REFERENCES

Hitchings, G. H., Elion, G. B., Falco, E. A. & Russell, P. B. (1949). *J. biol. Chem.* **177**, 357.
Hotchkiss, R. D. (1948). *J. biol. Chem.* **175**, 315.
Johnson, T. B. & Coghill, R. D. (1925). *J. Amer. chem. Soc.* **47**, 2838.
Levene, P. A. & Bass, L. W. (1931). *Nucleic Acids.* New York: Chemical Catalog Co., Inc.
Markham, R. & Smith, J. D. (1949). *Biochem. J.* **45**, 294.
Vischer, E., Zamenhof, S. & Chargaff, E. (1949). *J. biol. Chem.* **177**, 429.
Wheeler, H. L. & Johnson, T. B. (1904). *Amer. chem. J.* **31**, 591.
Wyatt, G. R. (1950). *Nature, Lond.*, **166**, 237.
Wyatt, G. R. (1951). *Biochem. J.* **48**, 584.

The Purine and Pyrimidine Composition of Deoxypentose Nucleic Acids

By G. R. WYATT*
Agricultural Research Council Plant Virus Research Unit, Molteno Institute, University of Cambridge

(*Received* 19 *June* 1950)

Although the composition of nucleic acids has been subject to frequent investigation since these compounds were first isolated by Miescher in the last century, it is only through the application of techniques developed in the past few years that their accurate quantitative analysis has become possible. Early analyses by macrochemical methods (Steudel, 1906; Levene & Mandel, 1908) indicated that nucleic acids contained two purine and two pyrimidine bases in approximately equimolecular proportions. This gave rise to the tetranucleotide hypothesis, upheld by Levene & Bass (1931) and widely accepted, according to which the unit of nucleic acid structure is a group of four nucleotides comprising one of each of the four bases. Recent micro-analyses, however, by such methods as filter-paper chromatography and ultraviolet spectrophotometry, have shown that the composition of nucleic acids is not consistent with any such simplified theory, a conclusion which is in line with biological evidence of their intimate function in cell physiology and of the specific activities of certain of them, e.g. in inducing mutations in bacteria (Avery, MacLeod & McCarty, 1944; Boivin, 1947).

The feasibility of separating nucleic acid derivatives by paper chromatography was demonstrated by Vischer & Chargaff (1948*a*) and by Hotchkiss (1948). A method for the quantitative analysis of ribonucleic acids (RNA) was developed by Vischer & Chargaff (1948*b*), and later applied to deoxypentose nucleic acids (DNA) (Chargaff, Vischer, Doniger, Green & Misani, 1949). This involves separation of the purines and the pyrimidines: the former are precipitated on methanolysis with dry hydrogen chloride, and liberation of the latter is completed by formic acid at 175°. The two groups are then estimated on separate chromatograms.

A simpler procedure for the analysis of RNA has been described by Smith & Markham (1950), who use a short hydrolysis in N-hydrochloric acid to liberate purines along with pyrimidine nucleotides, all of which are then separated on one-dimensional paper chromatograms. This method is not applicable to DNA, from which nucleotides or nucleosides cannot be obtained quantitatively by chemical hydrolysis because of the lability of the deoxy-sugar. In the method now described, DNA is hydrolysed by formic acid to purines and pyrimidines, which are separated on single paper strips. Preliminary results from this method have already been reported (Wyatt, 1950), and the identification and estimation of the pyrimidine 5-methylcytosine in DNA is described in the preceding paper (Wyatt, 1951).

Since this work was done, Daly, Allfrey & Mirsky (1950) have published analyses of DNA from a number of sources, based on separations obtained with starch columns. Their results differ from those now presented in that they failed to recognize 5-methylcytosine, and inclined to the view that the differences between various nucleic acids were not significant.

METHODS

Detection of the spots of chromatograms

For detection of the spots of purines and pyrimidines on paper chromatograms the photographic technique of Markham & Smith (1949) was used, which consists in making contact prints of the dried chromatograms on reflex document paper with filtered ultraviolet light of wavelengths 254 and 265 mμ.

