617. *Deoxypentosenucleic Acids. Part IV.* The Properties and Composition of the Deoxypentosenucleic Acids from Certain Animal, Plant, and Bacterial Sources.*

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A number of deoxypentosenucleic acids from certain animal, plant, and bacterial sources, have been analysed in various ways. An analytical study has also been made of the compositions of the enzyme-resistant " cores " obtained by the action of deoxyribonuclease on the nucleic acid preparations.

As part of an investigation of the composition and specificity of deoxypentosenucleic acids, the properties of a number of nucleic acid preparations have been studied by the usual well-established methods. The purine and pyrimidine contents of the deoxypentosenucleic acids and of their enzyme-resistant " cores " (obtained by the action of deoxyribonuclease) have been determined by the chromatographic technique.

Notwithstanding the fact that such analyses have already been carried out in a number of laboratories (cf., *e.g.*, Vischer and Chargaff *J. Biol. Chem.*, 1948, **176**, 715; Chargaff, Vischer, Doniger, Green, and Misani, *ibid.*, 1949, **177**, 405; Magasanik, Vischer, Doniger, Elson, and Chargaff, *ibid.*, 1950, **186**, 37; Wyatt, *Biochem. J.*, 1951, **48**, 581; 1951, **49**, 144;

* Part III, *J.*, 1952, 303.

Daly, Allfrey, and Mirsky, *J. Gen. Physiol.*, 1950, **33**, 497), the present results are presented here since it is considered that, with such ill-defined materials as deoxypentosenucleic acids, the full significance of the results can only be determined by comparison of the data accumulated in independent investigations.

Representative of the nucleic acids from animal tissues, deoxypentosenucleic acid preparations were isolated (in the frozen-dried state) from calf thymus (preparations T_1 and T_2) and beef spleen by essentially the methods of Mirsky and Pollister (*Proc. Nat. Acad. Sci. Wash.*, 1942, **144**, 383; *J. Gen. Physiol.*, 1946, **30**, 117) and Peterman and Lamb (*J. Biol. Chem.*, 1948, **176**, 685; but cf. Webb, *Nature*, 1952, **169**, 417). Additional preparations were derived from herring testis (preparations H_1 and H_2, isolated according to the method of Mirsky and Pollister, *loc. cit.*), and a transplantable mouse sarcoma, originally induced by the intramuscular injection of 1 : 2 : 5 : 6-dibenzanthracene (the authors are indebted to Dr. D. L. Woodhouse for the deoxypentosenucleic acid preparation from this source). Wheat-germ deoxypentosenucleic acid (isolated as described by Laland, Overend, and Webb, *Acta Chem. Scand.*, 1950, **4**, 885) was examined as a typical plant nucleic acid. Bacterial deoxypentosenucleic acids were represented by samples isolated from *Mycobacterium tuberculosis* (human strain) and *Mycobact. phlei* by a method (unpublished) developed in collaboration with Dr. A. S. Jones.

These preparations (with the exception of those derived from wheat germ, mouse-sarcoma tissue, and *Mycobact. phlei*) contained *ca.* 1% of pentosenucleic acid (Table 1) as determined by Euler and Hahn's method (*Svensk Kem. Tidskr.*, 1946, **10**, 251). Colour tests for proteins were negative.

Certain properties and characteristics of the preparations are recorded in Table 1. The significance of some of these results must be briefly discussed.

Nitrogen : Phosphorus Ratio.—Values (1·62) obtained for the deoxypentosenucleic acid preparations from calf thymus agree with those quoted by Gulland *et al.* (*J.*, 1947, 1129) and by Chargaff *et al.* (*J. Biol. Chem.* 1949, **177**, 429). Other values, with the exception of that found for the deoxypentosenucleic acid of *Mycobact. tuberculosis*, do not differ appreciably from each other. The low value (1·32) found for the N : P ratio of the *Mycobact. tuberculosis* deoxypentosenucleic acid is in agreement with the value (1·39) reported by Brown and Johnson (*J. Amer. Chem. Soc.*, 1923, **45**, 1823) for tuberculinic acid, but differs from that reported by Chargaff *et al.* (*loc. cit.*, p. 429) for a preparation of the deoxypentosenucleic acid of avian tubercle bacilli (*i.e.*, 1·80).

