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The Distribution of Nucleotides in Deoxyribonucleic Acid

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Deoxyribonucleic acid is degraded by diphenylamine in acid solution to pyrimidine oligonucleotides of the general formula $\text{Py}_n\text{P}_{n+1}$, where Py represents pyrimidine deoxynucleoside and p is phosphate (Burton, 1956; Burton & Petersen, 1957, 1960). The scission of the backbone chain requires an aromatic amine in addition to weak acid (Burton & Petersen, 1960) and the ultimate products are the same as the principal products of hydrolysis by mineral acids under more drastic conditions (Levene & Jacobs, 1912; Dekker, Michelson & Todd, 1953; Cohn & Volkin, 1957; Shapiro & Chargaff, 1957*a, b*). After terminal dephosphorylation with prostatic phosphomonoesterase, a number of these products can be separated by two-dimensional paper chromatography. In this way, the frequencies of occurrence of pyrimidine nucleotides in deoxyribonucleic acid derived from several sources have been determined (Burton & Petersen, 1960; Burton, 1960).

Petersen (1961) separated many more of the products by means of two-dimensional electrophoresis and chromatography on paper, as applied by Rushizky & Knight (1960*a, b*) to the separation of the products of pancreatic ribonuclease action.

This paper describes further improvements in the method and gives the results obtained from the degradation of deoxyribonucleic acid from several sources.

MATERIALS AND METHODS

Deoxyribonucleic acid. The calf-thymus DNA used in this study was the same preparation as described by Burton & Petersen (1960). Herring-testis DNA, prepared by the method of Emanuel & Chaikoff (1953), was a gift from Dr M. R. Lunt. Samples of diphenylamine-degraded DNA of *Alcaligenes faecalis* and *Pseudomonas aeruginosa* were those used by Burton (1960).

Phosphorus estimations. Inorganic and total phosphate of DNA digests and of material eluted from paper chromatograms were determined as described by Burton & Petersen (1960).

Paper for chromatography and electrophoresis. Sheets of Whatman no. 1 paper (47 cm. $\times$ 57 cm.) were washed chromatographically, six at a time, with N-HCl (2 l.) and water (3 l.) and dried at room temperature.

Paper electrophoresis. A Perspex apparatus, essentially the same as that described by Rushizky & Knight (1960*a*), was used. The formate buffer used in the apparatus was 2.5 times as concentrated as that used by Rushizky & Knight (1960*a*) and was prepared by adding 45 ml. of 98–100% formic acid to 6 l. of water and titrating to pH 2.7 at room temperature with approx. 8 ml. of aq. NH_3 , sp.gr.

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0.88. The electrophoresis apparatus was operated in the cold room at 4°.

Location of nucleotides on chromatograms. The ultraviolet-absorbing spots were located on chromatograms with a Chromatolamp (Hanovia Ltd., Slough, Bucks.) and Ilford 50M photographic paper, the shortest practicable exposure time (Markham & Smith, 1949) being used. Close contact between the chromatogram and the photographic paper was achieved through the use of a frame consisting of a convex plywood board over which a double thickness of 0.005 in. gauge polythene sheet was tightly stretched and held in place with a pair of spring fasteners. No exposure correction is required with the apparatus.

Desalting of nucleotide solutions. Acid solutions of pyrimidine oligonucleotides were desalted before rechromatography by adsorption on a mixture of 5 parts by wt. of Celite (grade 545) and 1 part of Darco G60 activated charcoal (Darco Corp., 60 East 42nd Street, New York, N.Y., U.S.A.) followed by elution with 50% (v/v) ethanol containing 2% (v/v) of aq. NH_3 , sp.gr. 0.88. Almost quantitative (98–100%) recovery of adsorbed nucleotides was obtained.

Degradation of deoxyribonucleic acid with diphenylamine. DNA was degraded with 2% (w/v) of diphenylamine in 66% (v/v) formic acid and the products were freed from purines, and terminally dephosphorylated with prostatic phosphomonoesterase, as described by Burton & Petersen (1960). The enzyme digests were deproteinized with CHCl_3 and concentrated to give solutions containing 130–150 $\mu\text{g.}$ atoms of DNA phosphorus/ml.

