CCLXXVII. ON THE PENTOSE POLYNUCLEOTIDE OF THE PANCREAS.

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THE prosthetic group of O. Hammarsten's nucleoprotein of pancreas [1894] has been the subject of many investigations. Until 1920 it was believed to consist of guanylic acid, which had been isolated twenty years earlier by Bang [1898]. E. Hammarsten [1920] and Feulgen [1919–20] simultaneously and independently advanced the view that the guanylic acid is only a single component of a polynucleotide, probably of a hitherto unknown nature. Contrary to the two well-known tetranucleotides, thymus and yeast nucleic acids, it was supposed to contain six (Hammarsten) or five (Feulgen) single nucleotides. The former author found a pentose content and a guanine : adenine ratio corresponding to a pentose hexanucleotide, while Feulgen concluded from the positive fuchsin sulphurous acid test that one molecule of guanylic acid combined with one molecule of thymus nucleic acid to form "eine Guanylnukleinsäure."

In the meantime, several findings accumulated which further demonstrated the occurrence of a pentose polynucleotide in the pancreas. Thus Berkeley [1921] found adenine pentose phosphoric acid together with guanylic acid in the pancreas of the dogfish. Jorpes [1924] prepared from ox pancreas crystalline brucine salts of nucleic acids having the composition of the salts of cytidine- and uridine-phosphoric acids, and in the same year crystalline adenine and cytosine pentose nucleotides were isolated from the same source by Jones and Perkins [1924–25]. In accordance herewith, Calvery [1928] found all the four pentose nucleotides of yeast nucleic acid in chicken embryos. A pentose polynucleotide therefore occurs not only in the pancreas but also to some extent in other organs.

The question of the nature of the pentose nucleic acid of the pancreas thus being undecided, Jorpes [1928] performed a series of analyses of the pancreas nucleic acid at different stages of its preparation. The material was analysed for pentoses, total and easily hydrolysed phosphorus and, when protein free, for total and purine nitrogen. The bulk of the evidence obtained supported the view that the nucleic acid in question really is a pentanucleotide containing the components of yeast nucleic acid chemically bound to one molecule of guanylic acid. This view was based on analyses of the nucleoprotein as well as of the barium and sodium salts and of the free polynucleotide.

The preparation and analysis of the pancreas nucleic acid.

A detailed description of the course of preparation and of the analysis of this polynucleotide has been given in a previous paper [Jorpes, 1928]. By extraction of the frozen and minced glands with 10 % sodium chloride at a temperature of $-3°$ and precipitation with alcohol a starting material containing 60–70 % of

the nucleic acid-P of the glands was obtained. Its P content was about 1·25 %, 53–59 % of the phosphoric acid being easily hydrolysed by heating with 5 % H_2SO_4 (by volume) in a water-bath for 2·5 hours. The amount of furfuraldehyde obtained in ordinary hydrolysis with 20 % HCl corresponded to 3 % pentoses. The ratio of pentose to purine nucelotide-P was found to be 4·5–5 to 1, whereas the figure calculated for a pentose purine nucleotide is 4·84 to 1.

When this dried material (N I) was suspended in cooled 10 % NaCl 50–60 (sometimes 70) % of the nucleic acid-P was obtained in 2–3 extractions. The denaturated protein material did not dissolve to any considerable degree and the precipitate obtained, when alcohol was added (N II), contained 5–6·5 % P; 50–59 % of the phosphoric acid hydrolysed easily and the ratio pentose : purine nucleotide-P was 4–4·86 : 1.

Protein-free nucleic acids were easily obtained by dissolving this material (N II) in distilled water and adding 0·25 vol. of 20 % barium acetate solution, the reaction of which had been adjusted to p_H 6·8. The pentose nucleic acid settled out and was obtained biuret-free after washing three times in centrifuge-tubes with barium acetate solution. A further advantage of this precipitation is that the main part of the thymus nucleic acid of the pancreas is removed with the mother-liquor. When barium hydroxide solution was added to distinctly alkaline reaction to the mother-liquor, a pentose nucleic acid settled out which was greatly contaminated with thymus nucleic acid.

