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Reexamination of the Association Between Melting Point, Buoyant Density, and Chemical Base Composition of Deoxyribonucleic Acid

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The equations currently used for the calculation of the chemical base composition of deoxyribonucleic acid (DNA), expressed as moles per cent guanine plus cytosine (% GC), from either buoyant density (ρ) or midpoint of thermal denaturation (T_m) were recalculated by using only sets of data on DNA determined with the same strains. All available information from the literature was screened and supplemented by unpublished data. The results were calculated by regression and correlation analysis and treated statistically. From the data on 96 strains of bacteria, it was calculated that % GC = 2.44 (T_m − 69.4). T_m appears to be unaffected by the substitution of cytosine by hydroxymethylcytosine. This equation is also valid for nonbacterial DNA. From the data on 84 strains of bacteria, the relation % GC = 1038.47 (−1.6616) was calculated. The constants in this equation are slightly modified when data on nonbacterial DNA are included. Both correlations differ only slightly from those currently used, but now they lean on a statistically sound basis. As a control, the relation between ρ and T_m was calculated from data of 197 strains; it agrees excellently with the above two equations.

Lee, Wahl, and Barbu (59) and Belozersky and Spirin (4) discovered that the base composition of bacterial deoxyribonucleic acid (DNA) varies from 25 to 75 moles per cent guanine plus cytosine (% GC). It has since been established that % GC values are very important for the identification and classification of bacteria. Through the excellent techniques of thermal denaturation (65) and buoyant density (ρ; 83) the determinations became less laborious and time-consuming, and apparently more precise.

A tentative correlation was calculated (65, 83) between the chemically determined % GC and either the midpoint of the thermal denaturation (T_m) in SSC buffer (0.15 M NaCl plus 0.015 M trisodium citrate, *p*H 7.0) or the buoyant density:

$$\% \text{ GC} = (T_m - 69.3)/0.41 \quad (1)$$

$$\% \text{ GC} = (\rho - 1.660)/0.00098 \quad (2)$$

Both equations, however, are uncertain because it is not clear whether the data on % GC, T_m, and ρ were derived from the same strains. Another factor of confusion is that the % GC values calculated from T_m in Tables 1, 2, and 3 (65) were not obtained by equation 1, but by another one, which we found by regression analysis to be

$$\% \text{ GC} = (T_m - 69.24)/0.42 \quad (3)$$

By using the same data as Marmur and Doty (65), we calculated for their regression lines

$$T_m = 0.415 \% \text{ GC} + 69.3 \quad (4)$$

and

$$\% \text{ GC} = 2.34 (T_m - 68.75) \quad (5)$$

both with a product moment correlation of 0.986. By using Schildkraut, Marmur, and Doty's (83) data, we calculated for their regression lines

$$\rho = 0.000945 \% \text{ GC} + 1.6618 \quad (6)$$

and

$$\% \text{ GC} = 1018.6 (\rho - 1.6600) \quad (7)$$

with a product moment correlation of 0.994. Other correlations have been suggested. McDonald et al. (66) used % GC = (T_m − 69) 2.439. Mandel et al. (18, 35) stressed the need to recheck correlations 1 and 2. In the former paper, a

slightly curved T_m versus % GC standard line was presented. In the latter paper the best correlation was

$$\% \text{ GC} = 1030.9\ (\rho - 1.662) \qquad (8)$$

for the lactic acid bacteria. The replacement of equation 2 by equation 8 was not yet recommended. It is clear that both equations 1 and 2 are uncertain and need to be recalculated.

In the present paper, we reexamine the correlations between chemical % GC determination versus T_m, chemical % GC determination versus ρ, and ρ versus T_m. The difference of the present treatment with previously reported ones (65, 83) is that we use only those sets of data which were determined on the same strain. Also, additional data are included. The organisms are mainly bacteria, some viruses, yeasts, algae, and protozoa. Some data on plant and animal DNA are also considered. We attempt to correlate T_m, ρ, and DNA base composition as accurately as possible because % GC is a chemical constant for an organism; it is one of the very few constants in biology of great importance for classification and identification, and it should be known and determined with the utmost care and precision.

MATERIALS AND METHODS

Most of the literature data on chemical % GC determinations, T_m, and ρ, on all kinds of organisms were cataloged. In addition, we included many unpublished data from current projects in our laboratory. Quite frequently, the same strain was investigated under different collection numbers; they were cross-checked against each other and against different culture collection catalogs. Only those strains were retained for which it was ascertained that the % GC was determined with at least two methods, when in the chemical method the molar per cent of guanine and cytosine corresponded at least reasonably well, and when T_m was determined in 1 SSC buffer. In the buoyant density method, the density of the sample DNA is determined by comparison against a known standard DNA. Schildkraut, Marmur, and Doty (83) used *Escherichia coli* K-12 DNA with $\rho = 1.710$ g/cm^3 as a reference. Nearly all values reported in the literature were compared either with K-12 DNA or with another reference DNA (e.g., 1.742 g/cm^3 from ^{15}*N-Pseudomonas aeruginosa*) which in its turn was calibrated against K-12 DNA. In very few cases (e.g., for *Bacterium paracoli*), the buoyant density of the reference DNA had to be corrected to make it comparable with K-12 DNA. The regression lines were calculated with the method of the least squares. Statistical analyses were carried out by standard procedures. All calculations were performed on a suitably programmed Olivetti Programma 101 electronic desk-top computer.

RESULTS AND DISCUSSION

Table 1 lists all strains and organisms retained after screening the literature, together with the available T_m, ρ, and chemical % GC values. Many unpublished data from our laboratory are also included.

The correlation between T_m and chemical % GC values for bacteria. The data from Table 1 are plotted in Fig. 1. The available data range from 30 to 75% GC. Both regression lines are (for 96 strains)

$$\% \text{ GC} = 2.44\, T_m - 169.25 \qquad (9)$$

or

$$= (T_m - 69.37)/0.41 \qquad (10)$$

and

$$T_m = 0.39\ \% \text{ GC} + 70.26 \qquad (11)$$

with a correlation coefficient of 0.98, indicating good linearity. The observed t_{94} is 47.7 (Student t test) in the significance test for b = 0. This confirms the linear relationship between T_m and % GC. Additional statistical information is compiled in Table 2. The denominator in equation 10 can range from 0.39 to 0.43. The limits of accuracy of prediction from linear regression at the 5% probability level are about ±4.5% GC for a single observed T_m (*see* Fig. 1 and Table 2). This includes the errors both on T_m and on the chemical % GC values from all observers. It may be expected that the accuracy obtainable by one observer will be greater. This is indeed the case. We repeated all the above calculations by comparing only T_m values obtained in our laboratory versus the chemical % GC values from both our and other laboratories, determined on the same strains. The limits of accuracy for one T_m determination are now ±3.7 to ±4.1 % GC, which represent the usual error in the paper chromatographic method. Of 96 strains, only the five following strains fall outside the 95% confidence limits: *Bacillus "stearothermophilus"* FJW, *Clostridium butyricum*, *Desulfovibrio desulfuricans* NCIB 8380, *E. coli* NCIB 8545, and *Micrococcus luteus* CCM 852. Two others are on or near the border, *Clostridium acidiurici* and *Moraxella osloensis* ATCC 19961. Their T_m and chemical % GC values, or both, should be determined again.