Chromatographic solvents

Although purines and pyrimidines can be separated by various neutral, ammoniacal or weakly acid solvents (a number are listed by Markham & Smith, 1949), such solvents

* Present address: Laboratory of Insect Pathology, Box 490, Sault Ste Marie, Ontario, Canada.

are not satisfactory for quantitative resolution of nucleic acid hydrolysates in amounts adequate for reliable estimation, because guanine 'tails' badly, owing to its low solubility. Many such solvents were tested. However, when Smith & Markham (1950) had shown that solvents containing relatively high concentrations of HCl could be used successfully on filter-paper chromatograms, systems of this type were tried. Increasing acidity has the effect of slowing the movement of all the substances and must be balanced by increasing the water content. The purines are held back more than the pyrimidines, and in solvents containing HCl stronger than about 1N, adenine moves more slowly than cytosine, reversing the positions they take in neutral systems. After many mixtures had been tested, that selected for resolution of the bases from DNA was an aqueous solution containing *iso*propanol 65% (v/v) and HCl 2·0N in the whole volume. This is made up as follows: to 65 ml. of absolute *iso*propanol (or 68 ml. of 95% *iso*propanol) at 16° add sufficient titrated conc. HCl to give 0·2 g.mol. of HCl, and water to 100 ml. The proportions are rather critical, and some empirical adjustment may be necessary; at higher temperatures the volume of *iso*propanol is reduced slightly. To obtain the best spacing of the spots the solvent should be used within a few days of mixing and the tank must be very well saturated with its vapour.

The R_F values of the common purines and pyrimidines in this solvent are given in Table 1, along with those of some nucleosides and nucleotides, to illustrate the effect on chromatographic movement of linkage to ribose and deoxyribose and of phosphorylation. These must be regarded merely as a guide to the relative positions of the spots, as absolute R_F's vary considerably.

Table 1. *R_F values in filter-paper chromatography of nucleic acid derivatives in* isopropanol (65%, *v/v*)-*water*-HCl (2·0N)

	R_F (Whatman no. 1 filter paper)
Purines:	
Guanine	0·25
Xanthine	0·25
Hypoxanthine	0·31
Adenine	0·36
Pyrimidines:	
Cytosine	0·47
5-Methylcytosine	0·55
Uracil	0·68
Thymine	0·77
Nucleosides and nucleotides:	
Cytosine riboside	0·50
Cytosine deoxyriboside	0·63
Cytidylic acid	0·61
Uridylic acid	0·79

Up to 75 μg. of each substance can be resolved from mixtures in 35 cm. movement of the solvent front. An incidental advantage of this solvent is that xanthine and hypoxanthine are estimated along with guanine and adenine respectively, so that the error caused by any deamination of the purines will be minimized. Before printing, the chromatograms must be dried until most of the HCl has evaporated. This can be done overnight by hanging them at room temperature, or for about 30 min. in a current of warm air, but excessive heat causes charring and must be avoided.

Estimation of the purines and pyrimidines

The procedure for estimation of the substances resolved on paper chromatograms is as follows. Triplicate spots of hydrolysate are applied to Whatman no. 1 filter paper from a micropipette (that used throughout these experiments contained 18·0 μl.); the chromatograms are run by the descending method until the front has moved about 35 cm. and are then dried and printed photographically. The positions of the spots are traced from the print on to the filter paper, and disks including them are cut out, along with blanks of equal size cut from a free 'lane' opposite the substances to be estimated; each is eluted in a test tube containing 5 ml. of 0·1N-HCl. After standing for several hours, the eluates are read at their absorption peaks against the corresponding blanks in the Beckman spectrophotometer.

When tested with solutions of pure substances, this method has given recoveries of 96–100%. The range of variation of replicate spots on one chromatogram and of subsequent chromatograms of one material is generally less than 5%; occasional greater discrepancies have been traced to remnants of ultraviolet-absorbing detergent or other dirt on the test tubes used for elution. A smaller error may result from the absorption of the silica Beckman cells themselves, which varies from cell to cell (cf. Morton, 1949). The set of cells used in this work have an absorption peak at 243 mμ. with an optical density averaging 0·014 when the three more opaque cells are read against the most transparent. Their absorption difference at 250 mμ. is sufficient to cause an error of up to 3% in estimations of guanine; this has been corrected for in results here presented.

A technique for the estimation of minor components, such as methylcytosine, by elution from one chromatogram and running again with another solvent, has already been described (Wyatt, 1951).

Table 2. *Ultraviolet absorption data on purines and pyrimidines in* 0·1N-*hydrochloric acid*

(mε is millimolecular extinction coefficient.)