Dische and Feulgen Values.—The Dische values obtained with the deoxypentosenucleic acids of calf thymus, beef spleen, and herring testis are all of similar magnitude. This is possibly correlated with the fact that the purine contents of these nucleic acids are similar (Table 1, cf. Mirsky, *Adv. Enzymol.*, 1943, **3**, 1). The Dische value given by the wheat-germ deoxypentosenucleic acid is slightly lower than those of the above nucleic acids, in agreement with the lower purine content of the preparation. However with the deoxypentosenucleic acid from *Mycobact. phlei* no relation is apparent between the Dische value and the purine content of the preparation. This discrepancy may be due to the fact that purines are not so readily liberated by acid from bacterial deoxypentosenucleic acids as from other deoxypentosenucleic acids (Vischer, Zamenhof, and Chargaff, *J. Biol. Chem.*, 1949, **177**, 429).

The Feulgen values recorded do not appear to show any significant differences.

Chromatographic Analysis of the Deoxypentosenucleic Acids.—Samples of the deoxypentosenucleic acids were hydrolysed at 175° for 50 minutes with formic acid, and the free bases then separated by paper chromatography. The chromatograms were run with a solvent mixture of *n*-butanol, 0·5N-hydrochloric acid and, 2-methoxyethanol (30 : 17 : 10; v/v/v). The bases were located on the chromatograms by the ultra-violet photographic technique (Markham and Smith, *Biochem. J.*, 1949, **45**, 295; 1950, **46**, 509). Control experiments with standard mixtures of authentic purines and pyrimidines showed that the method had an error not greater than $\pm 5\%$ with adenine, guanine, cytosine, and thymine, and $\pm 10\%$ with 5-methylcytosine.

Comparison of the results of these determinations with previously published values (Table 2) shows that the present results are in closest agreement with those of Wyatt

TABLE 1.

Source of deoxypentose nucleic acid	Nitrogen content (%) [1]	Phosphorus content (%) [2]	N/P	Pentose nucleic acid content (%) [3]	Ultra-violet absorption [4] $\varepsilon_{(P)259}$	$\varepsilon_{(P)230}$	$\varepsilon_{(P)280}$	$\varepsilon_{(P)257}$	Relative viscosity, η_r [5]	Purine-N / Pyrimidine-N [6]	Mols. of purines per 4 atoms of phosphorus	Dische value [7]	Feulgen value [8]
Calf thymus (T_1)	12·8	7·90	1·62	1·3	7700	3350	4450	—	3·60	1·60	1·76	2050	810
Calf thymus (T_2)	12·9	7·95	1·62	0·8	7900	3600	4600	—	4·80	—	1·78	2200	800
Herring testis (H_1)	13·4	8·35	1·61	0·4	7500	3070	4100	—	1·85	1·60	1·71	2030	790
Herring testis (H_2)	13·4	8·50	1·58	0·4	7600	3170	4250	—	1·80	—	1·70	2050	780
Beef spleen	12·0	7·75	1·55	1·8	6750	3200	3900	—	1·41	—	1·75	1950	740
Mouse sarcoma	12·7	8·10	1·57	15·7	7800	3550	4350	—	1·67	—	1·65	1780	750
Wheat germ	13·7	8·65	1·59	4·8	7300	3015	3900	—	1·33	1·57	1·57	1860	790
Mycobact. tuberculosis...	10·8	8·20	1·32	1·3	—	3500	4430	6550	—	—	1·28	1370	—
Mycobact. phlei	11·7	7·80	1·50	5·8	—	3200	3700	6750	1·90	—	1·57	1230	750

