THE COMPOSITION OF THE DESOXYPENTOSE NUCLEIC ACIDS OF THYMUS AND SPLEEN*

By ERWIN CHARGAFF, ERNST VISCHER,† RUTH DONIGER, CHARLOTTE GREEN, AND FERNANDA MISANI

(From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York)

(Received for publication, July 28, 1948)

While the early workers in this field, such as Miescher and Hoppe-Seyler, appear to have conjectured the macromolecular and complex character of the nucleic acids that they were the first to isolate, this view soon was abandoned in favor of the chemically more attractive tetranucleotide hypothesis, and those students of nucleic acid chemistry (*e.g.* (1, 2)) who still felt that much remained to be discovered worked against the current of their time. In the more recent past, the development of methods for the study and the characterization of high polymers has brought about a revival of interest in the chemistry of macro molecules occurring in nature. As regards the desoxyribonucleic acid of calf thymus, the high molecular character of this extremely asymmetric compound has been demonstrated repeatedly (3–6). That this nucleic acid was considered as the prototype of all desoxypentose nucleic acids is understandable, since it is the only compound of its kind readily available for a detailed chemical investigation, but the implicit assumption on the part of many workers that desoxypentose nucleic acid is a single chemical individual, regardless of the source from which it is obtained, is incorrect. A comparison of the results contained in the present communication with those submitted in an accompanying paper (7) will be of interest in this connection. A recent study from this laboratory (8), as well as a review article (9), has provided a fuller discussion of the pertinent literature. The problem of nucleic acid specificity also has been considered repeatedly (10, 11).

The great part in the activities of the living cell, ascribed at present to the desoxypentose nucleic acids, makes it imperative to perfect a foundation that will make possible the direct consideration of problems of structure, composition, and specificity. Whatever chemical changes are produced in nucleic acids in the course of cellular development or by radiations or mutagens, such as the compounds of the mustard series, will hardly be of the kind that can be revealed by the mere inspection of optical or other physical characteristics.

* This work has been supported by a research grant from the United States Public Health Service.

† Swiss-American Student Exchange Fellow.

A chemical comparison with respect to identity of the extremely complex compounds of cellular origin, such as nucleic acids, proteins, or polysaccharides, must be based on the nature and the proportions of their constituents, on the sequence in which these constituents are arranged in the molecule, and on the type of linkages which hold them together. Evidently, a decision will be much easier if, as in the proteins, the number of different constituents is very large, quite apart from the important aid rendered by immunochemical procedures. In the case of the nucleic acids, the relatively small number of different components made very attractive the postulation of identity and regularity which, however, has never been demonstrated adequately. It will be understood that analytical deviations from strictly integral, simple proportions, admissible for substances of comparatively small size, become very significant when applying to compounds whose molecular weights range in the millions.

The present study attempts to provide a survey of the distribution of the purines and pyrimidines in hydrolysates of the desoxypentose nucleic acids derived from calf thymus and beef spleen. It is based on the same microprocedures for the separation and estimation of the nitrogenous constituents of nucleic acids (12) that already have served for the investigation of the composition of pentose nucleic acids (8). The paper last mentioned also included a consideration of the hydrolysis methods employed and a critical discussion of the validity of the experimental results. These points, therefore, need not be reviewed here again.

A synopsis of the results with several nucleic acid preparations is later provided in Table V, which lists the molar proportions in which the four nitrogenous constituents were found. In contrast to the pentose nucleic acids (8), cytosine was the base present in the lowest concentration. Although, in respect to composition, the agreement between nucleic acids of thymus and of spleen is surprisingly good, no claim as to identity should be made. It will be seen that the distribution of purines and pyrimidines was far from that required by a tetranucleotide: for 10 molecules of cytosine, there were found about 16 molecules of adenine, 13 of guanine, 15 (or 13) of thymine.

The correlation of the results submitted here with previous findings in the literature is difficult for a number of reasons. Almost all preparations of desoxypentose nucleic acid that were made the object of detailed analytical studies had been isolated with the use of strong alkali and were probably degraded to some extent. Moreover, since "animal nucleic acids" were regarded as of one kind only, the older workers paid no attention to the possible contamination of their material with pentose nucleic acids, and this may, in preparations derived from sources other than thymus and fish sperm, have contributed to serious errors. For the same reason, prepa-

rations of different, and often unstated, origin were frequently analyzed indiscriminately. Two studies are usually adduced in support of the equimolar distribution of the four nitrogenous constituents. Steudel in 1906 (13) reported on the composition of the nucleic acids of thymus and of herring sperm. His percentage figures, though interpreted by him as evidence for the tetranucleotide structure, actually trend toward the values arrived at in the present study; they correspond to the following mole proportions: adenine 2.0, guanine 1.5, cytosine 1.0, thymine 1.7. Levene and Mandel (14), however, reported figures for a nucleic acid from an unnamed source, probably from spleen (15), that seemed to uphold the tetranucleotide hypothesis.

