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Infrared Spectrum of Deoxyribonucleic Acid —— Effects of Base Composition and of ^{15}N-Substitution ——

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Infrared absorption spectra have been observed in the 4000—500 cm^{-1} region of deuterated and undeuterated films of deoxyribonucleic acids (DNA's) obtained from *Micrococcus lysodeikticus* (guanine plus cytosine content or GC content 72%), from *Bacille de Calmette-Guérin* (GC 65%), from *Pseudomonas aeruginosa* (GC 65%, ^{15}N-substituted), from calf thymus (GC 44%), and from *Cancer borealis* (GC 3%). Of the undeuterated DNA, 14 absorption bands have been assigned to the GC pair and 17 absorption bands to the adenine-thymine (AT) pair. Of the deuterated DNA, 19 absorption bands have been assigned to the GC pair and 16 to the AT pair. For 4 bands of undeuterated DNA and 13 bands of deuterated DNA, appreciable ^{15}N isotopic shifts have been observed. A discussion has been given of a few of these bands on the basis of the observed ^{15}N shift and some other data.

This work is a part of our attempt to establish a basis of an infrared base-analysis which should eventually give the amount of guanine-cytosine, adenine-thymine, and adenine-uracil base pairs and the amounts of unpaired guanine, cytosine, adenine, thymine, and uracil base residues in a given polynucleotide sample. In our present knowledge, every native deoxyribonucleic acid (DNA) obtained from a bacterium or a higher organism contains only the guanine-cytosine (GC) and adenine-thymine (AT) pairs, and practically no unpaired bases. Some of such DNA's have high GC contents and some very low. Therefore, infrared absorption bands assignable to the GC and AT pairs are considered to be found by comparing infrared absorption spectra of these DNA's. This has been the case as will be described below. In additon, the ^{15}N-substitution effect on the spectrum of a DNA with a GC content 65% has been examined. From the result in combination with data previously obtained, some nature of the normal vibration of each of the absorption bands has been clarified. A preliminary account of this work appeared already elsewhere.[1,2] It should also be mentioned here that Fritzsche[3] made independently an observation of the infrared spectra of a few deuterated DNA's with different GC contents.

Experimental

The DNA samples used are listed in Table 1.

DNA from *Micrococcus lysodeikticus* was prepared by ourselves. Mostly, the procedure described by Marmur[4] was followed. For dissociating the nucleic acid from protein, however, not only the Marmur's method but also the method described by Kay, Simmons, and Dounce[5] was adopted. The molecular weight of the product was estimated to be 3.2×10^6 from the sedimentation coefficient. DNA from *Bacille de Calmette-Guérin* (B.C.G.) was pre-

TABLE 1. DNA SAMPLES USED IN THE PRESENT WORK

Source	GC content	
Micrococcus lysodeikticus	72%[a]	
Bacille de Calmette-Guérin	65 [b]	
Pseudomonas aeruginosa	65 [a]	(^{15}N-substituted)
Calf thymus	44 [c]	
Cancer borealis	3 [d]	(light component)

a) N. Sueoka and T. Y. Cheng, *J. Mol. Biol.*, **4**, 161 (1962).

b) Determined by Professor T. Tsumita (by hydrolysis).

c) E. Chargaff, "The Nucleic Acids," E. Chargaff and J. N. Davidson, Eds., Vol. 1, Academic Press, Inc., New York (1955), p. 354.

d) N. Sueoka and T. Y. Cheng.[7]

1) M. Tsuboi and K. Shuto, *Chem. Pharm. Bull.*, **14**, 784 (1966).

2) M. Tsuboi and K. Shuto, Paper read at 5th Annual Meeting of Biophys. Soc. Japan, 17 p-C-4, Kyoto, Dec., 1966; M. Tsuboi, K. Shuto and S. Higuchi, Paper read at 7th International Congress of Biochemistry, Tokyo, Aug., 1967 (Abstract B-38).

3) H. Fritzsche, *Studia biophysica, Berlin*, **1**, 273 (1966).

4) J. Marmur, *J. Mol. Biol.*, **3**, 208 (1961).

