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Determination of the Base Composition of Deoxyribonucleic Acid from its Thermal Denaturation Temperature

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The previously discovered linear relation between the base composition of DNA, expressed in terms of percentage of guanine plus cytosine bases, and the denaturation temperature, T_m, has been further investigated. By means of measurements on 41 samples of known base composition the previously observed relation has been confirmed. It can be summarized thus: for a solvent containing 0·2 M-Na^+, $T_m = 69{\cdot}3 + 0{\cdot}41$ (G–C) where T_m is in degrees Centigrade and G–C refers to the mole percentage of guanine plus cytosine. The deviations of experimental points from this relation are no more than that expected from the uncertainties of base analysis and the variations of a half degree in the reproducibility of determining the T_m. Consequently it appears that the measurement of the T_m is a satisfactory means of determining base composition in DNA. The T_m values are most simply measured by following the absorbance at 260 mμ as a function of temperature of the DNA solution and noting the midpoint of the hyperchromic rise. Only 10 to 50 μg of DNA are required.

A number of other DNA samples of unknown base composition have been examined in this manner and their base compositions recorded.

1. Introduction

The extensive chemical base analyses of DNA by Chargaff (1955) and his associates in which they have shown the equivalence of the base pairs adenine and thymine and guanine and cytosine, and the general acceptance of the Watson–Crick structure for DNA, have made it possible to determine the average base composition of DNA by correlating it with some physical property of the macromolecule. Such a relationship has been demonstrated between the mole percent guanine plus cytosine (G–C) and the buoyant density of DNA in a CsCl density gradient (Sueoka, Marmur & Doty, 1959; Rolfe & Meselson, 1959; Schildkraut, Marmur & Doty, 1962).

It has also been shown that the base composition of DNA is related to its thermal denaturation temperature. When DNA is heated in solution, a sharp increase in its extinction coefficient occurs at the temperature where the transition takes place from the native, double-stranded structure to the denatured state. The temperature corresponding to the midpoint of the absorbance rise, the T_m, is linearly related to the average DNA base composition; a higher G–C content confers a higher thermal stability (Marmur & Doty, 1959).

Because of its ease of determination and the reproducibility of the results, the thermal transition profile is useful in determining the mole percent G–C of polymerized DNA. Other properties of the DNA preparation, such as its heterogeneity

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with respect to base composition as well as the presence of denatured, contaminating regions can be detected and estimated.

2. Materials and Methods

DNA was isolated by the method of Marmur (1961) from micro-organisms harvested in the logarithmic phase of growth. The samples of DNA from salmon sperm and calf thymus were prepared by the method of Simmons (Litt, 1958). The bacteriophage DNA was a gift from Dr. H. Van Vunakis of Brandeis University. Samples of poly d-AT and d-GC were generously supplied by Drs. J. Adler, J. Josse and A. Kornberg of Stanford University. It is important that the DNA be in the native configuration. The protein content of the DNA samples was estimated to be approximately 0·2 to 0·5%. Small variations in the amount of protein contaminating the DNA preparations did not significantly alter the denaturation temperature.

The base compositions of the DNA, determined by chemical analyses, were gathered from published reports of other workers.

Determination of the T_m: Unless otherwise stated, the solvent used for the DNA was 0·15 M-NaCl plus 0·015 M-sodium citrate adjusted to pH 7·0 ± 0·3 and referred to hereafter as saline–citrate. The DNA was at a concentration of approximately 20 μg/ml. and contained in a 3 ml. glass-stoppered quartz cuvette having a 1 cm light path. Small (1 ml.) cuvettes were also used when a limited supply of the sample was available. The samples were placed in the Beckman DU spectrophotometer chamber whose temperature could be raised by circulating hot water through two thermal spacers on either side of it. To protect the photocell, spacers with slowly circulating tap water at room temperature are placed adjacent to the thermal spacers circulating the hot water. A special cover for the cuvette chamber can be easily fashioned with a small hole for a thermometer to fit snugly into the chamber with its bulb immersed in a cuvette occupying either end of the chamber. This arrangement leaves enough space for two samples and a blank. The temperature drop between the thermostat and the cuvette varies from 0 to 6°C over the operating temperature range 25 to 100°C.

