We now report the reversible blocking of the remaining amino groups of phenylthiocarbamoyl-phenylalanine (B-1) insulin with suitable protecting groups. These must survive the trifluoroacetic acid treatment employed to remove phenylalanine B-1 and expose the amino group of valine B-2. It is then possible to couple amino acids back into position B-1 with high efficiency and specificity of location, and we give examples of preliminary coupling experiments of this type. We intend to continue the degradation further to make a series of shortened insulins available for study and to recouple amino acids and peptides on to the shortened chain.

Several sets of conditions were used to produce the phenylthiocarbamoyl-insulin. Kinetic studies, monitored by isoelectric focusing in polyacrylamide gel (Wrigley, 1968), showed that the maximum yield of phenylthiocarbamoylphenylalanine B-1 insulin was obtained in pyridine-water (3:1, v/v) with a phenyl isothiocyanate/insulin ratio 1:2 (Brandenburg, 1969) after approx. 22h at 3°C. This derivative was separated from other phenylthiocarbamoyl-insulins by DEAE-cellulose chromatography at 3°C. The eluent was 0.01M-tris-1mM-EDTA-0.03M-NaCl-6.95M-urea, pH 7.7 (at room temperature). The phthaloyl and trifluoroacetyl groups were found to be resistant to trifluoroacetic acid. The former group was applied by using *N*-ethoxycarbonylphthalimide (Nefkens, Tesser & Nivand, 1960), but in our hands could not be removed easily. Phenyl trifluoroacetate (Weygand & Röpsch, 1960) was used for the second group (the more conventional thiol esters cause reduction of disulphide bridges). Conditions were: 0.04% solution of insulin in anhydrous dimethylformamide (obtained by rotary evaporation of an aqueous solution), 5% w/v MgO, 1.6% v/v reagent for 3h at 20°C. Removal was by *m*-NH₃ for 24h at 20°C. Trifluoroacetic acid treatment (1h at 20°C) of the trifluoroacetylated and phenylthiocarbamoylated derivative produced the required intermediate. Couplings with normal and radioactive trifluoroacetyl-amino acid active esters were carried out on the triethylamine salt of the derivative in anhydrous dimethylformamide.

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Chain Lengths of the Oligodeoxyribonucleotides Formed by the Nucleoside Triphosphate-Dependent Deoxyribonuclease of *Mycobacterium smegmatis*

By F. G. WINDER and M. LAVIN. (*Department of Biochemistry, Trinity College, Dublin 2, Irish Republic*)

The presence of a nucleoside triphosphate-dependent deoxyribonuclease in *Mycobacterium smegmatis* has been reported (Winder & Coughlan, 1967, 1969). The deoxyribonucleotides formed by this enzyme are largely short-chain oligonucleotides (Winder & Lavin, 1969). These have now been characterized further.

The enzyme was partially purified as described by Winder & Lavin (1969) and incubation mixtures were as described by these authors except that [³²P]DNA, prepared as described by Marmur (1967), was employed. Incubation periods ranging from 15 to 60min were used, the reaction was stopped with HClO₄, and the soluble fraction was neutralized with KOH.

This fraction was chromatographed on DEAE-Sephadex A-25 with 0.02M-tris-HCl buffer, pH 7.6, containing 7M-urea and a linear 0.1-0.27M-NaCl concentration gradient (Satoh & Inoue, 1969). This gave three major discrete peaks of ³²P radioactivity, corresponding to di-, tri- and tetra-nucleotides. Raising the NaCl concentration to 1M eluted some further material. The chain length of the oligonucleotides in each peak was determined by concentration (by using Dowex 1) followed by chromatography on Sephadex G-25 in 0.5M-NH₄HCO₃ containing 8M-urea (Stanley, 1967) and on DEAE-cellulose paper in one dimension (Furlong, 1969), and by determining the proportion of monoester phosphate (Furlong, 1969).