Equation 10 is essentially identical to the classically used equation 1 of Marmur and Doty (65). It might be argued that equation 11 is physically more correct, because T_m depends on the chemical composition and not vice versa.

TABLE 1. *Chemical base composition (expressed as % GC), buoyant density (ρ) in g/cm³ and "melting point" (T_m) of DNA from bacteria, viruses, some algae, protozoa, yeasts, plant and animal tissues*[a]

Strain	No.	T_m in C	Reference	ρ in g/cm³	Reference	Chemical determination as % GC	Reference
Bacteria							
Acetobacter peroxydans	NCIB 8618	95.0	28			61.0	28
A. rancens	NCIB 6428	93.5	28			58.8	28
A. mesoxydans var. *saccharovorans*	4	94.2	28			61.0	28
A. "cerinus"	22	92.2	28			56.5	28
A. mesoxydans	NCIB 8747	94.25	28			61.1	28
A. aceti	NCIB 9505	95.6	28			65.4	28
A. aceti	Ch 31	93.8	28			59.5	28
"Achromobacter" liquefaciens	ATCC 15716	87.6	This paper	1.700	14		
Acinetobacter anitratus	NCIB 8250	86.6	This paper			38.2	84
A. lwoffii	ATCC 9957	88.65	This paper			43.4	10
Aerobacter aerogenes	ATCC 14308	93.0	18	1.712	18		
A. aerogenes	ATCC 13048	91.8	M. P. Starr and M. Mandel, *unpublished data*	1.712	M. P. Starr and M. Mandel, *unpublished data*		
A. aerogenes	1088	93.5	65	1.716	83		
A. aerogenes	ATCC 13882	92.1	M. P. Starr and M. Mandel, *unpublished data*	1.715	M. P. Starr and M. Mandel, *unpublished data*		
A. cloacae	ATCC 13047	92.0	M. P. Starr and M. Mandel, *unpublished data*	1.713	M. P. Starr and M. Mandel, *unpublished data*		
A. lipolyticus	ATCC 14460	91.4	M. P. Starr and M. Mandel, *unpublished data*	1.7115	M. P. Starr and M. Mandel, *unpublished data*		
Aeromonas hydrophila	ATCC 9071	93.6	63			61.4	84
A. salmonicida	ATCC 14174	94.5	63			58.5	84
A. liquefaciens	NRRL B-966	95.4	63	1.721	63		
Agrobacterium tumefaciens	E III 9.6.1	94.8	B. J. Tinbergen, Ph.D. Thesis, State University of Leiden, The Netherlands, 1966	1.7186	B. J. Tinbergen, Ph.D. Thesis, State University of Leiden, The Netherlands, 1966		
A. tumefaciens	S1	94.7	24	1.719	Mandel, *personal communication*	58.0	Sebald; van der Plaat, *personal communication*
A. tumefaciens	A 6	94.6	B. J. Tinbergen, Ph.D. Thesis, State University of Leiden, The Netherlands, 1966	1.7177	B. J. Tinbergen, Ph.D. Thesis, State University of Leiden, The Netherlands, 1966		
A. tumefaciens	B 6	94.6	24	1.7186	B. J. Tinbergen, Ph.D. Thesis, State University of Leiden, The Netherlands, 1966; Mandel, *personal communication*	60.8	97
A. tumefaciens	ATCC 143	94.35	25			58.8	28
A. tumefaciens	SCA-1	94.2	This paper			59.7	97
A. tumefaciens	M 39	96.2	24	1.7235	Mandel, *personal communication*	62	Sebald; van der Plaat, *personal comm unication*

TABLE 1.—*Continued*

Strain	No.	T_m in C	Reference	ρ in g/cm³	Reference	Chemical determination as % CG	Reference
"A." ferrugineum	A 7	94.05	This paper			60	1
"A." luteum	A 61	93.15	This paper			57	1
A. radiobacter	Ra	94.8	B. J. Tinbergen, Ph.D. Thesis, State University of Leiden, The Netherlands, 1966	1.7183	B. J. Tinbergen, Ph.D. Thesis, State University of Leiden, The Netherlands, 1966		
"A." sanguineum	A 91	96.05	This paper			64	1
Alcaligenes faecalis	NCIB 8156	92.58	This paper	1.715	18		
A. haemolysans	ATCC 17988	87.6	This paper	1.7010	6		
Bacillus amyloliquefaciens	H	87.1	102	1.708	102		
B. amyloliquefaciens	SB	87.7	102	1.708	102		
B. amyloliquefaciens	F	87.6	102	1.708	102	44.0	102
B. amyloliquefaciens	T, P, N, W, K	87.5	102	1.707	102		
B. brevis	ATCC 9999	87.5	65	1.704	82		
B. cereus	MB 19	83	65	1.696	82		
B. licheniformis	ATCC 9789	88.55	65, 90	1.705	83	46.9	90
B. macerans	ATCC 7069	90.5	65	1.713	82		
B. megaterium	University of Pennsylvania	85	65	1.697	82		
B. natto	MB 275	87.5	65	1.703	82		
B. pumilus	ATCC 6631	87.8	90			45.1	90
B. "stearothermophilus"	FJW	90.2	90			56.0	90
B. stearothermophilus	10	91.0	90			52.9	90
B. stearothermophilus	2184	91.0	90			52.2	90
B. "stearothermophilus"	194	87.75	64, 65	1.705	83	46.7	Quoted in 64
B. subtilis	W 23	86.7	102	1.705	102	42.2	102
B. subtilis	168	87.6	38, 65, 66	1.703	82	41.95	38, 100
B. subtilis	Sc-22	96.0	38			65.0	38, 100
B. subtilis	MK 9	95.4	38			64.0	38
B. subtilis	MK 12	94.6	38			62.9	38
B. subtilis	168 I^-	86.7	102	1.706	102		
B. subtilis	ATCC 4529	87.1	102	1.706	102		
B. subtilis	ATCC 6051	86.7	102	1.705	102	42.6	43
B. subtilis	ATCC 7067	86.3	102	1.706	102		
B. subtilis	ATCC 9466	86.3	102	1.705	102		
B. thuringiensis	ATCC 10792	83.5	65	1.695	83		
Bacillus species	X 1	87.0	90			41.5	90
Bacterium paracoli	ATCC 23280	89.0	37	1.707	73		
B. paracoli	ATCC 23281	98.2	37	1.724	73		
B. paracoli		91.5	38			57.8	38
B. paracoli	Mutant 52-1	100.3	38			75.4	38
Bifidobacterium	Gr IV Dehnert; 456, IV			1.717	35	63.6	36
Bifidobacterium	Gr V Reuter; 12, V			1.717	35	60.8	36
Bifidobacterium	Gr IV Reuter; 50, IV			1.7165	35	59.4	36
Bifidobacterium	Gr IV Dehnert; 659, IV			1.7165	35	58.6	36
Bifidobacterium	Prevot Co, II B			1.716	35	57.2	36
Brucella abortus	2308	92.6	42	1.716	42		
B. abortus	19	92.5	65	1.715	83		
Chlamydia trachomatis	TE-55	87.7	50	1.7063	50		
C. trachomatis	MRC-1/G	87.6	50	1.7060	50		
C. trachomatis	Cal 1	87.7	50	1.7061	50		
C. psittaci	6B6	85.5	50	1.7030	50		
C. psittaci	MN	85.4	50	1.7025	50		
Clostridium acidiurici		79.7	94	1.6916	94	29.8	94
C. butyricum		82.1	94			37.4	94
C. chauvei		80.5	65	1.691	83		
C. cylindrosporum		82.3	94			32.4	94
C. madisonii	16	80.5	65	1.693	83		