In determining the T_m of the DNA, the temperature of the chamber can be raised quickly to about 5°C below the estimated onset of the melting region after first measuring the optical density at 25°C. When temperature equilibrium has been attained, the temperature is then raised about 1°C at a time, allowing about 10 min for equilibration at each temperature. The optical density is read at 260 mμ and corrected for thermal expansion of the solution. A sharp increase in the absorbance occurs in the transition range during which the DNA denatures. When no further increase occurs on raising the temperature, the denaturation can be considered to be complete. When the T_m is greater than 90°C, ethylene glycol is added to the thermostatted water. Temperatures of 104°C in the chamber can easily be attained in this manner. If one uses glass-stoppered cuvettes that fit well, the loss of water due to evaporation during the heating cycle can be kept to less than 2%.

The optical density at each temperature, corrected for thermal expansion, is divided by the value at 25°C and the ratio (relative absorbance) plotted *versus* the temperature of the solution. The temperature corresponding to half the increase in the relative absorbance is designated as the T_m.

When the thermometer used to record the cuvette temperature was standardized, its readings, in °C, compared to the corrected values (in parentheses) were as follows: 65 (65·5); 70 (70·5); 80 (80·5); 85 (85·8); 90 (91); 100 (101). The temperatures recorded in Figs. 1 to 3 of this and previous communications are uncorrected; those of the Tables 1 to 5 have been corrected, as have been the values of Fig. 4.

3. Experimental Results

Dependence of the T_m *on the ionic strength.* The thermal transition of DNA is greatly influenced by the ionic strength of the solution. Figures 1 and 2 clearly show this dependence for DNA isolated from *E. coli* (K12) and *D. pneumoniae* (R-36A). It is

also apparent that, because of its higher G+C content, the *E. coli* (G+C = 50%) has a higher T_m than the *D. pneumoniae* DNA (G+C = 39%) at equivalent KCl concentrations.

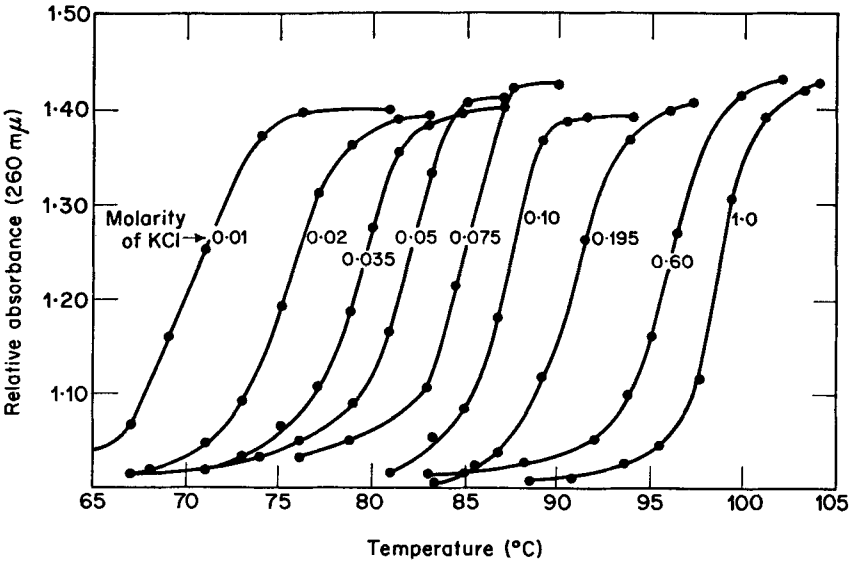

FIG. 1. Dependence of thermal denaturation of *E. coli* K12 DNA on ionic strength. *E. coli* DNA, suspended in various concentrations of KCl in glass-stoppered quartz cuvettes, was heated in the Beckman model DU spectrophotometer chamber and the relative absorbance (corrected for thermal expansion) measured at the elevated temperatures. The temperature readings are uncorrected.

