183. *The Nucleic Acid of Normal and Tumour Tissues. Part II.* The Preparation and Composition of Pentosenucleic Acids from the Fowl Sarcomata, Rous No. 1 and Duran-Reynals "D."*

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Pentosenucleic acids have been prepared from acetone-dried Rous No. 1 and Duran-Reynals "D" fowl sarcomata. The component pentose nucleosides of each have been isolated after quantitative separation by partition-chromatography on a starch column (see Part I *). The pentosides of adenine, guanine, cytosine, and uracil alone have been demonstrated. The molar proportions of these are different in each nucleic acid and from the proportions found for G.R.C.H. 15 fowl sarcoma pentosenucleic acid. The overall ratio of purine- to pyrimidine-pentosides shows only slight variation (G.R.C.H. 15, 1·5; Rous No. 1, 1·66; Duran-Reynals "D," 1·41), and the degree of enzymic degradation by ribonuclease is the same for each.

Part I * described the preparation and properties of the pentosenucleic acid from the non-filterable carcinogen-induced fowl sarcoma, G.R.C.H. 15. The investigation of tumour nucleic acids is now extended to the pentosenucleic acids from the virus-induced fowl sarcomata Rous No. 1 and Duran-Reynals "D."

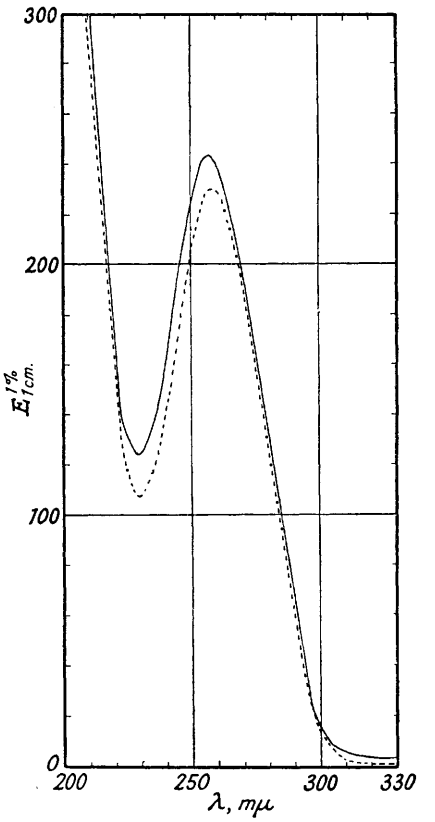
Since 1950 a number of other investigators have prepared "ribonucleic acids" from a variety of sources and analysed them by hydrolysis and separation of the component bases or nucleotides by chromatography on paper or by ion-exchange procedures. It is apparent that, in general, the estimation of the purine moieties is a simple matter and the figures obtained, by either of the above methods, or by differential spectrophotometry, are in good agreement. Greater discrepancies, however, appear in the determinations of uracil and cytosine. For instance, by paper chromatography of the bases Chargaff, Vischer, Doniger, Green, and Misani (*J. Biol. Chem.*, 1949, **177**, 405) found, for pig pancreas pentosenucleic acid, a molar ratio of cytosine to uracil of 4·5, whereas alkaline hydrolysis of the nucleic acid, followed by paper chromatography of the nucleotides, gave a cytidylic acid to uridylic acid ratio of 2·13 (Chargaff, Magasanike, Vischer, Green, Doniger, and Elson, *ibid.*, 1950, **186**, 51). In each case the molar ratio of purine to pyrimidine was 2·25. It is probable therefore that, as a result of alkaline hydrolysis, some 20% of the cytidylic acid had been deaminated to uridylic acid. This suggestion is supported by the observations of Marrian, Spicer, Earl Balis, and Brown (*ibid.*, 1951, **189**, 533), who found that hydrolysis of pentosenucleic acids at room temperature by concentrations of sodium hydroxide greater than N (0·3N at 37°) leads to extensive deamination of cytidylic acid. The common hydrolytic procedure using 0·2N-barium hydroxide at 100° gave 50—100% deamination in 2 hours. Allowance should possibly be made for this effect in the pyrimidine analyses given by Volkin and Carter (*J. Amer. Chem. Soc.*, 1951, **73**, 1516), who effected hydrolysis by 0·5N-sodium hydroxide at 37° for 17 hours, or in those of Marshak (*J. Biol. Chem.*, 1951, **189**, 607) who, before separation and determination of the bases, separated the pentosenucleic from deoxypentosenucleic acid by treatment of the mixture with N-potassium hydroxide at 30° overnight.