5) E. R. M. Kay, N. S. Simmons and A. L. Dounce, *J. Am. Chem. Soc.*, **74**, 1724 (1952).

pared by Professor Toru Tsumita in the Institute of Medical Science, University of Tokyo, and kindly placed at our disposal by him. ^{15}N-DNA from *Pseudomonas aeruginosa* was donated by Dr. Ts'ai-Ying Cheng, now at the Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania, U. S. A. This was obtained by the Marmur's method[4] from *P. aeruginosa* grown for many generations in $^{15}NH_4Cl$ as the sole nitrogen source. In this DNA sample, practically all of the nitrogen atoms is considered to be ^{15}N, on the basis of the manner of preparation. Calf thymus DNA was purchased from Sigma Chemical Company. The "light DNA" from *Cancer borealis*, which contains 97 mol percent of deoxyadenylate and deoxythymidylate in predominantly alternating sequence,[6,7] was again a gift from Dr. Cheng.

For the infrared absorption measurements oriented or unoriented films were prepared on AgCl plates. The control of the humidity of the air over the sample and deuteration of the sample were made by the method described by Sutherland and Tsuboi.[8] The instruments used were a model DS-402 G double-beam grating spectrometer of Japan Spectroscopic Company, a Perkin-Elmer model 12C Spectrometer with CaF_2, NaCl, and KBr prisms, and a Perkin-Elmer model 221 Spectrometer. For obtaining polarized radiation an AgCl polarizer was used.

Absorption Bands Assignable to the GC and AT Base Pairs

Infrared absorption spectra of an oriented film of the "light DNA" from *Cancer borealis* observed with the polarized radiation are shown in Fig. 1. As may be seen in the figure, many details of the spectra of this DNA are different from what are observed of calf thymus DNA.[8,9] Some parts of the infrared absorption spectra of unoriented films of the five DNA's (see Table 1) observed with the ordinary radiation are given in Figs. 2—6. The spectral features are found to depend

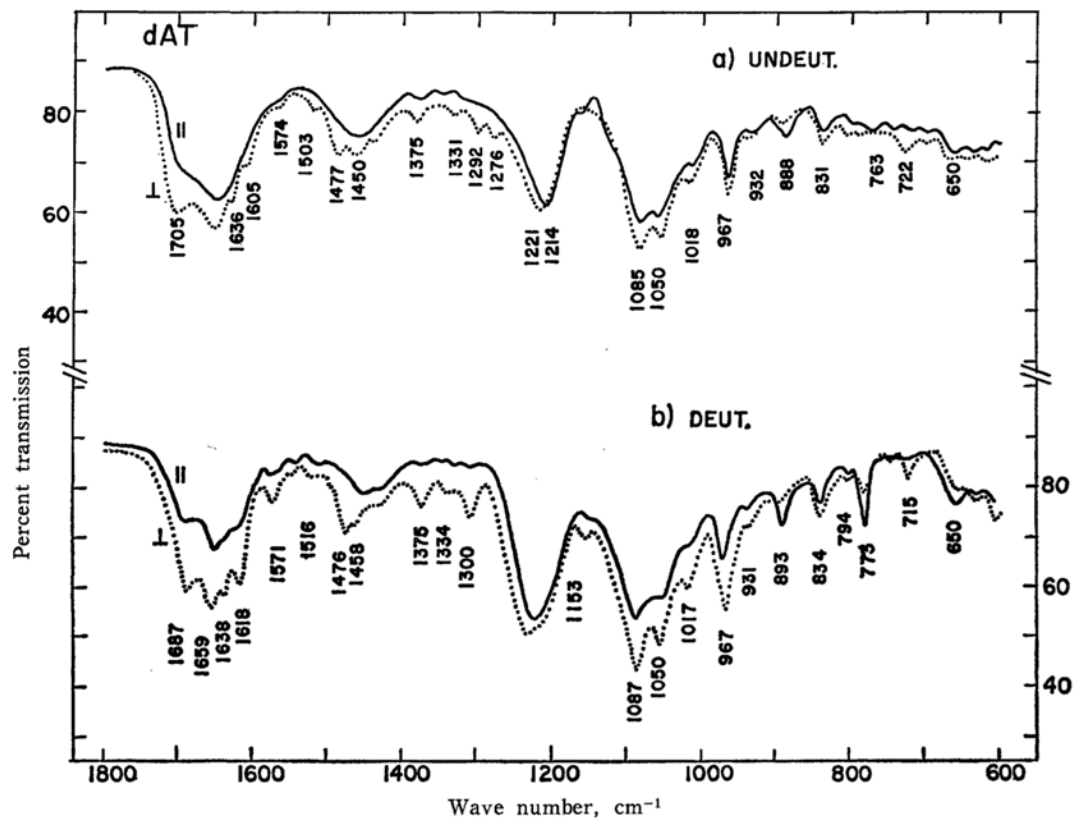

Fig. 1. Infrared absorption spectra of oriented films of the light DNA from *Cancer borealis* with GC 3%. (a) Undeuterated, at 92% relative humidity; (b) deuterated, at 92% relative humidity. Full line: electric vector of the incident radiation is parallel to the fiber axis. Broken line: electric vector the incident radiation is perpendicular to the fiber axis.

