts. The protecting or sensitizing effect of representative R factors (and of several protecting colib factors) was qualitatively the same in K12 of wild-type UV-sensitivity and in UV-sensitive mutants (uvrA, urvB, urvC and rec-) believed to have defects in reactions, presumably enzymic, needed for repair of UV-damaged DNA. UV killing of a multiple auxotroph was, as expected, much diminished if the irradiated cells were 'starved' for 2 hr in amino acid-deficient medium before plating on nutrient agar. In K12 of wild-type UV-sensitivity (but not in UV-sensitive mutants) the effect of protecting R factors was reversed (i.e. they caused decreased survival) if the irradiated cells were 'starved' before plating—but not otherwise. Some R factors decreased the ability of K12, wild-type or UV-sensitive, to effect host-cell-reactivation of irradiated phage T1.

Perhaps plasmids which change the UV-susceptibility and host-cell-reactivating ability of their bacterial hosts do so by determining production of endodeoxyribonuclease(s) active on DNA strands which, because of UV damage, are not readily susceptible to host endonuclease(s) used in the repair of UV-damaged DNA. Such additional endonuclease activity might in some circumstances permit repair of otherwise irreparably damaged DNA regions—and in other circumstances effect DNA degradation exceeding the capacity of the repair mechanisms of the host.

Transduction of try Genes by Phages \(\phi 80\) \(pt\) in Escherichia coli. By J. P. Gratia (Laboratory of Microbiology and Hygiene, University of Liège, Belgium)

Among the transducing particles of Bacteriophage \(\phi 80\), some transduce different segments of the tryptophan (try) operon and are still able to form plaques: \(\phi 80t_{b}\) transduces try \(O_{C}=E-D\) (Sato, K. & Matsushiro, A. (1965), J. Mol. Biol. 14, 608), \(\phi 80pt_{i}\) (same reference) and \(\phi 80ptd_{sa}\) (produced by a defective lysogen; Gratia, J. P. (1967), Life Sci. 6, 209) transduce try \(C-B-A\). Such phages have been used to transduce try genes to Escherichia coli K12 strains carrying either point-mutations or deletions extending from try \(A\) to try \(C\) or further.

When point-mutants are infected by complementary \(\phi 80\) \(pt\) particles, i.e. try \(E^{-}\) or try \(B^{-}\)mutants by \(\phi 80t_{b}\) or by \(\phi 80pt_{i}\) (or-\(ptd_{sa}\)) respectively, most transductants are non-lysogenic haploid recombinants. When deletion mutants are infected by \(\phi 80pt_{i}\) or by \(\phi 80ptd_{sa}\) (host-range derivatives have been used), transductants also appear, on minimal medium if the deletion does not overreach try \(C\) and on medium supplemented with indole if the deletion is further extended and not entirely complemented. But these transductants are partial diploids, sometimes very stable, segregating very few try \(^{-}\) cured cells, or sometimes quite unstable according to the recipient used. When the deletion strains carry a prophage in \(att_{so}\), the transduction of try \(C-B-A\) always results in the formation of stable diploids. Indole-requiring transductants, having inherited the T1-receptor through \(\phi 80pt_{i}\), have been superinfected by \(\phi 80pt_{b}\) which is carrying the complementary genes, giving rise to 'supertransduced' try \(^{+}\) \(ind^{+}\) cells. These are stable triploids carrying overlapping segments of the try operon each linked to a prophage. With \(\phi 80pt_{b}\)-transductants of a double-mutant marked by a try \(C-B-A\) deletion and in addition by a mutation in try \(E\), and thus anthranilate-requiring, 'supertransduction' of anthranilate-independence by \(\phi 80pt_{b}\) has also been achieved.

When recipients are carrying a prophage in \(att_{so}\), transductants appear at a lower frequency. The reduction is particularly pronounced with \(\phi 80\) \(pt\), or -\(ptd_{sa}\) transducing immune bacteria.
Inducible Cephalosporinase in Enterobacter cloacae. By T. D. Hennessey (Department of Bacteriology, Royal Postgraduate Medical School, London, W. 12)

The terms 'penicillinase' and 'cephalosporinase' are descriptive for \(\beta\)-lactamase, they denote the class of substrate for which the relative specificity of the enzyme is greatest. Distribution of cephalosporinase among Gram-negative bacteria, and the significance of the enzyme in terms of contribution to cephalosporin resistance, have been the subjects of much work. Presence of inducible \(\beta\)-lactamase in Pseudomonas aeruginosa was clearly shown by Sabath et al. ((1965), Biochem. J. 96, 739), but demonstration of inducible enzyme in other Gram-negative bacteria has not been as well defined.