Preparation of Pentosenucleic Acid from Rous No. 1 and from Duran-Reynals "D" Sarcoma.—These nucleic acids were prepared by a procedure similar to that already described (*J.*, 1950, 1397) for the pentosenucleic acid from G.R.C.H. 15 sarcoma. However, both tumours contain a mucoid substance which accompanies the pentosenucleic acid during separation from tissue proteins. This mucoid substance was found to be a substrate for hyaluronidase and was accordingly hydrolysed by preliminary treatment of the tissue with this enzyme. The nucleic acids were then extracted with 2% aqueous phenol instead of with physiological saline, and protein contaminants were removed by tryptic digestion in the usual way. Yields of pentosenucleic acid from both sarcomata were similar to

* Part I, *J.*, 1950, 1397.

those obtained from G.R.C.H. 15. The ultra-violet absorption spectra are shown in the figure.

Action of Ribonuclease.—Volkin and Carter (*loc. cit.*) showed that those mammalian pentosenucleic acids whose pyrimidine nucleotide content was about 50% of the total purine and pyrimidine nucleotide liberated 0·4 equiv. of phosphoric acid groups per molecule of phosphorus after treatment with ribonuclease whereas calf-thymus and calf-pancreas pentosenucleic acids, which contain less than 40% of pyrimidine nucleotide, gave a corresponding figure of 0·25 equiv. per molecule of phosphorus which was similar to that given by yeast nucleic acid (Allen and Eiler, *J. Biol. Chem.*, 1941, **137**, 757; Schmidt, Cubiles, Swartz, and Thannhauser, *ibid.*, 1947, **170**, 759). Criteria for the hydrolytic action of ribonuclease differ considerably. Wiener, Duggan, and Allen (*ibid.*, 1950, **185**, 163) found that yeast nucleic acid lost 61·7% of organically combined phosphorus, rendered dialysable by ribonuclease hydrolysis. Zittle (*ibid.*, 1946, **163**, 119) showed that 23—33% of the total phosphorus of this nucleic acid was rendered soluble in 0·125% uranyl chloride (final concentration) in 2·5% trichloroacetic acid, a reagent which, under the conditions prevailing, does not precipitate mononucleotides. However, Tsuboi and Stowell (*Biochim. Biophys. Acta*, 1950, **6**, 192) claimed a 65·5% enzymic hydrolysis of their specimen of yeast nucleic acid, using this method, and 75% for a pentosenucleic acid derived from mouse liver. Carter and Cohn (*J. Amer. Chem. Soc.*, 1950, **72**, 2604) separated hydrolytic products from enzymically undegraded yeast nucleic acid by adjustment of the pH to 3·0 with hydrochloric acid; 73% of the total phosphorus was acid-soluble but part of this was polynucleotide in character. It thus appears (if one may summarise work which includes some without adequate characterisation of the substrate) that hydrolysis of yeast pentosenucleic acid with ribonuclease gives about 40% of polynucleotide (dialysable, and soluble in hydrochloric acid at pH 3·0) together with 20—30% of mononucleotides (largely pyrimidine, dialysable, soluble in hydrochloric acid at pH 3·0 and in 2·5% trichloroacetic acid containing 0·125% of uranyl chloride). Under Zittle's conditions, the three avian sarcomata pentosenucleic acids gave 59·3% (G.R.C.H. 15), 56·5% (Duran-Reynals " D"), and 56·5% (Rous No. 1) hydrolysis. The high ratio of purine to pyrimidine bases in these nucleic acids, therefore, does not lead to the suspicion that enzymic degradation had occurred before or during isolation.



——— *Nucleic acid from Rous No.* 1 *sarcoma*, pH 7·32.
– – – – – *Nucleic acid from Duran-Reynals " D " sarcoma*, pH 6·81.

Separation, Identification, and Determination of Nucleosides.—The procedure for the quantitative hydrolysis of the nucleic acids to the component nucleosides followed exactly that described in Part I (*loc. cit.*), *viz.*, hydrolysis at 37° with 0·2N-sodium hydroxide followed by enzymic dephosphorylation of the nucleotides with prostatic acid phosphatase to nucleosides. The starch chromatograms were prepared and developed in the usual way. The nucleosides were identified by means of their absorption spectra in aqueous solution, by the characteristic spectral shifts on change of pH, and by their absolute and relative *R* values. The spectrophotometric methods available for the quantitative determination of the nucleosides have already been evaluated (Part I, *loc. cit.*), and for these analyses the method employing the combination of fractions comprising one chromato-

graphic band, evaporation to dryness *in vacuo*, and re-solution in 0·05N-hydrochloric acid was adopted. "Back-ground absorption" * was similarly determined. Table 1 compares the absolute and relative R values, and the analytical data are shown in Table 2.