Extinction coefficients. Theoretical values for the ratios of extinctions of pyrimidine oligonucleotides at 280 and 260 $\text{m}\mu$ were calculated from appropriate multiples of the extinction coefficients of the components. No allowance was made for any possible hypochromic effect. The molar extinction coefficients of deoxycytidylic acid at 260 and 280 $\text{m}\mu$ (pH 1–2) were taken as 6300 and 12 600 respectively. The corresponding values used for thymidylic acid were 9000 and 6300. The amounts of the products eluted from paper chromatograms were determined from the extinction of acid solutions at 267 $\text{m}\mu$ (the isosbestic point of the two pyrimidine nucleotides) by using a molar extinction coefficient of 9900 (Burton & Petersen, 1960). Extinction measurements were made with a Beckman model DU quartz spectrophotometer.

Abbreviated representation of pyrimidine oligonucleotides. The system used in this paper is that used by Burton & Petersen (1960) and Burton (1960), and is in accordance with the suggestions of the International Union of Pure and Applied Chemistry (1961) except that, since all the compounds described in this paper are deoxyribonucleoside derivatives with a regular 3'→5' internucleotide linkage, the recommended prefix 'de-3',5'' is omitted. C and T represent deoxycytidine and thymidine respectively, p represents phosphate esterified with the nucleosides. When p is to the right of the nucleoside symbol it is esterified with the 3'-hydroxyl group of the nucleoside; when it is to the left it forms a 5'-ester. Sequences that are expected to consist of mixtures of isomeric nucleotides in unknown proportions are written with the nucleoside symbols in parentheses. Thus CpT represents deoxycytidylyl-(3'→5')-thymidine, TpC represents thymidylyl-(3'→5')-deoxycytidine and (CT)p represents a mixture of these two isomers in unspecified proportions.

EXPERIMENTAL AND RESULTS

The separation of the products of the reaction of diphenylamine and phosphomonoesterase with DNA was carried out in two stages. Samples (0.1 ml.) of the digests were spotted on acid-washed Whatman no. 1 paper and chromatographed in two dimensions as described by Burton & Petersen (1960). In the present work the solvent used for the second development in the first dimension was propan-2-ol-aq. 5N-ammonia (65:35, v/v), instead of propan-2-ol-water (7:3, v/v) as previously used. This solvent, which has been used in the separation of products from bromouracil-containing DNA (Burton, 1962), gave improved separation of the pyrimidine oligonucleotides. A small amount (approx. 0.1 μC) of carrier-free [^{32}P]orthophosphate was added to the material spotted on each chromatogram. The inorganic phosphate spots on the developed chromatograms were detected by radioautography and eluted with 50 ml. of water. The amount of inorganic phosphate in the solution was determined, and this value was used to calculate the total DNA phosphorus applied to the paper, from the fact that 73% of the calf-thymus and 75.5% of the herring-testis DNA phosphorus in the digests was in this form. The portion of each chromatogram bearing the C_3P_2 , $(\text{C}_2\text{T})\text{P}_2$ and $(\text{C}_2\text{T}_2)\text{P}_3$ spots, together with all unresolved material running nearer to the origin, was cut out and eluted by descending chromatography with approx. 50 ml. of diethyl ether, followed by 250 ml. of water. The ether eluate, which contained non-nucleotide ultraviolet-absorbing material, was discarded. A standardized solution (0.1 ml.) of thymidine 5'-phosphate (California Corp. for Biochemical Research, Calif., U.S.A.), containing approx. 1.5 mg./ml., was then added to the water eluate with a micrometer syringe. The water solution was concentrated in a rotary evaporator, transferred to a small tube and evaporated to dryness in a stream of nitrogen at 37°. An air stream was unsuitable since it led to the formation of brown oxidation products, which interfered with the subsequent electrophoresis. The residue was dissolved in 0.05 ml. of water.