Substance	Wavelength (mμ.)	mε	$E^{0.001\%}_{1\text{ cm.}}$	$E^{0.001\%}_{1\text{ cm.}}$ (Hotchkiss, 1948)
Adenine	260	13·0	0·96	1·01
Guanine	250	11·0	0·73	0·665
Uracil	260	7·9	0·705	0·72
Cytosine	275	10·5	0·95	0·91
Thymine	265	7·95	0·63	0·61
5-Methylcytosine	283	9·8	0·785	—

For use in estimation of the purines and pyrimidines separated by chromatography, extinction coefficients have been determined from pure samples. The sources of the material used for this are: guanine and uracil Hoffmann-La Roche; adenine, British Drug Houses Ltd.; thymine, commercial sample recrystallized; cytosine, isolated from herring-sperm nucleic acid, purified through the picrate and recrystallized; 5-methylcytosine, the gift of Dr A. Pircio, University of Chicago, purified through the picrate and

recrystallized. Each gave only one spot on chromatography except cytosine, in which a trace of methylcytosine persisted; this, however, was too small to cause significant error. The samples were dried, weighed and dissolved in 0·1 N-HCl for extinction measurement. Following Hotchkiss (1948), readings were taken at the nearest multiple of 5 mμ. to the wavelength of maximum absorption, except for methylcytosine. Results are given in Table 2, along with values calculated from the data of Hotchkiss for comparison; all except guanine agree within 5% with Hotchkiss's values.

Quantitative hydrolysis of deoxypentose nucleic acid

Hydrolysis with formic acid. The value of pure formic acid in the hydrolysis of nucleic acids was shown by Vischer & Chargaff (1948*b*), who found that it liberated pyrimidines with much less deamination than was caused by strong mineral acids. For RNA, however, 2 hr. at 175° were required for complete splitting of the pyrimidine ribosides, and in this time significant destruction of the purines had occurred. This led to their using separate hydrolyses for the purines and the pyrimidines.

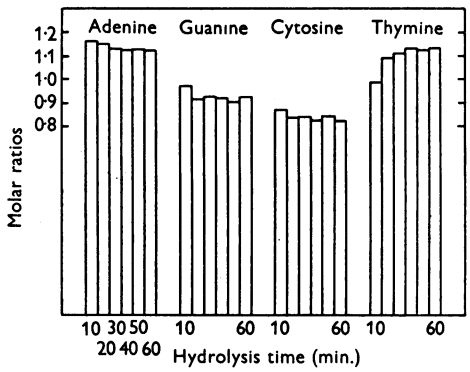

Fig. 1. Molar ratios of the purines and pyrimidines released from herring-sperm deoxypentose nucleic acid by hydrolysis in 98% formic acid at 175° for various lengths of time. For each hydrolysis time the yield of each base in molar units is expressed as a proportion of a total of 4·00.

It was known, however, that acid hydrolysis of DNA proceeds more rapidly than that of RNA, and in the hope of obtaining quantitative yields of all the bases from a single hydrolysis, shorter treatments with formic acid were tried. The purines are released from DNA by exceedingly short acid treatment, and of the pyrimidines cytosine is freed more quickly than thymine. At 165° in 98% formic acid recovery of cytosine reaches a maximum after 15 min., but the yield of thymine increases up to 2 hr. The results of a series of hydrolyses of herring-sperm DNA at 175° are shown in Fig. 1. Maximal yield of thymine is reached by 30 min., and there is no significant drop in recovery of any of the bases up to 60 min. (the higher initial ratios of adenine, guanine and cytosine are due to deficiency of thymine, since the four are calculated to constant totals). The experiment was repeated with a commercial thymus nucleic acid, with the same outcome. 30 min. at 175° in 98% formic acid therefore appears to give maximal yields of both purines and pyrimidines from DNA. As a further check on possible destruction of purines, the yield of adenine from DNA by this treatment was compared with that from 15 min. in aqueous N-HCl at 100°, and found to be the same.

To carry out this hydrolysis conveniently 1–30 mg. of DNA is weighed into a Pyrex tube of 13 mm. diameter, or a solution containing this amount is evaporated to dryness in the tube. Then 0·5 ml. of 98% formic acid is added and the tube is sealed off and heated in an electric muffle at 175° for 30 min. After cooling, the tube is opened cautiously in view of its contents being under pressure and the hydrolysate evaporated to dryness in the same tube. Aqueous N-HCl is then added in sufficient quantity to make a solution 3–4% (w/v) with respect to the original nucleic acid, allowance being made for the volume of the nucleic acid on the assumption that its specific volume is 0·6. The hydrolysate is allowed to dissolve and portions are applied to the chromatogram paper with a micropipette. It is important to use strong enough acid for dissolving the hydrolysate, as otherwise part of the guanine may remain out of solution.

