212. *Deoxypentose Nucleic Acids. Part I. Preparation of the Tetrasodium Salt of the Deoxypentose Nucleic Acid of Calf Thymus.*

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The preparation of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus is described by a method in which the nucleoprotein is first isolated and then decomposed; during these processes the reaction did not vary significantly from pH 7. The analytical data support the relative proportions of nucleotides indicated by titration (Gulland, Jordan, and Taylor, Part II, this vol., p. 1131).

Gulland, Barker, and Jordan (*Ann. Rev. Biochem.*, 1945, **14**, 175) have suggested that the direct isolation of nucleic acids from tissues may lead to a degraded product, and they consider that a more satisfactory procedure is to isolate and subsequently decompose the nucleoprotein. In the method which has been developed, therefore, the deoxypentose nucleoprotein of calf thymus was first isolated by making use of the remarkable difference in solubility of the nucleoprotein in sodium chloride solutions of different strengths described by Mirsky and Pollister (*Proc. Nat. Acad. Sci. U.S.*, 1942, **28**, 344; *Biol. Symposia*, 1943, **10**, 24). The deoxypentose nucleic acid was then separated from the protein by the method introduced by Sevag, Lackman, and Smolens (*J. Biol. Chem.*, 1938, **124**, 425).

The material obtained by this preparation gave satisfactory analytical figures for carbon, hydrogen, phosphorus, and sodium, but the nitrogen value was low, being 15·4% compared with the theoretical value of 15·9% based on a polynucleotide containing the four nucleotides in equimolecular quantities. Furthermore the ratio of the purine nitrogen content to that of pyrimidine nitrogen was low, a mean value of 1·6 being obtained in place of the theoretical value of 2·0. These data may be interpreted with the aid of the results of electrometric titration (Part II, *loc. cit.*), which, whilst indicating that for every four atoms of phosphorus there is 1·0 radical of thymine and 1·0 of guanine, suggest that the amount of cytosine may be as high as 1·2 radicals and of adenine as low as 0·8 radical. Re-calculation of the theoretical nitrogen analysis on this basis gives a value of 15·5%, and the purine nitrogen/pyrimidine nitrogen ratio has the value 1·6. These data are in satisfactory agreement with the observed results. The theoretical values for carbon, hydrogen, phosphorus, and sodium are not affected appreciably by this change in the nucleotide ratio in the statistical polynucleotide.

Experimental

Isolation of the Tetrasodium Salt of the Deoxypentose Nucleic Acid of Thymus Gland.—Fresh frozen calf thymus glands (54·5 kg.) were minced and suspended in 0·9% sodium chloride (54 l.) and milled to produce a fine suspension. This suspension was centrifuged (6300 r.p.m.) and the solid material resuspended in 0·9% sodium chloride (45·5 l.) and milled and centrifuged as before. The ribonucleoproteins, together with cytoplasmic material, were obtained from the combined extracts (120 l.) by adding an equal volume of industrial methylated spirit, and the precipitated solid was washed by decantation with 50%, then 100% industrial methylated spirit, collected, and dried in a vacuum at room temperature. Yield, 2·68 kg.

The tissues, which were now free of material containing pentose, were suspended in 10% sodium chloride (214 l.) with vigorous mechanical stirring at 0°. At this stage the viscosity of the solution increased considerably (cf. Mirsky and Pollister, *loc. cit.*). After extraction at 0° for 48 hours, the insoluble material was removed by centrifuging (6300 r.p.m.) and the deoxypentose nucleoprotein precipitated from the resultant solution (pH 6·5) by the addition of an equal volume of industrial methylated spirit. The precipitated solid was washed with 70%, then 100% industrial methylated spirit and dried in a vacuum at room temperature. Yield, 1·69 kg. of a very slightly yellow fibrous solid.

The nucleoprotein (500 g.) was powdered to assist solution and dissolved in 10% sodium chloride (45·5 l.) with vigorous mechanical stirring. The solution, which was viscous, was clarified by centrifuging (6300 r.p.m.). To the clear solution was added an equal volume of a mixture of chloroform (35 parts) and amyl alcohol (10 parts), and the mixture was emulsified by rapid mechanical stirring. The emulsion was then separated by centrifuging (DeLaval, model " 500 ", disc type bowl, 5500 r.p.m.) into three parts: (i) the chloroform–amyl alcohol mixture, (ii) a solution containing the sodium salt of deoxypentose nucleic acid and nucleoprotein, (iii) a gel of protein hydrochloride and the chloroform–amyl alcohol mixture. The protein gel remained in the bowl of the centrifuge whereas the chloroform–amyl alcohol mixture and the solution of nucleic acid and nucleoprotein were discharged from separate outlets. The last mentioned solution was again emulsified with the chloroform–amyl alcohol mixture and the process repeated until no gel was formed on emulsification; this required nine emulsifications. The sodium salt of the deoxypentose nucleic acid was precipitated by the addition of an equal volume of industrial methylated spirit, washed free from chloride with 70% industrial methylated spirit, then 100% ethyl alcohol, and finally ether, and dried in a vacuum at room temperature. Yields from two 500 g. quantities of nucleoprotein were 130 g. and 150 g. of a white fibrous solid giving negative biuret and Sakaguchi tests [Found on sample dried at 110° in a vacuum over phosphoric oxide: C, 35·5, 35·5,