A number of barium salts were analysed. The samples obtained at neutral reaction showed a uniform composition, as is evident from Table I.

Table I. *The composition of some barium salts.*

Sample	N %	Total P %	Ratio N : P	P split off by acid hydrolysis %	P in purine nucleotides in % of total-P (corr. made for hydrolysed pyrimidine-nucleotide-P)	Pentoses in purine nucleotides, %	
						Found after corr. for pyrimidine-nucleotide pentoses	Calculated (4·84 × purine-nucleotide-P)
1	10·52	5·73	—	3·57	59·9	15·11	16·59
2	10·91	5·77	—	3·40	56·2	15·38	15·67
3	10·28	5·50	1·87	3·40	59·3	13·90	15·77
4	—	4·93	—	3·09	60·2	12·48	14·36
5	8·24	4·70	1·75	2·92	59·6	—	—
6	4·45	2·48	1·79	1·51	58·5	—	—
7	7·77	4·26	1·82	2·56	57·5	—	—
8	—	4·02	—	2·40	57·0	—	—
9	5·41	3·09	1·75	1·83	56·6	—	—

Apparently the phosphoric acid is liberated during hydrolysis to a greater extent than might have been expected with a tetranucleotide containing two purine nucleotides, in which case 50 % of the phosphorus is liberated as found on analysing yeast and thymus nucleic acids. The pentose content corresponded very closely to that calculated for a pentanucleotide containing three pentose purine nucleotides. The barium salts still contained a few % thymus nucleic acid.

The barium salts were converted into sodium salts and then into the free nucleic acid. On treatment with sodium chloride, a mixed water-soluble sodium-barium salt was obtained, which was precipitated with alcohol. On the addition of sodium sulphate to its solution, pure sodium salts were obtained. When hydrochloric acid was added to a solution of the sodium salt the nucleic acid or an

acid salt thereof settled out owing to its insolubility in water. All these preparations showed a N : P ratio of 1·78 to 1·83. The phosphoric acid hydrolysed easily to 58–59 %.

In the next paper on this topic by Levene and Jorpes [1930] more stress was laid on the purine-N content and on the guanine : adenine ratio in the free pancreas nucleic acid. A new principle was introduced for the preparation of the free nucleic acid. The barium salts were suspended in ice-water and treated with hydrochloric acid. The free acid or a less soluble sodium barium salt settled out, forming a cake. This was repeatedly washed with ice-water in a mortar. After the addition of pieces of ice, the acid was dissolved in the smallest possible volume by the addition, drop by drop, of a strong sodium hydroxide or carbonate solution. An alkaline reaction is to be carefully avoided. The acid is precipitated on the addition of ten volumes of glacial acetic acid. If the barium salt is not quite protein-free, a precipitate appears on the addition of the first traces of the acetic acid. With this precipitate the last traces of protein can be removed before further glacial acetic acid is added. When the pentose nucleic acid is thrown down with glacial acetic acid, the thymus nucleic acid remains in the mother-liquor. The glacial acetic acid precipitate is practically free of it, whereas the precipitate obtained on adding alcohol to the mother-liquor contains about 10 % thymus nucleic acid.

Analysis of several specimens of the purified pancreas nucleic acid showed contents of purine-N, pentoses and easily hydrolysable P which corresponded very closely to the calculated figures for a pentose pentanucleotide. The purine-N as determined by Kjeldahl analysis on the silver precipitate from the acid hydrolysate checked particularly well with the theoretical figure for three purine bases with two pyrimidine bases. This fact very strongly supported the view about the pentanucleotide structure of the pancreas nucleic acid, since the purine bases are almost quantitatively precipitated with silver sulphate.

In one respect however the results were by no means conclusive. The guanine : adenine ratio found was consistently higher than 2 : 1. The same had been the case in all the earlier publications. Thus Hammarsten [1919] found three molecules of guanine for each molecule of adenine corresponding to a hexanucleotide structure of the pancreas nucleic acid. In the analysis of Levene and Jorpes a ratio between 3 : 1 and 4·6 : 1 was found depending on the technique of analysis.