TABLE 1.—*Continued*

Strain	No.	T_m in C	Reference	ρ in g/cm³	Reference	Chemical determination as % GC	Reference
C. pasteurianum		81.8	94	1.6911	94	30.8	94
C. perfringens	876	80.5	65	1.691	83		
C. tartarivorum	T 9-0	85.4	C. L. Irwin, *unpublished data*			40.3	C. L. Irwin, *unpublished data*
C. thermosaccharolyticum	3814	84.3	C. L. Irwin, *unpublished data*			35.8	C. L. Irwin, *unpublished data*
Comamonas cyclosites	NCIB 2581	95.8	This paper			63.9	84
C. neocistes	NCIB 2582	95.9	This paper			63.6	84
C. neocistes	RH 1810	95.7	17	1.723	17		
C. terrigena	NCIB 8193	95.6	This paper			64.6	84
C. percolans	RH 260	94.6	17	1.722	17		
Corynebacterium glutamicum	KY 9005	91.7	This paper			57.5	92
C. glutamicum	NRRL B 2243	91.9	This paper			56.8	92
C. glutamicum	ATCC 13032	91.8	This paper			56.8	92
C. xerosis	ATCC 9016	93.5	65	1.718	83		
Coxiella burnetii	Paretsky	87	65	1.704	83	44.5	105
Cytophaga species	ATCC 11947	83.5	18	1.689	16, 18		
Cytophaga species	NCMB 292	83.1	29	1.6931	26		
C. fermentans	ATCC 12470	86.2	62	1.698	62		
C. johnsonii	405	83.5	62	1.694	62		
Desulfotomaculum nigrificans	NCIB 8395			1.708	78, 83	45.4	85
D. orientis	NCIB 8382			1.704	83	42.2	85
Desulfovibrio desulfuricans	NCIB 8380	90.5	79	1.716	79, 83	56.3	85
D. desulfuricans	ATCC 13541			1.720	83	56.8	85
D. desulfuricans	NCIB 8393			1.719	83	57.4	85
D. gigas	NCIB 9332			1.724	79	64.6	85
D. vulgaris	NCIB 8303	93.6	79	1.724	79, 83	62.8	85
D. salexigens	NCIB 8403	87.1	79	1.709	79		
Diplococcus pneumoniae	R-36A	85.5	65	1.701	83		
Erwinia amylovora	ICPB EA 11	91.0	Starr and Mandel, *unpublished data*	1.7125	Starr and Mandel, *unpublished data*		
E. herbicola	G 150	92.2	This paper	1.7149	Starr and Mandel, *unpublished data*		
E. herbicola	G 151	92.25	This paper	1.714	Starr and Mandel, *unpublished data*		
E. herbicola	G 152	92.1	This paper	1.715	Starr and Mandel, *unpublished data*		
E. lathyri	ICPB EL 103	92.2	Starr and Mandel, *unpublished data*	1.714	Starr and Mandel, *unpublished data*		
E. milletiae	ICPB EM 102	92.0	Starr and Mandel, *unpublished data*	1.714	Starr and Mandel, *unpublished data*		
E. nigrifluens	ICPB EN 104	92.5	Starr and Mandel, *unpublished data*	1.715	Starr and Mandel, *unpublished data*		
E. oleraceae	ICPB EO 1	90.4	Starr and Mandel, *unpublished data*	1.7095	Starr and Mandel, *unpublished data*		
E. salicis	ICPB ES 4	90.8	Starr and Mandel, *unpublished data*	1.7103	Starr and Mandel, *unpublished data*		
E. tracheiphila	ICPB ET 106	90.9	Starr and Mandel, *unpublished data*	1.709	Starr and Mandel, *unpublished data*		
E. uredovora	NCPPB 802	91.4	This paper	1.712	Starr and Mandel, *unpublished data*		

TABLE 1.—*Continued*

Strain	No.	T_m in C	Reference	ρ in g/cm³	Reference	Chemical determination as % GC	Reference
Escherichia aurescens	ATCC 12814	90.8	63; Starr and Mandel, *unpublished data*	1.710	Starr and Mandel, *unpublished data*		
E. coli	K 12	90.6	57; 65; De Ley, *unpublished data;* Starr and Mandel, *unpublished data*	1.710	83	51.2	30, 34, 92
E. coli	W	90.5	65			51.7	12
E. coli	44B	91.5	63	1.710	82		
E. coli	B	90.7	42; De Ley, *unpublished data;* 65	1.710	83	50.9	30, 89
E. coli	NCIB 8545	91.9	94			50.0	94
E. coli	ATCC 11775	90.5	Starr and Mandel, *unpublished data*	1.710	Starr and Mandel, *unpublished data*		
E. freundii	5610-52	91.8	63	1.712	83		
E. freundii	ATCC 8090	90.9	Starr and Mandel, *unpublished data*	1.7115	Starr and Mandel, *unpublished data*		
Franciscella tularensis	(Detrick)	83	65	1.695	83		
Gluconobacter oxydans	SU	92.8	28			58.1	28
G. oxydans	2G	92	65	1.714	83		
G. oxydans	26	94.85	28			61.0	28
G. oxydans	NCIB 4943	94.75	28			61.3	28
G. oxydans	NCIB 8086	94.2	28			61.0	28
G. oxydans	NCIB 8131	95.35	28			62.1	28
Haemophilus aegyptius		86	65	1.698	83		
H. influenzae	Rd	85.6	65; De Ley, *unpublished data*	1.696	5, 83		
H. parainfluenzae		85.5	65	1.698	83		
Klebsiella edwardsii var. *atlantae*	ATCC 13887	92.6	Starr and Mandel, *unpublished data*	1.715	Starr and Mandel, *unpublished data*		
K. edwardsii var. *atlantae*	ATCC 13886	92.5	Starr and Mandel, *unpublished data*	1.7155	Starr and Mandel, *unpublished*		
K. pneumoniae	23	92.5	65	1.715	83		
K. pneumoniae	ATCC 13883	92.9	Starr and Mandel, *unpublished data*	1.7123	Starr and Mandel, *unpublished data*		
K. rhinoscleromatis	ATCC 13884	92.7	Starr and Mandel, *unpublished data*	1.7145	Starr and Mandel, *unpublished data*		
Lactobacillus brevis	ATCC 8007			1.7018	35	43.2	36
L. brevis	V_7			1.706	35	43.9	36
L. buchneri	NCIB 8007			1.7040	35	42.6	36
L. buchneri	ATCC 9460			1.7040	35	42.0	36
L. cellobiosus	ATCC 11740			1.7125	35	49.8	36
L. cellobiosus	ATCC 11739			1.7115	35	52.5	36
L. fermenti	ATCC 9338			1.712	35	49.5	36
L. viridescens	NCDO S40(E_3)			1.695	35	41.0	36
L. viridescens	NIRD 403			1.7015	35	37.9	36
L. casei var. *alactosus*	B 51			1.7055	35	44.3	36
L. casei var. *casei*	NIRD 151			1.7045	35	46.2	36
L. casei var. *casei*	NIRD 152			1.7055	35	47.0	36
L. casei var. *casei*	NIRD 155			1.705	35	43.4	36
L. casei var. *casei*	61 BG3			1.706	35	45.5	36
L. casei var. *casei*	65 M			1.7055	35	49.4	36
L. casei var. *rhamnosus*	64 H			1.7063	35	47.4	36
L. plantarum	NCDO 343			1.704	35	43.4	36
L. plantarum var. *rudensis*	NIRD 773			1.704	35	42.0	36

