Table 1. Nuclear magnetic resonance spectra of hydrogen atoms in imidazole- and pyrazole-ring systems

Compound	Shift to low field with respect to reference line* (cyc./sec.)	Separation of peaks (cyc./sec.)	Multiplet structure	Relative areas	Assignment (position)
Imidazole	73	25	Singlet	2	4:5
	98		Singlet	1	2
<i>N</i> -Methylimidazole	74	21	Singlet	2	4:5
	95		Singlet	1	2
Pyrazole	46	52	Triplet	1	4
	98		Doublet	2	3:5
<i>N</i> -Methylpyrazole	46	49	Triplet	1	4
	95		Doublet	2	3:5
Isolated material†	64	49	Triplet	1	—
	113		(Unresolved doublet?)	2	—

* Where the peaks show fine structure, this shift refers to the centre of the multiplet.

† Examined as a solution in 3M-NaOD in D₂O; the other compounds were studied in D₂O alone.

The fact that two lines of relative areas 1:2 are obtained shows that any substitution in the heterocyclic ring must have occurred at the nitrogen atom. In a *C*-substituted molecule, the spectrum would consist of either a single line or of two lines of equal areas. The separation of 49 cyc./sec. between the peaks, the fine structure and the relative positions of the peaks (the peak of smaller area being closer to the reference line) all indicate that the heterocyclic system is a pyrazole ring and not an

imidazole ring. The remainder of the spectrum, which consists of a doublet and triplet in the region characteristic of hydrogen atoms attached to saturated carbon atoms, indicates the presence of a $-\text{CH}_2-\text{CH}<$ group; this is consistent with the presence of an alanine side chain attached to the heterocyclic nitrogen atom.

The spectrum of synthetic β -pyrazol-1-ylalanine was identical with that of the isolated material.

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Frequencies of Nucleotide Sequences in Deoxyribonucleic Acids

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Burton & Petersen (1957, 1960) have studied the degradation of deoxyribonucleic acid from calf thymus by the action of diphenylamine in aqueous formic acid at 30°. The products include a complex mixture of pyrimidine nucleotides that represent the sequences of pyrimidine nucleotides which were originally attached at each end to a purine nucleotide. Several of these nucleotides can be separated by paper chromatography after treatment with phosphomonoesterase. Since the degradation and enzymic dephosphorylation appear to go to completion without complicated side reactions or exchange processes these procedures constitute a simple method for determining the frequencies of a number of sequences in the deoxyribonucleic acid. This paper

describes additional results obtained with calf-thymus deoxyribonucleic acid as well as with preparations of deoxyribonucleic acid from four species of bacteria, from herring testes and from the sperm of salmon and of a sea urchin (*Echinus esculentus*). Some of the results have been communicated to The Biochemical Society (Burton, 1960).

MATERIALS AND METHODS

Nucleases. Pancreatic deoxyribonuclease and ribonuclease were obtained from Sigma Chemical Co., St Louis 18, Mo., U.S.A.

Deoxyribonucleic acids. A preparation from calf thymus was obtained by virtually the same procedure as that described by Burton & Petersen (1960). Salmon-sperm

deoxyribonucleic acid (DNA) was purchased from California Corporation for Biochemical Research, Los Angeles 63, Calif., U.S.A. It had been prepared according to Emanuel & Chaikoff (1953) but with certain unspecified modifications. Herring-testes DNA was prepared according to Emanuel & Chaikoff (1953).

Sperm of *Echinus esculentus* were mixed with 2 vol. of ethanol for transport to the laboratory. With the aid of a blender, the insoluble material was suspended in 2.5 l. of 2M-NaCl containing 5mm-disodium ethylenediaminetetraacetic acid. The suspension was filtered through a thin layer of Celite and the filtrate was treated with NaCl and then filtered according to Emanuel & Chaikoff (1953). The filtrate thus obtained was treated with chloroform in a blender and the resulting denatured protein was removed by centrifuging at 15 000 g. The DNA was precipitated by ethanol. To remove NaCl, the DNA was dissolved in water at 0° and reprecipitated with ethanol, and the solution and precipitation were repeated.