[1] Estimated by the micro-Kjeldahl method (Markham, *Biochem. J.*, 1942, **36**, 790).
[2] Determined by Allen's method (*Biochem. J.*, 1940, **34**, 858) as modified by Jones, Lee, and Peacocke (*J.*, 1951, 623).
[3] Method of Euler and Hahn (*Svensk Kem. Tidskr.*, 1946, **10**, 251).
[4] Determined on nucleic acid solutions (containing *ca.* 0·06 mg./c.c.) with a Unicam spectrophotometer. The values reported are atomic extinction coefficients with respect to the phosphorus content of the nucleic acid (cf. Chargaff and Zamenhof, *J. Biol. Chem.*, 1948, **173**, 327); all preparations exhibited maximum absorption at 259 mμ with the exception of the two microbial deoxyribonucleic acids which had maximum absorption at 257 mμ.
[5] The values recorded were obtained in Ostwald viscometers at 25° with 0·05% aqueous solutions of the nucleic acids (previously dried at 80°/20 mm. over phosphoric oxide).
[6] Method of Gulland *et al.* (*J.*, 1947, 1129) (cf. the differences in the values determined by this method and the chromatographic technique, Table 2).
[7] The deoxypentosenucleic acid solution (0·5 c.c. of a solution containing 6 mg./10 c.c.) was heated with water (1·5 c.c.) and the Dische reagent (4 c.c.) (cf. Stacey *et al.*, *J.*, 1949, 1222) for 15 mins. at 100°. The intensity of the colour developed was measured in the Spekker absorptiometer with Ilford filters No. 606. Values recorded are atomic extinction coefficients with respect to the phosphorus contents of the nucleic acids.
[8] Values are calculated on same basis as the Dische values. Colour developed according to Widström's procedure (*Biochem. Z.*, 1928, **199**, 298) with Tobie's modification (*Ind. Eng. Chem., Anal.*, 1942, **14**, 405) of Schiff's reagent.

TABLE 2.

Source of deoxyribonucleic acid	Investigator	Molar proportions of bases.[a] A/C	G/C	T/C	G/T	Mols. of bases/4 atoms of phosphorus A	G	C	T	Mc	Total	Ratio mols. purines/ mols. pyrimidines	Purine-N / Pyrimidine-N	N/P	Nitrogen recovery (%)
Calf thymus	This paper (prepn. T_1)	1·30	1·01	1·31	0·77	0·99	0·77	0·76	1·00	0·07	3·59	0·96	1·98	1·59	96·5
	,, (prepn. T_2)	1·31	0·97	1·29	0·75	1·02	0·76	0·78	1·01	0·07	3·64	0·96	1·96	1·61	94·0
	Wyatt [1]	1·29	0·98	1·30	0·75	0·98	0·75	0·76	0·99	0·05					
	Chargaff *et al.*[2]	1·47	1·16	1·31	0·88	1·12	0·88	0·76	1·00	—					
	Mirsky *et al.*[3]	1·40	1·19	1·42	0·84	1·10	0·94	0·79	1·12	—					
Beef spleen	This paper	1·38	1·01	1·37	0·74	1·01	0·74	0·73	1·00	0·05	3·53	0·99	2·03	1·54	96·5
	Wyatt [1]	1·34	1·00	1·34	0·75	1·02	0·76	0·76	1·02	0·05					
	Chargaff *et al.*[2]	1·51	1·19	1·41	0·84	1·04	0·82	0·69	0·97	—					
Herring testis	This paper (prepn. H_1)	1·29	0·90	1·31	0·70	1·00	0·71	0·82	1·05	0·10	3·68	0·89	1·74	1·56	97·5
	,, (prepn. H_2)	1·30	0·91	1·31	0·69	1·00	0·70	0·77	1·01	0·10	3·58	0·91	1·84	1·57	94·5
	Wyatt [1]	1·31	1·06	1·31	0·81	1·00	0·81	0·76	1·00	0·07					
Wheat germ	This paper	1·38	1·03	1·40	0·74	0·90	0·67	0·65	0·91	0·19	3·32	0·88	1·80	1·55	89·5
	Wyatt [1]	1·51	1·34	1·54	0·86	0·89	0·79	0·59	0·91	0·19					
	Brawerman and Chargaff [4]	1·62	1·34	1·61	0·83	1·05	0·87	0·65	1·04	0·23					
	Mirsky *et al.* [3]	1·67	1·42	1·64	0·87	1·10	0·94	0·66	1·08	—					
Mycobact. tuberculosis, human strain	This paper	0·55	0·81	0·50	1·62	0·52	0·76	0·94	0·47	—	2·69	0·91	1·70	1·27	94·0
	Wyatt [5]	0·54	0·85	0·60	1·43										
Mycobact. tuberculosis, avian strain	Chargaff *et al.*[2]	0·47	1·10	0·40	2·75	0·48	1·12	1·02	0·41	—					
Mycobact. phlei	This paper	0·52	0·91	0·45	2·04	0·57	1·00	1·10	0·49	—	3·16	0·98	1·85	1·50	92·5
Mouse sarcoma	This paper	1·30	0·96	1·41	0·85	0·95	0·70	0·73	0·82	—	3·20	1·05	2·16	1·57	87·5