Hydrolysis with perchloric acid. Since most of this work was done, Marshak & Vogel (1950) have reported quantitative hydrolysis of both RNA and DNA by 12 N-$HClO_4$ at 100° for 1 hr. This method has been tried. In the experiment summarized in Table 3, equal quantities of ox-spleen DNA were hydrolysed with formic acid as described above and with 72% (w/w) $HClO_4$ (11·1 N) at 100° for 1 hr., and analysed by chromatography simultaneously. The differences were small, and the total recovery of bases was nearly the same by each method. By repeating the comparative experiment, it was confirmed that $HClO_4$ gives consistently a slightly higher molar ratio of guanine and a lower one of thymine than formic acid; the reasons for this are not clear, but slight destruction of guanine by treatment with formic acid is probable.

Hydrolysis with $HClO_4$ has also been tried on RNA. A sample of the purified yeast nucleic acid which had been analysed by Smith & Markham (1950) was hydrolysed in

Table 3. *Recovery of bases from ox-spleen deoxypentose nucleic acid after hydrolysis with formic and with perchloric acids*

	Mol./atom P in hydrolysis with			Molar ratios* in hydrolysis with	
	HCOOH (*A*)	$HClO_4$ (*B*)	*A*/*B*	HCOOH	$HClO_4$
Adenine	0·257	0·259	0·99	1·13	1·12
Guanine	0·191	0·207	0·92	0·84	0·90
Cytosine	0·198	0·195	1·02	0·87	0·85
Thymine	0·254	0·250	1·02	1·12	1·08
Total	0·900	0·911	0·99	3·96	3·95

* Calculated to total 3·95, making 4·00 when 0·05 is added for 5-methylcytosine (cf. Table 8).

this way; the molar ratios found are compared in Table 4 with ratios calculated from Smith & Markham's (1950) published values by applying certain corrections recently determined by Dr R. Markham (personal communication). Considering that the two methods involve different hydrolyses, different chromatographic solvents, and the use of independently determined extinction values, the closeness of agreement is remarkable.

Table 4. *Composition of yeast ribonucleic acid hydrolysates*

	Molar ratios found by Smith & Markham (1950) (corrected—see Text)	Molar ratios found after hydrolysis with $HClO_4$ (means of 3 estimations)
Adenine	1·02±0·01*	1·04±0·01*
Guanine	1·19±0·01	1·22±0·005
Cytosine	0·83±0·02	0·82±0·01
Uracil	0·96±0·04	0·92±0·015

* Standard error of the mean.

Hydrolysis with $HClO_4$ is conveniently carried out as follows. To the air-dry nucleic acid in a small Pyrex tube is added a measured volume of 72% $HClO_4$ such that the concentration of nucleic acid in solution is 6–8%. The tube is closed with a glass stopper bound in place and is heated in a boiling-water bath for 1 hr. After cooling, the hydrolysate is diluted with an equal volume of water, and the tube is briefly centrifuged to sediment the residue of carbon. Samples can then be pipetted from the supernatant solution directly on to the chromatogram paper. In the *iso*propanol-aqueous HCl solvent, $HClO_4$ remains at the starting point and does not interfere with separation of the bases. Owing to the very hygroscopic nature of conc. $HClO_4$, it is advisable to determine the concentration of nucleic acid in the hydrolysate by estimation of P in samples taken at the same time as those taken for chromatography.

As a further check on possible destruction of the bases by either formic or perchloric acid, some experiments were performed to test the recovery of known amounts of pure bases added to nucleic acid and then subjected to hydrolysis. Table 5 shows the results of one such test. Portions of a solution containing adenine, guanine and thymine in 90% (w/w) formic acid (which is an excellent solvent for sparingly soluble bases such as guanine) were added to samples of ox-spleen DNA and evaporated to dryness, and the mixtures hydrolysed by both the formic acid and the $HClO_4$ techniques. Samples of the nucleic acid without the added bases were hydrolysed at the same time, and the quantities of the bases in the various hydrolysates and in the unhydrolysed solution of pure bases estimated by chromatography. P estimations on the hydrolysates gave a check on volume change by the $HClO_4$ solutions. The amounts recovered of the three added bases were all better than 95% by both methods of hydrolysis, so that little destruction during hydrolysis is indicated. It should be pointed out, however, that great accuracy cannot be expected from an experiment of this sort, which depends upon the comparison of three estimated quantities, each subject to experimental error, and the true yields may not be quite as nearly quantitative as these figures suggest. A previous similar experiment gave less satisfactory results, although recovery of each base was still better than 90%.