Seeing that all the other analytical data, particularly the content of purine-N, indicated the presence of two molecules of guanine for each molecule of adenine it seemed necessary to subject the analytical methods for the analysis of the single purine bases to a critical examination. Evidently the difference in solubility between guanine and adenine is too small both in neutral and in ammoniacal solution to permit a separation of the two purine bases in the usual way. This assumption also proved correct when the principle outlined in the previous paper [Jorpes, 1934] for the determination of the single purine bases was applied to the pancreas nucleic acid.

The guanine : adenine ratio in the pancreas nucleic acid.

Having thus the possibility of determining the individual purine bases fairly accurately, the question of the structure of the pancreas nucleic acid was taken up again. This time two different lots of nucleoprotein (N II) were available as the starting material. They were worked up in portions of 50–100 g. The protein-free barium salts precipitated from a neutral solution (p_H 6·8) with barium acetate contained 47–55 % of the P present in the starting material. From the

washings and the mother-liquor barium salts of nucleic acids had previously been isolated, showing a similar composition except for the higher content of thymus nucleic acid. Therefore only the directly precipitated main fraction of the barium salts was further analysed here.

Out of nine preparations of the barium salt obtained at neutral reaction five were analysed for purine-N after acid hydrolysis by precipitation with silver sulphate in acid solution. The figures found were, in % of total N: (1) 73·9; (2) 72·7, 73·0; (3) 74·1, 74·5, 74·8; (4) 75·2, 76·6; (5) 76·5, 76·6, 76·7. Four preparations were analysed after preparation of the free acid and its precipitation with glacial acetic acid. The corresponding figures obtained were: (6) 76·1, 76·3, 76·4; (7) 75·8, 75·5; (8) 75·2; (9) 74·2, 73·9, 74·0. On four of these, the easily hydrolysed phosphates were determined; 62·6 to 63·9 % of the phosphates were split off. These figures again checked very well with those calculated for a pentanucleotide with three purine nucleotides (75 % purine-N and 63 % hydrolysable phosphates, without any correction for phosphates hydrolysed from pyrimidine nucleotides). The corresponding figures for yeast nucleic acid are 66·7 and 53 %, and for a hexanucleotide containing four purine nucleotides 80 and 69 %.

Furthermore, a larger fraction of barium salts (240 g.) containing 5·4 % P was prepared and divided into lots of 50 g. for the preparation of the free acid or its acid salts insoluble in glacial acetic acid. Four such specimens (Nos. 10, 11, 12 and 13) were prepared. Yield in each case 12–15 g. air-dried substance containing about 8·5 % P. Sample 12 was further suspended in ice-water and dissolved by addition of concentrated bicarbonate solution drop by drop, an alkaline reaction being carefully avoided. The traces of barium still present were removed with sodium sulphate, and the nucleic acid was again precipitated with glacial acetic acid. Yield 7·3 g. air-dried substance.

These samples were now subjected to purine analysis, the main object being to find the guanine : adenine ratio. On applying the technique of analysis outlined in the previous paper the exact ratio 2 : 1 was found both on sample 6 and on the samples 10–13, including sample 12 after reprecipitation with glacial acetic acid.

For the purine analysis 1·5 g. of the air-dried substance were hydrolysed and treated as described for yeast nucleic acid. In 75 ml. of the hydrolysate the purine bases were precipitated with ammoniacal silver solution. After washing, removal of the silver and dilution to 100 ml. with *N* HCl, the total purine bases in 3×8 ml. were precipitated with the copper-bisulphite reagent. 24 ml. were taken for analysis of adenine after previous oxidation with permanganate.

As stress is presently to be laid on the guanine : adenine ratio alone rather than on the content of total purine bases, the results are given in Table II as found directly on analysis. A guanine : adenine ratio 2 : 1, in which case adenine makes up a third of the amount of purine bases present, requires the same nitrogen content in both series. This was in fact the finding, though allowance was made for the varying completeness of the oxidation with permanganate on different days. For this reason the date of analysis is given. On 4th July the simultaneously analysed yeast nucleic acid gave the almost theoretical guanine : adenine ratio 1 : 1, *viz.* purine-N in 10 ml. 9·33 and 9·33 mg. and adenine-N in 20 ml. 9·13 and 9·02 mg.