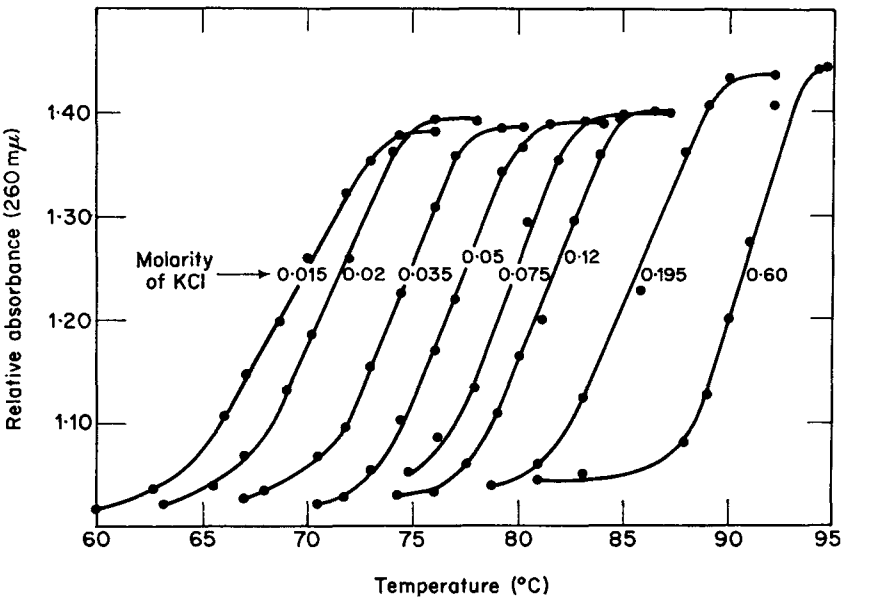

FIG. 2. Dependence of thermal denaturation of *D. pneumoniae* (R-36A) DNA on ionic strength. The experiments were carried out in an identical manner to that described in Fig. 1.

Effect of molecular weight on the T_m. Calf thymus DNA subjected to sonic disintegration (Doty, McGill & Rice, 1958) to a molecular weight of 620,000 was compared to native DNA of a higher molecular weight (8×10^6) with respect to their T_m values in saline–citrate. It can be seen from Fig. 3 that the T_m is essentially unaltered by degrading the double-stranded molecule.

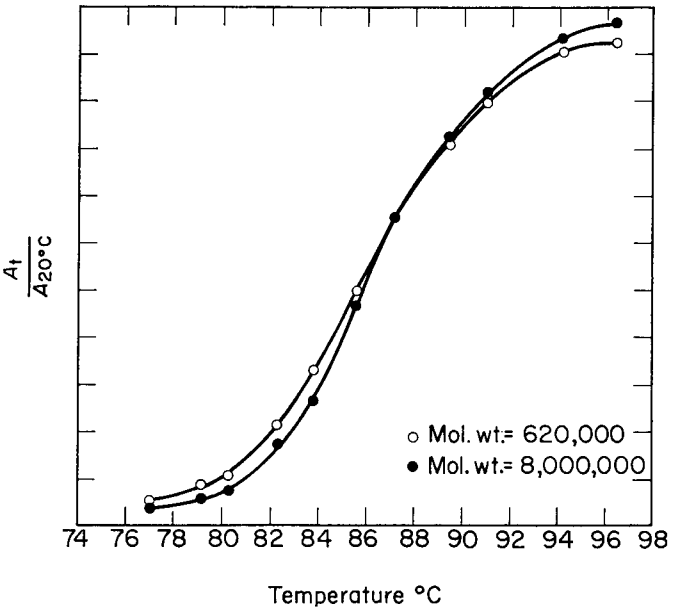

FIG. 3. Effect of molecular weight on the thermal denaturation of calf thymus DNA in 0·15 M-NaCl plus 0·015 M-sodium citrate. Sonically treated and untreated samples of DNA were thermally denatured and the absorbance at the elevated temperatures relative to that of native material at 20°C ($A_t/A_{20°C}$) was plotted as a function of the temperature to which the DNA solutions were exposed. The temperature readings are uncorrected.

