THE FEULGEN NUCLEAL REACTION

Acid Degradation of Sperm Deoxyribonucleic Acid

WE have been seeking methods for preparing deoxyribonucleotides and nucleosides by chemical degradation of sperm deoxyribonucleic acid under acid conditions, and thought it of interest to test for liberated aldehydo groups on the various products. The results have proved of some interest in regard to the views of various workers on the interpretation of results of the Feulgen reaction[1] and more particularly in the light of views described by one of us with another colleague in the following communication.

Sperm deoxyribonucleic acid, prepared in typically fibrous form from soft herring roe by the elegant method of Mirsky and Pollister[2], can be freed from all but traces of protein by chloroform/butanol treatment and appears to be combined with protein in sperm by electrovalent linkages. Such material slowly becomes Feulgen-positive on being steeped for two to three hours in Schiff's reagent at *p*H 3. This deoxyribonucleic acid can be irreversibly depolymerized by dialysis of a solution of its sodium salt against tap water for several days, or more rapidly by autolysis (*p*H 3) at 100° for 3 minutes. The product, prepared by the dialysis method, when precipitated by acid ethanol (*p*H 3·5), loses all semblance of its fibrous nature and can be obtained as a white powder of almost crystalline appearance. It is insoluble at an acid *p*H and is converted into an insoluble gum on contact with water. On treatment with Schiff's reagent, the gum-like product instantly gives a strongly positive Feulgen-staining reaction which reaches its maximum intensity in about three hours. The dye so formed is completely non-soluble and non-diffusible, the reagent above the dyed nucleic acid remaining clear for more than thirty hours (cf. Stedman[3]).

The most significant finding is that this partly degraded product having a slightly higher nitrogen content than the original (N/P = 11/3) still retained almost completely its content of purine bases, so we have shown, in contrast to Feulgen's original ideas, that it is unnecessary to remove all purine bases to obtain Feulgen-positive material. We believe that polymeric linkages, by which the maximum macromolecular state (such as is possessed by material isolated in the fibrous form) of native deoxyribonucleic acid is maintained, involve highly labile sugar aldehydo linkages, and these can readily be broken by acid or depolymerase enzymes. For more profound chemical degradation under acid conditions we have used sperm deoxyribonucleic acid provided by the Glaxo Laboratories, Ltd., and have treated it initially with 1·5 per cent methanolic hydrogen chloride at room temperature. We anticipated that such a method would protect any sugar aldehydo groups by acetal or glycoside formation, would not convert deoxyribose into ω-hydroxy lævulinic aldehyde and would not remove organically bound phosphate residues.

We have obtained clear evidence that the first components to be liberated are, indeed, the purine-containing fractions, which pass into the methanolic hydrogen chloride solution, in which they can be estimated by ultra-violet absorption methods, and then isolated in crystalline form or as the picrates. The methanol-insoluble residue remaining after the bulk of the purine bases have been removed corresponds in properties to a methyl derivative (N, 7–8; OMe 6–7 per cent) of the well-known thymic acid[4]. It is soluble in water, $[\alpha]_D + 30°$, and after mild acid treatment (*p*H 3) to remove methoxyl groups, or standing in Feulgen's reagent, it gives an intense 'Feulgen-positive' stain with Schiff's reagent. The dye formed is completely soluble in water, and if diffused into protein material is strongly adsorbed by the protein as in Stedman's experiments[3]. From the modified thymic acid, by increasing step-wise the acid concentration in the methanolic hydrogen chloride treatment up to *p*H 3·6, we have obtained a variety of soluble and insoluble fractions which are difficult to characterize precisely.

Using elementary analysis for carbon, hydrogen, nitrogen and phosphorus, Feulgen and Dische[5] estimations, ultra-violet absorption and the usual methods for estimating purine bases, we have obtained evidence of the presence of guanine, adenine, 'sperm guanosine', 'sperm adenylic' acid and deoxyribose phosphates. There invariably remained a residue completely insoluble in methanolic hydrogen chloride consisting apparently almost completely of the more stable 'sperm thymylic' acid, from which crystalline thymine in good yield can readily be obtained by sublimation methods.

We think it highly improbable from the proportion of the nucleotides so far estimated that sperm deoxyribonucleic acid contains a simple tetranucleotide repeating unit, and consider that Levene's[6] formula will need to be revised to apply to this deoxyribonucleic acid. From the ease of liberation of sugar aldehydo groups, there would appear to be no reason to doubt that the Feulgen reaction in the hands of cytologists does locate deoxyribonucleic acid at the precise site of its occurrence. On the other hand, the varying intensities of the Feulgen colour, as noted particularly by Bauer[7] and Hillary[8] (see also Stefano[9]), can readily be accounted for by the high lability under acid conditions of deoxyribonucleic acid and more particularly of its deoxyribofuranose component (see following communication).

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Mechanism of the Feulgen Nucleal Reaction

In a previous paper[1] from these laboratories, it was shown that the mechanism for the Dische[2] diphenylamine reaction for deoxyribonucleic acid depends upon the conversion by acid treatment of the deoxyribose component into *w*-hydroxy lævulinic aldehyde, which then coupled with diphenylamine to give a blue dye with a characteristic absorption band. Extension of this work with Dr. Ethel Teece has led

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