The composition of a highly polymerized preparation of thymus desoxyribonucleic acid appears to have been considered in one instance only, and this by a quite indirect method. Gulland *et al.* (16) interpreted the results of electrometric titrations (17) in conjunction with the determination of the ratio of purine nitrogen to non-purine nitrogen in the hydrolysate (1.6) as signifying that their product contained, for every 4 gm.-atoms of phosphorus, 1.0 mole each of thymine and of guanine, 1.2 moles of cytosine, and 0.8 mole of adenine. These deductions, which are based on a comparison of the titration curves of the nucleic acid itself and of the so called thymic acid to which it gives rise by acid degradation, are not in accord with the findings presented here. But it should be pointed out that the actual results of the titration of Gulland's nucleic acid corresponded to three dissociations attributable to the amino group and to two dissociations of the purine-pyrimidine hydroxyl per 4 P atoms (17, 18), which is in excellent agreement with the molar proportions calculated for Preparation 3 in Table V (3.9 amino groups for 2.6 purine-pyrimidine hydroxyls) and in satisfactory agreement with the other preparations.

The present study concludes with an attempt to characterize the sugar component of the desoxypentose nucleic acid of spleen, hitherto unidentified, by comparing its partition behavior with that of desoxyribose from thymus nucleic acid. The techniques employed have been discussed in a previous paper (8), but because of the instability of the desoxy sugars the hydrolysis was carried out in a different manner. The nucleic acids were in part degraded enzymatically to the nucleoside stage and the sugars liberated by a short treatment with very dilute acid. The two carbohydrates agreed completely in their partition behavior in three different solvents. It may be tentatively concluded that the desoxypentose nucleic acid of spleen contains 2-desoxyribose.

In conclusion, a few words should be said about a matter of more general concern. To what extent constancy of composition applies to macro molecules of biological origin is unknown and can, perhaps, not even be ascer-

tained by existing methods. It is not impossible that many ostensibly homogeneous preparations of cellular origin actually are mixtures of closely related chemical individuals, representative of different positions within the cell. This may be particularly true of the desoxypentose nucleic acids derived from the cell nucleus, if current conceptions of the specific structure of chromosomes and genes are taken into account. Since, in that case, the constant composition of the nucleic acids of the same cell type is merely a statistical expression of the unchanged state of the cell, it becomes a matter of real interest to compare the distribution of constituents in desoxypentose nucleic acids derived from different deficient mutants of the same species.

EXPERIMENTAL

Material

Desoxyribonucleic Acid of Calf Thymus—Three highly polymerized preparations were used. The figures for their N and P contents, and the other data which are given in Table I, refer to the dry substances. *Preparation 1* was isolated as the sodium salt from thymus by a method which followed, with a few modifications, that of Hammarsten (1).[1]

Preparation 2 was a potassium salt derived from the sodium salt, Preparation 1, by a purification method that made use of the well known precipitating action of lanthanum salts (1, 19). One experiment only will be described. The lanthanum salt, produced by the addition of 6 cc. of 2 per cent aqueous lanthanum acetate to a solution of 200.8 mg. of the sodium nucleate in 400 cc. of saline, was collected by centrifugation in the cold and washed twice with 0.2 per cent lanthanum acetate. It was then suspended in 50 cc. of M potassium chloride containing 5 per cent potassium oxalate (pH 7.2), and the mixture was shaken mechanically for 43 hours. The bulky conglomerate of fibers, produced by the addition of 150 cc. of absolute alcohol to the viscous milk, was filtered off without delay, washed with 66 per cent and with absolute alcohol, and taken up in 40 cc. of water. The very turbid solution was freed of lanthanum oxalate by centrifugation for 1 hour at 1900 *g*, and the supernatant was dialyzed, filtered through infusorial earth, and again dialyzed for 10 days against many changes of ice-cold distilled water. After one more centrifugation the solution was frozen and evaporated in a vacuum. The *potassium desoxyribonucleate* (Preparation 2, Table I) weighed 166.1 mg. (83 per cent of the starting material) and formed a white fiber felt which gave a clear, very viscous solution in water. This potassium nucleate served as the standard in the determinations (20) of the desoxypentose contents of the other preparations which, in terms of this one, varied from 95 to 102 per cent.