It is therefore concluded that essentially quantitative yields of both purines and pyrimidines from DNA can be obtained by treatment either with 98% formic acid at 175° for 30 min., or with 72% $HClO_4$ at 100° for 1 hr., and that the second method is suitable for RNA also. Since the latter method was not tried until its publication by Marshak & Vogel (1950), the former has been used in most of the analyses described in this paper. The use of $HClO_4$ has certain practical advantages, since the use of sealed tubes is not necessary, but difficulty has been experienced in hydrolysing impure nucleic acids with $HClO_4$, for the presence of protein interferes, retarding splitting of pyrimidine ribosides.

Preparation of nucleic acids

For standardization of the analytical technique two preparations of DNA were made by methods avoiding the use of acid, alkali or heat. One was prepared from ox spleen (calf thymus not being available) by a method similar to that of Mirsky & Pollister (1946). The minced fresh spleen tissue was washed three times with cold 0·15 M-NaCl, then extracted with M-NaCl. Nucleoprotein was precipitated from the centrifuged extract by dilution with 6 vol. of water, then redissolved in M-NaCl and deproteinized by emulsification with $CHCl_3$-commercial octanol mixture (8:1, v/v) and centrifugation. After dialysis against water the nucleic acid was precipitated by addition of 1 vol. ethanol (90%, v/v) and washed successively with 90% (v/v) and

Table 5. *Recovery of added bases subjected to hydrolytic conditions in the presence of ox-spleen deoxypentose nucleic acid*

	μg./chromatogram spot			
	Adenine	Guanine	Cytosine	Thymine
Formic acid hydrolysis:				
Found in hydrolysed mixture	65·8	63·8	19·4	55·0
Accounted for by NA	30·7	25·4	19·3	28·4
Extra base found, by difference	35·1	38·4	—	26·6
Extra base added	36·6	39·2	0·0	26·3
Percentage recovery	96%	98%	—	101%
Perchloric acid hydrolysis:				
Found in hydrolysed mixture	67·2	67·2	19·4	53·1
Accounted for by NA	31·1	27·6	19·1	27·9
Extra base found, by difference	36·1	39·6	—	25·2
Extra base added	36·6	39·2	0·0	26·3
Percentage recovery	99%	101%	—	96%

absolute ethanol and ether. A sample for weighing was dried at 110° over P_2O_5 *in vacuo*.

DNA was prepared from herring testes by a similar procedure, except that papain was used for removal of the protamine, which fails to form a gel with $CHCl_3$. Some protein remained in this preparation.

The method of Mirsky & Pollister (1946) for preparation of crude nucleoprotein by extraction with M-NaCl and precipitation by dilution with water is applicable to most tissues (though not to mammalian sperm) and has been used in preparing most of the DNA preparations here studied. When the ratio of RNA to DNA in the original tissue is high, however, preparation of the latter in the pure state requires tedious reprecipitations accompanied by considerable loss. In making DNA from locusts, even when the minced tissue was washed four times with 0·15M-NaCl and the nucleoprotein twice precipitated with water, some RNA remained.

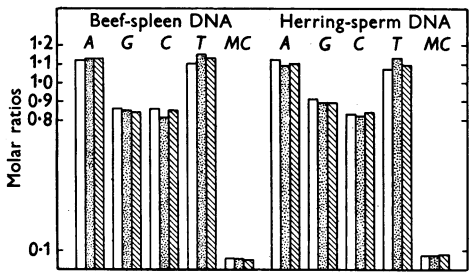

Fig. 2. Molar ratios of purines and pyrimidines from ox-spleen and herring-sperm deoxypentose nucleic acids, untreated (□), after treatment with N-NaOH (▦), and after treatment with ribonuclease (▨). *A*, adenine; *G*, guanine; *C*, cytosine; *T*, thymine; *MC*, 5-methylcytosine.