[a] A = Adenine, G = guanine, C = cytosine, T = thymine, Mc = 5-methylcytosine, N/P = nitrogen/phosphorus ratio of the nucleic acid hydrolysate placed on chromatogram.

[1] Wyatt, *Biochem. J.*, 1951, **48**, 584.
[2] Chargaff *et al.*, *J. Biol. Chem.*, 1949, **177**, 429; 1949, **177**, 405; 1950, **187**, 1.
[3] Daly, Mirsky, and Allfrey, *J. Gen. Physiol.*, 1950, **33**, 497.
[4] Brawerman and Chargaff, *J. Amer. Chem. Soc.*, 1951, **73**, 4052.
[5] Wyatt, *Biochem. J.*, 1951, **49**, 144.

Chargaff *et al.* have published results obtained with several preparations of calf-thymus deoxypentosenucleic acid (which exhibit some variation in composition) and with two preparations of beef-spleen deoxypentosenucleic acid; Mirsky *et al.* have recorded the results on three preparations of calf-thymus deoxypentosenucleic acid.

(*loc. cit.*). Table 2 shows, however, that the nitrogen recoveries for all the deoxpentosenucleic acids examined were low and ranged from 87·5% (mouse-sarcoma deoxypentosenucleic acid) to 97·5% (beef-spleen deoxypentosenucleic acid). Low nitrogen-recovery figures are also characteristic of results obtained by other workers (cf. Chargaff *et al.*, *loc. cit.*, p. 405; Daly *et al.*, Wyatt, *locc. cit.*) and may be due to such factors as, *e.g.*, incomplete hydrolysis, destruction of the bases during hydrolysis, incomplete recovery of the bases from the chromatograms, and the presence of nitrogenous contaminants (*e.g.*, protein) in the nucleic acid preparations. Moreover, small amounts of nitrogenous components in the deoxypentosenucleic acids may have so far escaped detection. In the present experiments it is considered unlikely that incomplete hydrolysis is the cause of the low nitrogen-recovery figures, since longer times of hydrolysis did not alter the results obtained. Destruction of bases during hydrolysis, and incomplete recovery of the bases from the chromatograms, are also considered unlikely, since experiments with standard mixtures gave satisfactory results within the limit of experimental error.

The content of 5-methylcytosine found for the wheat-germ deoxypentosenucleic acid (29·2; expressed as a molar percentage of the cytosine content) agrees well with the value (33) reported by Wyatt (*loc. cit.*) for a similar preparation. Values (expressed as above) for the 5-methylcytosine content of the deoxypentosenucleic acids of calf thymus, herring testis, and beef spleen are somewhat higher than those reported by Wyatt (*loc. cit.*). In agreement with the results obtained by Chargaff *et al.* (*J. Biol. Chem.*, 1949, **177**, 429) with the deoxypentosenucleic acid of avian tubercle bacilli, and of Wyatt (*loc. cit.*), the deoxypentosenucleic acids isolated from *Mycobact. tuberculosis* (human strain) and *Mycobact. phlei* contained no detectable amount of 5-methycytosine.

The results (Table 2) also show that the deoxypentosenucleic acid isolated from wheat germ is similar in composition to the animal nucleic acids, but contains slightly less of all four bases per four atoms of phosphorus, and is characterised by a much higher 5-methylcytosine content. The considerable differences in composition between the bacterial nucleic acids and the other nucleic acids examined, are also apparent from the Table.

Enzymic Degradation of the Deoxypentosenucleic Acids and Isolation of the Enzyme-resistant Residues ("*Cores*") (cf. Zamenhof and Chargaff, *J. Biol. Chem.*, 1949, **178**, 531; 1950, **187**, 1; Overend and Webb, *J.*, 1950, 2746).