6) N. Sueoka, *J. Mol. Biol.*, **3**, 31 (1961).
7) N. Sueoka, and T. Y. Cheng, *Proc. Nat. Acad. Sci. (U. S. A.)*, **48**, 1851 (1962).
8) G. B. B. M. Sutherland and M. Tsuboi, *Proc. Roy. Soc.*, **A 239**, 446 (1957).
9) E. M. Bradbury, W. C. Price and G. R. Wilkinson, *J. Mol. Biol.*, **3**, 301 (1961).

markedly upon the base compositions of the DNA samples. All of these spectra were observed at 92% relative humidity. At this humidity, the DNA fiber takes the B-form irrespective of the base compositions.[10,11] The geometrical structures of DNA's with different base compositions are so similar that practically no difference can be found in their X-ray diffraction patterns.[10,11] Therefore, the marked difference in their infrared spectra are to be ascribed solely to the difference in the normal vibrations of the structure with the GC pair and that with the AT pair. In most of the vibrational modes corresponding to the absorptions in the 4000—500 cm^{-1} region, the displacements of atoms are localized within relatively small atomic groups. In addition, the vibrational coupling between the adjacent two nucleotide residues would not be great. Therefore, most of the absorption bands of

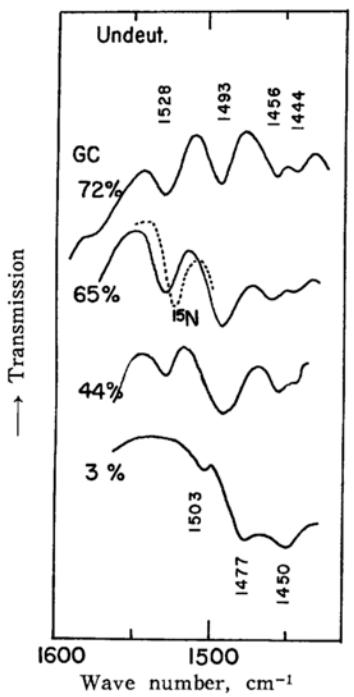

Fig. 2. Infrared absorption spectra in the 1560—1440 cm^{-1} region of undeuterated films of the five DNA's listed in Table 1. Observed at 92% relative humidity.

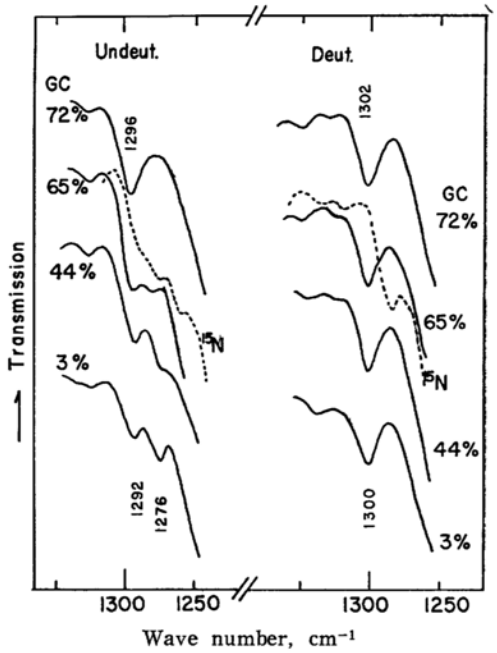

Fig. 3. Infrared absorption spectra in the 1340—1250 cm^{-1} region of undeuterated and deuterated films of the five DNA's listed in Table 1. Observed at 92% relative humidity.

DNA in the spectral region now in question are expected to be classified into three groups: (i) bands assignable to some vibrations localized in the guanine-cytosine (GC) base pair, (ii) bands assignable to some vibrations localized in the adenine-thymine (AT) base pair, and (iii) bands assignable to some vibrations localized in the phosphate-deoxyribose main-chain. An examination of the spectra was made first with this expectation in mind.