[1] This preparation was made in collaboration with Dr. A. Bendich.

Preparation 3 was isolated by a procedure somewhat similar to that of Gulland *et al.* (16).[2]

Desoxypentose Nucleic Acid of Beef Spleen—1800 gm. of cleaned, fresh beef spleen were ground mechanically and washed by suspension in several 2 liter portions of ice-cold physiological saline. The tissue material was removed by pressing through four layers of cheese-cloth, suspended in 1500 cc. of cold M sodium chloride solution (21), and kept overnight in the refrigerator. The insoluble residue was again extracted at 4°, this time with 10 per cent NaCl solution. The combined extracts were centrifuged at 1900*g* for 1 hour and 1.5 volumes of 95 per cent ethanol were added to the supernatant. The sediment, obtained by the centrifugation of the chilled

TABLE I

Desoxypentose Nucleic Acids

Preparation No.*	Source	N	P	Atomic N:P ratio	Ultraviolet absorption				Specific viscosity ($\eta_{sp.}$)†		
					Maximum		Minimum		0.2 per cent solution	0.1 per cent solution	0.05 per cent solution
		per cent	*per cent*		*mμ*	$\epsilon(P)$	*mμ*	$\epsilon(P)$			
1	Calf thymus	13.4	8.0	3.7					17	5.2	1.8
2	" "	13.7	8.0	3.8	259	6400	232	2700	23	3.8	1.5
3	" "	13.5	7.6	3.9					12	4.2	2.4
4	Beef spleen	14.7	8.8	3.7	259	6500	231	2900	3.3	1.5	0.7
5	" "	14.1	8.6	3.6							

* Preparations 1, 3, 4, and 5 were sodium salts, Preparation 2 the potassium salt. N was determined by the Dumas method, P by the gravimetric Pregl-Lieb procedure.

† Ostwald-Fenske viscosimeter; distilled water, 30.3°.

mixture, was taken up in 800 cc. of cold 10 per cent NaCl, the solution was clarified at 1900*g*, and the nucleoprotein precipitated by the addition of 1.5 volumes of ethyl alcohol. The stringy precipitate was spooled on a glass hook and thereby separated from the granular material, which was discarded. It then was suspended in physiological saline and deproteinized (22) in the usual manner by nine treatments with chloroform-octanol (8:1). The threads, precipitated by the addition of 2 parts of ethanol, were lifted and the simultaneously produced granular sediment reworked and converted largely to threads by four precipitations from saline with alcohol (*cf.* (23)). The viscous saline solution of the combined threads was diluted sufficiently to permit clarification by centrifuging, and the nucleic acid again precipitated with alcohol. Its saline solution was then dialyzed against running tap water for 60 hours, against ice-cold distilled water for

[2] We are indebted to Dr. S. Zamenhof for help with this preparation.

24 hours, and evaporated in the frozen state in a vacuum, when the *sodium desoxypentose nucleate* was obtained as a white fluff weighing 1.13 gm. (Preparation 4, Table I).

Another sodium desoxypentose nucleate preparation from beef spleen is listed as Preparation 5 in Table I. This fraction was prepared by a combination of the procedures described previously (1, 16, 21).

Physical Properties—All nucleic acid preparations formed white fluffs or fibers and yielded viscous solutions in water. Preparations 2, 3, and 4 (Table I) were free from pentose and protein; Preparations 1 and 5 contained traces of the latter. They showed the characteristic absorption spectrum in the ultraviolet with the maxima and minima listed in Table I (distilled water as the solvent). The reasons for the use of the expression $\epsilon(P)$, *i.e.* the atomic extinction coefficient with respect to phosphorus, have been explained previously (23). Viscosity data for some of the preparations likewise are given in Table I. It will be seen that solutions stronger than 0.1 per cent exhibited anomalously high viscosities. The preparations from spleen gave solutions that were less viscous than those of the products from thymus.

Composition of Desoxypentose Nucleic Acids

Methods—The procedures for the liberation, separation, and estimation of the purines and pyrimidines, described in detail with respect to ribonucleic acids in a recent publication (8), were followed exactly.