In the barium salts a higher figure for the adenine-N was consistently found, which was due, in part at least, to their content of thymus nucleic acid. This was fairly well demonstrated when the barium salt was transformed into the free acid. (See samples Nos. 6 and 10–13.)

Table II. *Total purine-N and adenine-N in some samples of the pancreas nucleic acid.*

Sample	6		8	9	10	
	Barium salt	Free acid	Free acid	Free acid	Free acid	
Date of analysis	4. vii	15. vii	4. vii	2. vii	23. v	4. vii
mg. purine-N in 8 ml.	9·78	9·55	11·50	9·55	10·80	11·12
	9·76	9·41	11·40	9·76	10·70	11·10
	9·73	—	—	—	10·35	11·01
mg. adenine-N in 24 ml.	12·21	9·44	12·97	10·19	10·12	12·08
	12·26	9·60	12·82	10·13	10·70	11·81
	12·08	8·95	—	—	—	11·35

Sample	11		12	13	10–13	14
	Free acid		Free acid reprecipitated with glacial acetic acid	Free acid	Barium salt from which the free acid of samples 10–13 had been prepared	Free acid precipitated with alcohol from the glacial acetic acid mother-liquors of samples 10–13
Date of analysis	23. v	15. vii	4. vii	4. vii	23. v	4. vii
mg. purine-N in 8 ml.	11·48	9·35	10·80	11·55	8·88	9·46
	11·45	9·50	10·74	11·15	8·88	9·43
	—	9·07	—	11·58	8·77	9·41
mg. adenine-N in 24 ml.	11·08	8·80	10·43	11·75	9·41	12·83
	10·82	8·55	10·39	12·01	9·88	12·90
	11·18	8·30	—	—	—	—

A higher adenine content was also obtained in one sample (No. 14) precipitated with alcohol from the glacial acetic acid mother-liquor, where it is known that the thymus nucleic acid of the barium salts accumulates.

The guanine : adenine ratio in the different samples of the free acid was rather uniform. Even in sample 11 the composition was but little changed, if at all, by the following treatment. 9·1 g. air-dried substance were dissolved by adding sodium carbonate in the cold and reprecipitated with barium acetate. The barium salt was washed in the ordinary way and treated with hydrochloric acid. The free acid was then dissolved in alkali and precipitated with glacial acetic acid. After dissolving the precipitate by adding sodium carbonate the sodium salt was precipitated with alcohol. Yield 3·9 g. air-dried substance. (See analysis 15. vii.)

On the other hand a mixture of yeast nucleic acid and guanylic acid behaves quite differently when subjected to the same treatment. Only the yeast nucleic acid is partly precipitated when hydrochloric acid is added and from the mother-liquor a precipitate is obtained with alcohol showing a guanine : adenine ratio 3 : 1.

The titration curve of the pancreas nucleic acid.

Because of the unusual nature of this nucleic acid it is desirable to exclude the possibility of its being a mixture of yeast nucleic acid and guanylic acid. The latter might easily arise from enzymic hydrolysis of yeast nucleic acid in the gland or during the preparation. In order to prevent such an eventuality the preparation was carried out in the earlier stages at a temperature below zero. Further, the nucleoprotein (N II) was in some preparations dissolved in boiling water and the solution boiled before precipitation of the nucleic acid

with barium acetate without causing any change in the composition of the barium salts and of the free acid.

It is indeed very unlikely that the samples would have such a uniform composition at the different stages of the preparation if the guanylic acid were only admixed. This is particularly the case after precipitation of the pancreas nucleic acid from diluted hydrochloric acid and later with 90 % acetic acid in which media guanylic acid is easily soluble.

