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Composition of Mammalian Desoxyribonucleic Acids[1]

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Desoxyribonucleic acids were isolated from tissues of four mammalian genera. Ox thymus and liver, sheep thymus and liver, pig thymus, liver, spleen, thyroid, and human thymus and liver (including carcinomatous tissue) served as the sources of a total of 38 preparations. The contents in individual purines and pyrimidines were determined quantitatively and compared. The significance of the differences in composition, most marked when bovine and human preparations were compared, and a number of regularities are discussed. All nucleic acids appeared to yield the same sugar constituent, tentatively identified as 2-desoxyribose.

We compare here the composition of the desoxypentose nucleic acids isolated, in a highly polymerized form, from different tissues of ox, sheep, pig and man. Altogether, 38 preparations were analyzed in 85 hydrolysis experiments. The number of individual determinations of the nitrogenous constituents performed with each hydrolysate averaged 18.

The arithmetic means of the proportions of adenine, guanine, cytosine, thymine, and their standard errors actually found in all preparations from the same species are listed in Table I. In Table II the results are corrected for a 100% recovery, in order to facilitate comparison, and the significance of differences is indicated. It will be seen that the adenine and thymine contents increase, and the guanine and cytosine contents decrease, in the following order of genera: ox, sheep, pig, man. The differences between two adjoining columns are, in general, not significant or of doubtful significance, when analyzed statistically; but the probability of identity becomes very small, when the desoxypentose nucleic acids from bovine and human tissues are compared. The molar relationships are summarized in Table III.

In the preceding presentation all values obtained for preparations of the same species were pooled, regardless of the organ that had served as the source. This is permissible only if specimens from different tissues of the same host show no significant

TABLE I

DISTRIBUTION OF NITROGENOUS CONSTITUENTS IN MAMMALIAN DESOXYRIBONUCLEIC ACIDS

Proportions in moles of nitrogenous constituent per 100 g.-atoms of P in hydrolysate.

		Ox	Sheep	Pig	Man
Sources and no. of preparations		Thymus (21), liver (2)	Thymus (2), liver (2)	Thymus (2), liver (2), spleen (1), thyroid (1)	Thymus (1), liver (4)[a]
Number of hydrolyses		33	12	19	21
Adenine	mean proportion	27.1	27.2	28.4	29.0
	standard error	0.2	0.2	0.2	0.2
Guanine	mean proportion	19.8	19.6	19.4	18.7
	standard error	0.2	0.3	0.3	0.3
Cytosine	mean proportion	19.8	19.4	19.7	19.0
	standard error	0.2	0.2	0.2	0.2
Thymine	mean proportion	26.8	26.6	27.7	28.7
	standard error	0.2	0.2	0.3	0.2
Total recovery		93.5	92.8	95.2	95.4

[a] Two preparations from normal human liver and one each from the metastasis portion and the unaffected portion of a carcinomatous liver.

TABLE II

COMPARISON BETWEEN DESOXYRIBONUCLEIC ACIDS FROM DIFFERENT MAMMALS

Proportions in moles of nitrogenous constituent per 100 g. atoms of P in hydrolysate, corrected for a 100% recovery.

	Ox	Sheep	Pig	Man	Significance of differences[a] beween DNA of: Ox and sheep	Ox and pig	Ox and man	Sheep and pig	Sheep and man	Pig and man
Adenine	29.0	29.3	29.8	30.4	−	+	++	−	++	±
Guanine	21.2	21.1	20.4	19.6	−	±	++	−	+	−
Cytosine	21.2	20.9	20.7	19.9	−	±	++	−	+	+
Thymine	28.7	28.7	29.1	30.1	−	−	++	−	++	+

[a] The following notation was adopted to indicate the degree of significance of the differences in composition, based on the statistical estimate (by the t method) of P, the probability of identity: $P < 0.1\%$, ++; $P < 1\%$, +; $P < 5\%$, ±; $P > 5\%$, −.

TABLE III

MAMMALIAN DESOXYRIBONUCLEIC ACIDS; MOLAR RELATIONSHIPS[a]

Molar ratio	Source: Ox	Sheep	Pig	Man
Adenine to guanine	1.37	1.39	1.46	1.55
Thymine to cytosine	1.35	1.37	1.41	1.51
Adenine to thymine	1.01	1.02	1.02	1.01
Guanine to cytosine	1.00	1.01	0.98	0.98
Purines to pyrimidines	1.01	1.02	1.01	1.00
Amino groups to enolic hydroxyls	1.43	1.43	1.43	1.41

[a] Based on the mean proportions of each nitrogenous constituent (Table I).