This solution was subjected to two-dimensional electrophoresis and chromatography on acid-washed Whatman no. 1 paper, as described by Petersen (1961). The solvent for the chromatographic step was 2-methylpropan-2-ol-ammonium formate, as used by Rushizky & Knight (1960a). A better separation of the components was obtained by developing the chromatograms twice in the same direction in the buffered solvent. The separation of the products is illustrated in Fig. 1. By this procedure several more pyrimidine oligo-

Fig. 1. Tracing of a contact print of a chromatogram made in ultraviolet light, illustrating the separation of some of the reaction products. Paper electrophoresis and chromatography were carried out as described by Petersen (1961). The short vertical interrupted line indicates the position of the electrophoresis origin, which was 15 cm. from the negative end of the sheet. The horizontal line A indicates the line to which the bands were concentrated before chromatography. The solvent front is marked B. Tp represents the thymidine 5'-phosphate marker. The components shown are: 1, C₃P₂; 2, C₄P₃; 3, (C₃T)P₃; 4, (C₄T)P₄; 5, (C₂T₂)P₃; 6, (C₃T₂)P₄; 7, (C₄T₂)P₅; 8, (C₂T₃)P₄; 9, (C₃T₃)P₅; 10, (C₄T₃)P₆; 11, (CT₄)P₄; 12, (C₂T₄)P₅; 13, (C₃T₄)P₆; 14, (C₄T₄)P₇; 15, (CT₅)P₅; 16, (C₂T₅)P₆; 17, (C₃T₅)P₇; 18, (C₄T₅)P₈; 19, (CT₆)P₆; 20, (C₂T₆)P₇; 21, (C₃T₆)P₈.

nucleotides are separated than by electrophoresis of the whole digest without prior paper chromatography (Petersen, 1961). The amount of thymidine 5'-phosphate marker on each chromatogram was determined and used as an internal standard to compensate for losses on transfer.

In other experiments with more heavily loaded chromatograms of a calf-thymus digest, additional faint spots occupying positions corresponding to (C₅T₂)P₆, (C₅T₃)P₇ and (C₅T₄)P₈ were seen, but the amounts were too small to permit accurate measurement or identification. In no case was C₅P₄ or any higher polycytidylic acid homologue seen. Pooled samples from several chromatograms of those components not previously isolated from diphenylamine digests of DNA were desalted on charcoal, rechromatographed in 2-methylpropan-2-ol-ammonium formate and eluted with 0.03N-hydrochloric acid, along with appropriate paper blanks. The ratios of extinctions at 280 and 260 mμ are given in Table 1, together with the measured extinction coefficients and the corresponding calculated values. The identification of the various

Table 1. Extinction characteristics of pyrimidine oligonucleotide sequences isolated from calf-thymus deoxyribonucleic acid

$E(p)_{267}$ is the extinction coefficient at 267 mμ/mg.atom of phosphorus. The theoretical values were calculated by using an extinction coefficient at 267 mμ of 9.9/m-mole of pyrimidine and ignoring any possible hypochromic effect.

Sequence	E_{280}/E_{260} (pH 1-2)		$E(p)_{267}$	
	Found	Calc.	Found	Calc.
C ₄ P ₃	1.92	2.0	11.6	13.2
(C ₃ T)P ₃	1.50	1.58	12.5	13.2
(C ₄ T)P ₄	1.63	1.66	10.5	12.4
(C ₃ T ₂)P ₄	1.31	1.36	11.6	12.4
(C ₂ T ₃)P ₄	1.07	1.11	11.4	12.4
(C ₄ T ₂)P ₅	1.42	1.46	11.6	11.9
(C ₃ T ₃)P ₅	1.20	1.24	10.4	11.9
(C ₂ T ₄)P ₅	1.00	1.04	10.2	11.9
(C ₄ T ₃)P ₆	1.30	1.33	10.4	11.5
(C ₃ T ₄)P ₆	1.14	1.15	10.0	11.5
(C ₂ T ₅)P ₆	0.99	0.99	10.3	11.5
(CT ₆)P ₆	0.81	0.84	—	—
(C ₄ T ₄)P ₇	1.17	1.24	10.5	11.3
(C ₃ T ₅)P ₇	1.04	1.08	10.0	11.3
(C ₂ T ₆)P ₇	0.90	0.95	11.0	11.3
(C ₄ T ₅)P ₈	0.96	1.17	—	—