Three strains of Enterobacter cloacae (P99, 214, and 256) have been investigated with respect to inducibility of cephalosporinase. Their levels of resistance to cephaloridine were all about the same, but their activity in hydrolysing the drug, measured iodometrically in disrupted, log-phase cultures, was 1300, 200 and 2 enzyme units per mg. dry wt, respectively. It was argued that if production of the enzyme in strain 256 contributed to cephaloridine resistance to the same extent as in P99 and 214, the enzyme was likely to be inducible. To test this hypothesis strain 256 was grown in the presence of penicillin, in concentrations of 10 \(\mu\)g.–12 \(\mu\)g./ml. Cells were harvested and hydrolysis of cephaloridine by disrupted cells was compared with untreated controls. It was found that induction of cephalosporinase took place maximally when the penicillin concentration was 500 \(\mu\)g./ml., and that the ratio of induced:uninduced activities was 117. Using the same penicillin concentration, cephalosporinase activity of strain 214 was increased 5 times but there was no increase with P99 under similar conditions.

Ampicillin, cephaloridine and methicillin were also tested as inducers of cephalosporinase in strain 256. With a concentration of 500 \(\mu\)g./ml., both ampicillin and cephaloridine increased whilst methicillin decreased the rate of hydrolysis of cephaloridine.

Some Properties of a Phenol Oxidase Isolated from Aspergillus nidulans. By B. L. A. Carter and A. T. Bull (Department of Microbiology, Queen Elizabeth College, London, W. 8)

The melanin deposited in the hyphal walls of Aspergillus nidulans is a heterophenolic polymer composed, in part, of indolic residues (Bull, A. T. (1966), Abstr., IXth Int. Congr. Microbiol., p. 201, Moscow). This report details some properties of the phenol oxidase concerned in melanogenesis. Low and poor reproducibility of enzyme activity in cell-free extracts lead to a search for endogenous inhibitors, possibly released on cell disruption (\(^{14}\)C-pigment precursors are readily incorporated into melanin by whole mycelia (Bull, A. T., loc. cit.)). Products of phenol oxidase activity were examined as potential causes of the low enzyme levels but experiments designed to remove such phenolic/quinonoid metabolites produced on mycelial disruption did not enhance enzyme recovery. Acetone powders of cell-free extracts occasionally had activity suggesting that protein separation techniques might yield active material. The phenol oxidase was resolved eventually by DEAE-cellulose chromatography, being strongly adsorbed and only eluted with a buffer system containing 1-0 m-NaCl, while the inhibitor, also a protein, was eluted at low molarities. A reversed pattern of elution resulted when cationic exchangers were used.

A variety of phenol oxidase assay systems have been examined but we have found only those based upon coupled oxidation-reduction and oxygen uptake will allow accurate determination of initial rates and be unaffected by secondary reactions. The phenol oxidase confirmed to be of the tyrosinase type, has a high specific activity and its capacity for \(\alpha\)-hydroxylation appears to be much higher than that reported for other sources of tyrosinase. Investigations of the kinetics, electrophoresis and cellular location will be considered together with the enzyme's behaviour on dextran gels. The activity peak (E\(^1\)) from DEAE-cellulose columns is a protein of high M.W. (ca. 500,000) which dissociates into a polypeptide (E\(^2\)) of one-quarter this size. Kinetic data on these two components will be discussed. Tyrosinase production and regulation in A. nidulans and other aspects of metabolism are being studied under conditions of continuous culture; preliminary results of this work will be presented.
Isolation of DNA from Kinetoplasts of *Crithidia fasciculata*. By B. A. Newton* (Sub-Department of Chemical Microbiology, Department of Biochemistry, University of Cambridge)

The kinetoplast is located at the proximal end of the flagellum in members of the protozoan family Trypanosomatidae. This organelle has long been known to contain Feulgen-positive material and is now thought to resemble a mitochondrion in some respects. DNA extracted from trypanosomid flagellates can be separated into a major and a minor component by equilibrium centrifugation (Schildkraut et al. (1962), *Nature*, Lond. no. 196, 795). This communication will describe the isolation and some of the characteristics of the minor DNA component from *Crithidia fasciculata* and provide evidence that it originates from the kinetoplast.

Organisms were grown in a chemically defined medium (Kidder & Dutta (1958), *J. gen. Microbiol.* 18, 621), washed, suspended in 0-25 M sucrose containing 0-01 M sodium citrate and broken by shaking with glass beads. A fraction (P) containing cell pellicles, kinetoplasts and damaged nuclei was obtained by centrifugation at 3000 g for 10 min.; RNA was removed from this fraction by RNase and DNA extracted by the method of Marmur ((1961), *J. Mol. Biol.* 3, 208). This DNA banded as a single component \( \rho = 1.716 \text{g./c.c.} \) in a caesium chloride gradient. Treatment of P with pronase before DNA extraction yields material which can be separated into a major \( \rho = 1.716 \text{g./c.c.} \) and a minor \( \rho = 1.688 \text{g./c.c.} \) component by equilibrium centrifugation. The major component can be removed selectively by controlled DNase digestion of P before treatment with pronase; acridine orange staining has shown that this digestion does not remove kinetoplasts from the preparation.