DNA was prepared from *Escherichia coli* according to Burton (1956), except that the treatment of the bacteria with sodium dodecyl sulphate was omitted.

Strain C10 of *Pseudomonas aeruginosa* (Dickinson & Codd, 1952) was grown overnight in a medium of 0.8% of tryptone, 0.5% of NaCl and 1% of glucose at 30° with aeration. The cells from 16 l. were treated in a blender with 200 ml. of 0.5M-NaCl containing 0.25% of sodium dodecyl sulphate in order to remove any extracellular DNA. (Later experiments with pancreatic deoxyribonuclease showed that the amounts of extracellular DNA were negligible in similar cultures.) The mixture was centrifuged, the cells were broken in a press (Hughes, 1951), suspended in 500 ml. of M-NaCl and treated in a blender with 50 ml. of chloroform for about 5 min. After centrifuging, the precipitate was suspended in M-NaCl and the treatment in the blender was repeated. The supernatants from the two treatments in the blender were combined and mixed with 1.5 vol. of ethanol. The resulting precipitate was dissolved in water, reprecipitated with ethanol and treated again in the blender with M-NaCl and chloroform. After centrifuging, the supernatant was mixed with ethanol and the resulting fibrous precipitate was suspended in 100 ml. of 0.07M-triethanolamine buffer, pH 7.4. Most of the insoluble material was removed by high-speed centrifuging and the supernatant was incubated at 40° for 1 hr. with 1 mg. of five-times crystallized

ribonuclease. After agitation with chloroform, centrifuging and precipitation with ethanol, the DNA was dialysed, first against 2M-NaCl and then against two lots of 20 vol. of water. The material was clarified by centrifuging and the DNA was precipitated by ethanol and dried over CaCl₂.

A culture of *Alcaligenes faecalis* (N.C.T.C. 1347) was grown overnight in a medium of 1% of peptone, 1% of tryptone and 0.5% of yeast extract with aeration at 30°. The cells from 10 l. were treated with crystalline deoxyribonuclease to remove any extracellular DNA and then they were washed twice with water and broken in a press (Hughes, 1951). The material was treated in a blender for about 5 min. with 50 ml. of chloroform and 180 ml. of ice-cold M-NaCl containing 0.5% of sodium dodecyl sulphate. The precipitate obtained on centrifuging was then extracted twice by stirring with 300 ml. of 0.5M-NaCl. The extracts were combined with the supernatants obtained after the two treatments in the blender and nucleic acids were then precipitated by ethanol. They were treated with ribonuclease and the DNA was isolated in the same way as with the material from *P. aeruginosa*.

A sample of DNA from *Micrococcus lysodeikticus* strain 53-20 was very kindly given by Dr H. B. Naylor (cf. Scaletti & Naylor, 1959).

Purity of deoxyribonucleic acids. Except for the salmon DNA, which contained 8.3% of P, the samples contained only 5.0-7.5% of P and were therefore not pure. Traces of protein were present in most of the samples but the following evidence indicates that less than 2% of the P was present as ribonucleic acid. The mixtures obtained by degradation of the DNA samples with diphenylamine and removal of the reagents by ether were found to react in the orcinol reaction as if they contained 5-6 moles of ribose per 100 g. atoms of P. The procedure of Ogur & Rosen (1950) was followed. Most of this apparent ribose was presumably due to the pyrimidine deoxyribonucleotides, since the conditions are drastic enough to hydrolyse their glycosidic links and since deoxyribose itself or each of the four common deoxyribonucleosides (California Corporation for Biochemical Research, Los Angeles 63, Calif., U.S.A.) reacted as if they contained 8-10 moles of ribose/100 moles of deoxyribose.

As mentioned in the Discussion, the results obtained with the DNA from *M. lysodeikticus* can be taken to suggest that the material may have contained 10-20% of the P as an unidentified impurity.

Table 1. *Base compositions*

Amounts are given as moles/100 moles of the four bases $\pm$ s.e.m. The values for *A. faecalis* DNA are those given by Spirin, Belozersky, Shugayeva & Vanyushin (1957) and by Catlin & Cunningham (1958) respectively. The figures in parentheses indicate the number of values.