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TABLE IV

DISTRIBUTION OF NITROGENOUS CONSTITUENTS IN DESOXYRIBONUCLEIC ACIDS FROM DIFFERENT ORGANS[a]

	Ox		Sheep		Pig				Man	
	Thymus	Liver	Thymus	Liver	Thymus	Liver	Spleen	Thyroid	Thymus	Liver
Total recovery	93.3	94.1	94.3	89.9	93.9	95.3	96.0	97.4	94.6	95.4
	Cor. mean proportions									
Adenine	29.0	28.8	29.3	29.3	30.0	29.4	29.6	30.0	30.9	30.3
	(0.2)	(0.3)	(0.2)	(0.2)	(0.2)	(0.3)	(0.3)	(0.4)	(0.6)	(0.2)
Guanine	21.2	21.0	21.4	20.7	20.4	20.5	20.4	20.8	19.9	19.5
	(0.2)	(0.6)	(0.3)	(0.2)	(0.3)	(0.4)	(0.9)	(0.4)	(0.5)	(0.2)
Cytosine	21.2	21.1	21.0	20.8	20.7	20.5	20.8	20.7	19.8	19.9
	(0.2)	(0.2)	(0.2)	(0.3)	(0.2)	(0.3)	(0.2)	(0.3)	(0.7)	(0.2)
Thymine	28.5	29.0	28.3	29.2	28.9	29.7	29.2	28.5	29.4	30.3
	(0.2)	(0.3)	(0.2)	(0.3)	(0.4)	(0.4)	(0.7)	(0.7)	(0.4)	(0.2)
	Molar ratios									
Adenine to guanine	1.37	1.37	1.37	1.41	1.47	1.44	1.45	1.45	1.55	1.55
Thymine to cytosine	1.34	1.37	1.35	1.41	1.40	1.45	1.40	1.38	1.48	1.52
Purines to pyrimidines	1.01	0.99	1.03	1.00	1.02	0.99	1.00	1.03	1.03	0.99

[a] The number of preparations from each tissue subjected to analysis is listed in Table I. The total average recovery of moles of nitrogenous constituents per 100 g. atoms of P is given in the first line. The mean proportions of each constituent (with their standard errors in parentheses) have been corrected for a 100% recovery.

TABLE V

COMPARISON BETWEEN DESOXYRIBONUCLEIC ACIDS FROM HUMAN NORMAL AND CARCINOMATOUS LIVER[a]

				Significance of differences between DNA of		
	Normal liver	Unaffected portion of carcinomatous liver	Metastasis portion of carcinomatous liver	Normal human liver and ox liver	Human liver metastases and ox liver	Human liver metastases and normal human liver
Total recovery	95.2	96.3	96.9			
		Cor. mean proportions				
Adenine	30.1 (0.2)	30.5	30.2(0.2)	+	+	−
Guanine	19.7 (0.2)	19.5	19.8	−		
Cytosine	19.7 (0.2)	20.2	19.8 (0.2)	++	+	−
Thymine	30.4 (0.3)	29.7	30.1 (0.3)	+	±	−
		Molar ratios				
Adenine to guanine	1.53	1.56	1.53			
Thymine to cytosine	1.54	1.47	1.52			
Purines to pyrimidines	1.00	1.00	1.00			

[a] For explanations, see footnotes in Tables II and IV.

analytical differences. The correctness of this observation, discussed in our earlier work on the composition of desoxypentose nucleic acids,[2,3] is borne out by the results given in Table IV in which the distribution of purines and pyrimidines is listed separately for the specimens isolated from each organ. No significant differences in composition were found, when the desoxyribonucleic acids isolated from the thymus and liver of the same genus were compared statistically. Finally, a comparison is provided in Table V between the specimens from normal human liver and from carcinomatous human liver. Though it should be borne in mind that the information is as yet only fragmentary, it will be seen that the desoxyribonucleic acids from human liver, both from normal and pathological tissue, showed a very similar composition, whereas they differed, more or less significantly, from the specimens isolated from ox liver. For reasons which will be mentioned later the comparison of the guanine values of liver specimens presented difficulties.