components is further supported by the positions that these compounds occupy on the chromatograms, suggesting their relationship in homologous series. Compounds increasingly rich in thymine, and thus with greater net negative charge at pH 2.7, show increasing mobilities on electrophoresis, whereas the R_F values in the chromatographic direction decrease with increasing chain length.

The efficiency of the elution step in the procedure was tested on chromatograms of a dephosphorylated diphenylamine digest of the DNA of *Micrococcus lysodeikticus* labelled with ³²P (given by Dr K. Burton). After elution with 190 ml. of water, the papers were heated in 2N-hydrochloric acid at 100° for 30 min., and samples of the acid eluate counted. Less than 0.07% of the total activity placed on the chromatograms remained to be extracted with hot hydrochloric acid.

Amounts of nucleotides found in deoxyribonucleic acids from different sources. The amounts of Py_nP_{n-1} products isolated from the DNA of two species are given in Table 2, together with the amounts of these products that would have been expected if the nucleotides were randomly arranged in the nucleic acid molecule. The random values were calculated by the method of Jones, Stacey & Watson (1957), and are based on thymine/cytosine ratios 1.32 and 1.40 for calf-thymus DNA and herring-testis DNA respectively (Burton, 1960).

Sequences of polydeoxycytidylic acid. The sequence C₄P₃ was found in both DNA samples studied, contrary to the results of Spencer & Chargaff (1963), who were unable to detect this sequence in an acid

hydrolysate of calf-thymus DNA. Spots corresponding to sequences of deoxycytidylic acid longer than C_4p_3 were not found, however, even on heavily loaded papers. As an additional check, those parts of the paper that would be expected to contain this spot and any higher homologues were eluted with acid, and the absorption of the eluates was compared with the absorption of a corresponding blank cut from an adjacent part of the paper nearer to the cathode end of the sheet. In no case was there any absorption attributable to deoxycytidylic acid. The amounts of C_5p_4 that would be expected to occur in calf-thymus and herring-testis DNA on the basis of a random distribution of nucleotides are 0.056 and 0.049 mole of cytosine/100 g.atoms of DNA phosphorus respectively. Since the present methods would be capable of detecting less than 5 μ m-moles of deoxycytidylic

acid on the paper, it is concluded that the sequence C_5p_4 accounts for less than 0.03 mole of cytosine/100 g.atoms of DNA phosphorus.

In an attempt to establish whether the absence of $Pu-p-(Cp)_5-Pu$ sequences is a general feature of DNA, a preliminary examination was made of two bacterial DNA samples that are rich in cytosine. Terminally dephosphorylated diphenylamine digests of the DNA of *Alcaligenes faecalis* (thymine/cytosine 0.48) and the DNA of *Pseudomonas aeruginosa* (thymine/cytosine 0.49) were subjected to paper electrophoresis without preliminary chromatography (Petersen, 1961). The C_2p , C_3p_2 and C_4p_3 areas were eluted and measured. The amount of DNA phosphorus placed on the papers was determined from the amount of $(C_2T)p_2$ present, by using the values found by Burton (1960) for this component. The results of several

Table 2. Amounts of various pyrimidine oligonucleotides isolated from deoxyribonucleic acid, compared with the amounts expected from a randomly arranged polymer of the same base composition

Values are expressed as moles of pyrimidine/100 g.atoms of DNA phosphorus $\pm$ s.e.m. with the number of estimations given in parentheses. Values for T_4p_3 and T_7p_6 of calf thymus were obtained from another sample of DNA, more heavily loaded paper chromatograms (approx. 18.5 μ g.atoms of DNA phosphorus/paper) being used.