In Fig. 2, an absorption band at 1528 cm^{-1} is found to become weaker with the GC content. Therefore, this band may be assigned to the GC base-pair. The weak band at 1503 cm^{-1} and another band at 1477 cm^{-1} of the light DNA of cancer are not observed in DNA's with higher GC contents. Therefore, these should be assigned to the AT pair. The occurrence of a broad band at about 1490 cm^{-1} of the calf thymus DNA may be interpreted as a result of a superposition of 1503, 1477, and the 1493 cm^{-1} bands. The last one is to be assigned to the GC pair.

As has been pointed out previously,[1] the 1276 cm^{-1} band is attributed solely to the AT pair (see Fig. 3). On the other hand, the absorptions around the 1292—1296 cm^{-1} region are caused by both of the AT and GC pairs. Here, however, the absorption bands due to the AT and GC pairs seem to be situated at

10) L. D. Hamilton, R. K. Barclay, M. H. F. Wilkins, G. L. Brown, H. R. Wilson, D. A. Marvin, H. Ephrussi-Taylor and N. S. Simmons, *J. Biophys. Biochem. Cytol.*, **5**, 397 (1959); R. Langridge, H. R. Wilson, C. W. Hooper M. H. F. Wilkins and L. D. Hamilton, *J. Mol. Biol.*, **2**, 19 (1960); R. Langridge, D. A. Marvin, W. E. Seeds, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins and L. D. Hamilton, *ibid.*, **2**, 38 (1960).

11) D. R. Davies and R. L. Baldwin, *J. Mol. Biol.*, **6**, 251 (1963).

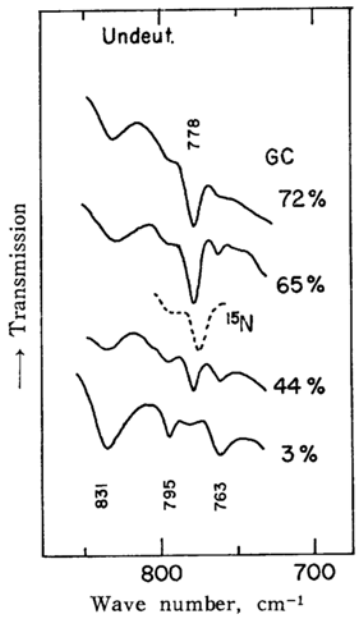

Fig. 4. Infrared absorption spectra in the 850—740 cm^{-1} region of undeuterated films of the five DNA's listed in Table 1. Observed at 92% relative humidity.

slightly different frequencies. On deteration, both of the 1296 and 1276 cm^{-1} bands disappear, and a slightly stronger band appears at about 1300 cm^{-1}. Both of the AT and GC pairs give a band here, but again at slightly different frequencies.

In a similar way, we may pick up the absorption band at 831, 795, and 763 cm^{-1} (see Fig. 4) as those assignable to the AT pair and the absorption band at 778 cm^{-1} as one assignable to the GC pair.

The absorption spectra of deuterated DNA's in the 1700—1450 cm^{-1} region are rather complicated (Fig. 5). As has been mentioned by Sutherland and Tsuboi,[8] almost all the absorption bands in this region are assignable to in-plance vibrations of bases. It was difficult, however, to proceed a further analysis by examining the calf thymus DNA only. It is now safely concluded that the absorptions at 1687, 1659, 1638, 1618, 1571, 1476, and 1458 cm^{-1} are due to the AT base pair, because at these frequencies the AT-DNA of cancer

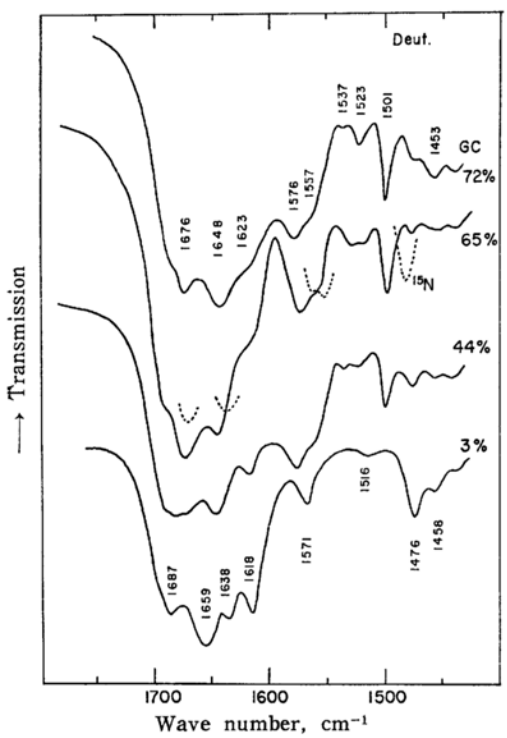

Fig. 5. Infrared adsorption spectra in the 1800—1430 cm^{-1} region of deuterated films of the five DNA's listed in Table 1. Observed at 92% relative humidity.