The results submitted here exemplify with particular clarity some of the regularities in the composition of desoxypentose nucleic acids observed in earlier studies. All mammalian sources examined in this paper yielded desoxypentose nucleic acids of the AT type.[4] The sum of purine nucleotides equaled that of pyrimidine nucleotides, and the ratios of adenine to thymine and of guanine to cytosine were 1.[3] These relationships are, with respect to the different genera, illustrated in Fig. 1. Another relationship that proved again remarkably constant was the ratio of amino groups to enolic hydroxy groups (Table III).[3b]

If it is taken for granted that desoxypentose nucleic acids are composed entirely of nucleotides, 100% of the nucleic acid phosphorus will, consequently, be accounted for, in equal parts, by the molar sums of the purine and of the pyrimidine nucleotides. Actually, the total recovery of nitrogenous constituents, expressed as mole % of P, amounts at best to 96 to 98%. This is probably due to several reasons, *e.g.*, the summation of small experimental errors and the presence of minute

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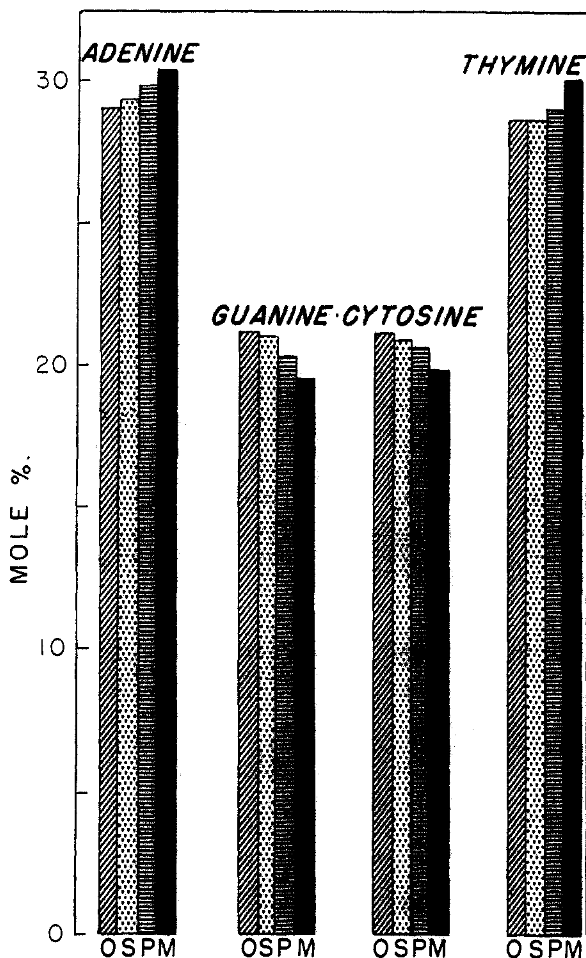

Fig. 1.—Proportions of nitrogenous constituents (per 100 g. atoms of phosphorus) in the desoxyribonucleic acids of the ox (O), sheep (S), pig (P) and man (M).

quantities of 5-methylcytosine,[5,6] which in the case of the nucleic acid from calf thymus amounts to about 1.2% of the total bases,[7,8] or of other as yet undiscovered trace constituents.

The analytical procedures employed in the present study permit the quantitative determination of all major nitrogenous constituents on one chromatogram. This arrangement, which has already served in several recent studies on different desoxypentose nucleic acids,[9,10] is believed superior to the one followed in our earlier work, in which the purines and the pyrimidines were determined separately.[3a] The results presented here, therefore, provide more reliable information on the composition of the most widely studied desoxyribonucleic acid, *viz.*, that from calf thymus of which 21 different preparations were analyzed, and correct previously published data on this substance[2] and on human desoxyribonucleic acid,[11] without, however, affecting the conclusions discussed in earlier papers. The numerous analyses carried out in the present study permitted the computation of the standard error of each average, as has already been done in other recent work,[9,10] and made it possible to compare the values for each nitrogenous constituent in different nucleic acids. We believe this to be a more stringent measure of differentiation than the comparison of ratios.

The study of the nature of the sugar components of the desoxypentose nucleic acids of sheep, pig and man showed them to be indistinguishable chromatographically from the 2-desoxyribose present in the bovine specimens. All desoxypentose nucleic acids examined in this and in previous work have apparently yielded the same desoxy sugar.