Sequence	Calf-thymus DNA			Herring-testis DNA		
	Found	Random	% of random	Found	Random	% of random
C	3.92 $\pm$ 0.06 (8)	5.35	73	6.02 $\pm$ 0.05 (9)	5.20	116
T	6.23 $\pm$ 0.05 (6)	7.08	88	7.76 $\pm$ 0.1 (8)	7.30	106
C_2p	1.99 $\pm$ 0.01 (8)	2.29	87	2.07 $\pm$ 0.01 (8)	2.16	96
CpT	2.96 $\pm$ 0.06 (7)	3.03	98	3.14 $\pm$ 0.01 (9)	3.04	103
TpC	2.23 $\pm$ 0.04 (7)	3.03	77	2.00 $\pm$ 0.04 (8)	3.04	66
T_2p	2.62 $\pm$ 0.02 (8)	4.01	65	3.02 $\pm$ 0.03 (9)	4.26	71
C_3p_2	0.76 $\pm$ 0.02 (7)	0.74	103	0.63 $\pm$ 0.03 (6)	0.68	93
$(C_2T)p_2$	2.73 $\pm$ 0.05 (7)	2.92	94	2.48 $\pm$ 0.02 (9)	2.84	87
$(CT_2)p_2$	2.64 $\pm$ 0.03 (8)	3.86	68	2.81 $\pm$ 0.05 (9)	3.99	70
T_3p_2	1.28 $\pm$ 0.03 (8)	1.70	75	1.71 $\pm$ 0.02 (8)	1.87	91
C_4p_3	0.31 $\pm$ 0.02 (8)	0.21	148	0.11 $\pm$ 0.01 (5)	0.19	58
$(C_2T)p_3$	1.39 $\pm$ 0.02 (7)	1.11	125	1.03 $\pm$ 0.04 (7)	1.05	98
$(C_2T_2)p_3$	1.99 $\pm$ 0.04 (8)	2.20	91	1.57 $\pm$ 0.05 (6)	2.21	71
$(CT_2)p_3$	1.53 $\pm$ 0.04 (7)	1.94	79	1.36 $\pm$ 0.04 (9)	2.07	66
T_4p_3	0.55 $\pm$ 0.03 (8)	0.64	86	0.61 $\pm$ 0.01 (9)	0.73	84
$(CT_4)p_4$	0.39, 0.39 (2)	0.37	105	0.35 $\pm$ 0.03 (4)	0.34	103
$(C_3T_2)p_4$	1.06 $\pm$ 0.04 (8)	0.98	108	0.84 $\pm$ 0.01 (7)	0.96	88
$(C_2T_3)p_4$	1.27 $\pm$ 0.03 (7)	1.30	98	0.94 $\pm$ 0.03 (6)	1.35	70
$(CT_4)p_4$	0.82 $\pm$ 0.02 (7)	0.86	95	0.59 $\pm$ 0.02 (6)	0.94	63
T_5p_4	0.19 $\pm$ 0.01 (7)	0.23	83	0.19 $\pm$ 0.02 (7)	0.27	70
$(C_2T_2)p_5$	0.40 $\pm$ 0.02 (5)	0.38	105	0.25, 0.21 (2)	0.36	64
$(C_3T_3)p_5$	0.85 $\pm$ 0.02 (8)	0.67	128	0.51 $\pm$ 0.03 (7)	0.67	76
$(C_2T_4)p_5$	0.79 $\pm$ 0.02 (7)	0.66	120	0.41 $\pm$ 0.02 (7)	0.71	58
$(CT_5)p_5$	0.42 $\pm$ 0.01 (6)	0.35	120	0.14 $\pm$ 0.03 (6)	0.40	35
T_6p_5	0.083 $\pm$ 0.003 (9)	0.077	107	0.04, 0.04 (2)	0.09	43
$(C_4T_2)p_6$	0.41 $\pm$ 0.01 (7)	0.29	141	0.23 $\pm$ 0.01 (7)	0.29	122
$(C_3T_4)p_6$	0.39 $\pm$ 0.01 (6)	0.38	103	0.26 $\pm$ 0.02 (7)	0.40	65
$(C_2T_5)p_6$	0.29 $\pm$ 0.01 (7)	0.31	94	0.15 $\pm$ 0.01 (6)	0.34	44
$(CT_6)p_6$	0.11 $\pm$ 0.01 (6)	0.13	85	0.07 (1)	0.16	(44)
T_7p_6	0.034 $\pm$ 0.002 (5)	0.025	136	—	—	—
$(C_4T_4)p_7$	0.26 $\pm$ 0.01 (7)	0.19	137	0.13 $\pm$ 0.01 (7)	0.19	68
$(C_3T_5)p_7$	0.22 $\pm$ 0.01 (7)	0.20	110	0.08 $\pm$ 0.01 (6)	0.21	38
$(C_2T_6)p_7$	0.13 $\pm$ 0.02 (7)	0.13	100	0.04 (1)	0.15	(27)
$(C_4T_5)p_8$	0.10 $\pm$ 0.01 (5)	0.11	91	0.05 $\pm$ 0.02 (4)	0.11	46
$(C_3T_6)p_8$	0.08 $\pm$ 0.01 (5)	0.10	79	—	—	—

