Acid Phosphatase Activity in *Klebsiella* species

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Acid phosphatase activity was determined in cultures of *Klebsiella* grown in semisynthetic media containing glucose and succinate as source of carbon. The phosphatase substrate was 7.6 m*M para*-nitrophenyl-phosphate in a pH 4.9, 0.09 *M* citrate buffer solution. Bacterial cultures were diluted out in buffer and adjusted with means of an EEL nephelometer to 58% of the deviation of the galvanometer (100% was the ground glass standard). After minutes contact of aliquots of suspension and reagent followed by centrifugation, the supernatant was read in a Spectronic 20 at 405 nm. The distribution of the values obtained within each of the 3 main species of *Klebsiella* in this study exhibited a single peak. The medians were as follow: *K. species* 0.3–0.4; *K. aerogenes* 0.2–0.3; *K. oxytoca* 0–0.2. The strains had been isolated from clinical cases in the university hospital. Criteria defining the severity of cases were established. There was an agreement between phosphatase activity and severity of cases as if the strains possessing high phosphatase activity were more virulent than the others.

Staphylococcus epidermidis with Positive Coagulase

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BAIRD-PARKER has defined in 1963 the coagulase as the main character of *Staphylococcus aureus*; the lack of coagulase being characteristic of *Staphylococcus epidermidis*. In 1967–68 BROWN and JEFFRIES have isolated a strain that presented the biochemical characters of *Staphylococcus epidermidis* although it was producing a coagulase. An identical phenomenon has been discovered in this laboratory upon the isolation of *Staphylococcus* from Swiss milks and various types of cheese, such as Vacherin 'Mont-d'Or'. 16 investigated strains have shown the following results according to Bergey's manual, 8th edn. 1 strain — *St. aureus*; 1 strain — *St. epidermidis* biotype 2; 1 strain — *St. aureus* with serotype 52.260; 13 strains — *St. epidermidis*, biotype 1 with positive coagulase. It is surprising that 81% of our strains do present this anomaly.

Comparison of the Reversed Passive Hemagglutination with the Electroimmunodiffusion Method for Hepatitis B Antigen

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In order to find the most rapid, effective and accurate HB Ag detection method, we employed comparatively the electroimmunodiffusion technic (EID) and the reversed passive hemagglutination one, to reveal HB Ag in 483 sera of 100 clinical patients admitted for renal epuration. 14% are found to have HB Ag with EID against 16% with hemagglutination, a method which gives us higher rates as EID and allows us to follow up the patients with more precision. Moreover, this technic requires control-tests in order to detect non specific positives (8%) and the results are given after 2 days against 1 day with EID. Finally, hemagglutination is three times more expensive as EID. The EID method is then preferable for a systematic detection.

Comparison of the Sensitivity of Electroimmunodiffusion, Reversed Passive Hemagglutination, Latex Test and Radioimmunoassay for Hepatitis B Antigen

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In order to compare the sensitivity of 4 technics, electroimmunodiffusion (EID), reversed passive hemagglutination (RPH), latex test and radioimmunoassay (RIA) for the detection of Hepatitis B Antigen, we tested 191 sera of clinical patients (endoscopy service). Included in this study was the detection of antibodies anti HB Ag with RIA and EID. 43 sera (22%) were found to be positive; 29 (15%) with antibodies and 17 (8,9%) with HB Ag. 3 sera contained both antibodies and Ag. The RIA test gave us 40 positive sera (29 with antibodies, 15 with Ag). EID: 15 positive sera (11 with antibodies, 5 with Ag). RPH and latex test, each 9 positive sera (9 HB Ag). Therefore, the RIA test is preferable in view of its sensitivity to detect HB Ag; EID shows the least sensitivity. For the detection of antibodies anti HB Ag, our results indicated that RIA also is the best method when compared to EID.

New Instruments for the Laboratory Diagnosis of Hog Cholera Using the Fluorescent Antibody Technique

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The hog cholera virus (HCV) multiplies in tissue culture usually without cytopathic effect. Due to this fact only indirect methods are applicable in demonstrating the virus. Immunofluorescent techniques showed to be most convenient to mark the virus in diagnostic methods such as isolation and neutralization tests. As the fluorescent antibody technique is very laborious we have looked for instruments to facilitate the technique. Two kind of chambers, the 'Liess Kammer' and the 'Lab-Tek-Chamber', have been found to be suitable. These tools make it possible to culture the cells, to incubate the virus, to fix, to stain and to wash the specimen slides without need to transfer them. The danger of damage or breakage is greatly reduced; at the same time the handling of the specimens has speeded up and is less difficult. These chambers are applicable time saving not only with non-CPE causing viruses, e.g. viruses isolated from diagnostic material can already be characterized by immunofluorescence in the first passage.

Molecular Structure of the R-Factor P111-ACS: Aggregate and Cointegrate

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The transferable R-factor P111-ACS (Ampicillin, Chloramphenicol, Streptomycin) and one of its infectious segregants P111-A have been physically characterized. *E. coli* strains harboring these plasmids produce a type I β-lactamase, as defined by PITTON (Rev. Phys. *65*, 15–93, 1972). Preliminary genetic observations suggest that P111-ACS is composed of independent replicons allowing the segregation of R-determinants during con-

jugation, with a preferential transfer of the A-determinant. Following these observations, the physical structure of P111-ACS and of its P111-A segregant has been determined. The plasmids were isolated by CsCl gradient centrifugation in presence of ethidium bromide. After removal of the dye, the contour length of the plasmids was measured by electron microscopy, using SV40 DNA molecules as internal standard. In *E. coli* K12 F⁻, P111-ACS shows both the structures of: 1. a plasmid aggregate formed by 19.2 μm molecules (inferred to be the transfer factor) and 3.2 μm molecules (inferred to be the R-determinants, A, C or S); 2. a plasmid cointegrate of 29.6 μm; occasionally a plasmid cointegrate of 22.8 μm was also observed. In the same host-cell P111-A shows only one structure: a molecule of 22.8 μm in length inferred to be the plasmid cointegrate TF-A. These results are in agreement with a cointegrate state of the R-determinants and the TF during the transfer the A-determinant probably having a preferential attachment to the TF compared to the other replicons.