TABLE 1.—*Continued*

Strain	No.	T_m in C	Reference	ρ in g/cm³	Reference	Chemical determination as % GC	Reference
L. plantarum	64 L			1.7045	35	43.1	36
L. plantarum	61 D			1.704	35	43.0	36
L. acidophilus	64 N			1.6995	35	34.9	36
L. acidophilus	61 Z			1.6965	35	36.7	36
L. acidophilus	65 K			1.696	35	36.8	36
L. acidophilus	63 E			1.6955	35	34.4	36
L. acidophilus	ATCC 9857			1.6963	35	36.6	36
L. acidophilus	NCTC 1723			1.696	35	34.2	36
L. acidophilus	(Blechman)	85.5	65	1.701	83		
L. bulgaricus	ATCC 11842			1.709	35	49.4	36
L. bulgaricus	CNRZ 36			1.7095	35	48.3	36
L. helveticus	ATCC 10386			1.6985	35	37.1	36
L. jugurti	ATCC 521			1.6985	35	36.5	36
L. jugurti	NIRD 99			1.6978	35	37.5	36
L. jugurti	ATCC 10812			1.698	35	37.1	36
L. jugurti	J 2			1.699	35	37.9	36
L. lactis	ATCC 8000			1.7085	35	48.3	36
L. lactis	L 1			1.710	35	48.2	36
L. leichmannii	ATCC 4797			1.7095	35	49.4	36
L. leichmannii	ATCC 7830			1.710	35	49.2	36
L. salivarius	ATCC 11742			1.694	35	36.6	36
L. salivarius	63 AJ			1.6933	35	33.0	36
L. salivarius	61 AK			1.695	35	35.0	36
Leuconostoc mesenteroides	ATCC 12291	85.5	65	1.701	83		
Listeria monocytogenes		85.3	65	1.697	83		
Micrococcus luteus	NCTC 7011	97.3	3			69.0	75
M. luteus	NCTC 7503	98.9	38			74.3	38
M. luteus	Mutant 44	98.9	38			72.8	38
M. luteus	Mutant 22	97.1	38			70.0	38
M. luteus	CCM 856	95.1	3			65.4	75
M. luteus	CCM 852	95.7	3			69.0	75
M. luteus	CCM 851	97.25	3			69.1	75
M. luteus	CCM 853	97.3	3			71.1	75
M. luteus	NRRL B-287	99.5	65	1.731	83		
M. luteus	26 C	98	65	1.731	83		
M. radiodurans		96.6	80	1.728	80	66.6	80
Moraxella bovis	ATCC 17949			1.703	6	44.6	10
M. lacunata	ATCC 10900	88.9	This paper	1.7025	6		
M. lacunata	ATCC 19991	88.0	This paper			42.47	10
M. lacunata	ATCC 17956	89.2	This paper	1.703	6		
M. duplex liquefaciens	ATCC 17952	88.5	This paper	1.7015	6	44.3	10
M. nonliquefaciens	ATCC 17953	88.25	This paper	1.701	6		
M. osloensis	ATCC 10973	89.0	This paper			44.6	10
M. osloensis	ATCC 19963	88.2	This paper			44.35	10
M. osloensis	ATCC 19961	89.35	This paper	1.7035	6	44.2	10
M. lwoffii var. *bacteroides*	ATCC 17985	88.1	This paper	1.7030	6		
M. lwoffii var. *brevis*	ATCC 17987	88.75	This paper	1.7035	6		
Mycobacterium phlei		97	65	1.732	83		
M. tuberculosis				1.724	99	65.0	99
Mycoplasma gallisepticum	PPLO 5969	84	65	1.6935	67, 83		
M. laidlawii	A	82.0	68	1.695	68		
M. pneumoniae		85.3	68	1.700	68		
Mycoplasma species	Kid	79.2	68	1.685	68		
Mycoplasma species	Calif. calf	79.0	68	1.686	68		
Myxococcus fulvus		97.9	62	1.728	62		
M. fulvus		98.2	62	1.730	62		
M. virescens		98.5	62	1.727	62		
M. virescens		98.5	62	1.728	62		
M. virescens		98.0	62	1.728	62		
M. virescens		98.4	62	1.729	62		
M. xanthus		98.5	62	1.729	62		