The results indicate that the minor DNA component is largely associated with the kinetoplast but the possibility that small amounts exist in the nucleus cannot yet be eliminated. The minor component forms a very compact band in caesium chloride which reaches equilibrium after 2 hr centrifugation at 44,000 r.p.m., whereas the major component and most other types of DNA require 15-17 hr centrifugation at this speed to reach equilibrium. A similar rapidly banding DNA has recently been obtained from *Leishmania enriettii* (Du Buy, Mattern & Riley (1966), *Biochem. Biophys. Acta* 123, 298).

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An Infrared Study of the Effects of Partial Desiccation and Radiation on Nucleic Acids. By S. J. Webb and M. D. Dutasia (Department of Bacteriology, University of Saskatchewan, Saskatoon, Saskatchewan)

A study has been made of changes in the infrared (IR) spectra of RNA and DNA due to desiccation and irradiation and the modifying effects of myo-inositol on these changes.

Judging from changes in the depth and shifts in the various IR absorption bands, the desiccation of DNA between 75 and 55 % relative humidity (RH) removed water from -N, -NH, -NH\(_2\), and -OH groups and between 55 and 10 % RH from the C-O, and P=O groups. These observations agree with those of Bradbury Price & Wilkinson ((1961), *J. Mol. Biol.* 3, 301), Falk Hartman & Lord (1963), *J. Am. Chem. Soc.* 85, 387). However, the DNA of cells grown in a minimal salt medium was less hydrated than that of cells grown in an enriched medium and changes in its IR spectrum due to desiccation were less pronounced. Changes in the spectra of polyuridylic and polycytidylic acids were small compared to those of polyinosinic and polyadenylic acids. Severe alterations in the IR spectrum of DNA occurred as a result of irradiation with ultraviolet light and most of these changes appeared to be due to the loss of hydration sites.

All of the spectral changes observed were completely or partially prevented by myo-inositol but this protection was inhibited by the presence of NaCl. In addition, inositol was less able to prevent changes in polyuridylic and polycytidylic acids than it was changed in polyadenylic and polyinosinic acids. The results of this study add strong support to the

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Endogenous Metabolism and Survival of Azotobacter insigne vi5. By H. Stockdale, E. A. Dawes and D. W. Ribbons* (Department of Biochemistry, The University of Hull)

As a result of a survey of poly-β-hydroxybutyrate (PHB) in the Azotobacteriaceae (Stockdale, H., Ribbons, D. W. & Dawes, E. A. (1965), J. gen. Microbiol. 41, xviii), Azotobacter insigne strain vi5, a non-encysting organism of low carbohydrate content, was selected for further investigation. Its survival is influenced by the nutritional status of the cell and the conditions of starvation. Cells grown on a modified Norris's nitrogen-free medium containing 2% glucose died at a rate of about 1.5% of the initial viable population per hr when suspended in a basal salts medium with aeration. During this period the PHB content of the cells falls from 10% to <1% and the endogenous Q0, declines from 8 to less than unity. The initial r.q. is 0.89±0.025. After 18–20 hr starvation, the rate of viablility loss is accelerated to 4% of the initial viable population per hr and only 5% of the initially viable cells survive at 45–50 hr.

No protective effect could be shown when cells were suspended in sterile starvation medium from which cells had been removed by Millipore filtration, or in the supernatant of carbon-limited growth medium.

Rapid death (50% decline in viability in 40 min.) occurs when cells are suspended in distilled water. The effect is not mitigated by the presence of a carbon source (glucose) or nitrogen source (ammonium nitrate). Sodium chloride of the same ionic strength as basal medium decreases the death rate (18% decline in viability in 40 min.). The presence of a dialysed supernatant fraction of a cell sonicate in distilled water produces a death curve similar to that observed in basal medium. No such effect was seen with bovine serum albumin (Sigma Chemical Co.).

These observations substantiate the findings of Sobek, Charba & Foust ((1966), J. Bact. 92, 687).