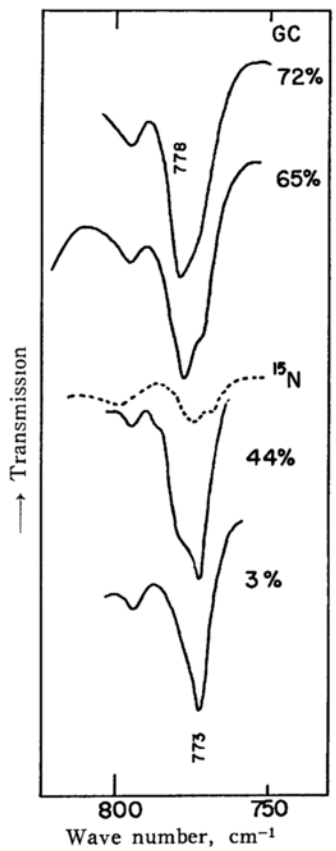

Fig. 6. Infrared absorption spectra in the 800—750 cm^{-1} region of deuterated films of the five DNA's listed in Table 1. Observed at 92% relative humidity.

gives absorption peaks (Figs. 1b and 5) but DNA's with higher GC contents do not (Fig. 5). The absorptions at 1676, 1648, 1576, 1523, 1501, and 1453 cm^{-1} are ascribed to the GC pair, because the Micrococcus DNA with GC 72% gives absorption peaks at these frequencies. The absorption curves of DNA's with the GC contents of 65% and 44% are interpreted in an approximation as proper superpositions of the absorption curves of DNA's with the GC contents 72% and 3% (Fig. 5).

As may be seen in Fig. 6, both of the GC and AT pairs give sharp peaks around 780 cm^{-1}. The former, however, gives a peak at a slightly higher frequency (778 cm^{-1}) then the latter (773 cm^{-1}). Therefore, the fact[8] that the calf thymus DNA gives a shoulder at 778 cm^{-1} besides the sharp peak at 773 cm^{-1} is now quite understandable on the basis of its base compositions.

Absorption bands at 1218, 1087, 1069, 1050, 1018, 967, and 650 cm^{-1} appear in every DNA and show almost no changes in their frequencies and intensities on deuteration. Therefore, they are assignable to some vibrations of atomic groups other than the bases.

As has been illustrated so far nearly all the absorption bands of DNA observed in the 1800—500 cm^{-1} region can be classified into the three groups, (i), (ii), and (iii), defined above, and a spectrum of any DNA appears, at least qualitatively, to be an assembly of the absorption bands of these groups with a proper set of intensities which depends upon the GC content of the DNA. The frequencies of the absorption bands belonging to these three groups are listed in Tables 2 and 3, with their intensities.

Most of the absorption bands assignable to the GC or AT base pair are found to be strongly polarized, if they are observed in the oriented films, either along the perpendicular direction to the fiber axis or along the parallel direction to it. This fact is understandable, because, in the B-form of DNA, base residues are arranged with their planes perpendicular to the fiber axis,[10] and because the normal vibrations localized in the base residues should take place with their transition moments either in their plane or perpendicular to the plane. Among the absorption bands of bases, what are strongly polarized along the perpendicular

TABLE 2. INFRARED ABSORPTION BANDS ASSIGNABLE TO THE GC BASE PAIR, AT BASE PAIR, AND OTHER PARTS OF THE UNDEUTERATED DNA MOLECULE