TABLE 1.—*Continued*

Strain	No.	T_m in C	Reference	ρ in g/cm³	Reference	Chemical determination as % GC	Reference
M. xanthus		97.7	62	1.727	62		
Neisseria catarrhalis	Ne 13			1.701	83	40.1	8
N. catarrhalis	NCTC 4103			1.7025	6, 9	45.1	9, 55
N. catarrhalis	ATCC 8176	87.95	This paper	1.7020	6	42.3	55
N. catarrhalis	ATCC 8193	88.45	This paper			42.25	9
N. catarrhalis	Ne 11	86.5	65	1.7010	6	41.0	8, 9
N. catarrhalis	NIH	86.5	D. T. Kingsbury and E. Weiss, *unpublished data*	1.7030	D. T. Kingsbury and E. Weiss, *unpublished data*		
N. flavescens	ATCC 13120	90	65	1.7067	6, 83	50.1	8
N. gonorrheae	WRAIR 116	89.5	Kingsbury and Weiss, *unpublished data*	1.7100	Kingsbury and Weiss, *unpublished data*		
N. meningitidis	Ne 15	91	65			51.3	8
N. meningitidis	SD 6	91.0	Kingsbury and Weiss, *unpublished data*	1.7100	Kingsbury and Weiss, *unpublished data*		
N. perflava	Ne 20	90	65			49.8	8
N. sicca	Ne 12	90	65	1.710	83	51.5	8
Nitrosomonas europaea		89.65	This paper	1.711	47		
N. europaea		90.5	2			51.6	2
Nocardia species	IMET 7801	96.6	70			68.6	71
Paracolobactrum aerogenoides	McK	92.5	65	1.713	83		
Pasteurella pestis	EV 6	88.5	65	1.706	83		
Pectobacterium aroideae	ICPB EA 14	91.5	Starr and Mandel, *unpublished data*	1.712	Starr and Mandel, *unpublished data*		
P. carotovora	ATCC 8061	91.5	63	1.709	82		
P. carotovora	ICPB EC 138	91.2	Starr and Mandel, *unpublished data*	1.711	Starr and Mandel, *unpublished data*		
P. chrysanthemi	ICPB EC 16	92.1	Starr and Mandel, *unpublished data*	1.714	Starr and Mandel, *unpublished data*		
P. dissolvens	ICPB ED 106	92.7	Starr and Mandel, *unpublished data*	1.716	Starr and Mandel, *unpublished data*		
P. nimipressuralis	ICPB EN 1	92.0	Starr and Mandel, *unpublished data*	1.714	Starr and Mandel, *unpublished data*		
P. rhapontici	ICPB ER 1	90.2	Starr and Mandel, *unpublished data*	1.710	Starr and Mandel, *unpublished data*		
Proteus mirabilis	35	85.3	65	1.700	83		
P. morganii	ATCC 8019	91	65	1.710	83		
P. rettgerii	3478	86	65	1.701	83		
P. vulgaris	ATCC 9484	85	65	1.698	83		
Pseudomonas acidovorans	RH 2167	95.6	17	1.724	17		
P. acidovorans	RH 2168	94.6	17	1.721	17		
P. acidovorans	RH 2169	94.8	17	1.720	17		
P. acidovorans	ATCC 9355	96.5	17	1.7248	17, 60		
P. acidovorans	ATCC 15005	96.3	17	1.7243	17, 60		
P. aeruginosa	NRRL B 23	97	65	1.727	60		
P. aeruginosa	ATCC 8689	97.7	18	1.726	60		
P. aeruginosa	ATCC 8707	97.5	18	1.7265	18, 60		
P. aureofaciens	ATCC 13985	95.1	27	1.7225	60		
P. chlororaphis	NCIB 9402	96.1	18	1.723	18		
P. cuneata	NCIB 8194	94.3	This paper			62.7	84
P. denitrificans	ATCC 12133	92.9	17	1.716	17		
P. diminuta	NCIB 9393	96.9	27	1.724	17		
P. fluorescens	NCIB 9392	95.3	27	1.7215	60		
P. fluorescens	CCEB 488	94.95	27			59.5	28

TABLE 1.—*Continued*

Strain	No.	T_m in C	Reference	ρ in g/cm³	Reference	Chemical determination as % GC	Reference
P. fluorescens	ATCC 13034 T	95.3	17	1.724	17		
P. fluorescens	ATCC 949	94.5	65	1.721	83		
P. fragi	ATCC 4973	94.5	17, 27	1.717	17		
P. iodinum	ATCC 9897	95.3	17	1.723	17		
P. iodinum	ATCC 15728	94.5	17	1.722	17		
P. iodinum	ATCC 15729	94.3	17	1.722	17		
P. maltophilia	NCIB 9203	96.2	This paper	1.724	17		
P. marginalis	ATCC 10858	92.9	17	1.718	17		
P. ovalis	ATCC 950	95.8	17	1.724	17		
P. putida	ATCC 12633	96.0	18	1.722	18, 58, 60	63.7	58
P. putida	ATCC 4359	93.4	17	1.719	17		
P. reptilovora	ATCC 11252	95.5	17	1.721	17		
P. stutzeri		95.8	17	1.724	17		
P. syncyanea	ATCC 9979	94.9	17	1.721	17		
P. testosteroni	RH 1104	94.2	17	1.719	17		
"*P.*" *cruciviae*	ATCC 13262	84.1	17	1.697	17		
"*P.*" *putrefaciens*	ATCC 8071	87.9	17	1.703	17		
Rhizobium japonicum	555	95	65	1.722	83		
Rhodospirillum rubrum	S-1	94.5	65	1.726	83		
Salmonella arizona	PC 145	90.5	65	1.712	82		
S. typhimurium	LT 2	91.8	63	1.712	82		
S. typhosa	643	90.5	65	1.711	82		
Serratia marcescens	Harvard Medical School	93.5	65	1.718	82		
S. marcescens	E. Eltinge, 1946	94.9	20, 63	1.717	63		
Shigella dysenteriae	15	90.5	65	1.710	82		
Sporocytophaga myxococcoides	Mass.	84.2	62	1.695	62		
Staphylococcus aureus	NCIB 8625	83.5	65	1.693	83	37.7	7
S. aureus	209	82.9	38			32.4	38
S. aureus	Mutant UV-2	98.1	38			71.0	38
S. aureus	Mutant UV-15	98.1	38			62.9	38
S. aureus	Mutant UV-16	97.9	38			70.9	38
Streptococcus cremoris	C 3	86.3	M. D. Kittel, W. E. Sandine, and P. R. Elliker, Bacteriol. Proc., p. 41, 1964			40.2	M. D. Kittel, W. E. Sandine, and P. R. Elliker, Bacteriol. Proc., p. 41, 1964
S. salivarius	I-R14 Sm^r	85.5	65	1.701	83		
Streptomyces albus	ATCC 618			1.730	33	72.3	107
S. albus	G	100.5	65	1.730	83		
S. fradiae	IMRU 3535			1.7304	93	74.5	107
S. griseolus	ATCC 3325			1.729	33	72.4	107
S. griseus	ATCC 10137			1.730	33	72.1	107
S. scabies	L 272 V	98.9	56			72.3	56
S. scabies	L 272 A	98.9	56			71.8	56
S. bobiliae	ATCC 3310			1.729	33	71.2	107
S. viridochromogenes	93	100.5	65	1.729	83		
Vibrio cholerae	20 A 10	89	65	1.708	19, 83		
V. cholerae	NIH-35A3	88.7	15	1.706	15		
V. cholerae	ATCC 14035	88.5	15	1.706	15		
V. El Tor	ATCC 14033	88.5	15	1.706	15		
V. marinus	MP-1	86.0	19	1.699	19		
V. metschnikovii	ATCC 7708	88.6	18	1.703	18, 19		
Vibrio species	NCTC 4715	88.3	15			47.7	84
Vibrio species	NCTC 4711	89.1	15			46.8	84
Vibrio species	MB 22	87.8	15	1.705	15		
Wolbachia persica		81.6	50	1.6900	50		
Xanthomonas begoniae	ICPB B3	96.85	29	1.7261	32		
X. campestris	ICPB C 129	97.3	29	1.7272	32		
X. carotae	ICPB C 104	96.8	29	1.7260	32		
X. hederae	ICPB H 1	96.9	29	1.7265	32		
X. juglandis	ICPB J 107	96.5	29	1.7253	32		
X. pelargonii	ICPB P 121	96.6	29	1.7255	32		