DNA	Adenine	Guanine	Cytosine	Thymine	Adenine + thymine	
					Guanine + cytosine	Thymine Cytosine
<i>M. lysodeikticus</i>	13.1	33.0	39.6	14.2	0.38	0.30
<i>A. faecalis</i>	16.5	33.9	32.9	16.8	0.49 $\pm$ 0.01 (range)	0.48 $\pm$ 0.03 (range)
	15.9	31.5	36	16.2		
<i>P. aeruginosa</i>	16.0 $\pm$ 0.2 (9)	29.6 $\pm$ 0.6 (8)	36.3 $\pm$ 0.3 (9)	17.8 $\pm$ 0.6 (9)	0.50 $\pm$ 0.03	0.49 $\pm$ 0.02
<i>Escherichia coli</i>	26.8 $\pm$ 0.6 (4)	23.2 $\pm$ 0.4 (4)	25 $\pm$ 0.4 (4)	25 $\pm$ 0.4 (4)	1.08 $\pm$ 0.04	1.0 $\pm$ 0.03
Salmon	27.8 $\pm$ 0.4 (5)	22.7 $\pm$ 0.3 (4)	26.5 $\pm$ 0.2 (8)	28 $\pm$ 0.4 (8)	1.26 $\pm$ 0.02	1.30 $\pm$ 0.02
Calf	28.6 $\pm$ 0.3 (11)	21.2 $\pm$ 0.4 (11)	21.7 $\pm$ 0.4 (13)	28.5 $\pm$ 0.6 (13)	1.33 $\pm$ 0.02	1.32 $\pm$ 0.04
Herring	31 $\pm$ 0.4 (5)	20 $\pm$ 0.4 (5)	21 $\pm$ 0.05 (5)	28 $\pm$ 0.6 (5)	1.44 $\pm$ 0.04	1.40 $\pm$ 0.02
<i>Echinus esculentus</i>	31.6 $\pm$ 0.4 (8)	18 $\pm$ 0.6 (8)	19.2 $\pm$ 0.3 (8)	31.6 $\pm$ 0.7 (8)	1.70 $\pm$ 0.03	1.65 $\pm$ 0.05

Base analyses. These were performed by hydrolysis in 7.5N-perchloric acid and paper chromatography with propan-2-ol-conc. HCl-water (65:20:10, by vol.; Wyatt, 1955). The values found are shown in Table 1. In all the analyses, between 90 and 100 moles of base were recovered per 100 g.atoms of P. The results agree well with the appropriate literature values (Chargaff, 1955; Lee, Wahl & Barbu, 1956; Spirin, Belozersky, Shugayeva & Vanyushin, 1957), although there are some small differences for the herring, *Echinus* and *Pseudomonas* preparations of DNA. The values found for salmon DNA are slightly different from those given by Chargaff (1955) but they agree very closely with those of Emanuel & Chaikoff (1958).

There was not enough of the DNA from *A. faecalis* or *M. lysodeikticus* to permit measurement of the base compositions. The values shown for *A. faecalis* are those given by Spirin *et al.* (1957) and by Catlin & Cunningham (1958). The values for *M. lysodeikticus* are those given by Scaletti & Naylor (1959) for the same strain.

Nomenclature. As in the previous paper (Burton & Petersen, 1960), C and T represent deoxycytidine and thymidine respectively and p represents orthophosphate esterified with these deoxynucleosides. A p symbol to the left of a nucleoside symbol is esterified to the 5' position. A p symbol to the right of a nucleoside symbol is at the 3' position, except in empirical formulae such as CTp or CT₂P₂, which do not indicate the sequence of nucleosides or the position(s) of the phosphate groups.

RESULTS

Following the procedures of Burton & Petersen (1960), the eight samples of DNA were degraded with diphenylamine in 66 % (v/v) formic acid, the amine and formic acid were removed by ether extraction and the purines were removed by Dowex-50 cation-exchange resin in the H⁺-ion form. The pyrimidine nucleotides were treated with purified phosphomonoesterase until no more inorganic phosphate was released and the amounts of the various nucleotides were determined after separation by two-dimensional chromatography on paper. It was desirable to cut out the thymidine area before

Table 2. Amounts of inorganic phosphate after diphenylamine treatment and after subsequent treatment with phosphomonoesterase

Figures in parentheses are the number of measurements obtained. Each measurement was performed in duplicate or triplicate.

