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: O⁶-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.

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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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tinization of urinary-tract mucosa was seen on histopathological examination.

The intake of animal protein, fat and vitamin A in stone-former adults was low. The amount of phosphate in the diet was found to be high in stone-former adults and the amount of protein and carbohydrate was found to be low in stone-former children. The aetiological factors contributing to the process of calculogenesis seem to be lack of control of urinary pH, crystalluria of calcium oxalate dihydrate, hypercalciuria, raised serum and urine mucoprotein, low protein and vitamin A intake and poor nutrition.

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The Uptake of Calcium from Urine *in vitro* by Powdered Phosphates and Oxalates

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Measurements of the quantitative uptake of ions from urine by the crystalline constituents of renal calculi might provide information about the degree of saturation of various urines. Also, information might be obtained on the presence of inhibitors of stone formation.

Experiments were performed in which powdered minerals and renal calculi were added to urine at controlled pH at 37°C. At pH 6.5 some sort of uptake occurs rapidly on to powdered phosphate calculus and a sample of hydroxyapatite. This occurs at all concentrations of urine calcium above about 0.25 mmol/l. It is not possible from these experiments to say whether the uptake was due to crystallization or entry into the liquid film surrounding the crystals. If it was the former then there seems to be little activity from inhibitors in urine. If it was the latter then from the high degree of uptake it seems that in the renal tract the local high concentration round a microlith may allow incorporation of salts into the stone by subsequent molecular rearrangement.

At pH 5.5 there is virtually no uptake on to phosphates. Moreover, there is very little uptake on to oxalate powder at this pH and at pH 6.5. This suggests either that oxalate of this particular structure is very much slower at growing, or that inhibitors are more effective.

The Detection of Heterozygotes for Homocystinuria by Oral Loading with L-Methionine

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Homocystinuria is an inborn error of methionine metabolism caused by deficiency of cystathionine synthase (EC 4.2.1.21). Up to now no conclusive distinction has been demonstrated between heterozygotes for homocystinuria and normal subjects after loading tests with L-methionine (see, e.g., Brenton, Cusworth & Gaull, 1965; Chase, Goodman & O'Brien, 1967).

In the present study oral L-methionine loads have been administered to six parents of homocystinuric patients and to seven normal adult subjects. Measurement of plasma concentrations of methionine, cysteine-homocysteine disulphide and homocysteine at specific times after methionine load were performed by automatic ion-exchange chromatography (Piez & Morris, 1960). The use of an automatic continuous-flow analysis system, in which the column effluent was monitored with iodoplatinate ($\text{H}_2\text{PtCl}_6\text{-KI}$) reagent (Awwad & Adelstein, 1966) and ninhydrin in parallel, facilitated the identification and quantitative determination of sulphur-containing amino acids in the urine of subjects loaded with L-methionine (Fowler, B. & Robins, A. J., unpublished work). The identity of sulphur-containing amino acids in urine was confirmed by analysis of relevant fractions of the column effluent by paper chromatography before and after treatment with Raney nickel.

Plasma amino acid analyses revealed significant differences in methionine concentration between heterozygotes and normal subjects at 4 h post load ($P < 0.01$) whereas differences in cysteine-homocysteine disulphide were highly significant ($P < 0.001$) at 12 h post load. Homocystine (0.002–0.011 $\mu\text{mol/ml}$) was detected in some post-load plasma samples from heterozygotes but not in normal subjects. The urine of each heterozygote contained significant amounts of homocystine (0.011–0.077 mmol/24 h) whereas only one normal subject excreted homocystine, but in an amount substantially less than that excreted by heterozygotes.

It was concluded that, in the subjects so far studied, the plasma concentration of cysteine-homocysteine disulphide after methionine load provided the most reliable biochemical parameter for distinction between heterozygotes for homocystinuria and normal subjects.