Reproducibility of the T_m *within strains.* In order to evaluate the reproducibility of the T_m values, a number of determinations was carried out on portions of the same sample and on different preparations from the same source. Eight determinations of a single sample of *E. coli* DNA and measurements of twelve different *D. pneumoniae* preparations showed the same degree of reproducibility of the T_m values. The standard deviation was $\pm 0{\cdot}4$°C. From the slope of the curve (Fig. 4) relating mole percent G–C to T_m, the maximum error in the estimation of the base composition from the T_m is seen to be of the order of ± 1 mole per cent G–C. Thus, by determining the T_m value several times on the same preparation, the uncertainty arising from the measurement itself can be kept within the equivalent of 1 mole per cent.

When seven different strains of *E. coli* (B, C, K12, TAU$^-$, I, 44B, and Crooks) were used as sources of DNA, it was found that the variability of their denaturation temperatures was approximately the same as the T_m values of different DNA preparations isolated from the same strain as well as of repeated determinations carried out on a single sample of DNA.

Relationship between T_m *and G–C contents.* In Fig. 4 we have plotted the values of T_m for samples isolated from a variety of sources as a function of the G–C content of the sample for two ionic strengths. The line at the lower ionic strength

(0·01 M-phosphate plus 0·001 M-EDTA) is parallel to the line at the higher ionic strength (0·15 M-NaCl plus 0·015 M-sodium citrate). The lines have a slope of 0·41°C per 1% rise in the G–C content and are separated by 20°C. The relation in saline–citrate