Experimental

Preparations.—With the exceptions noted below, all specimens from thymus were prepared by the procedures previously employed in this Laboratory.[2,8-12] These procedures, which represent an adaptation, with modifications, of several published methods,[13] involve the washing of the fresh, ground tissue with citrate-saline to remove pentose-containing material, extraction with 10% NaCl solution, precipitation of the nucleoprotein with alcohol, deproteinization with $CHCl_3$-amyl alcohol, reprecipitation with alcohol, dialysis and lyophilization. All operations were carried out in the cold. The same procedure was used for the nucleic acid preparation from pig spleen. For the isolation of three of the calf thymus preparations and of the specimen from pig thyroid a recently published method employing the detergent Duponol was followed.[14]

Liver presents a special problem owing to its high content in ribonucleic acid. The initial operations for the isolation of desoxypentose nucleic acid from ox, sheep and pig liver and from the samples of human carcinomatous liver have been detailed previously in connection with the description of the preparation of ribonucleic acid from these sources.[15] The final purification steps for these preparations and the isolation of the specimens from normal human liver were similar to the procedures mentioned above, particular attention being paid to the removal of granular material from the precipitated desoxypentose nucleic acid fibers. The complete elimination of contaminating ribonucleic acid was, finally, achieved by treatment with charcoal.[16]

All preparations were subjected to analysis for phosphorus, color yield with diphenylamine and orcinol, and absorption spectrum; in many specimens N (Dumas) and viscosity also were determined. The samples contained less than 2% of protein (biuret). The preparations from liver were free of pentose, after being treated with charcoal; the highest extent of contamination with ribonucleic acid in the specimens from thymus was 1.5%.

Elementary analyses and other characteristics of calf thymus desoxyribonucleic acid have been supplied frequently, *e.g.*, in previous papers from this Laboratory,[2,8] and will not be repeated here. It may be of interest to mention that for 8 preparations from ox tissues (7 from thymus, 1 from spleen) the average atomic extinction coefficient with respect to phosphorus, as defined previously,[17] was found, at the absorption maximum at 259 mμ, as $\epsilon(P) = 6650$ with a standard error of 50. The preparation from human thymus has been described before.[11] The characteristics of other preparations are assembled in Table VI. There was little difference in the spectroscopic properties

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of the various nucleic acids: their absorption maximum lay between 259 and 260 mμ, their ε(P) between 6600 and 6850.

TABLE VI

ANALYTICAL PROPERTIES OF DESOXYRIBONUCLEIC ACIDS[a]

Source	Tissue	N, %	P, %	Desoxypentose nucleic acid, %	Pentose nucleic acid, %
Sheep	Thymus	15.0	8.9	104	1.4
	Thymus	15.3	8.9	100	1.2
	Liver	15.7	9.2	105	...
	Liver	14.0	9.3	97	...
Pig	Thymus	13.8	8.5	95	1.5
	Thymus	14.7	8.8	98	1.5
	Liver	15.4	9.1	105	...
	Liver	15.2	8.7	100	...
	Spleen		8.7	97	...
	Thyroid		8.6	94	...
Man	Liver	14.3	8.1	92	...
	Liver	14.9	8.3	101	...
	Liver (carcinomatous, metastasis portion)	15.4	8.9	101	...
	Liver (carcinomatous, unaffected portion)	13.8	8.3		...

[a] The preparations were dried for 3 hours at 66° in a high vacuum. The values in the last two columns were obtained colorimetrically by means of the reactions with diphenylamine and orcinol, respectively, and are expressed in terms of highly purified reference standards, as described previously.[2,15]

Hydrolysis and Analytical Procedures.—The majority of the hydrolysis experiments was carried out with concd. formic acid as the hydrolytic agent.[2] The chromatographic procedures[9] and the spectrophotometric methods[18] have been fully described before. Eleven hydrolysis runs, mostly with liver preparations, were performed with perchloric acid[19] (5 mg. nucleic acid, 0.08 ml. 7.5 N $HClO_4$, 1 hr. at 100°). In many instances, additional determinations of the purines were made in which N H_2SO_4 served as the hydrolyzing agent.[20] These results were generally in very good agreement with the data presented here. In accordance with previous observations on occasional differences shown by different nucleic acid specimens in their hydrolysis behavior[2,21,22] it was noticed in the course of the present study that, in contrast to all nucleic acid preparations from thymus which were easily hydrolyzed regardless of the procedures used, those from liver yielded lower guanine values in formic than in perchloric acid.

The computation of the standard deviation and the standard error of the figures for individual nitrogenous constituents and the estimate of the significance of differences between different genera made use of customary statistical techniques.[23]

Sugar Component.—One specimen from each of the following sources: sheep thymus and liver, pig thymus and liver, human thymus, normal liver and metastasis portion of carcinomatous liver was examined for the sugar that could be liberated by mild acid hydrolysis of the nucleosides produced by enzymatic digestion. The procedures and the chromatographic arrangement have been described before.[2] A calf thymus specimen was examined simultaneously. All preparations yielded one sugar component which occupied the same position on the chromatograms in the three different solvent systems as 2-desoxyribose liberated from calf thymus nucleic acid.

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