Purines and Pyrimidines—The purines found in the hydrolysates were adenine (absorption maximum at 262.5 mμ) and guanine (249 mμ); the pyrimidines were cytosine (267.5 mμ) and thymine (264.5 mμ). In no case was uracil demonstrated on the chromatograms. The values found for the purine content are arranged in Table II, those for pyrimidines in Table III.

It was pointed out in a preceding paper (8) that the pyrimidine figures that served for the calculation of proportions and composition carried an upward correction of 5 per cent. This was done in order to take into account the retention of a small quantity of pyrimidine compounds in the purine hydrochloride precipitate that was produced by the treatment of the nucleic acid with methanolic HCl, preliminary to the liberation of the pyrimidines by means of concentrated formic acid. The reasons for this procedure have been given (8). With the ribonucleic acids, the extent of the loss in pyrimidines thus incurred could not be estimated easily, since some adenine, carried over into the pyrimidine fraction by the thorough washing of the purine hydrochlorides, would have contaminated the uracil fraction (12). With desoxypentose nucleic acids, however, such estimations could be performed, as adenine and thymine are separated without difficulty.

TABLE II

*Purine Content of Desoxypentose Nucleic Acids**

Preparation No.†	Experiment No.	Duration of hydrolysis with N H_2SO_4	Adenine	Guanine
		min.	*per cent*	*per cent*
1	1	60	10.0	6.4
	2	60	10.7	7.4
2	3	60	9.0	7.9
	4	60	9.2	8.1
3	5	30	9.4	8.8
	6	60	9.0	7.5
	7	60	9.2	8.4
	8	60	9.0	
	9	120	8.6	7.4
	10	180	8.6	7.1
4	11	60	9.4	8.3
	12	60	8.8	8.5
5	13	60	9.7	8.6

* Each value represents the average of at least six parallel determinations on the same hydrolysate. The solvent system used for the separations was *n*-butanol-diethylene glycol-water (in NH_3 atmosphere) in Experiments 4, 7, 12, and 13; in all others, *n*-butanol-morpholine-diethylene glycol-water was employed (12).

† The preparations are numbered as in Table I. Preparations 1, 2, and 3 were derived from calf thymus, Preparations 4 and 5 from beef spleen.

TABLE III

*Pyrimidine Content of Desoxypentose Nucleic Acids**

Preparation No.†	Experiment No.	Cytosine	Thymine
		per cent	*per cent*
1	1	4.5	8.0
2	2	4.5	7.7
3	3‡	4.3	6.8
	4	4.7	7.0
4	5	4.6	8.0
5	6	5.1	8.1

* Each value represents the average of at least six parallel determinations on the same hydrolysate. *n*-Butanol-water served as the solvent system.

† See Table II for the explanation.

‡ This hydrolysate was also examined for uracil, but none was found. The chromatogram segment removed at the location of uracil yielded an extract that absorbed no ultraviolet light.

When parallel pyrimidine determinations were carried out on two samples of thymus nucleic acid (Preparation 3, Table I), the values obtained by the customary technique (8), which avoided the washing of the purine hydrochlorides, were cytosine 4.3, thymine 6.8 per cent (Experiment 3,

Table III).[3] In the other experiment, the nucleic acid sample was degraded with the aid of methanolic HCl and the purine hydrochlorides were centrifuged and separated from the supernatant in the usual manner.