A New Kanamycin/Neomycin Phosphotransferase Found in Staphylococci

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Aminoglycoside-phosphotransferases I, II and III have been reported to be involved in aminoglycoside resistance of gramnegative bacteria. In two strains of *Staph. aureus* and one strain of *Staph. epidermidis* a phosphorylating enzyme was observed, differing from these enzymes in the substrate profile and in pH optimum. Kanamycin/neomycin phosphotransferase IV rapidly phosphorylated and inactivated kanamycin A, B and C, neomycin B and C, paromomycin, gentamycin A and B, butirosin, lividomycin and ribostamycin. After two hours of incubation amikacin was completely inactivated, but phosphorylation was only slow. This certainly is the reason for the susceptibility of the strains against amikacin. Over the range 25–45 °C, there was significant phosphorylation with optimal activity at 37 °C. A temperature of 55 °C for 15 min inactivated the enzyme completely. Enzymatic activity generally was found over the pH range 5 to 9. For the kanamycins and the ribostamycin group, the optimal pH was 5.5 to 6.0 in citrate phosphate buffer, for the neomycin group 8.0 to 8.5 in *Tris*-maleate buffer. In two strains, resistance to aminoglycosides was found to be plasmid-mediated. The characterization of the resistance plasmids by sucrose gradient centrifugation and electron microscopy revealed molecular sizes of 36.5 (*Staph. aureus* E 142) and 21.5 (*Staph. epidermidis* 147) megadaltons respectively. Preliminary experiments indicate that resistance in *Staph. aureus* 170 might be governed by chromosomally located genes.

Mutual Influence Between λ-Phages and R-Factors

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A few years ago we stated that MS_2-phage-infection of cells carrying R-factors does diminish or even prevent the R-transfer (Path. Microbiol. *40*, 153, 1974, and *41*, 194, 1974). Now we examined the influence of fi⁺ R-factors on λ-lysogeny and vice-versa and obtained the following result: If *E. coli* K12 carrying R192 grown in Columbia broth were infected with phage λ at a multiplicity of infection of 10 PfU and incubated for 24 h at 37 °C, we could not detect afterwards any lysogenic clones out of one thousand. If *E. coli* K12, which are not carrying an R-factor, were infected and incubated under the same conditions, we found about 70% out of all cells lysogenic after 24 h. After further incubation for 24 h the R-free progeny was 100% lysogenic, the cells carrying an R-factor however only for 30%. If λ-lysogenic and λ-sensitive cells of the same strain were R-infected under equal conditions, both transferred the R-factor in the same frequency. If, however, R-carrying cells were λ-lysogenic, we detected a serious reduction of the frequency of R-transfer. With derepressed fi⁺ R-factors in doing so the ability for building sex-pili was lost. Therefore the interactions between fi⁺ R-factors and λ-phages seems to depend on which of the two genomes is in the cell first. As these investigations were also done with wild-R-factors and wild-strains of *E. coli*, they allow allusions to the influence of the epidemiology of R-factors. The molecular biological explanation of these phenomenona is at work.

Proteus mirabilis Wild-Strains as Donors and Recipients of Wild R-Factors

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At the microbiological examination of urine isolates coli bacteria are isolated beside cells of *Proteus mirabilis*. Thereby most of all the coli bacteria are containing infectious R-factors while the *Proteus* strains are R-negative and do only possess the well-known chromosomal resistance against polymyxin B and tetracycline. This observation shows that – considering the reception of R-factors – *Proteus* does behave differently from the rest of the *Enterobacteriaceae*. Therefore we investigated the chloramphenicol-resistance-transfer of multiple-resistant *Proteus* into antibiotic-susceptible *Proteus* cells, the R-infection of *E. coli* into *Proteus mirabilis* and vice-versa. We obtained the following results: 2 out of 8 multiple-resistant *Proteus* wild-strains did not transfer their resistances on the 39 recipient-strains. The other 6 donor-strains transferred their resistance on the following number of the recipient-strains: 2, 6, 8, 10, 11 and 31. Six of the 39 recipient-strains did not act as recipients with any of the 8 donor strains, 10 strains conjugated with only 1, 10 with only 2 and the rest with 3 to 5 of the donor strains. The frequency of transfer came to 10^{-6} and 10^{-7}. Besides the resistance for chloramphenicol mostly also the other resistances of the donor strains were transferred. From the 39 *Proteus* recipient strains only 12 accepted – with a low frequency – R192 from *E. coli* K12, and out of 8 *Proteus* donor strains only 1 was able to transfer the resistance into restriction-free cells of *E. coli* K12 with the low frequency of 5×10^{-7}. From these results we conclude that *Proteus mirabilis* is a bad donor and recipient for R-factors.

Rifampicin-Resistance in *E. coli*: Comparison of Microbiological and Enzymatic Properties

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The antibiotic rifampicin inhibits the growth of *Escherichia coli* by forming a tight complex with the bacterial RNA polymerase and thus inhibiting the enzyme. Cells