Solutions of the deoxypentosenucleic acids at pH 7·1 were incubated for 15 hours at 37° with deoxyribonuclease under the conditions defined in the Experimental section, and then dialysed against frequent changes of distilled water until free from dialysable products. The non-diffusible residues were evaporated in the frozen state. Fractions of the residues (or "cores") were hydrolysed and analysed by the chromatographic technique as described in the preceding section. The results obtained are summarised in Table 3. The figures recorded for adenine, guanine, cytosine, and thymine are the means of five estimations, and those for 5-methylcytosine the means of two estimations.

The non-diffusible "cores" obtained after one treatment with the enzyme and subsequent dialysis comprised a relatively large residue, which in most cases amounted to at least 50% of the original nucleic acid. A second treatment of certain of the "cores" with deoxyribonuclease followed by dialysis of the enzymic degradation products afforded further non-diffusible residues, which amounted to approximately 20% of the original nucleic acid. These "cores" differ from those obtained by Zamenhof and Chargaff (*loc. cit.*) who, in some cases, obtained from calf-thymus deoxypentosenucleic acid after one treatment with deoxyribonuclease a non-diffusible "core" comprising only 7% of the original nucleic acid. These differences may be due to the fact that in the present experiments dialysis of the enzyme degradation products was carried out at room temperature against distilled water changed at noted intervals of time, and not against buffer solutions at 37°. Repeated changes of distilled water serve to remove metallic ions, which are known to favour the dialysis of polynucleotides (Lehmann-Echternacht, *Z. physiol. Chem.*, 1941, **269**, 169).

Some comparison of the present results with those reported by Zamenhof and Chargaff (*loc. cit.*) has been obtained by graphical representation of the latter. Thus, two preparations of the "cores" from calf-thymus deoxypentosenucleic acid (which amounted

to 53 and 59·5% of the weight of the original nucleic acid) showed increases in the purine : pyrimidine ratio compared with the original nucleic acid of 12·5 and 10% respectively. Corresponding figures from Zamenhof and Chargaff's results are 9·5 and 6·7%.

Similar increases in the purine : pyrimidine ratio are observed with the cores prepared

TABLE 3.

Source of non-diffusible "core" and original nucleic acid (DNA)	Mols. of bases per 4 atoms of phosphorus					Mols. of purine bases/mols. of pyrimidine bases	Purine-N / Pyrimidine-N
	A	G	Cy	T	MC		
Calf thymus "core" (53%) ...	0·94	0·80	0·65	0·89	0·07	1·08	2·23
Calf thymus "core" (59·5%)	0·98	0·75	0·72	0·85	0·07	1·06	2·13
Calf thymus "core" (20%) ...	0·98	0·74	0·63	0·91	0·05	1·08	2·23
Calf thymus (core" (22%) ...	1·05	0·75	0·65	0·99	0·05	1·07	2·19
Calf thymus DNA	0·99	0·77	0·76	1·00	0·07	0·96	1·98
M. phlei "core" (67%)	0·53	0·99	1·09	0·52	—	0·94	1·76
M. phlei "core" (24%)	0·41	0·89	0·86	0·51	—	0·95	1·80
M. phlei DNA	0·57	1·00	1·10	0·49	—	0·98	1·85
M. tuberculosis "core" (54%)	0·50	0·93	1·07	0·60	—	0·86	1·62
M. tuberculosis "core" (24%)	0·45	0·68	0·88	0·48	—	0·83	1·56
M. tuberculosis DNA	0·52	0·76	0·94	0·47	—	0·91	1·70
Wheat germ "core" (20%) ...	1·10	0·86	0·69	1·03	0·17	1·04	2·12
Wheat germ DNA	0·90	0·67	0·65	0·91	0·19	0·88	1·80
Herring testis "core" (50%)	1·14	0·91	0·83	1·16	0·06	1·00	2·05
Herring testis DNA	1·00	0·71	0·82	1·05	0·10	0·87	1·74
Mouse sarcoma "core" (50%)	1·04	0·71	0·69	1·08	—	0·99	2·06
Mouse sarcoma DNA	0·95	0·70	0·73	0·82	—	1·06	2·16

A = Adenine, G = guanine, Cy = cytosine, T = thymine, MC = 5-methylcytosine, N = nitrogen. The "cores" are expressed as percentages of the original nucleic acids.

from herring testis, wheat germ, and possibly mouse sarcoma. This increase in the purine : pyrimidine ratio is not, however a characteristic of the larger polynucleotide fragments resulting from the action of deoxyribonuclease on all deoxypentosenucleic acids, since this ratio is slightly reduced in the "cores" obtained from the bacterial deoxypentosenucleic acids.