TABLE IV

*Desoxypentose Nucleic Acids; Proportions and Balances**

	Constituent	Preparation 1	Preparation 2	Preparation 3	Preparation 4	Preparation 5
Content in nucleic acid, %	A.	10.0	9.2	9.4	9.4	9.7
	G.	7.4	8.1	8.8	8.5	8.6
	C.	4.7	4.7	4.9	4.8	5.3
	T.	8.4	8.1	7.3	8.4	8.5
Nitrogen in nucleic acid, %	A.	5.2	4.8	4.9	4.9	5.0
	G.	3.4	3.8	4.1	3.9	4.0
	C.	1.8	1.8	1.9	1.8	2.0
	T.	1.9	1.8	1.6	1.9	1.9
N accounted for as % of nucleic acid N	A.	38.7	34.8	36.1	33.1	35.8
	G.	25.6	27.4	30.2	26.8	28.3
	C.	13.3	13.0	13.7	12.4	14.2
	T.	13.9	13.1	12.0	12.7	13.4
	N. A.	91.5	88.3	92.0	85.0	91.7
$\frac{\text{Purine N}}{\text{Pyrimidine N}}$	"	2.3	2.4	2.6	2.4	2.3
Mole per mole P	A.	0.287	0.264	0.284	0.245	0.259
	G.	0.190	0.207	0.237	0.198	0.205
	C.	0.164	0.164	0.180	0.152	0.172
	T.	0.258	0.249	0.236	0.235	0.243
P accounted for as % of nucleic acid P	A.	28.7	26.4	28.4	24.5	25.9
	G.	19.0	20.7	23.7	19.8	20.5
	C.	16.4	16.4	18.0	15.2	17.2
	T.	25.8	24.9	23.6	23.5	24.3
	N. A.	89.9	88.4	93.7	83.0	87.9
Moles per 4 moles P	A.	1.15	1.06	1.14	0.98	1.04
	G.	0.76	0.83	0.95	0.79	0.82
	C.	0.66	0.66	0.72	0.61	0.69
	T.	1.03	1.00	0.94	0.94	0.97

* The preparations are numbered as in Table I. The following abbreviations are employed in this table: A. = adenine; G. = guanine; C. = cytosine; T. = thymine; N. A. = total nucleic acid.

They were then washed by suspending them in 0.5 cc. of methanol, and gaseous HCl was again passed through the mixture for 3 hours. Following centrifugation, the united supernatants were evaporated and the resi-

[3] The purine hydrochloride mixture isolated in this experiment was subjected to an analysis (*cf.* foot-note 3 (8)). The values found, *viz.* adenine 9.7, guanine 8.6 per cent, were in good agreement with the data assembled in Table II.

due was hydrolyzed with formic acid as described previously (8). The chromatogram now exhibited four spots, belonging to cytosine and thymine and to some of the adenine (34 per cent of the total) and guanine (20.5 per cent of the total) that had been transferred to the pyrimidine fraction by the washing process. The values found were cytosine 5.2, thymine 7.2 per cent. Since thymine is the pyrimidine best separated from mixtures contaminated with purines (12), it served for the computation of the relative pyrimidine deficit which, in this particular experiment, was 5.9 per cent.

Proportions and Balances—The results obtained with the several preparations are compared in Tables IV and V. With respect to the expressions used, reference may be made to the study of ribonucleic acids recently

TABLE V

*Desoxypentose Nucleic Acids; Molar Relationships**

	Constituent	Preparation 1	Preparation 2	Preparation 3	Preparation 4	Preparation 5
Molar proportions	Adenine	1.7	1.6	1.6	1.6	1.5
	Guanine	1.2	1.3	1.3	1.3	1.2
	Cytosine	1.0	1.0	1.0	1.0	1.0
	Thymine	1.6	1.5	1.3	1.5	1.4
Average No. of gm.-atoms N per mole constituent		3.8	3.8	3.9	3.8	3.8
Atomic N:P ratio in nucleic acid		3.7	3.8	3.9	3.7	3.6

* The preparations are numbered as in Table I.

published from this laboratory (8). Preparations 2, 3, and 4 are considered slightly more reliable than Preparations 1 and 5.

Rate of Purine Liberation—The rate of cleavage of the purine nucleotides of thymus nucleic acid by heating with N sulfuric acid was followed with Preparation 3 (Table I). The results assembled as Experiments 5 to 10 in Table II are indicative of the relative ease with which the purines are set free; maximal values for adenine and guanine were obtained after 30 minutes. Prolonged heating brought about a certain amount of destruction (Experiments 9 and 10).[4]

[4] One gains the impression that different nucleic acids differ in their readiness to split off purines on treatment with methanolic HCl, which is the first step in the determination of pyrimidines (8). Whereas with the ribonucleic acids and with the desoxyribonucleic acid of thymus 15 to 30 minutes were sufficient to bring about the appearance of purine hydrochlorides, the corresponding preparations from spleen and from tubercle bacilli (7) were much more sluggish, requiring about 2.5 hours.