TABLE 1.—*Continued*

Strain	No.	T_m in C	Reference	ρ in g/cm³	Reference	Chemical determination as % GC	Reference
X. phaseoli	ICPB P 162	96.4	29	1.7251	32		
X. tamarindi	ICPB T 20	97.1	29	1.7268	32		
X. vesicatoria	ICPB V 136	96.55	29	1.7253	32		
Poly d(T-G)·poly d(A-C)[b]		91.5	103			50.0	
Poly d(A-T)·poly d(A-T)[b]		66.0	44, 65	1.6785	83; Szybalski as quoted by 35	0	
Poly dA·poly dT[b]		72.6	72	1.647	Szybalski as quoted by 35	0	
Poly dG·poly dC[b]		105.85	45, 65	1.795	83; Szybalski as quoted by 35	100	
Viruses							
Coliphage	T 1			1.705	83	48	22
Coliphage	T 2	83.0	65	1.700	83	34.6	22, 106
Coliphage	T 3	90	65	1.712	83	49.6	53
Coliphage	T 4	84	65	1.698	83	34.5	106
Coliphage	T 5			1.702	83	39.0	106
Coliphage	T 6	83	65	1.707	83	34.5	106
Coliphage	T 7	89.5	65	1.710	83	47.4	96
Coliphage	λ	89	65	1.710	83	48.6	48
Pseudomonas putida phage	gh-1			1.716	58	57.0	58
Yaba tumor pox virus		82.3	F. J. Gallagher and D. S. Yohn, Bacteriol. Proc., p. 169, 1967	1.6905	F. J. Gallagher and D. S. Yohn, Bacteriol. Proc., p. 169, 1967		
Shope rabbit papilloma virus		89.5	98	1.711	98	48.0	98
Herpesvirus		97	77	1.727	77		
Polyoma virus		89.2	21	1.709	101		
Adenovirus	Type 1	92.8	69	1.718	69		
Adenovirus	Type 2	92.5	69	1.716	69	56	39
Adenovirus	Type 3	90.3	69	1.714	69		
Adenovirus	Type 4	92.5	69	1.717	69	57	41
Adenovirus	Type 5	92.6	69	1.717	69		
Adenovirus	Type 6	93.6	69	1.718	69		
Adenovirus	Type 7	90.3	69	1.713	69		
Adenovirus	Type 8	90.8	69	1.717	69		
Adenovirus	Type 9	93.4	69	1.720	69		
Adenovirus	Type 10	93.6	69	1.720	69		
Adenovirus	Type 11	90.0	69	1.712	69		
Adenovirus	Type 12	89.5	69	1.708	69		
Adenovirus	Type 13	93.1	69	1.719	69		
Adenovirus	Type 14	91.0	69	1.715	69		
Adenovirus	Type 15	93.3	69	1.718	69		
Adenovirus	Type 16	90.9	69	1.714	69		
Adenovirus	Type 17	93.0	69	1.718	69		
Adenovirus	Type 18	88.8	69	1.708	69		
Adenovirus	Type 19	93.1	69	1.719	69		
Adenovirus	Type 20	94.2	69	1.719	69		
Adenovirus	Type 21	90.8	69	1.714	69		
Adenovirus	Type 22	92.9	69	1.718	69		
Adenovirus	Type 23	93.2	69	1.719	69		
Adenovirus	Type 24	93.7	69	1.719	69		
Adenovirus	Type 25	93.8	69	1.720	69		
Adenovirus	Type 26	93.7	69	1.719	69		
Adenovirus	Type 27	94.1	69	1.719	69		
Adenovirus	Type 28	94.1	69	1.719	69		
Algae, Protozoa, Yeasts, Plants, Animals							
Anacystis nidulans				1.714	31	54.3	B. B. Biswas, Plant Physiol. Proc., 35: XXX, 1960

TABLE 1.—*Continued*

Strain	No.	T_m in C	Reference	ρ in g/cm³	Reference	Chemical determination as % GC	Reference
Plectonema boryanum	IU 594	88.7	49	1.706	49		
Chlorella ellipsoidea				1.716	13	58.5	46, 95, B. B. Biswas, Plant Physiol. Proc., 35: XXX, 1960
Euglena gracilis		90	65	1.7055	81, 83		
Tetrahymena patula	LFF 1	77	81	1.684	81		
T. pyriformis	W	81.2	81	1.690	81		
Crithidia fasciculata		97.4	81	1.717	81		
C. lucilliae		94.1	81	1.716	81		
Strigomonas oncopelti		95.5	81	1.713	81		
Dictyostelium discoideum	NC-4	79.5	81	1.6815	81, 83		
Trichomonas gallinae	YG	80.6	61	1.693	61		
T. gallinae	JB	82.85	61	1.693	61		
T. vaginalis	C 1	79	61	1.689	61		
Saccharomyces cerevisiae var. *ellipsoideus*	H 36	85.2	74			41.0	74
S. pombe	H 28	87.0	74			44.8	74
Antirrhinum majus (chloroplast)				1.696	40	37.7	76
Nicotiana tabacum (nucleus)		85.5	65	1.6955	40, 83		
Triticum vulgare (germ)		88.5	65	1.702	83	45.8	11; Josse, as quoted in 65
Chick (embryo, liver, chorioallantoic membrane)		87.5	65	1.701	52, 83	42.1	51
Calf thymus		87	2, 65	1.699	83	43.4	11, 54
Human spleen		86.5	65	1.698	83	41.4	11
Human liver				1.700	52	39.4	11
Bull sperm				1.700	83	44.2	104
Mouse spleen		86.5	65	1.704	91, 83	41.9	51
Rat (various organs)		86.5	65	1.700	52, 83	42.3	11, 51
Salmon sperm		87.5	65	1.703	83	41.2	11
Turtle				1.703	52	43.3	11
Acantholithodes hispidus		84.5	87	1.702	87		
Balanus nubilis		85.5	87	1.706	87		
Cancer antennarius		84.0	86, 87	1.700	86, 87		
C. gracilis		84.0	86	1.700	86		
C. magister		84.6	86	1.701	86		
C. oregonensis		85.2	87	1.701	87		
C. productus		84.2	86	1.701	86		
Chionoecetes bairdii		85.8	87	1.702	87		
Chorilia longipes		83.0	87	1.701	87		
Munida quadrispina		85.6	87	1.705	87		
Paralithodes camtschatica		85.1	87	1.701	87		
Drosophila melanogaster		86.5	65	1.702	83		
Haliotis camtschatkana		84.5	88	1.702	88		
Polinices lewisii		85.6	88	1.704	88		
Clinocardium nuttalli		82.8	88	1.696	88		
Crassostrea gigas		80.7	88	1.693	88		
Protothaca staminea		82.6	88	1.694	88		
Saxidomas giganteus		82.5	88	1.693	88		

[a] The strains of bacteria are listed as much as possible with an international collection number and not necessarily with the number used in the original paper when the base composition or biophysical data were given. Likewise, wherever possible, the names of bacteria were adapted according to more recent taxonomic knowledge. Our own unpublished data are referred to by "this paper." When two or more data on the same organisms are known, they are reported as the average.

[b] Double-stranded polynucleotide of perfectly alternating deoxythymidylate and deoxyadenylate poly d(A-T)·poly d(A-T) or deoxyguanylate and deoxycytidylate poly d(G-C)·poly d(G-C); double-stranded homopolymers of the same nucleotides, poly dA·poly dT and poly dG·poly dC.

Nevertheless, we prefered to use equation 9 = 10, because it is statistically correct when the % GC is calculated from experimental T_m values and because the latter are more reproducible. Anyway, the difference between both equations 9 and 11 is small, being at most some 1.5% GC at the extremes of 75 and 30% GC.