EXPERIMENTAL

Chromatographic Analysis of the Deoxypentosenucleic Acids.—(a) *Standard materials.* Adenine (supplied by B.D.H. Ltd.) was purified by recystallisation from hot water (Found : N, 52·2. Calc. for $C_5H_5N_5$: N, 51·8%). Guanine (supplied by B.D.H. Ltd.) was recrystallised according to the procedure of Horbaczewski (*Z. physiol. Chem.*, 1897, **23**, 229) (Found : N, 45·9. Calc. for $C_5H_5ON_5$: N, 46·5%). Hypoxanthine was prepared by deamination of adenine (Reichard, *J. Biol. Chem.*, 1949, **179**, 773) and recrystallised twice from hot water (Found : N, 41·4. Calc for $C_5H_4ON_4$: N, 41·2%). Xanthine, prepared from guanine by Reichard's method (*loc. cit.*), exhibited an ultra-violet absorption spectrum identical with that reported in the literature. A sample of cytosine kindly provided by Dr. D. L. Woodhouse was purified by recrystallisation from water (Found : N, 37·7. Calc. for $C_4H_5ON_3$: N, 37·8%). 5-Methylcytosine was synthesised according to Wheeler and Johnson (*Amer. Chem. J.*, 1904, **31**, 591). After recrystallisation from hot water, the base was obtained as prismatic crystals, m. p. 270° (decomp.) (Found : N, 33·1. Calc. for $C_5H_7ON_3$: N, 33·6%). *iso*Cytosine was recrystallised twice from hot water and showed m. p. 275—280° (Found : N, 37·9. Calc. for $C_4H_5ON_3$: N, 37·8%) (Caldwell and Kline, *J. Amer. Chem. Soc.*, 1940, **72**, 2365, give m. p. 276°). Uracil (supplied by B.D.H. Ltd.) was recrystallised from 0·1N-hydrochloric acid (Found : N, 25·3. Calc. for $C_4H_4O_2N_2$: N, 25·0%). Thymine (supplied by B.D.H. Ltd.) was recrystallised twice from water (Found : N, 22·8. Calc. for $C_5H_6O_2N_2$: N, 22·3%). Except where otherwise stated, the m. p. of the above bases were as reported in the literature.

(*b*) *Identification and estimation of the amounts of purines and pyrimidines on the chromatograms.* All chromatograms were run on Whatman No. 1 filter paper with a mixture of *n*-butanol, 0·5N-hydrochloric acid, and 2-methoxyethanol (30·5 : 17·7 : 10·1, v/v/v) as solvent. The positions of the bases on the chromatograms were determined by the ultra-violet photographic procedure (Markham and Smith, *Biochem. J.*, 1949, **45**, 295) (for the detection of small amounts of

5-methylcytosine ultra-violet light of wave-length 265 mμ was used). The following R_F values were determined for the standard materials in the above solvent mixture :

Base :	Adenine	Guanine	Hypoxanthine	Xanthine	Cytosine
R_F	0·49	0·345	0·43	0·52	0·49