Table 3. Amounts of cytosine-containing fragments found in deoxyribonucleic acid samples from two bacteria

Results of several determinations are given in each case and are expressed as moles of cytosine/100 g.atoms of DNA phosphorus.

Sequence	<i>Alcaligenes faecalis</i>			<i>Pseudomonas aeruginosa</i>		
	Found	Random	Mean % of random	Found	Random	Mean % of random
C ₂ p	5.05, 5.21, 5.01	5.63	90	7.09, 7.15, 7.34, 7.38	5.71	127
C ₃ p ₂	1.54, 1.60, 1.53	2.83	55	1.90, 1.85, 1.82, 1.94	2.90	65
C ₄ p ₃	0.28, 0.38, 0.24	1.27	24	0.31, 0.32	1.31	24

experiments are given in Table 3, together with the amounts expected on the basis of a random distribution. Although the sequence C₄p₃ was found in both DNA samples in amounts considerably less than those to be expected on a random basis, no ultraviolet-absorbing spot that might correspond to C₅p₄ was seen on any of the chromatograms. The portions of the papers that would be expected to bear C₅p₄ and higher homologues were eluted with acid and the extinctions of the eluates compared with those of appropriate blanks. In some cases a small net increase in extinction over the blank value was observed but the areas of paper eluted were large (approx. 50 cm.²) and the extinctions of the eluates were small. The eluates (3 ml.) with the highest absorption had E 0.026 at 280 m μ and E_{280}/E_{260} approx. 1. In no case was it possible to identify the ultraviolet-absorbing material as polydeoxycytidylic acid. However, even if all the extinction at 280 m μ was due to this compound, the amounts of C₅p₄ would be less than 17% of the random values (which are 0.53 and 0.55 mole of cytosine/100 g.atoms of DNA phosphorus for *Alcaligenes faecalis* and *Pseudomonas aeruginosa* respectively). The actual values are probably very much lower than this.

DISCUSSION

In all, at least 34 pyrimidine nucleotide products were separated, compared with 16 sequences previously isolated by paper chromatography alone. With few exceptions, the values obtained in this study are in agreement with those found by Burton & Petersen (1960) and Burton (1960). This is also true of sequences previously separated by paper chromatography alone but now obtained after paper electrophoresis. This demonstrates the efficiency of the elution step and the validity of using thymidine 5'-phosphate as an internal standard. The higher values obtained for (C₂T₂)p₃ of calf-thymus DNA and (CT₃)p₃ of herring-testis DNA are probably the result of a cleaner separation from other components in the new system. A number of the new pyrimidine nucleotide sequences

separated from both of the DNA samples studied are distributed in amounts significantly different from those expected on the basis of a random distribution of bases in the polymer. In agreement with the conclusion of Burton (1960), however, no general trend common to both DNA samples can be seen.