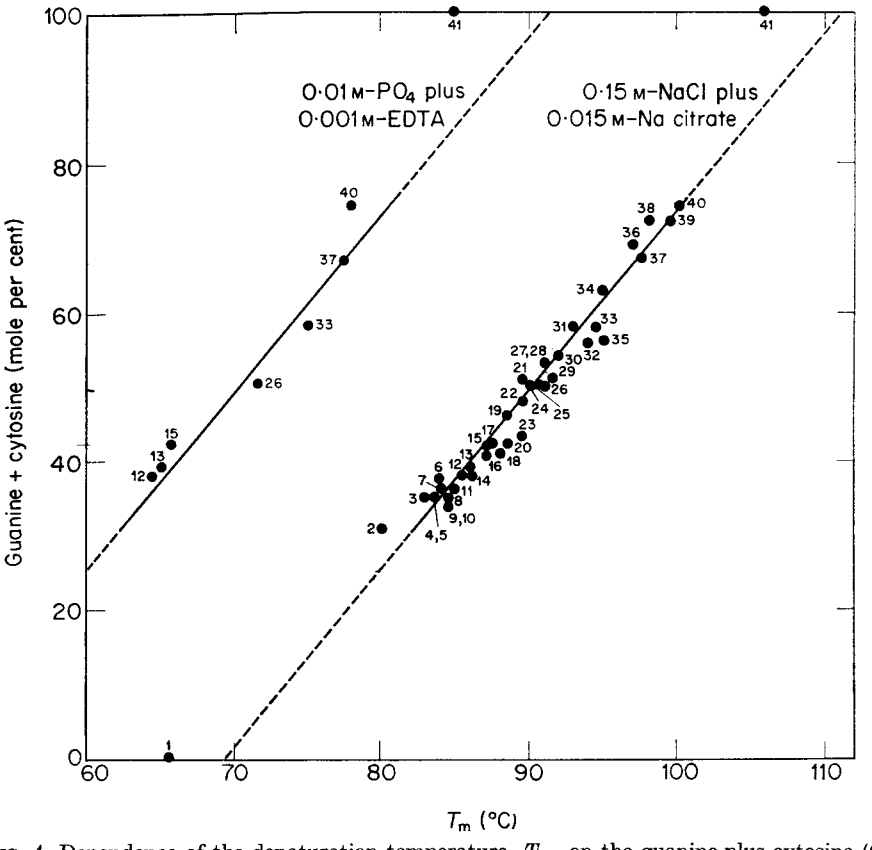

FIG. 4. Dependence of the denaturation temperature, T_m, on the guanine plus cytosine (G–C) content of various samples of DNA. The DNA samples were dissolved in either of the two solvents shown in the Fig. The T_m represents the midpoint of the hyperchromic increase of individual DNA absorbance–temperature profiles, carried out and plotted as shown in Figs. 1 to 3, and has been plotted as a function of the G–C content. The numbers next to each T_m value in the Fig. refer to the DNA extracted from the following organisms: 1, poly d-AT; 2, *Cl. perfringens*; 3, *Past. tularensis*; 4, *M. pyogenes* var. *aureus*; 5, *B. cereus*; 6, *Pr. vulgaris*; 7, *B. thuringiensis*; 8, T2r^+; 9, T4r^+; 10,T6r^+; 11, *S. cerevisiae*; 12, *B. megaterium*; 13, *D. pneumoniae*; 14, *H. influenzae*; 15, Calf thymus; 16, *N. catarrhalis*; 17, Chicken liver; 18, Salmon sperm; 19, Wheat germ; 20, *B. subtilis*; 21, *B. licheniformis*; 22, T7; 23, *V. cholerae*; 24, T3; 25, *N. flavescens*; 26, *E. coli*; 27, *Salm. typhosa*; 28, *Sh. dysenteriae*; 29, *N. meningitidis*; 30, *Salm. typhimurium*; 31, *Br. abortus*; 32, *A. aerogenes*; 33, *S. marcescens*; 34, *Ps. fluorescens*; 35, *Azot. vinelandii*; 36, *Myco. phlei*; 37, *Ps. aeruginosa*; 38, *Sar. lutea*; 39, *M. lysodeikticus*; 40, *Strep. viridochromogenes*; 41, poly d-GC. The first and last samples, the enzymatically prepared polymers, were obtained through the generosity of Drs. A. Kornberg, J. Josse and J. Adler.

The T_m values have been corrected and are those marked with an asterisk in Tables 1 to 3. The line shown to the right was fitted to the points shown by the method of least squares.

can be represented by the equation $T_m = 69{\cdot}3 + 0{\cdot}41$ (G–C). The T_m values for enzymatically synthesized poly d-GC and *S. viridochromogenes* could only be determined at the lower ionic strength. The expected values of T_m for these samples