Base :	5-Methylcytosine	*iso*Cytosine	Thymine	Uracil
R_F	0·54	0·54	0·77	0·685

After the spots had been located on the chromatograms by means of the photographic print, the appropriate areas of the paper were cut out and divided into halves. Corresponding areas of paper were cut out from regions on either side of the spot containing the base. The control areas and the purine and pyrimidine spots were cut into small pieces and extracted with 0·1N-hydrochloric acid at 37° for 24 hours. The ultra-violet absorption spectra of the extracts were then determined in the Unicam spectrophotometer. The extracts from the control spots served to correct for any other absorbing material present in the paper and hydrochloric acid. The extinction values were measured at the experimentally determined absorption maxima of the standard bases (in 0·1N-hydrochloric acid) (*i.e.*, adenine at 262·5, guanine at 249, cytosine at 276, 5-methylcytosine at 282, and thymine at 264 mμ). In addition the absorption of the extracts was measured at different wave-lengths (220—290 mμ) in order to effect a comparison between the spectra of the extracts and of the corresponding standard purine and pyrimidine solutions. From the extinction coefficient at the absorption maxima the amount of base in a given extract was calculated from the formula given by Chargaff *et al.* (*J. Biol. Chem.*, 1948, **176**, 703). Since adenine and cytosine had the same R_F value in the solvent mixture used and were thus at the same position on the chromatograms, they were determined from the extinction values at 262·5 mμ (maximum absorption of adenine) and 275 mμ (maximum absorption of cytosine) according to formulæ derived from those given by Chargaff *et al.* (*ibid.*, 1950, **186**, 37; cf. Loring and Ploeser, *ibid.*, 1949, **178**, 439).

(c) *Procedure for the complete chromatographic analysis of the deoxypentosenucleic acids.* The deoxypentosenucleic acid (5 mg.) was heated in a sealed tube (10 × 0·6 cm. internal diam; wall thickness, 2·5 mm.) with formic acid ("AnalaR;" 0·6 c.c.) for 50 minutes at 175—180°. Aliquots (0·05 c.c.) of the dark brown hydrolysate were placed on filter paper strips (10 × 52 cm.). Five chromatograms were developed for each hydrolysate. If no 5-methylcytosine was present a separation sufficient for the estimation of adenine, guanine, cytosine, and thymine was achieved after 15 hours at 18—20° (total length of flow, 40 cm.). However, if preliminary experiments revealed the presence of 5-methylcytosine adequate resolution was only obtained after 24 hours (total length of flow, 60—65 cm.). The chromatograms were dried in a stream of warm air, the positions of the bases were located by ultra-violet photography, and the quantitative estimations then performed as described above.

When small amounts of 5-methylcytosine were present in the hydrolysates the appropriate segments from 3—4 chromatograms were combined and extracted with 0·1N-hydrochloric acid (5 c.c.). The amount of the base present in the extract was calculated from the ultra-violet absorption measurements at 282 mμ.

The following results obtained from the analysis of calf-thymus deoxypentosenucleic (prep. T_1) are recorded as illustrative of the accuracy obtained with the chromatographic method of analysis : Three main spots were visible on the chromatogram corresponding to guanine, adenine + cytosine, and thymine. Estimation of the bases in these spots gave the following results (in μg.) :

	Chromatogram number				
Base :	1	2	3	4	5
Thymine	43·2	45·8	40·5	39·0	38·0
Guanine	44·0	39·5	39·5	37·5	38·7
Adenine	43·8	43·0	44·2	43·5	45·0
Cytosine	28·5	27·2	27·2	29·2	28·7

5-Methylcytosine (estimated by combining four chromatograms) = 3·25 μg.

Enzymic Degradation of the Deoxypentosenucleic Acids.—A solution (0·14%; 1 vol.) of the deoxypentosenucleic acid (of known phosphorus content), 0·1M-veronal buffer (0·5 vol.; pH 7·1) containing 0·01M-$Mg^{\cdot\cdot}$, and a solution (0·01%; 0·5 vol.) of deoxyribonuclease (isolated from pancreas according to McCarty's method, *J. Gen. Physiol.*, 1946, **29**, 123) were incubated together at 37° for 15 hours. The enzyme digest was then dialysed at room temperature in a Cellophane bag against distilled water (1 l. per 10 c.c. of digest) containing 0·0002% of merthiol-

ate. The external aqueous phase was changed after 7, 24, 48, 72, and 144 hours. The combined dialysates were evaporated and analysed for total phosphorus. The non-dialysable " cores " were obtained by evaporation of the solution remaining in the dialysis sac in the frozen state. In Table 3 these " cores " are characterised as percentages of the original nucleic acids (based on the phosphorus contents of the dialysates and the original nucleic acids).

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