At first glance, the correlation (Fig. 1) appears to be not perfectly linear and to curve off gently towards poly d(A-T)·poly d(A-T) and poly dG·poly dC. The same type of curvature is seen in Fig. 4 of Marmur and Doty (65), suggesting a faint hyperbola-like correlation. Colwell and Mandel (18) likewise presented a curved standard line. Crothers, Kallenbach, and Zimm (23) tentatively calculated the correlation between T_m and the GC fraction ν as

$$T_m - T_{AT} = -\nu R T_m T_{AT} \cdot \ln k/\Delta H \quad (12)$$

where k is a constant, R is the gas constant, T_{AT} is the "melting point" of the double-stranded polynucleotide of randomly distributed deoxythymidylate and deoxyadenylate poly d(A, T)·poly d(A, T), and ΔH is the heat of formation of DNA helix from coil DNA. It can be rewritten as

$$\%\,GC = -100\,\Delta H\,(1/T_{AT} - 1/T_m)/R \ln k \quad (13)$$

We plotted $1/T_m$ versus % GC. The entire correlation is decidedly more curved than a % GC – T_m plot. It is only apparently linear within the range 40 to 70% GC according to the equation

$$\%\ GC = 294.9 - 22.051/T_m \quad (14)$$

with a correlation coefficient of −0.976 for linearity, from which it follows that T_{AT} would be 74.8 C and $\Delta H = -220.5$ R ln k.

When the data for all the other organisms (viruses and phages, algae, protozoa, yeasts, plants, animals, but not the three T-even phages) are included in the calculation, equation 10 is not perceptibly changed. Substitution of cytosine by small amounts of methylated cytosine thus have no effect on T_m. From equation 10, it follows that the expected T_m for a pure AT-DNA is 69.37. This value is perfectly centered between those of poly dA·poly dT (T_m 72.6 C) and poly d(A-T)·poly d(A-T) (T_m 66 C). An AT-DNA might thus be considered as a heteropolymer with a sequence different from the regular alternation ...ATATATAT... as pointed out already (72). The expected T_m for a pure GC-DNA is 110.4 C, which is quite different from the homopolymer poly dG·poly dC (T_m = 105.8 C). If this GC-DNA would also hold an intermediate position, the alternating poly d(G-C)·poly d(G-C) might be expected to have a T_m value of approximately 115 C.

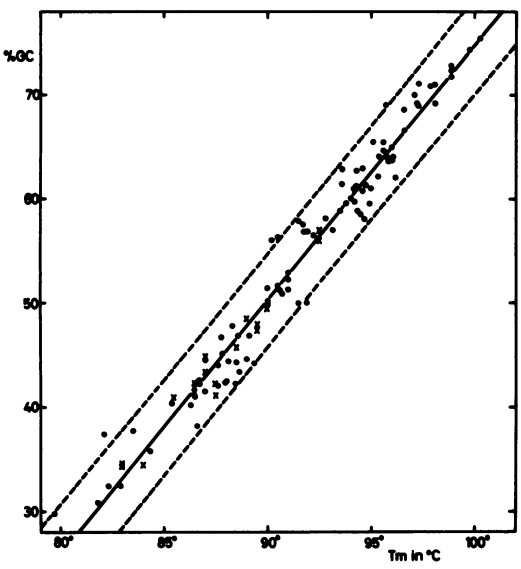

FIG. 1. *Correlation between the DNA base composition (expressed as % GC) determined chemically and the midpoint of the thermal denaturation (T_m) determined in SSC buffer. Each point corresponds to a bacterial strain, each cross to another type of organism. All data are taken from Table 1. The full line is the regression according to equation 9 = 10. The broken lines represent the limits of accuracy at the 5% probability level.*

The correlation between ρ and chemical % GC values for bacteria. The data are plotted in Fig. 2. The available data range again from 30 to 75% GC. Both regression lines are (for 84 strains)

$$\%\,GC = 1038.47\,(\rho - 1.6616) \quad (15)$$

or

$$= (\rho - 1.6616)/0.000963 \quad (16)$$

and

$$\rho = 0.000925\ \%GC + 1.6634 \quad (17)$$

Additional statistical information is given in Table 2. The denominator in equation 16 can range from 0.000909 to 0.001023. The confidence limits of approximately ±4.3% GC are nearly identical to the ones calculated from the regression of % GC on T_m. They did not noticeably decrease when the ρ data determined in the same laboratory were compared with chemical % GC values. Three strains fall definitely out of the limits [*Bifidobacterium* strain GrIV Dehnert 456, IV; *Lactobacillus viridescens* NCDO S40(E_3); and *Staphylococcus aureus* NCIB 8625]; *Bacillus amyloliquefaciens* F is just at the border. Their ρ

TABLE 2. *Correlation and regression between chemical base composition of DNA (expressed as % GC), buoyant density (ρ) in g/cm³, and "melting point" (T_m) in C of a variety of organisms, mainly bacteria*[a]

	Equation 9: % GC = 2.44 T_m − 169.25		Equation 15: % GC = 1038.47 (ρ − 1.6616); for bacteria only	Equation 18: % GC = 1020.6 (ρ − 1.6606); for all organisms	Equation 20: ρ = 0.00222 T_m + 1.5096; for bacteria only	Equation 21: T_m = 429.76 (ρ − 1.5002); for bacteria only
	For bacteria only	For all organisms				
Degrees of freedom	94	109	82	103	195	195
Correlation of coefficient	0.98	0.98	0.98	0.98	0.98	0.98
Residual variance $s^2 = (S_{yy} - S_{xy}^2/S_{xx})/(n-2)$	5.0515	4.6273	4.5216	4.831	5.303	1.02497
Variance of average % GC = s^2 n	0.05262	0.04149	0.05296	0.04594		
Variance of the slope = s^2/S_{xx}	0.00261	0.00213	538.6	512		
Variance of average ρ					118×10^{-11}	44.0
Variance of average T_m					269×10^{-10}	
Interval of the slope $S_{xy}/S_{xx} \pm t\sqrt{s^2/S_{xx}}$						0.005192
at 5% probability level	2.44 ± 0.10	2.44 ± 0.09	1038.47 ± 46	1020.6 ± 45	0.00222 ± 0.000068	429.76 ± 13.06
at 1% probability level	2.44 ± 0.13	2.44 ± 0.12	1038.47 ± 61	1020.6 ± 60	0.00222 ± 0.00009	429.76 ± 17.25
Interval for average $y \pm t\sqrt{s^2/n}$						
at 5% probability level	54.94 ± 0.46 % GC	53.74 ± 0.40 % GC	47.98 ± 0.46 % GC	47.69 ± 0.43 % GC	1.7116 ± 0.00032 ρ	90.87 ± 0.14 T_m
at 1% probability level	54.94 ± 0.61 % GC	53.74 ± 0.53 % GC	47.98 ± 0.61 % GC	47.69 ± 0.57 % GC	1.7116 ± 0.00043 ρ	90.87 ± 0.19 T_m
Limits of accuracy of prediction from linear regression at 5% probability level for a single observed y $\pm ts\sqrt{1 + 1/n + (x - \bar{x})^2/S_{xx}}$	± (4.5 to 4.6) % GC	± (4.27 to 4.34) % GC	± (4.23 to 4.35) % GC	± (4.39 to 4.56) % GC	± 0.0045 ρ	± (1.99 to 2.02) C
for the average of many y values $\pm ts\sqrt{1/n + (x - \bar{x})^2/S_{xx}}$	± (0.5 to 0.9) % GC	± (0.42 to 0.88) % GC	± (0.47 to 1.12) % GC	± (0.44 to 1.31) % GC	± 0.0005 ρ	± (0.14 to 0.37) C

[a] Data from Table 1 were used for calculations.

and chemical % GC values, or both, should be redetermined.