DNA	Inorganic P (% of total P ± s.e.m.)	
	After diphenylamine	After phosphatase
<i>M. lysodeikticus</i>	22, 21	71, 75
<i>A. faecalis</i>	24.5	75
<i>P. aeruginosa</i>	24.5, 23.5	75, 76
<i>Escherichia coli</i>	25.7 ± 0.4 (3)	71 ± 1.2 (3)
Salmon	25.2, 24.9	72.5
Calf	27.8 ± 0.4 (4)	72.2 ± 1.5 (3)
Herring	25.1 ± 0.5 (5)	72
<i>Echinus esculentus</i>	24.6 ± 0.6 (4)	73 ± 2 (3)

Table 3. Amounts of nucleotides after treatments with diphenylamine and phosphatase

The amounts were determined from the ultraviolet absorption of the eluates from two-dimensional paper chromatograms (Burton & Petersen, 1960). The number of eluates measured are shown in parentheses and the mean amounts ± s.e.m. are given in moles of pyrimidine/100 g.atoms of P on the chromatograms.

	Source of DNA							
	<i>M. lysodeikticus</i>	<i>A. faecalis</i>	<i>P. aeruginosa</i>	<i>Escherichia coli</i>	Salmon	Calf	Herring	<i>Echinus esculentus</i>
T	2.96 ± 0.1 (8)	5.5 ± 0.08 (6)	3.9 ± 0.05 (9)	5.3 ± 0.06 (6)	6.6 ± 0.19 (4)	6.53 ± 0.16 (8)	8.6 ± 0.15 (4)	8.4 ± 0.10 (4)
T ₂ P	0.22 ± 0.04 (5)	1.52 ± 0.08 (8)	1.15 ± 0.04 (11)	2.82 ± 0.06 (15)	3.15 ± 0.13 (5)	2.60 ± 0.06 (14)	3.1 ± 0.05 (5)	4.3 ± 0.30 (4)
T ₃ P ₂	—	0.5 ± 0.05 (6)	—	0.96 ± 0.05 (11)	1.23 ± 0.04 (5)	1.21 ± 0.05 (14)	1.5 ± 0.1 (6)	2.14 ± 0.06 (5)
T ₄ P ₃	—	0.1	—	0.32 ± 0.04 (10)	0.54 ± 0.04 (4)	0.57 ± 0.08 (11)	0.55 ± 0.03 (5)	0.90 ± 0.03 (4)
T ₅ P ₄	—	—	—	0.14 ± 0.02 (5)	—	0.20 ± 0.04 (4)	—	0.3
C	6.9 ± 0.09 (8)	8.3 ± 0.3 (8)	8.9 ± 0.13 (10)	6.3 ± 0.12 (14)	4.05 ± 0.12 (6)	3.85 ± 0.06 (4)	5.9 ± 0.14 (4)	5.1 ± 0.09 (5)
C ₂ P	4.9 ± 0.18 (9)	5.08 ± 0.06 (8)	7.1 ± 0.08 (8)	3.83 ± 0.1 (10)	1.71 ± 0.18 (4)	1.77 ± 0.05 (12)	1.8 ± 0.06 (5)	1.9 ± 0.04 (5)
C ₃ P ₂	1.7 ± 0.11 (9)	1.4 ± 0.1 (8)	1.4 ± 0.23 (9)	0.97 ± 0.04 (10)	0.44	0.77 ± 0.05 (7)	0.64 ± 0.03 (5)	0.5 ± 0.02 (3)
C ₄ P ₃	1.45 ± 0.03 (8)	3.5 ± 0.15 (6)	3.25 ± 0.1 (10)	2.78 ± 0.08 (12)	3.56 ± 0.15 (6)	3.04 ± 0.06 (11)	3.2 ± 0.05 (4)	2.3 ± 0.09 (3)
C ₅ P ₄	2.5 ± 0.07 (8)	2.23 ± 0.03 (6)	3.9 ± 0.05 (10)	3.15 ± 0.07 (12)	2.11 ± 0.13 (6)	2.21 ± 0.05 (11)	2.0 ± 0.01 (5)	2.9 ± 0.07 (3)
TpC	0.96 ± 0.04 (8)	2.65 ± 0.11 (8)	1.9 ± 0.08 (12)	2.20 ± 0.07 (13)	2.80 ± 0.20 (5)	2.73 ± 0.11 (10)	2.8 ± 0.09 (5)	3.1 ± 0.05 (5)
CT ₂ P ₂	—	1.1 ± 0.1 (7)	0.5 ± 0.04 (9)	1.14 ± 0.04 (11)	1.24 ± 0.09 (5)	1.48 ± 0.07 (7)	0.98 ± 0.07 (4)	1.7 ± 0.09 (5)
CT ₃ P ₃	—	0.5 ± 0.1 (4)	—	0.50 ± 0.02 (6)	0.7, 0.96	0.92 ± 0.11 (3)	0.70 ± 0.04 (4)	1.1, 1.1
CT ₄ P ₄	—	—	—	—	—	0.49	0.8	—
CT ₅ P ₅	—	—	—	—	—	2.90 ± 0.11 (4)	2.3 ± 0.10 (5)	2.0 ± 0.10 (4)
C ₂ T ₂ P ₂	3.15 ± 0.03 (9)	3.4 ± 0.08 (8)	4.6 ± 0.1 (8)	2.34 ± 0.10 (6)	3.11 ± 0.15 (4)	1.63 ± 0.17 (3)	2.3 ± 0.10 (5)	2.0 ± 0.10 (4)
C ₃ T ₃ P ₃	1.0 ± 0.10 (5)	2.1 ± 0.2 (5)	2.6 ± 0.13 (8)	1.13 ± 0.04 (6)	2.25, 1.42	—	1.6 ± 0.17 (5)	1.3, 1.1
C ₄ T ₄ P ₄	—	—	0.97	—	—	—	1.05 ± 0.10 (3)	1.8
C ₅ T ₅ P ₅	1.5 ± 0.21 (6)	—	2.6, 2.8	—	—	—	1.07, 1.1	—