TABLE 1

Mole per cent G–C and T_m of DNA from micro-organisms

Organism	Strain or source	T_m (°C)	Mole per cent G–C From T_m	Mole per cent G–C Literature
	Enterobacteriaceae†			
**Proteus vulgaris*	ATCC 9484	85	37	36·5‡
Pr. mirabilis	35	85·3	38	
Pr. rettgeri	3478	86	39·5	
**Shigella dysenteriae*	15	90·5	50	53·4‡
**Salmonella typhosa*	643, ETS9	90·5	50	53·3‡
Salm. arizona	PC 145	90·5	50	
Escherichia freundii	5619–52	90·5	50	
**E. coli*	B, C, W, K12	90·5	50	50·1‡
Salm. ballerup	Walter Reed	91	51·5	
"Aerobacter"	1041	91	51·5	
Pr. morganii	ATCC 8019	91	51·5	
**Salm. typhimurium*	LT-2	91	51·5	50·2‡
Erwinia carotovora	ATCC 8061	91	51·5	
Paracolobactrum aerogenoides	McK	92·5	55	
Klebsiella pneumoniae	23	92·5	55	
**Aerobacter aerogenes*	1088	93·5	57·5	57·1‡
**Serratia marcescens*	Harvard Med.	93·5	57·5	58‡
	Lactobacillaceae			
**Diplococcus pneumoniae*	R-36A	85·5	39	38·5‡
Lactobacillus acidophilus	Blechman	85·5	39	
Streptococcus salivarius	I-R14Sm^r	85·5	39	
Leuconostoc mesenteroides	ATCC 12291	85·5	39	
	Bacillaceae			
**Clostridium perfringens*	876	80·5	26·5	31‡
Cl. tetani	Mandel	80·5	26·5	
Cl. chauvei	Mandel	80·5	26·5	
Cl. madisonii	Seeley	80·5	26·5	
Bacillus alvei	ATCC 6344	83	33	
**B. cereus*	MB 19	83	33	35‡
**B. thuringiensis*	ATCC 10792	83·5	34	35·9‡
B. megaterium-cereus	ATCC 14B22	83·5	34	
B. circulans	ATCC 4513	84	35	
**B. megaterium*	U. of Penn.	85	37	37·6‡
B. lentus	ATCC 10840	85	37	
B. sphaericus	ATCC 4525	85	37	
B. pumilus	NRS 236	85·5	39	
B. laterosporus	ATCC 64	86	40	
B. firmus		86·5	41	
B. subtilis var. *atterimus*	ATCC 6460	87·5	43	
B. brevis	ATCC 9999	87·5	43	
**B. subtilis*	168	87·5	43	42·4‡
B. natto	MB 275	87·5	43	
B. niger	ATCC 6454	87·5	43	
B. subtilis var. *niger*	ATCC 6455	87·5	43	
B. stearothermophilus	194	88	44	
B. polymyxa	ATCC 842	88	44	
**B. licheniformis*	NRS 243	88·5	46	50·7§
B. macerans	ATCC 7069	90·5	50	

TABLE 1 (*continued*)

Organism	Strain or source	T_m (°C)	Mole per cent G–C From T_m	Mole per cent G–C Literature
	Brucellaceae			
**Pasteurella tularensis*	Detrick	83	33	34·7‡
Hemophilus suis	3090	85·5	39	
**H. influenzae*	RD	85·5	39	
H. parainfluenzae	G. Leidy	85·5	39	
H. aegypti	G. Leidy	86	40	
Moraxella bovis	M. Mandel	86·5	41	
P. pestis	AVO_2, EV6	88·5	46	
**Brucella abortus*	19	92·5	55	57·9‡
	Spirillaceae			
**Vibrio cholerae*	20A10	89	47	43·3‡
	Pseudomonadaceae			
Acetobacter gluconicum	2G	92	54	
Acetobacter ascendens	ATCC 9323	92·5	55	
**Pseudomonas fluorescens*	ATCC 949	94·5	60	63·0‡
**Ps. aeruginosa*	NRRL B-23	97	66	67‡
	Corynebacteriaceae			
Listeria monocytogenes	Detrick	85·3	38	
Corynebacterium xerosis	ATCC 9016	93·5	57·5	
	Azotobacteriaceae			
**Azotobacter vinelandii*	ATCC 9104	94·5	60	56·3‡
	Mycobacteriaceae			
**Mycobacterium phlei*	A. Brodie	97	66	66·4‡
	Micrococcaceae			
**M. pyogenes* var. *aureus*	NRRL B-313	83·5	34	34‡
**Sarcina lutea*	26C (Mandel)	98	68	72‡
**M. lysodeikticus*	NRRL B-287	99·5	72	71·9‡
	Athiorhodaceae			
Rhodospirillum rubrum	S-1	94·5	60	
	Neisseriaceae			
**Neisseria catarrhalis*	Ne 11 (Catlin)	86·5	41	40·7¶
N. perflava	Ne 20 (Catlin)	90	49	50·4¶
N. sicca	Ne 12 (Catlin)	90	49	51·5¶
**N. flavescens*	13120 (Catlin)	90	49	49·2¶
**N. meningitidis*	Ne 15 (Catlin)	91	51·5	51·3¶
	Rhizobiaceae			
Rhizobium japonicum	555	95	61	
	Streptomycetaceae			
Streptomyces albus	G	100·5	74	
**S. viridochromogenes*	93	100·5	74	73·8‡
	Rickettsiaceae			
Coxiella burnetii	Paretsky	87	42	
	Mycoplasmataceae			
Mycoplasma gallisepticum	PPLO 5969	84	35	