Since the fractionation of Schmidt & Thannhauser (1945) with N-NaOH has been widely used as an analytical procedure, it seemed worth while to discover whether this treatment might safely be applied to the preparation of DNA for analysis. Samples of the ox-spleen and herring-sperm DNA were dissolved in N-NaOH, left overnight at 37°, precipitated by acidification with glacial acetic acid and the addition of 1 vol. of ethanol, and analysed. In case this treatment might alter the composition by removal of traces of RNA, identical samples were incubated with ribonuclease and precipitated. The composition after each treatment is compared with the original composition in Fig. 2. The only significant change caused by the action of both alkali and ribonuclease on both nucleic acids is a slight increase in the ratio of thymine to the other bases, and this could easily result from the removal of a trace of contaminant RNA. The composition is otherwise not significantly altered, and the characteristic differences between the two nucleic acids are retained. Treatment with N-NaOH overnight at 37° has therefore been used wherever removal of RNA was necessary, and with preparations containing much RNA (such as nucleic acid from whole locusts) has been found much more effective than ribonuclease. Of the nucleic acids whose analysis is given below, NaOH was used in the preparation of those from bull sperm, ram sperm, rat-bone marrow, locusts and wheat germ.

The finding that the composition of DNA is unaltered by alkaline treatment does not, of course, diminish the importance of using mild methods of preparation when macromolecular properties are to be studied.

A sample of calf-thymus nucleic acid prepared by a modification of the method of Hammarsten (1924) was the gift of Dr K. Bailey, DNA from rat-bone marrow was prepared by Dr C. Lutwak-Mann, and that from ram sperm in collaboration with Dr T. Mann, to all of whom thanks are due.

Elementary micro-analyses

Total N was estimated by the micro-Kjeldahl method, and P by the colorimetric methods of Allen (1940) and Tisdall (1922).

RESULTS

In Table 6 are shown the results of an analysis of calf-thymus nucleic acid with respect to purines and pyrimidines (after hydrolysis with formic acid), P and N. The P and N accounted for by the estimated purines and pyrimidines have been integrated, the former on the assumption of simple polynucleotide structure, and the calculated values expressed as percentages of total P and N.

The results of similar analyses of other nucleic acids with respect to P and N are summarized in Table 7.

Table 6. *Composition of calf-thymus nucleic acid* (Na *salt*)

		μg. mol./mg. dry wt.			
	% of dry wt.	Found	Calculated from bases	% of total accounted for	Molar* ratios
Adenine	10·00	0·741	—	—	1·11
Guanine	8·51	0·563	—	—	0·84
Cytosine	6·37	0·574	—	—	0·86
Thymine	9·46	0·751	—	—	1·13
Methylcytosine	0·46	0·037	—	—	0·056
Total bases	34·80	2·666	—	—	4·00
Phosphorus	9·4	3·03	2·67†	88	—
Nitrogen	14·5	10·36	9·86	95	—

* Calculated to an arbitrary total of 4·00.
† Assuming simple polynucleotide structure.

Table 7. *P and N contents of nucleic acid preparations with percentages of total P and N accounted for by estimated purines and pyrimidines*

Nucleic acid of	% of dry wt.		% of total accounted for by estimated bases (means of all experiments)	
	P	N	P	N
Calf thymus	9·4	14·5	90	94·5
Ox spleen	9·35	15·0	90	92·5
Ram sperm	7·4	15·9	88·5	68
Herring sperm	8·7	15·1	91	88
Wheat germ	9·7	14·9	84·5	92
Bull sperm	7·0*	—	90	—
Echinus sperm	8·6*	—	92	—

* Not specially dried.

The mean molar ratios of purines and pyrimidines in eight animal and one plant DNA are listed in Table 8, calculated in each case to an arbitrary total of 4·00. Where three or more independent analyses (separate hydrolyses and separate chromatograms) have been made, the standard error of the mean is shown.

Noting that Smith & Markham (1950) accounted for more than the total P of yeast RNA by their estimated bases, I have analysed a sample of their purified yeast nucleic acid by hydrolysis with perchloric acid, and although the ratios of the bases came very close to theirs (Table 4), the P accounted for by the bases was only 92 % of the total P. It seems most likely that both types of nucleic acid do contain bases and P in the 1:1 relationship usually assumed, and that the deficiency of estimated bases may be due to undiscovered error in the analytical techniques.