The results showed that the amounts of mononucleotide and P_i released from the DNA were small. The acid-soluble products consisted on average of 23% of dinucleotide, 40% of trinucleotide, 20% of tetranucleotide and 10% of oligonucleotides of greater chain length. Two-dimensional paper chromatography (Sulkowski & Laskowski, 1962) showed that at least eight dinucleotides were formed. Susceptibility to specific phosphatases showed that the oligonucleotides were 5'-phosphate-terminated.

These results, taken together with the equivalence of ATP used to deoxyribose solubilized, indicated that about 3 molecules of ATP were used/phosphodiester bond split. The findings were similar to

those of Anai, Hirahashi & Takagi (1970) with the corresponding enzyme from *Micrococcus lyso-deikticus*.

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Role of Converting Bacteriophage in Antibiotic Resistance of Strains of *Staphylococcus aureus*

By F. A. CLYNES and L. K. DUNICAN. (*Department of Microbiology, University College, Galway, Irish Republic*)

Bacteriophage has been found to be a significant vector of antibiotic-resistance among strains of *Staphylococcus aureus* (Ritz & Baldwin, 1958; Mitsuhashi, Hasimoto, Kono & Morimura, 1965; Mitsuhashi, Oshima, Kawaharada & Hasimoto, 1965). However, its role in antibiotic-resistance within the recipient organism has not been clarified.

The phenomenon of bacteriophage conversion has been established in *Corynebacterium diphtheriae* (Freeman, 1951), where toxin production by the organism is dependent on the lysogenic state. The present study indicates that a somewhat similar relationship exists between probacteriophage and antibiotic-resistance in some strains of *S. aureus*.

S. aureus strain B was used as donor organism with drug-sensitive strains 8325 (containing P11, P12, P13), 8325-4, 8325-4 (P11), 8325-4 (P12) and 8325-4 (P13, P11 de) as recipients. Mitomycin C induction of *S. aureus* strain B yielded probacteriophage GU capable of transferring resistance to penicillin, tetracycline, cadmium, arsenate, lead and mercury in different combinations; loss of a β -haemolysin sometimes accompanied the acquisition of antibiotic-resistance. In addition, the transductants had required immunity to pro-

bacteriophage GU and to induced lysates of itself and of the other transductants prepared. Transductants were now capable of acting as donors, their induced lysates transferring their acquired resistances to new recipients.

Growth of transductants at 43°C, treatment known to eliminate some extrachromosomal DNA, yielded a low percentage of sensitive variants; these variants had simultaneously lost their antibiotic resistances and their immunity to bacteriophage GU.

Transduction of drug-sensitive hospital strains of *S. aureus* also gave cultures showing immunity to bacteriophage GU. Although probacteriophage pattern of these strains is not defined, as in Novick's strains, these results indicate that the lysogenic state and antibiotic-resistance are interdependent here also.

It was concluded that transducing bacteriophage and resistance markers are inseparable in these strains of *S. aureus* and either borne as a plasmid-bacteriophage association or within the bacteriophage genome itself.

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The Isolation of Rat Liver Phenol Sulphotransferase

By F. A. McEVOY and J. CARROLL. (*Department of Biochemistry, Trinity College, Dublin 2, Irish Republic*.)

The sulphotransferases catalyse the transfer of the sulphuryl group of adenosine 3'-phosphate 5'-sulphatophosphate to an acceptor to form the sulphate ester and adenosine 3',5'-diphosphate. The acceptors include hydroxyl-containing compounds, and the sulphurylation of simple phenols, steroids and tyrosine esters is of particular interest. Using ion-exchange chromatography Banerjee & Roy (1966) separated the steroid from the phenol-sulphurylating activity of guinea-pig liver, and this latter fraction was also active towards tyrosyl esters (Banerjee & Roy, 1968). Mattock & Jones (1970) purified the L-tyrosine methyl ester-sulphurylating activity of female rat liver 70-fold, but this preparation was contaminated with phenol sulphotransferase (adenosine 3'-phosphate 5'-sulphatophosphate-phenol sulphotransferase, EC 2.8.2.1). Studies carried out in this laboratory on the susceptibility to denaturation, together with the effects of