The possibility of such an admixture of guanylic acid is however to be taken into consideration. Jorpes [1928] therefore performed sodium analyses on four samples of the sodium salt of the pancreas nucleic acid precipitated with alcohol from a neutral solution. For five equivalents of P, five equivalents of Na were found instead of six, as calculated when the two hydroxyl groups of one molecule of guanylic acid were free. Nor could any precipitate be obtained when sodium acetate was added to saturation, in which case any free guanylic acid would have been precipitated.

The study of the course of the titration curve at least, seems to be fully convincing, since the guanylic acid, owing to its two hydroxyl groups, binds much alkali, and its titration curve shows a strongly marked rise in the p_H-range 5–7. Any free guanylic acid in the samples of the pancreas nucleic acid might consequently be expected to influence the titration curve in the same way. Titration curves were therefore made on the pancreas nucleic acid as well as on yeast nucleic acid (Merck), thymus nucleic acid (Hammarsten, E.) and a mixture containing one molecule of guanylic acid to one molecule of yeast nucleic acid. The results are given in Fig. 1. For each experiment an amount of the nucleic

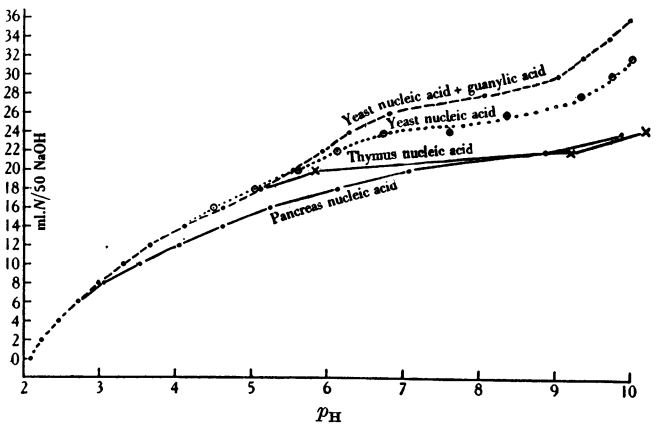

Fig. 1.

acid or of the mixture containing 14·4 mg. P was taken. It was dissolved or suspended in 15 ml. distilled water and the p_H adjusted with alkali and hydrochloric acid to 2·1. Then $N/50$ NaOH was added and the p_H determined with a glass electrode, the potentials being measured with an electrometer valve potentiometer from the Cambridge Instrument Co. Three samples of the pancreas nucleic acid were titrated and all of them gave the same titration curve.

The curve of pancreas nucleic acid resembles that of thymus nucleic acid, except in the more acid range, where it is probably less soluble. The yeast nucleic acid binds somewhat more alkali than the thymus nucleic acid, a

difference which may be due to less mild treatment of the former during the preparation. The mixture of guanylic acid and yeast nucleic acid in the same proportions as in pancreas nucleic acid shows a different course of the titration curve. These findings as well as the chemical analysis thus support the view expressed above as to the structure of the pancreas nucleic acid.

Summary.

An outline is given of the work previously done on the pentose nucleic acid of the pancreas gland. This nucleic acid, which occurs together with a certain amount of thymus nucleic acid, has a structure differing from that of the ordinary tetranucleotides thymus nucleic and yeast nucleic acids. Its composition corresponds to a pentanucleotide structure, containing three molecules of purine pentose nucleotides and two molecules of pyrimidine nucleotides. Its content of pentoses as found on ordinary acid hydrolysis, of purine-N and of easily hydrolysed phosphates, *i.e.* phosphates in purine nucelotides, agrees closely with the figures calculated for a pentose pentanucleotide.

In this paper the guanine : adenine ratio is shown to be 2 : 1. Hence the pancreas nucleic acid contains two molecules of guanylic acid for each molecule of adenylic acid.

The preparation and some of the properties of the pancreas nucleic acid are described.

Its titration curve is compared with that of the ordinary tetranucleotides. The base-binding is equal to that of thymus nucleic acid. This result and the insolubility of the pancreas nucleic acid in water and in 90 % acetic acid exclude the possibility of an admixture of free guanylic acid with yeast nucleic acid.

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