Significance of the molar ratios

The conclusion has been drawn by Chargaff, Zamenhof & Green (1950) that the composition of deoxyribonucleic acids varies in different species but not in different tissues of one species. The results of the present study tend to confirm this generalization and to strengthen it by the inclusion of 5-methylcytosine in the analyses.

Among the three bovine DNA preparations analysed those of thymus and spleen show no significant differences. The slightly increased purine/pyrimidine ratio in bull-sperm DNA as compared with the other two may be due to incipient autolysis, as the sperm was incubated with papain in an attempt to extract the nucleic acid before this was done with alkali; there is evidence that nucleases detach pyrimidine nucleotides preferentially (Overend & Webb, 1950). The methylcytosine ratio, however, is identical in the three bovine DNA preparations, whereas it is significantly different in each of the other nucleic acids analysed. As the ratios of the other bases are very similar in cow, sheep and rat DNA, the content of methylcytosine appears to be one of the most characteristic differences between nucleic acids from different species.

When more distantly related species are compared the divergences become greater. Herring DNA differs significantly from bovine DNA with respect to guanine as well as methylcytosine, and in locust and sea-urchin nucleic acids the differences are greater again.

Table 8. *Ratios of purines and pyrimidines from deoxypentose nucleic acids, after hydrolysis with formic acid*

Source of nucleic acid	No. of analyses	Molar ratios calculated to total 4·00				
		Adenine	Guanine	Cytosine	Thymine	Methylcytosine
Calf thymus	3	1·13±0·013	0·86±0·008	0·85±0·008	1·11±0·011	0·052±0·004
Ox spleen	7	1·13±0·006	0·85±0·006	0·84±0·011	1·13±0·008	0·054±0·002
Bull sperm	2	1·15	0·89	0·83	1·09	0·052
Ram sperm	3	1·15	0·88	0·84	1·09	0·039±0·001
Rat bone marrow	2	1·15	0·86	0·82	1·14	0·044
Herring sperm	6	1·11±0·015	0·89±0·009	0·83±0·006	1·10±0·021	0·075±0·002
Locusta migratoria (whole)	3	1·17±0·016	0·82±0·025	0·83±0·015	1·17±0·010	0·008±0·002
Echinus esculentus sperm	3	1·24±0·019	0·78±0·003	0·74±0·014	1·18±0·004	0·071
Wheat germ	2	1·06	0·94	0·69	1·08	0·23

DISCUSSION

The relation between bases and phosphorus

In all the nucleic acids analysed, more P is present than can be accounted for by assigning one phosphate radical to each molecule of estimated purine and pyrimidine. The fraction of the total P accounted for in this way averages 90 % (Table 7), and does not differ materially whether based on P estimated directly in samples of hydrolysate or on the P content of the original material. This discrepancy from 100 % seems greater than can be accounted for by destruction of the bases during hydrolysis, and suggests the presence of some phosphate additional to that in a simple polynucleotide structure. In an attempt to remove any loosely bound phosphate contaminant, a portion of ox-spleen DNA was reprecipitated ten times and analysed, but the fraction of total P accounted for by the bases was still only 91 %, so that little if any extraneous phosphate had been removed.

Remarkable, however, is the constant pattern of composition in all the animal DNA so far analysed (the 'molar ratios' of adenine and thymine being greater, and those of guanine and cytosine less than unity), in contrast with the widely divergent compositions of microbial DNA's (Vischer, Zamenhof & Chargaff, 1949; Smith & Wyatt, 1951). The only DNA of a higher plant so far analysed, that of wheat germ, has a pattern of composition similar to that of the animal nucleic acids, but is marked by an exceptionally high content of methylcytosine.

These characteristic ratios of the purines and pyrimidines in nucleic acids from different sources are not consistent with any simple theory of nucleic acid composition, but indicate a highly complex structure. In view of the localization of DNA in the chromosomes and the indications that DNA composition is more similar among related than unrelated species, it is tempting to speculate whether DNA composition may bear some direct relation to genetic structure.

There is very little evidence whether nucleic acids, as prepared, are homogeneous, or whether they contain mixtures of different molecular types whose total composition is constant from a given source. If the molecules in a given nucleic acid preparation are all of one type, the proportion of methylcytosine (e.g., 1 nucleotide in 75 in ox, 1 in 53 in herring, 1 in 500 in locusts) sets a minimum repeating unit of structure which, although less than the particle weight estimated for thymus nucleic acid by physical methods ($0{\cdot}5$–2×10^6, e.g., Cecil & Ogston, 1948), implies a remarkably elaborate molecule. Though this may be the case, it is equally possible that the preparation contains a number of particle types which, although differing in composition, are similar in size and net charge and so appear homogeneous when examined by electrophoresis or centrifugation.