Discussion.—The results leave no doubt that, like the other pentosenucleic acids which have been investigated, the two avian tumour nucleic acids described contain the four pentosides, adenosine, guanosine, cytidine, and uridine. The degree of recovery obtained in terms of hydrolysate nitrogen and phosphorus, and the sensitivity of the chromatographic procedure, render it extremely improbable that any component has been overlooked. There is little difference in the adenosine and guanosine content of the three nucleic acids. However, there is a considerable (29%) decrease in uridine content of the nucleic acid from Rous No. 1 sarcoma and a 17·5% increase in the cytidine content of that from Duran-Reynals "D" sarcoma compared with the G.R.C.H. 15 sarcoma. It is difficult to assess the significance, if any, of such variations. The other interesting feature of the three avian nucleic acids is the high guanine content which is thus far paralleled among mammalian pentosenucleic acids by that from calf-pancreas and from human-liver carcinoma (Volkin and Carter, *loc. cit.*; Chargaff *et al.*, 1950, *loc. cit.*). The general similarity between the composition of the latter and those here described may be significant, although the nucleic acid derived by Volkin and Carter (*loc. cit.*) from mouse hepatoma does not correspond. Should the preponderance of guanine be found to be characteristic of nucleic acids of some tumours in some animal species but not of others, a clue may be provided to the action of 8-azaguanine which has an inhibitory action on the growth of a number of transplantable mouse carcinomata and on some transplantable mouse lymphoid leukæmias. This inhibitory action was not manifested against mouse sarcomata, melanomata, or lymphosarcomata or against rat tumours (Kidder, Dewey, Parks, and Woodside, *Cancer Res.*, 1951, **11**, 204; Sugiura, Hitchings, Cavalieri, and Stock, *ibid.*, 1950, **10**, 178).

A preponderance of guanine in the isolated pentosenucleic acid may be presumed to imply a high demand for guanine, or guanine precursors, during cell growth, and it will be interesting to know whether the pentosenucleic acids of the mouse tumours which are inhibited in growth by 8-azaguanine have a different purine : pyrimidine composition from those derived from non-susceptible mouse tumours and whether such differences are paralleled in the deoxypentosenucleic acids from the same tissues.

Experimental

(With the assistance of J. F. Thomas.)

Preparation of Rous No. 1 Sarcoma Pentosenucleic Acid.—Acetone-dried sarcoma tissue (50 g.) was mixed with water (1 l.) in a Waring blendor for 1—2 minutes, and a few mg. of purified bovine-testis hyaluronidase (Armour Laboratories) in 2 c.c. of water were added. The action of the enzyme, which is extremely rapid at room temperature, was interrupted after a few minutes by addition of 1 l. of 4% aqueous phenol, and the tissue was allowed to undergo extraction for 24 hours at room temperature. The suspension was then centrifuged, and the deposit re-extracted with 1 l. of 2% phenol under the same conditions. The combined supernatant liquids were adjusted to 1% with respect to sodium chloride by addition of 20% sodium chloride and treated with alcohol (2 vols.), and the precipitate was collected, after chilling, by centrifugation. The deposit was thoroughly washed with 70% alcohol to remove traces of phenol, redissolved in 100 c.c. of 0·9% saline, the pH adjusted to 7·5 (glass electrode), and crystalline trypsin (30 mg., containing 50% of magnesium sulphate) added. The flask was "layered" with toluene and incubated at room temperature for 36 hours. The further purification of the nucleic acid then followed exactly the procedure already described for G.R.C.H. 15 pentosenucleic acid (Part I, *loc. cit.*). Two preparations gave: (1) (150 mg.) N (micro-Kjeldahl), 15·0%; P (micro-colorimetric), 8·1%; (2) (220 mg.) N, 14·9%; P, 7·6%. Both preparations were qualitatively free from protein and from deoxypentose.

Preparation of Duran-Reynals "D" Sarcoma Pentosenucleic Acid.—Two samples of this nucleic acid were prepared by the above method. Preparation 1 (166 mg.) gave N, 14·3; P, 7·9%. Preparation 2 (175 mg.) gave N, 14·3; P, 7·8%.

