Effect of Proteolytic Enzymes on Deoxyribonucleohistone

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The susceptibility to attack of histone and deoxyribonucleohistone (DNH) by trypsin and chymotrypsin are markedly different. Trypsin and chymotrypsin rapidly degrade histone with loss of all secondary structure in the protein, as monitored by changes in circular dichroism. On the other hand, the only fraction to be attacked by trypsin in DNH is F1. The action of trypsin renders DNH soluble in 0.2M-NaCl.

Chymotrypsin attacks fractions F1, F3 and F2b in DNH and produces a loss of secondary structure of the protein but little dissociation from the DNA. The dissociation properties in salt are, however, greatly modified and this enables a nucleohistone complex containing only the F2a2, F2b and F2a1 fractions to be obtained. The properties of this complex show that the differences in the near-u.v. circular dichroism between DNA and DNH are due to fraction F3, but that the differences in viscosity are caused by fraction F2b.

Methylation of $\mu 2$ Bacteriophage Ribonucleic Acid by the Carcinogen N-Methyl-N-nitrosourea: Evidence for O-Methylation

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Carcinogens of the alkyl nitrosamide type may be distinguished from typical alkylating agents such as dimethyl sulphate by their ability to attack O atoms in nucleosides (Loveless, 1969) or in nucleic acids (Lawley & Thatcher, 1970).

Messenger RNA may be a significant target of such carcinogens (cf. Villa-Treviño, 1967), and the methylation of a model single-stranded RNA with biological activity was therefore studied.

Bacteriophage $\mu 2$ RNA treated with $N[^{14}C]$ -methyl-N-nitrosourea was degraded to purine bases with acid or to nucleosides enzymically, and chromatographed on Dowex 50 (H^+ form or NH_4^+ form), together with appropriate marker methylated bases or nucleosides; separation of nucleosides was well achieved at pH 8.9 (Junowicz & Spencer, 1969).

Methylnitrosourea was distinguished from dimethyl sulphate in yielding a different distribution of minor products: O^6 -methylguanosine was formed, and more 7-methyladenine, but less 3-methylcytosine and 1-methyladenine; 7-methylguanine was the major product from both. Also, some

degradation of RNA accompanied methylation by methylnitrosourea as shown by analytical ultracentrifugation of RNA liberated from methylated bacteriophage; this did not occur with bacteriophage alkylated extensively with mono- or difunctional sulphur mustards (Edwards, Shooter & Lawley, 1970). A small number (about ten) of alkylations of RNA in bacteriophage by all agents inactivated, and degradation by methylnitrosourea could account for less than one-fifth of lethal events; 7-alkylguanines (except for mustard-induced cross-links) appeared not to be lesions.

Edwards, P. A., Shooter, K. V. & Lawley, P. D. (1970). *Rep. Br. Emp. Cancer Campn.*, **47**, 21.

Junowicz, E. & Spencer, J. H. (1969). *J. Chromat.* **44**, 342.

Lawley, P. D. & Thatcher, C. J. (1970). *Biochem. J.* **116**, 693.

Loveless, A. (1969). *Nature, Lond.*, **223**, 206.

Villa-Treviño, S. (1967). *Biochem. J.* **105**, 625.

Studies on Aetiology of Urolithiasis in Karachi

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The incidence of urinary calculus disease in West Pakistan appears to be particularly high, but little data are available on its aetiology. Incidence of vesical calculi is common in boys under 10 years of age and renal calculi occurred in male adults (Ilahi, 1967; Jehan & Rahman, 1970). Ureteric and urethral stones were found exclusively in males. The present work was undertaken to investigate the type and composition of urinary stones and calculogenic factors. Thirty stone-formers and 17 normal cases were investigated. Most calculi on analysis were found to be mixed-type. Oxalates and phosphates of calcium, ammonium and urates were the common constituents and calcium oxalate stones were more common in the kidney and ammonium urates in the bladder. The nuclei of renal and vesical calculi were similar, suggesting the renal origin of vesical calculi. Urinary pH varied in stone-formers on either side of pH 7. A correlation was found between surface layers of the stone and pH of the urine; uric acid stones were found in an acid medium and phosphate stones in an alkaline medium. Crystalluria of calcium oxalate dihydrate was most commonly seen in stone-formers. Hypercalciuria was noted and serum protein and vitamin A concentrations were found to be low in stone-formers. Mucoproteins in serum and urine were higher in stone-former adults whereas uric acid excretion was decreased. No hyperplasia or kera-

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