As a further check, the purine nitrogen was determined in one preparation by precipitation with silver sulfate (24), the experimental procedure of Gulland *et al.* (16) being followed. The purine nitrogen, thus estimated in the sodium nucleate, Preparation 3, Table I, following hydrolysis with N H_2SO_4 for 1 hour at 115–125°, was found as 8.7 per cent, in good agreement with the value of 9.0 per cent recorded in Table IV.[5]

Sugar Component of Desoxypentose Nucleic Acids

The procedure for the identification by chromatography of the sugar component of the purine nucleotides following acid hydrolysis, as applied to the study of the pentose nucleic acids (8), could not be extended directly to the investigation of the desoxypentoses because of the great lability of these sugars. It was first necessary to convert the nucleic acids into a mixture of desoxypentose nucleosides. Substances of this type have, in the past, served for the isolation of the pure desoxy sugar (25, 26). The enzymatic degradation of the nucleic acids to the nucleoside stage was carried out by a mixture of enzymes consisting of purified desoxyribonuclease of pancreas (27)[6] and of an enzyme preparation, derived from *Aspergillus oryzae*, which is designated as "mylase P" (Wallerstein Laboratories, New York).[7] Preliminary assays with this enzyme mixture showed that, under the conditions of the experiments described below, about 65 per cent of the nucleic acid phosphorus was converted to inorganic phosphate within 11 hours.

10 mg. of each nucleic acid preparation were dissolved in 1.5 cc. of veronal buffer of pH 6.7 (containing 15 micromoles of magnesium sulfate), 0.5 cc. of the enzyme solution (containing a total of 100 γ of desoxyribonuclease and 5 mg. of mylase P) was added, and the mixture, protected with 0.01 per cent of ethyl mercurithiosalicylate, incubated for 20 hours at 30°. It was then deproteinized by being shaken with chloroform-octanol and centrifuged. Samples of the solutions were removed in order to test, by chro-

[5] It might be mentioned here that the attempts to characterize all nitrogenous constituents in the same nucleic acid sample, based on the precipitation of the pyrimidine nucleotides as uranium salts (8), were extended to the desoxypentose nucleic acids. However, this procedure yielded low figures for the pyrimidines, in confirmation of similar findings with ribonucleic acids (8). For Preparation 2 (Table I) cytosine 3.2, thymine 6.3 per cent were found; for Preparation 4, cytosine 3.7, thymine 6.1 per cent. In the case of the desoxypentose nucleic acids, a portion of the uranium precipitates was insoluble in 2 N HCl and concentrated formic acid.

[6] We are very grateful to Dr. M. McCarty of the Rockefeller Institute for a specimen of this enzyme.

[7] We should like to thank Mr. Philip P. Gray, Wallerstein Laboratories, New York, for this preparation.

matography, for the presence of free sugars at this stage, but none were found. The remaining solutions were adjusted to pH 1.5 by means of N HCl and heated in boiling water for exactly 12 minutes. The hydrolysates were, in portions of 0.01 and 0.02 cc., neutralized on the filter paper with gaseous NH_3 and subjected to chromatography in three different solvent systems (28). The development was carried out by means of *m*-phenylenediamine dihydrochloride as described previously (29), and the fluorescent zones that formed were observed in ultraviolet light.

Only one, very fast moving sugar component was observed in all hydrolysates, whose position on the chromatogram was, regardless of the solvent system used, identical for Preparations 1 and 3 from thymus and for Preparation 4 from spleen (Table I). The R_F values, *i.e.* the proportion of the distances of the starting point from the adsorbate and from the solvent front (30), found (at about 28°) for the sugar component of both the thymus and spleen nucleic acids were as follows: (*a*) in isobutyric acid (saturated with H_2O), 0.55; (*b*) in butanol-pyridine (to the upper layer, resulting from the mixture of 1 volume of pyridine, 1.5 volumes of water, and 3 volumes of *n*-butanol, 1 volume of pyridine was added), 0.60; (*c*) in *n*-butanol-ethanol-water (4:1:5), 0.45.[8] The R_F values for D-ribose in these solvents, listed in the same order, were 0.44, 0.49, 0.30; for L-rhamnose 0.48, 0.56, 0.40. The carbohydrate present in the desoxypentose nucleic acid of spleen was, therefore, in all likelihood identical with the sugar of thymus desoxyribonucleic acid, *viz. 2-desoxyribose.*

We are indebted to Mr. W. Saschek and Miss R. Rother for the microanalyses.

SUMMARY

The present paper continues the study of the composition of nucleic acids. The distribution of the purines (adenine, guanine) and the pyrimidines (cytosine, thymine) in the hydrolysates of several highly polymerized preparations of the desoxypentose nucleic acids of calf thymus and beef spleen was investigated with the aid of a method recently published for the separation and estimation of these nitrogenous constituents in minute amounts.