development with the 2-methylpropan-2-ol-HCl-water mixture because otherwise it tended to become contaminated with impurities which absorbed ultraviolet light.

The amounts of phosphate released before and after the phosphatase treatment are shown in Table 2 and the amounts of the various nucleotides are shown in Table 3. Except for the salmon and *A. faecalis* preparations of DNA, the results in Table 3 were obtained from two or three different degradations of each DNA. The results obtained with different portions of the same DNA were in good agreement with each other.

DISCUSSION

Some of the results are presented diagrammatically in Fig. 1 and compared with the amounts of nucleotides which should have been found for randomly arranged polynucleotides of the same thymine/cytosine ratios. More detailed comparisons are shown in Table 4. The expected amounts were calculated according to Jones, Stacey & Watson (1957), and it was assumed that the thymine and cytosine together represented 50 % of the bases. Each DNA differs markedly from each other and from the randomly arranged polynucleotide, and there is no obviously consistent pattern. Although the distributions reflect the variations in thymine/cytosine ratios there are considerable differences between the amounts of the nucleotides in DNA preparations of closely similar ratios. For all the DNA preparations except *A. faecalis*, T_2P and CT_2P_2 are obtained in amounts which are less than the random value.

Isomeric dinucleotides. The two sequential isomers CpT and TpC were separated in the two-dimensional

chromatograms and, as shown in Fig. 2, the relative amounts differ for the various DNA preparations. The ratio shows no relation to the base composition and ranges from 0.6 for *M. lysodeikticus* to 1.7 for salmon DNA. It is clearly desirable to attempt to determine the proportion of isomers in the tri- and tetra-nucleotide components.

Bunching of pyrimidines and purines. Tamm, Shapiro, Lipshitz & Chargaff (1953) and Shapiro & Chargaff (1957, 1960) have claimed that there is a pronounced tendency for the nucleotides to be arranged in blocks of pyrimidines followed by blocks of purines. On the basis of the present results, such a tendency can be conveniently assessed in two somewhat independent ways: (i) from the amounts of inorganic phosphate; (ii) from the amounts of mono- and di-pyrimidine fragments.