† According to the classification in Bergey's *Manual of Determinative Bacteriology*, Seventh Edition.

* Values used to plot Fig. 4.

‡ Belozersky & Spirin (1960).

§ Envreinova, Bunina & Kusnetzova (1959).

¶ Catlin & Cunningham (1961).

in saline-citrate are obtained by adding the above difference. Both sets of values are included in Fig. 4.

It is interesting to note that the presence of glucose and hydroxymethylcytosine in the DNA (bacteriophages T2, T4 and T6) does not displace their T_m values very much from the line. Also, DNA isolated from a thermophile grown at an elevated temperature (*B. stearothermophilus*) exhibits a T_m which is compatible with its G–C content (Marmur, 1960). T_m values are greatly influenced by the presence of certain polyamines (Mahler, Mehrotra & Sharp, 1961), denaturing agents and extremes in pH.

It has also been found that T_m values are unchanged when the denaturation is carried out in D_2O as the solvent (Kucera, unpublished results). The presence of deuterium in the non-exchangeable positions of DNA also has no influence on its T_m (Marmur & Schildkraut, 1961).

T_m values that fall off the line may arise from the use of strains different from those whose mole per cent G–C are listed in the literature, inaccuracies in the chemical determinations of their G–C content or some yet unknown or unevaluated variables. The values listed for the chemical analyses of the DNA base compositions from the same tissue vary to a greater extent than that obtained from the T_m. Thus, the reported guanine plus cytosine content of calf thymus DNA, compiled by Chargaff (1955), varies from the extremes of 41·3 to 45·0 mole per cent.

Determination of the G+C content from the T_m. DNA was isolated from a number of organisms whose G–C content have not yet been recorded or whose published values show considerable variance. The mole per cent G–C was then evaluated from Fig. 4 using their T_m values determined in 0·15 M-NaCl plus 0·015 M-sodium citrate. The base compositions thus determined are listed in Tables 1 to 3. A careful examination of the G–C values will show that, in general, those organisms which are genetically and/or taxonomically related have similar base compositions (Lee, Wahl & Barbu, 1956).

4. Discussion

Since the results of this further investigation of the dependence of T_m on the composition of DNA are quite straightforward, only two further comments are necessary: one regarding the applicability of the method to the determination of the base composition in DNA and another concerning the relation of these results to the related study of the dependence of buoyant density on the composition of DNA (Schildkraut *et al.*, 1962).

The extension of the correlation between T_m and G–C to more than forty samples indicates the high reliability of the method for independent base composition determination and the absence of unexpected interfering situations. Moreover, the generality of the relation clearly supports the original interpretation of the molecular melting phenomenon being of a co-operative type that averaged out the fluctuations of base composition along the chain and responded to the mean composition of the chain (Doty, 1956; Marmur & Doty, 1959).

The advantages of the use of T_m determinations are clear. The result can be obtained easily on a very small amount of DNA, ordinarily 50 μg although 15 μg suffices with special cuvettes. Of course, care must be taken to insure that the DNA was not denatured in preparation, that the cation concentration is maintained at 0·2 M-Na^+ without divalent metal ion contamination and that the heating is not carried out too rapidly.