When the available data for all the organisms except T-even phages are included, the equation

$$\% \text{ GC} = 1020.6\ (\rho - 1.6606) \quad (18)$$

is obtained, which is nearly indistinguishable from Schildkraut's original proposal (*see* equation 2). It was already pointed out (83) that substitution of cytosine by hydroxymethyl cytosine (as in the T-even phages) changes the buoyant density considerably. The difference between equations 15 and 18 is probably largely a result of the small amount of hydroxymethyl cytosine in plant and animal tissues. The % GC values from equation 15 are 0.5 to 1.5% GC lower than from the currently used equation 2. The former has a greater probability of being correct.

According to equation 15, the buoyant density for a pure AT-DNA would be 1.6616 g/cm³, which is again very close to the middle between ρ of poly d(A-T)·poly d(A-T) and poly dA· poly dT. For pure GC-DNA, ρ would be 1.7579 g/cm³, which is much lower than the observed ρ of 1.795 g/cm³ for poly dG·poly dC, and suggests that the buoyant density of a hydrogen-bonded alternating polymer would be quite different from the homopolymer.

There is a reasonably good agreement between equations 10 and 15. This is apparent when one compares the DNA base composition of the reference *E. coli* K-12. From ρ = 1.710 g/cm³ it is 50.3% GC. From T_m it is approximately 51.8% GC. The average of the chemical determinations for this strain is 51.2% GC. The slight discrepancy between both equations 10 and 15 is mainly a reflection of the inaccurate chemical determinations.

The correlation between ρ and T_m. From the equations 10 and 15, it can be calculated that the relation between ρ and T_m is

$$T_m = 425.62\ (\rho - 1.4986) \quad (19)$$

An independent control on the latter equation, and thus also on the general working equations 10 and 15 is possible because the values for ρ and T_m are known for 197 strains of bacteria. The results are plotted in Fig. 3. The regression lines are

$$\rho = 0.00222\ T_m + 1.5096 \quad (20)$$

$$T_m = 429.76\ (\rho - 1.5002) \quad (21)$$

Equation 19 is very close to the orthogonal regression line between 20 and 21; its resemblance with equation 21 is quite clear. It can thus be concluded that equations 10 and 15 are reliable for deter-

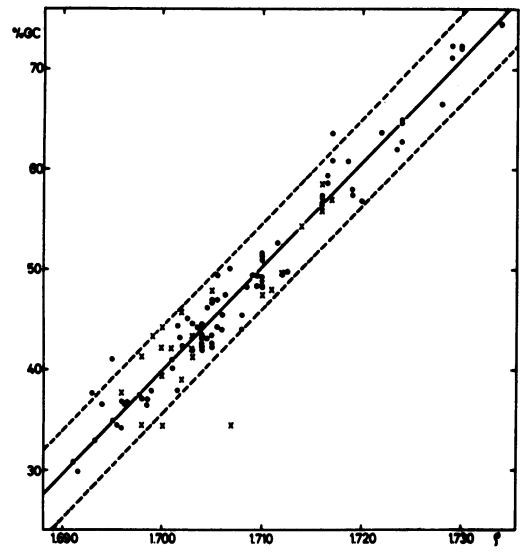

FIG. 2. *Correlation between the DNA base composition (expressed as % GC) determined chemically and the buoyant density, (ρ) in g/cm³. The full line is the regression according to equation 15 = 16. All other information as in legend to Fig. 1.*

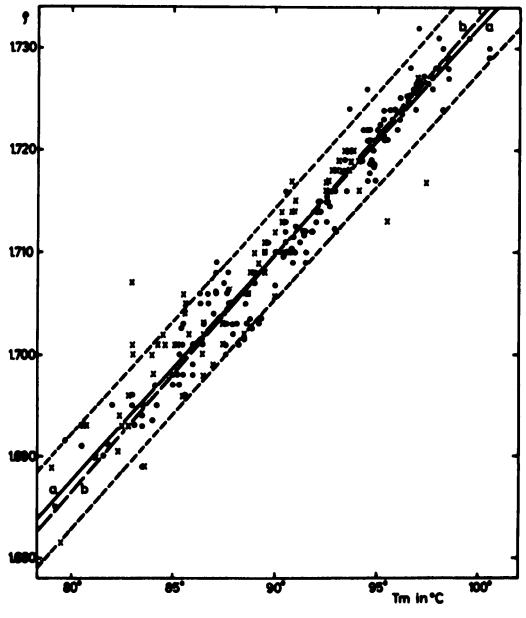

FIG. 3. *Correlation between the buoyant density (ρ) in g/cm³ and the midpoint of the thermal denaturation (T_m) determined in SSC buffer. The full line a is the regression of ρ on T_m, according to equation 20. The central broken line b is the regression of T_m on ρ, according to equation 21. All other information as in legend to Fig. 1.*

mining DNA base composition. The scatter (Fig. 3) is enormous for methods which are claimed to be very precise and reproducible. The wide limits show that ρ and T_m values are not always determined in the best possible conditions. The ρ and T_m values, or both, of several strains are seen (Fig. 3) to be outside or just on the border of the safety limits; they should be reexamined for *Mycobacterium phlei*, *Rhodospirillum rubrum* S-1, *Desulfovibrio vulgaris* NCIB 8303, *D. desulfuricans* NCIB 8380 (*see* also above), *D. salexigens* NCIB 8403, *Bacillus amyloliquefaciens* H, *B. subtilis* ATCC 7067, *Clostridium acidiurici*, *C. madisonii*, *Streptomyces viridochromogenes* 93, "*Achromobacter*" *liquefaciens* ATCC 15716, *Cytophaga* species ATCC 11947, and *Aerobacter aerogenes* ATCC 14308. Some of these deviations can be readily understood. For *Streptomyces* and *Mycobacterium*, the T_m values are rather high and are measured with some technical difficulty.

The plot of the ρ versus T_m values for all organisms except bacteria shows a much greater scatter; 19% of the data fall on or outside the safety limits, whereas for bacteria alone it is 7%. This much greater heterogeneity is very likely the result of the effect of unusual bases (hydroxymethylcytosine, etc.) on the buoyant density, and to the concomitance of nuclear, mitochondrial, and chloroplast DNA. Although they are thus less reliable, we give here both regression lines for sake of completeness, calculated from 272 organisms

$$\rho = 0.00217\ T_m + 1.5150 \quad (22)$$

$$T_m = 435.44\ (\rho - 1.5033) \quad (23)$$

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