The overall recovery of pyrimidine bases was 41.4 and 41.6 moles of pyrimidine/100 g.atoms of DNA phosphorus for calf-thymus DNA and herring-testis DNA respectively, compared with the corresponding random values of 48.0 and 48.7 moles/100 g.atoms of DNA phosphorus. The reason for this low recovery probably lies partly in the difficulty of compensating for the ultraviolet absorption of the paper. It is likely that the method used in this study tended to over-estimate this background absorption. In addition to this source of error, the values presented in Table 1 reveal that many of the sequences display a hypochromicity that can be as high as 15%. Many of the values given in Table 2 must therefore be considered as minimum values. The use of radioactive DNA in studies of this type would obviate both sources of error.

Calf-thymus DNA and herring-testis DNA have been shown to contain approx. 1.9 and 2.8 moles of 5-methylcytosine/100 g.atoms of DNA phosphorus respectively (Laland, Overend & Webb, 1952). Pyrimidine oligonucleotides containing this base are not separated from their cytosine-containing analogues in the present system. Since at 267 m μ the molar extinction coefficient of 5-methyldeoxycytidylic acid is approximately one half of that of deoxycytidylic acid and thymidylic acid (Cohn, 1951), the percentage recovery of total pyrimidines based on extinction measurements at 267 m μ may be low by an amount of the order of 1.9 and 2.8% for calf-thymus DNA and herring-testis DNA respectively.

SUMMARY

1. A combination of paper chromatography and paper electrophoresis has been used to separate the pyrimidine oligonucleotides obtained through the

action of acid diphenylamine solution and phosphomonoesterase on deoxyribonucleic acid. Thirty-four components, consisting of oligonucleotides containing up to nine pyrimidine residues, have been separated and identified with respect to pyrimidine composition and chain length. Many of these, however, consist of mixtures of isomeric sequences in unknown proportions.

2. The amounts of these components in digests of calf-thymus deoxyribonucleic acid and herring-testis deoxyribonucleic acid have been measured. Many of the sequences were present in amounts significantly different from those expected on the basis of a random distribution of nucleotides in deoxyribonucleic acid, but, in accordance with earlier conclusions, no general trend common to both nucleic acids was seen.

3. Sequences of four consecutive deoxycytidylic acid residues were found in digests of deoxyribonucleic acid from calf thymus, herring testis. *Alcaligenes faecalis* and *Pseudomonas aeruginosa*. Sequences of five consecutive deoxycytidylic acid residues were not detected in any of these four preparations and therefore may be absent.

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Lipids of Human Adrenals

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Numerous studies have demonstrated that the adrenal gland is extremely rich in lipids, especially cholesterol, and that stress or administration of corticotrophin causes depletion of lipids, particularly cholesterol (Rogers & Williams, 1947; Adams & Baxter, 1949; Sayers, 1950). The range of conditions likely to cause 'stress' as used in this context is wide and includes severe infections, injuries, especially severe burns, exposure to cold and severe emotional upsets of the degree of severity associated with suicidal states.

Symington & Davidson (1956), Symington, Duguid & Davidson (1956) and Symington (1960), working with human glands obtained at autopsy

and from patients undergoing two-stage adrenalectomy for cancer of the breast, showed that stress or stimulation with corticotrophin caused disappearance of the sudanophil globules from the clear cells, which were replaced by compact cells.

There is now good evidence that cholesterol is utilized by the adrenal as a precursor of the steroid hormones (Hechter, 1958), but Grant (1960) has drawn attention to the fact that none of the schemes proposed envisages the use of cholesterol esters despite the evidence that these are involved.

It was clear that a detailed study of the lipids of the adrenal under varying conditions of stress or stimulation would be of value. In the present