TABLE 2

Mole per cent G–C and T_m of DNA from bacteriophage

Bacteriophage	Host	T_m (°C)	Mole per cent G–C From T_m	Mole per cent G–C Literature†
*T2r^+	*E. coli*	83	33	35
*T4r^+	*E. coli*	84	35	34·4
*T6r^+	*E. coli*	83	33	34·2
α	"*B. megaterium*"	86·5	41	
λ^c	*E. coli*	89	47	50·0
*T7	*E. coli*	89·5	48	48·0
*T3	*E. coli*	90	49	49·6
S20 v	*S. marcescens*	92·5	55	

* Values used to plot Fig. 4.
† Sinsheimer (1960).

TABLE 3

Mole per cent G–C and T_m of DNA from plants and animals

Organism	Tissue	Method of preparation and/or source	T_m (°C)	Mole per cent G–C† From T_m	Literature
		Plants			
Tobacco	Leaf	Marmur	85·5	39	
*Wheat	Germ	Josse	88·5	46	46·6†
		Animals			
Human	Spleen	Kirby	86·5	41	41·4§
Mouse	Spleen	Kirby	86·5	41	41·9‖
Rat	Liver	Kirby	86·5	41	41·4‖
Drosophila melanogaster	Whole animal	Kirby	86·5	41	
*Calf	Thymus	Simmons	87	42	41·9§
*Chicken	Liver	Kirby	87·5	43	41·7‖
Chicken	Embryo liver	Kirby	87·5	43	41·7‖
*Salmon	Sperm	Simmons	87·5	43	41·2§
		Miscellaneous			
**Saccharomyces cerevisiae*			85	37	35·7¶
Euglena gracilis			90	49	

* Values used to plot Fig. 4.
† Includes the substitution of cytosine by methylcytosine.
‡ Josse (personal communication).
§ Chargaff (1955).
‖ Kirby (1959).
¶ Belozersky & Spirin (1960).

The limitations of the method are its obvious insensitivity to other than the four normally occurring bases and its restriction to the range of composition for which it has been established, that is from 25 to 75 mole per cent G–C. This latter point is emphasized by the fact that the helical complexes made from synthetically prepared homologous polynucleotides do not fit the extrapolation of the linear relation.

Indeed, the divergences for poly GC and poly AT correspond to 5°C or 12 mole per cent G–C. This is quite outside probable error and suggests that a slightly different structure is taken up when only one kind of base pair needs to be accommodated in the helix. Conversely, when two base pairs, which have slightly different dimensions, occur in the helical framework, it is quite likely that a different adjustment of the parameters is required for optimal fit from those that best satisfy the case where only one base pair is involved.

The linear relation between T_m and G–C obtained here and that derived between buoyant density and G–C (Schildkraut *et al.*, 1962) are, of course, expected to be mutually consistent. A direct examination of compositions deduced from the measurement of T_m and buoyant density on identical samples shows that the base compositions deduced from the linear relations are generally within the expected probable error. A few deviations occur in the very low G–C region (25 to 33%). However, in no case was the difference greater from that corresponding to either 2·5° or 0·006 g/c.cm. Thus, although some further work remains to be done when samples of very low G–C content become available, the agreement can be taken as satisfactory over the range of 30 to 75% and compositions estimated from either T_m or buoyant density taken as interchangeable.

The authors are indebted to Drs. C. L. Schildkraut and N. Sueoka for discussions during the course of this work and to Dr. J. Josse for a sample of wheat germ DNA, to Dr. B. W. Catlin for DNA from *Neisseria* and to Dr. K. S. Kirby for DNA samples from animal sources prepared by the phenol method. Dr. D. Paretsky supplied the DNA from *Coxiella burnetii*. The *Mycoplasma* was grown for us by Dr. R. Cleverdon. Several samples of DNA and strains of bacteria were supplied by Drs. M. Mandel and S. Falkow. They also contributed greatly in rewarding discussions concerning the taxonomic classification of several bacterial groups..

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