The composition of both the thymus and spleen desoxypentose nucleic acids was found closely similar, but it was not in accord with the expec-

[8] When thymus nucleic acid was subjected to the treatment customary for the diphenylamine reaction (20), but with the omission of diphenylamine, no fluorescent band was observed in the chromatogram following the application of *m*-phenylenediamine. Control tests were also performed to ascertain the absence of chromatographically demonstrable sugars from the enzyme mixture employed.

tations derived from the tetranucleotide hypothesis. For 10 molecules of cytosine, 16 molecules of adenine, 13 of guanine, and 15 (or 13) of thymine were found.

The sugar component of spleen desoxypentose nucleic acid, which was liberated from a portion of the nucleosides obtained by enzymatic digestion, closely resembled the carbohydrate similarly released from thymus desoxyribonucleic acid in its chromatographic behavior in three different solvents and was tentatively identified as 2-desoxyribose.

BIBLIOGRAPHY

1. Hammarsten, E., *Biochem. Z.*, **144,** 383 (1924).
2. Feulgen, R., *Z. physiol. Chem.*, **237,** 261 (1935); **238,** 105 (1936).
3. Signer, R., Caspersson, T., and Hammarsten, E., *Nature*, **141,** 122 (1938).
4. Astbury, W. T., and Bell, F. O., *Nature*, **141,** 747 (1938).
5. Schmidt, G., Pickels, E. G., and Levene, P. A., *J. Biol. Chem.*, **127,** 251 (1939).
6. Tennent, H. G., and Vilbrandt, C. F., *J. Am. Chem. Soc.*, **65,** 424 (1943).
7. Vischer, E., Zamenhof, S., and Chargaff, E., *J. Biol. Chem.*, **177,** 429 (1949).
8. Vischer, E., and Chargaff, E., *J. Biol. Chem.*, **176,** 715 (1948).
9. Chargaff, E., and Vischer, E., in Annual review of biochemistry, Stanford University, **17,** 201 (1948).
10. Gulland, J. M., in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, **12,** 95 (1947).
11. Chargaff, E., in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, **12,** 28 (1947).
12. Vischer, E., and Chargaff, E., *J. Biol. Chem.*, **176,** 703 (1948).
13. Steudel, H., *Z. physiol. Chem.*, **49,** 406 (1906).
14. Levene, P. A., and Mandel, J. A., *Biochem. Z.*, **10,** 215 (1908).
15. Levene, P. A., *J. Biol. Chem.*, **48,** 177 (1921).
16. Gulland, J. M., Jordan, D. O., and Threlfall, C. J., *J. Chem. Soc.*, 1129 (1947).
17. Gulland, J. M., Jordan, D. O., and Taylor, H. F. W., *J. Chem. Soc.*, 1131 (1947).
18. Gulland, J. M., in Nucleic acid, Symposia of the Society for Experimental Biology, Cambridge, **1,** 1 (1947).
19. Hammarsten, E., Hammarsten, G., and Teorell, T., *Acta med. Scand.*, **68,** 219 (1928).
20. Dische, Z., *Mikrochemie*, **8,** 4 (1930).
21. Mirsky, A. E., and Pollister, A. W., *Proc. Nat. Acad. Sc.*, **28,** 344 (1942).
22. Sevag, M. G., *Biochem. Z.*, **273,** 419 (1934).
23. Chargaff, E., and Zamenhof, S., *J. Biol. Chem.*, **173,** 327 (1948).
24. Schmidt, G., and Levene, P. A., *J. Biol. Chem.*, **126,** 423 (1938).
25. Levene, P. A., and London, E. S., *J. Biol. Chem.*, **81,** 711 (1929); **83,** 793 (1929).
26. Klein, W., *Z. physiol. Chem.*, **255,** 82 (1938).
27. McCarty, M., *J. Gen. Physiol.*, **29,** 123 (1946).
28. Partridge, S. M., *Nature*, **158,** 270 (1946).
29. Chargaff, E., Levine, C., and Green, C., *J. Biol. Chem.*, **175,** 67 (1948).
30. Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38,** 224 (1944).

THE COMPOSITION OF THE DESOXYPENTOSE NUCLEIC ACIDS OF THYMUS AND SPLEEN

Erwin Chargaff, Ernst Vischer, Ruth Doniger, Charlotte Green and Fernanda Misani

J. Biol. Chem. 1949, 177:405-416.