SUMMARY

1. A micromethod has been devised for the estimation of the purine and pyrimidine bases in deoxypentose nucleic acids. Both purines and pyrimidines are released by a single hydrolysis with formic or perchloric acid and separated on a single paper chromatogram.

2. Extinction coefficients of the common purines and pyrimidines have been determined at their absorption maxima in the ultraviolet.

3. The composition of deoxypentose nucleic acid is unaltered by treatment with N-sodium hydroxide (Schmidt & Thannhauser, 1945) and this fractionation can satisfactorily be used for obtaining specimens free from ribonucleic acid for analysis.

4. Simple polynucleotides calculated from the estimated purines and pyrimidines account for about 90% of the total phosphorus and up to 94% of the total nitrogen of the various deoxypentose nucleic acid preparations analysed.

5. In analyses of deoxypentose nucleic acids from eight animal sources and from one plant the ratios of the purines and pyrimidines vary with the species source of the nucleic acid, but not with different tissues from one species. The content of 5-methylcytosine shows particularly characteristic variations.

I am indebted to the Anti-Locust Research Centre for supplies of locusts, and to Lord Rothschild for samples of bull semen and *Echinus* semen. I wish also to thank Dr R. Markham and Dr J. D. Smith for helpful discussion of the work. The work was carried out while I was on transfer of research from the Forest Insect Investigations Unit, Division of Entomology, Department of Agriculture, Canada, to the Agricultural Research Council Plant Virus Research Unit, Molteno Institute, Cambridge. This paper is Contribution No. 2702, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.

REFERENCES

Allen, R. J. L. (1940). *Biochem. J.* **34**, 858.

Avery, O. T., MacLeod, C. M. & McCarty, M. (1944). *J. exp. Med.* **79**, 137.

Boivin, A. (1947). *Cold. Spr. Harb. Sym. quant. Biol.* **12**, 7.

Cecil, R. & Ogston, A. G. (1948). *J. chem. Soc.* p. 1382.

Chargaff, E., Vischer, E., Doniger, R., Green, C. & Misani, F. (1949). *J. biol. Chem.* **177**, 405.

Chargaff, E., Zamenhof, S. & Green, C. (1950). *Nature, Lond.*, **165**, 756.

Daly, M. M., Allfrey, V. G. & Mirsky, A. E. (1950). *J. gen. Physiol.* **33**, 497.

Hammarsten, E. (1924). *Biochem. Z.* **144**, 383.

Hotchkiss, R. D. (1948). *J. biol. Chem.* **175**, 315.

Levene, P. A. & Bass, L. W. (1931). *Nucleic Acids.* New York: Chemical Catalog Co.

Levene, P. A. & Mandel, J. A. (1908). *Biochem. Z.* **10**, 215.

Markham, R. & Smith, J. D. (1949). *Biochem. J.* **45**, 294.

Marshak, A. & Vogel, H. J. (1950). *Fed. Proc.* **9**, 85.

Mirsky, A. E. & Pollister, A. W. (1946). *J. gen. Physiol.* **30**, 117.

Morton, R. A. (1949). *Photoelectric Spectrometry Group Bull.* **1**, 15.

Overend, W. G. & Webb, M. (1950). *Research*, **3**, 238.

Schmidt, G. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 83.

Smith, J. D. & Markham, R. (1950). *Biochem. J.* **46**, 509.

Smith, J. D. & Wyatt, G. R. (1951). Unpublished observation.

Steudel, H. (1906). *Hoppe-Seyl. Z.* **49**, 406.

Tisdall, F. F. (1922). *J. biol. Chem.* **50**, 329.

Vischer, E. & Chargaff, E. (1948*a*). *J. biol. Chem.* **176**, 703.

Vischer, E. & Chargaff, E. (1948*b*). *J. biol. Chem.* **176**, 715.

Vischer, E., Zamenhof, S. & Chargaff, E. (1949). *J. biol. Chem.* **177**, 429.

Wyatt, G. R. (1950). *Biochem. J.* **47**, vii.

Wyatt, G. R. (1951). *Biochem. J.* **48**, 581.