The inorganic phosphate released by the action of diphenylamine was originally present as phosphodiester bridges between consecutive purine nucleosides. A randomly arranged polynucleotide with equimolar amounts of purines and pyrimidines would contain 25 % of its phosphate in the inter-purine positions. The results in Table 2 therefore show that there is no general tendency for purines to be bunched together, since it is only in calf-thymus DNA that appreciably more than 25 % of the phosphate is released by diphenylamine.

Although the amounts of interpurine phosphate agree within analytical error with the amounts of organic phosphate remaining after the phosphatase treatment, it is not possible exactly to equate amounts of the latter with the interpyrimidine phosphate because the DNA samples could have contained up to 2 % of ribonucleic acid as well as other phosphorus compounds. Indeed the agreements found can perhaps be taken as evidence to

Table 4. Comparison (on percentage basis) of amounts of nucleotides found with those expected for randomly arranged polynucleotides of the same base composition

The deviations are relatively larger than those in Table 3. They were calculated by the formulae $100(f/\phi)\sqrt{(s_1^2/f^2 + s_2^2/\phi^2)}$, where $f \pm s_1$ is the observed frequency and s.e.m. of the nucleotides (Table 3). ϕ is the frequency calculated for a randomly arranged polynucleotide and s_2 is the s.e. introduced by the base analyses.

Source of DNA ...	<i>A. faecalis</i>	<i>P. aeruginosa</i>	<i>Escherichia coli</i>	Salmon	Calf	Herring	<i>Echinus esculentus</i>
T	136 ± 6	94 ± 4	85 ± 3	93 ± 4	91 ± 4	118 ± 3	107 ± 4
T_2P	112 ± 11	85 ± 8	90 ± 6	81 ± 5	65 ± 2	73 ± 3	89 ± 8
T_2P_2	146 ± 20	—	82 ± 9	73 ± 5	70 ± 2	81 ± 7	95 ± 9
T_2P_3	—	—	82 ± 10	85 ± 10	88 ± 16	77 ± 6	99 ± 13
C	99 ± 6	106 ± 4	101 ± 4	75 ± 4	72 ± 3	113 ± 3	93 ± 3
C_2P	89 ± 8	128 ± 11	123 ± 9	73 ± 9	77 ± 6	83 ± 4	107 ± 7
C_2P_2	49 ± 7	50 ± 10	83 ± 8	—	103 ± 12	94 ± 7	100 ± 10
CpT	128 ± 6	118 ± 4	89 ± 3	116 ± 5	100 ± 3	105 ± 2	78 ± 3
TpC	83 ± 1	142 ± 3	101 ± 3	69 ± 5	72 ± 2	66 ± 1	99 ± 3
CT_2P_2	133 ± 9	93 ± 6	62 ± 4	71 ± 6	69 ± 4	70 ± 1	76 ± 4
CT_2P_3	185 ± 24	83 ± 10	73 ± 6	64 ± 6	75 ± 6	48 ± 5	75 ± 7
CT_2P_4	—	—	81 ± 9	—	102 ± 16	74 ± 8	—
C_2Tp_2	82 ± 5	111 ± 6	66 ± 4	103 ± 6	98 ± 6	80 ± 4	80 ± 6
$C_2T_2P_2$	113 ± 11	138 ± 8	48 ± 3	—	72 ± 8	72 ± 8	—

suggest that any such impurities were present in only small amounts except in the DNA from *M. lysodeikticus*.

The second way of assessing the degree of bunching is to examine whether the amounts of pyrimidines in the mono- and di-pyrimidine fragments are less than the amount which is expected for a randomly arranged polynucleotide, namely 50 % of the total pyrimidines. The values found are 38 % for the DNA of *M. lysodeikticus*, 40 % for calf-thymus DNA, 42.6 % for salmon DNA and between 48 and 56 % for the other five DNA preparations.