35·3; H, 3·68, 3·83, 3·56; N, 15·3, 15·3, 15·4; P, 9·35, 9·32, 9·32; Na, 6·9, 6·8 (gravimetric), 7·1 (colorimetric). Calc. for a large polynucleotide consisting of the tetrasodium salts of tetranucleotides containing on average 1 mol. each of guanine, adenine, cytosine, and thymine deoxypentose nucleotides, *i.e.* $(C_{39}H_{45}O_{24}N_{15}P_4Na_4)_x$: C, 35·4; H, 3·4; N, 15·9; P, 9·4; Na, 6·95. Calc. for a large polynucleotide consisting of the tetrasodium salts of tetranucleotides containing on average 1 mol. each of guanine and thymine, and 1·2 mols. of cytosine and 0·8 mol. of adenine deoxypentose nucleotides, *i.e.* $(C_{38\cdot8}H_{45}O_{24\cdot2}N_{14\cdot6}P_4Na_4)_x$: C, 35·4; H, 3·4; N, 15·5; P, 9·4; Na, 6·98%. In both cases the additional HONa atoms of the two terminal nucleotides of a straight-chain polynucleotide have been ignored, since their contribution is negligible if the size of the polynucleotide is large].

Determination of Purine Nitrogen/Pyrimidine Nitrogen Ratio.—The method used was that of precipitation of purines with silver sulphate (Schmidt and Levene, *J. Biol. Chem.*, 1938, **126**, 423). The sodium salt of deoxypentose nucleic acid (*ca.* 30 mg.) was hydrolysed by boiling under reflux with 5% sulphuric acid (7 ml.) for 1 hour. When cold, the solution was diluted to 25 ml., the total nitrogen was determined on a portion (2 ml.), and 2 ml. samples were treated with an equal volume of saturated silver sulphate. The solution containing the precipitate of the silver salts of guanine and adenine was cooled at 0° for ½ hour. The precipitate was separated by centrifuging and washed twice by stirring at 0° with a saturated solution of silver sulphate (2 ml.) followed by centrifuging. The solution was combined with the washings and analysed for pyrimidine nitrogen. The precipitate was decomposed with dilute hydrochloric acid (2 ml.), and the silver chloride removed by centrifuging and washed twice with dilute hydrochloric acid (2 ml.). The solution was combined with the washings and analysed for purine nitrogen. The purine nitrogen/pyrimidine nitrogen ratio was found to be 1·60, 1·63, 1·60, 1·58, 1·57 for five separate hydrolyses; mean, 1·60. The discrepancy between this value and the theoretical of 2 for a statistical polytetranucleotide is greater than the experimental error indicated by the Table. Nevertheless we should not have inclined to attribute undue significance to the ratio, were it not for the agreement between it, the analytical data, and the results of electrometric titration; this uniformity of trend does suggest that the purine/pyrimidine nitrogen now recorded has real meaning.

In order to confirm the accuracy of the method claimed by Schmidt and Levene (*loc. cit.*), a series of analyses of known purine and pyrimidine derivatives was performed; representative results are recorded in the Table. Determination of the purine/pyrimidine nitrogen ratio of two samples of yeast ribonucleic acid was made for comparison, the results being 1·84, 1·86; 1·84, 1·90.

Substance.	Total N, mg.	Purine N, mg.	Pyrimidine N, mg.	Recovery, %.
Guanine	10·42, 10·17, 10·38, 9·80	10·48, 10·29, 10·56, 9·70	—	100·6, 101·2, 101·7, 99·0
Adenine	10·50	10·22	—	97·3
Hypoxanthine	8·06	7·90	—	98·1
Guanosine	9·53, 8·78	9·62, 8·91	—	101·0, 101·4
Adenosine	8·49, 9·40	8·43, 9·30	—	99·3, 98·9
Thymine	1·46, 7·20	—	1·39, 7·40	94·3, 102·8
Cytidine	6·45	—	6·44	99·9
Uridine	2·98	—	2·99	100·3
Guanine * }	9·40	9·30	—	{ 98·0
Uracil * }	6·95	—	7·15	{ 102·9

* Mixed.

Analytical Methods.—The sodium salt of deoxypentose nucleic acid is extremely hygroscopic when dried, as in this instance, at 110°/0·1 mm. over phosphoric oxide, and special technique was required in order to minimise exposure of the dried material to atmospheric moisture. Estimations of carbon by combustion presented some difficulty owing to the occlusion of carbon by the sodium metaphosphate residue in the boat, but this problem was solved by the addition of small quantities of anhydrous potassium chlorate to the fused mass at the end of the combustion. Determinations of nitrogen were made by a modified micro-Kjeldahl technique embodying the methods of Elek and Sobotka (*J. Amer. Chem. Soc.*, 1926, **48**, 501) and Friedrich ("Die Praxis der quantitativen organischen Mikroanalyse," 1933, Deuticke, Leipzig and Vienna); of phosphorus by a modification of the method of Embden (*Z. physiol. Chem.*, 1920, **113**, 138); and of sodium either gravimetrically (as sodium sulphate after removal of phosphate as barium phosphate) or colorimetrically (Hoffman and Osgood, *J. Biol. Chem.*, 1938, **124**, 347).

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