* *Errata.* In Part I, p. 1402, l. 3 from bottom, *for* "as above" *read* "unity"; p. 1403, l. 2, *for* "$E_1 = AB$; $E_2 = DE$" *read* "$xE_1 = AB$ and $xE_2 = DE$."

Action of Ribonuclease.—A solution (12 c.c.) of the nucleic acid (about 20 mg.) at pH 7·1 (glass electrode) was treated with a solution (1 c.c.) of crystalline ribonuclease (3 mg.). An aliquot part (1 c.c.) was taken immediately, 1 c.c. of 0·25% uranyl acetate in 5% trichloroacetic acid added, the mixture rapidly chilled, and the precipitated polynucleotide collected by centrifugation and washed. Analysis of both deposit and pooled supernatant liquids for organic phosphorus at zero time, after 1 hour's incubation at room temperature, and after an over-night treatment under the same conditions gave :

Time	Total precipitable P (mg.)	Hydrolysis, %	Time	Total precipitable P (mg.)	Hydrolysis, %	Time	Total precipitable P (mg.)	Hydrolysis, %
	Rous No. 1 sarcoma			G.R.C.H. 15 sarcoma			Duran-Reynals " D " sarcoma	
0	1·82	0	0	1·70	0	0	1·79	0
1 Hour	—	—	1 Hour	0·70	58·7	1 Hour	0·915	49·0
Overnight	0·79	56·5	Overnight	0·69	59·3	Overnight	0·665	56·5

Hydrolysis and Chromatography of the Pentosenucleic Acid of Rous No. 1 Sarcoma.—(A) The procedures used were exactly the same as those described in Part I (*loc. cit.*). The nucleoside hydrolysate was derived from 9·2 mg. of nucleic acid (Preparation 1). Flow data are given in Table 1 and the results in Table 2 (a).

(B) The nucleoside hydrolysate was here derived from 4·6 mg. of nucleic acid. Flow data are given in Table 1 and the results in Table 2 (b).

Hydrolysis and Chromatography of the Pentosenucleic Acid of Duran-Reynals " D " Sarcoma.—The nucleoside hydrolysate was derived from 4·55 mg. of nucleic acid. Flow data are given in Table 1 and the results in Table 2 (c).

TABLE 1. *Absolute and relative* R *values.*

Pentosenucleic acid	Starch col. (cm.)	Flow (c.c./h.)	Vol. (c.c.) at which band maximum occurred : Adenosine	Uridine	Guanosine	Cytidine
Rous A	19·5	2·2 ± 0·3	82	126	205	282
Rous B	20·0	2·6 ± 0·2	82	132	212	280
Duran-Reynals " D "	20·5	3·5 ± 0·2	84	138	214	302

	R (absolute)				*R* (relative)		
	Adenosine	Uridine	Guanosine	Cytidine	$R_U : R_A$	$R_G : R_A$	$R_C : R_A$
Rous A	0·75	0·49	0·30	0·22	0·65	0·40	0·29
Rous B	0·77	0·48	0·30	0·23	0·62	0·39	0·29
Duran-Reynals " D "	0·77	0·47	0·30	0·21	0·61	0·39	0·28

TABLE 2.

Nucleoside calc. as	Mg.	% of hydrolysate	N, as % of hydrolysate N	P, as % of hydrolysate P	Molar ratios	Mols. purine / Mols. pyrimidine
(a) *Analysis of Rous No. 1 pentosenucleic acid* (A, 9·2 mg.).						
Adenosine	0·843	9·17	16·03	13·1	1·0	4·45
Guanosine	3·085	33·58	55·40	45·3	3·45	
Uridine	0·475	5·17	3·96	8·1	0·62	2·72
Cytidine	1·588	17·29	19·94	27·3	2·10	
			95·3	93·8	—	1·64
(b) *Analysis of Rous No. 1 pentosenucleic acid* (B, 4·6 mg.).						
Adenosine	0·374	8·12	14·15	11·6	1·0	4·35
Guanosine	1·329	28·8	47·6	39·0	3·35	
Uridine	0·225	4·88	3·75	7·65	0·66	2·58
Cytidine	0·655	14·2	16·4	22·4	1·92	
			81·9	80·7	—	1·68
(c) *Analysis of Duran-Reynals " D " pentosenucleic acid* (4·55 mg.).						
Adenosine	0·311	6·85	12·6	10·05	1·0	4·61
Guanosine	1·192	26·30	45·4	36·32	3·61	
Uridine	0·258	5·68	4·57	9·13	0·91	3·27
Cytidine	0·669	14·70	17·80	23·80	2·36	
			80·4	79·3	—	1·41

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