Thus both assessments indicate that there is in calf-thymus DNA a slight but definite tendency for bunching of nucleotides into blocks of pyrimidines

and blocks of purines. In salmon DNA there seems to be a slight bias against mono- and di-pyrimidine runs, although the total amount of interpurine phosphate is still 25 %. Since five of the other six DNA preparations show no appreciable tendency towards such a bunching of nucleotides, it can be concluded that this is not a general property of DNA. Even with calf-thymus DNA, the degree of bunching appears to be much less than that which was claimed by Tamm *et al.* (1953) and by Shapiro & Chargaff (1957).

The results obtained with *M. lysodeikticus* DNA are somewhat contradictory in suggesting a strong tendency against bunching by the first assessment and a strong tendency towards bunching by the second. They could be more easily understood if 10–20 % of its phosphate were present as an unidentified impurity which is incompletely dephosphorylated by the phosphatase. A correction of 15 % has therefore been arbitrarily applied to the values shown for this DNA in Figs. 1 and 2. The figures in Table 1 have not been corrected in this way and values for this DNA are not included in Table 4.

Josse & Kornberg (1960; and personal communication) have used the deoxyribonucleotide-polymerase system to study the frequencies of the sixteen kinds of consecutive pairs of nucleotides in the primer DNA. Their results agree with those given here in showing that there is a slight tendency

Fig. 1. Amounts of nucleotides obtained. For each nucleotide, the eight rectangles indicate the amounts for the respective DNA samples: A, *Micrococcus lysodeikticus*; B, *Alcaligenes faecalis*; C, *Pseudomonas aeruginosa*; D, *Escherichia coli*; E, salmon sperm; F, calf thymus; G, herring testes; H, sea-urchin sperm (*Echinus esculentus*). For reasons given in the Discussion, the amounts for A as given in Table 3 have been increased by 15 %. ●, Amounts expected for randomly arranged polynucleotides of the same base composition as the corresponding DNA. CTp, Sum of the two isomers CpT and TpC.

Fig. 2. Amounts of the isomers, deoxycytidylyl-(3' → 5')-thymidine (CpT) and thymidylyl-(3' → 5')-deoxycytidine (TpC). □, CpT; ■, TpC; ●, random values. DNA samples A–H are as identified in Fig. 1. The values for *M. lysodeikticus* from Table 3 have been increased by 15 %.

for purines to be bunched in calf-thymus DNA and essentially no tendency in the other five types of DNA which were examined. Since these five sources include *M. lysodeikticus*, their results are evidence for the validity of the correction of 15% which has been applied to the results for this DNA in Figs. 1 and 2.

SUMMARY

1. Preparations of deoxyribonucleic acid (DNA) from eight sources (four bacterial species and four animal species) have been degraded with diphenylamine in formic acid. The amounts of inorganic phosphate and of several pyrimidine nucleotides have been measured.

2. The results have been compared with those expected for randomly arranged polynucleotides of the same base composition. Each DNA differs from the others and from the randomly arranged polynucleotide but there is no obviously consistent pattern. There appears to be a tendency for T_2p and CT_2p_2 to be obtained in amounts which are less than the random value.

3. There is no general tendency for purines or pyrimidines to be bunched together in a non-random fashion.

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Levels and Distribution of the Acid-Soluble Ribonucleotides in the Mammary Glands of Pregnant and Lactating Rats

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The striking increases in the rate of metabolic and synthetic activity which are known to occur in the mammary gland in the transition from the relative quiescence of late pregnancy to the fully active state obtaining in late lactation could be expected to be reflected in similar changes occurring in either the level or turnover (or both) of the acid-soluble ribonucleotides.

Some studies on the distribution of the acid-soluble ribonucleotides in the mammary gland have already been reported. For instance, Forrest,

Wilken & Hansen (1960) have examined the nucleotide levels in rat mammary tissue at late lactation and Manson (1956) has made similar studies in the cow and goat. Both of these studies found a pattern of distribution essentially similar to that found in rat liver by Hurlbert, Schmitz, Brumm & Potter (1954). Unfortunately, these investigations covered only one stage of lactation and did not attempt to correlate the level of ribonucleotides with lactational activity. Further Denamur, Fauconneau & Guntz (1958) have