Inhibition of Growth of Azotobacter by Oxygen. By H. Dalton and J. R. Postgate (A.R.C. Unit of Nitrogen Fixation, University of Sussex, Brighton, Sussex)

High oxygen tensions inhibit or delay growth of many aerobic and facultatively anaerobic bacteria (e.g. Moore, B. & Williams, R. S. (1911), Biochem. J. 5, 181) including Azotobacter spp. (Tschaepke, M. & Giambiagi, N. (1955), Arch. Mikrobiol. 21, 376). Inhibition or delay of growth of aerobes sometimes occurs when cultures are aerated very efficiently with normal air; it is generally attributed to depletion of CO2 known to be necessary for the initiation of growth of many aerobes (Walker, H. H. (1932), Science, 76, 602; Gladstone, G. P., Fildes, P. & Richardson, G. D. (1935), Br. J. Exp. Path. 16, 335). We report here a case in which inhibition of growth by efficient aeration seems attributable to oxygen.

Azotobacter chroococcum (NCIB 8003) was grown in continuous culture (D = 0.25 hr−1) in a nitrogen-free mannitol medium. Batch cultures (200 ml.) of a similar medium were inoculated with about 60 μg. dry wt organisms and stirred rapidly (about 2500 r.p.m.; O2 solution rate about 42 m-mole/l./hr) or slowly (about 300 r.p.m.; O2 solution rate about 3.2 m-mole/l./hr) under 400 ml./min. of CO2-free air. Growth was always delayed, and usually prevented absolutely, at the high stirring rate; it proceeded normally at the lower rate. Addition of 2% CO2 to the atmosphere did not prevent inhibition by rapid stirring. A similar effect was observed using air enriched with oxygen to 0.4 atm.

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The Repair of DNA in *Micrococcus radiodurans* following Ultra-violet Irradiation. By B. E. B. MOSELEY (Molteno Institute, University of Cambridge)

*Micrococcus radiodurans* is extremely resistant to ultraviolet (UV) and ionizing radiation. This resistance is caused by its ability enzymically to remove potentially lethal irradiation products from its DNA. For example, adjacent thymine bases in its DNA, dimered during UV irradiation, are lethal unless removed and replaced by new bases.

Two radiation sensitive mutants of *M. radiodurans* have been isolated and their excision and repair processes following UV irradiation compared with those of the wild-type. The UV radiation dose used was one which reduced the viability of the mutants by a factor exceeding 10⁶ though not affecting the viability of the wild-type.

Excision was studied by labelling cellular DNA with H²-thymidine, irradiating the cells and measuring the loss of label from DNA and its appearance in the incubation medium. In all three strains 0-6% of the thymine was dimerized and the dimer excised during the first hour of incubation. In the mutants, however, the excision rate of labelled thymine was five times faster than in the wild-type, after 4 hr incubation more than 90% of the DNA label being in the medium. In the wild-type about 20% of the DNA label was lost during this period.

Repair was studied by irradiating unlabelled cells, incubating them in a medium containing §H-thymidine and measuring incorporation of label into DNA. All three strains began to incorporate label but whereas in the wild type incorporation kept pace with excision, in the mutants the process stopped after less than 30 min.

It is concluded that the mutants, though they can recognize defects in DNA and excise them, are sensitive to radiation because the rate of excision of bases grossly exceeds the rate of re-incorporation and the DNA molecule disintegrates.

Chlortetracycline and Polyribosomes. By E. CUNDLiffe (Sub-Department of Chemical Microbiology, Department of Biochemistry, University of Cambridge)

*Bacillus megaterium* KM was steady-state labelled with §P and then converted to protoplasts which were incubated until the optical density began to increase exponentially; chlortetracycline (CTC) was then added and the incubation continued. Samples were then taken into chilled glass vials containing Triton X 100, a neutral detergent, and deoxyribonuclease, and the resulting lysates were layered directly on to sucrose density gradients (15–40% sucrose). Polyribosome profiles were visualized by counting the radioactivity in successive gradient fractions after centrifugation. With low concentrations of CTC (5 μg./ml.) a rapid breakdown of polyribosomes occurred, at higher concentrations there was less breakdown. It seems probable that CTC acts by interfering with the binding of amino-acyl-transfer RNA to the ribosome-messenger RNA complex. It is suggested that this has the effect of fixing an affected ribosome *in situ* on the messenger (or at least of inhibiting its movement temporarily) thereby also hindering the movement of those ribosomes further towards the 5' end of the messenger. Those ribosomes to the 3' side of the blockage are still able to proceed and to be released. Raising the concentration of CTC would be expected to affect more ribosomes per polyribosome and would therefore give less release.

With low concentrations of CTC, breakdown of polyribosomes was followed by a slower re-aggregation process which resulted in much ribosomal material being pelleted in the gradient tubes. The re-aggregation was sensitive to actinomycin D but was not inhibited by
concentrations of CTC sufficient to prevent all protein synthesis. The re-aggregated material contained many of the 70s ribosomes originally present as was shown by ribonuclease treatment of lysates. The re-aggregation process and the nature of its product are not clearly understood, although some species of RNA other than that in mature 70s ribosomes would appear to be necessary for the formation of the aggregates.

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