Bands due to GC				Bands due to AT			Others		
Frequency	Characterization[a]	^{15}N shift		Frequency	Characterization[a]	^{15}N shift	Frequency	Intensity	^{15}N shift
		Frequency shift	$\Delta\lambda/\lambda°$						
cm^{-1}		cm^{-1}		cm^{-1}			cm^{-1}		cm^{-1}
1705	s, *i*	−5*	−0.006*	1705	s, *i*				
							1220	s	0*
				1636	m, *i*		1087	s	0*
1609	m, *i*			1605	m, *i*				
1576	w, *i*			1574	w, *i*		1050	s	0*
1528	m, *i*	−8	−0.010	1503	w		1018	m	0*
1493	m, *i*			1477	m, *i*		967	s	0*
1456	w, *i*			1450	m, *i*		650	m	
1444	w, *i*			1375	w,				
1414	w, *i*			1331	w,				
				1292	w, *i*				
1380	m, *i*			1276	w, *i*				
1331	w, *i*			932	m, *i*				
1296	m, *i*	−20*	−0.031*	888	m, *o*				
893	m, *o*	0	0	831	m				
860	w, *o*			795	w				
				763	w, *o*				
				722	w,				
778	m, *o*	−3	−0.008						

a) s : strong, m : medium, w : weak, *i* : in-plane vibration, *o* : out-of-plane vibration,
* less accurate than others.

TABLE 3. INFRARED ABSORPTION BANDS ASSIGNABLE TO THE GC BASE PAIR, AT BASE PAIR, AND OTHER PARTS OF THE DEUTERATED DNA MOLECULE

Bands due to GC				Bands due to AT				Others		
Frequency	Characterization[a]	^{15}N shift		Frequency	Characterization[a]	^{15}N shift		Frequency	Intensity	^{15}N shift
		Frequency shift	$\Delta\lambda/\lambda^\circ$			Frequency shift	$\Delta\lambda/\lambda^\circ$			
cm^{-1}		cm^{-1}		cm^{-1}		cm^{-1}		cm^{-1}		cm^{-1}
1676	s, *i*	0	0	1687	s, *i*			1087	s	
1648	s, *i*	−6	−0.007	1659	s, *i*			1050	s	0
1623	m, *i*	−6	−0.007	1638	m, *i*			1017	m	0
1576	s, *i*	−17*	−0.021*	1618	s, *i*			967	s	0
1557	m, *i*									
1537	w, *i*	−20	−0.026	1571	m, *i*					
1523	w, *i*	−18	−0.023							
1501	m, *i*	−14	−0.019	1516	w, *i*					
1453	w, *i*			1476	m, *i*			650	m	
1439	w, *i*			1458	w, *i*					
1377	w, *i*	0	0	1375	w, *i*					
1350	w	−14	−0.021	1334	w, *i*					
1302	m, *i*	−17	−0.026	1300	w, *i*					
851	w,			1153	w, *i*					
831	m,			834	m					
797	w,	+3*	+0.008*	794	w,					
778	s, *o*	−3	−0.008	773	m, *o*	−3*	−0.008*			
727	w, *o*	−8	−0.022	715	w, *i*					
698	w, *o*	−5	−0.014							

a) s : strong, m : medium, w : weak, *i* : in-plane vibration, *o* : out-of-plane vibration.
* less accurate than others.

direction to the fiber axis are assigned to some in-plane (*i*) vibrations, and what are strongly polarized along the parallel direction to some out-of-plane (*o*) vibrations. These are also indicated in Tables 2 and 3.

^{15}N Isotope Shifts in the Vibrational Frequencies

The ^{15}N-DNA from *Pseudomonas aeruginosa* (GC 65%) shows an infrared absorption spectrum similar in its general feature to that of the DNA from *Bacille de Calmette-Guérin* (GC 65%). Many of the absorption maxima of the former DNA, however, are found to be shifted towards the lower frequencies from the corresponding maxima of the latter DNA (Figs. 2—7).

In principle, the ^{15}N isotope effect on an infrared spectrum of DNA should be found by comparing the ^{15}N-DNA and ^{14}N-DNA obtained from the same organism. Unfortunately, these were not available in our laboratory, and the ^{15}N-^{14}N comparison has been made between different species of bacteria having the same GC content as above. Practically, however, this is justified, because the DNA's from *Pseudomonas* and *B.C.G.* are expected to make no difference in their infrared spectra. These DNA's are different only in the sequence and arrangement in the GC and AT base pairs. The infrared spectrum in the spectral region now in question is not sensitive to the sequence and arrangement. Absorptions of a base residue might appreciably be different depending upon the neighbours. Such a difference, however, should disappear in an average, unless the nearest neighbour sequence frequencies of the two DNA's are greatly different; the nearest neighbour sequence frequencies have been found to be rather similar for natural DNA's from different sources but with almost equal GC contents.[12]

The significance of the observed shift may be judged, not only on the basis of the above consideration, but also by examining the experimental results given in Figs. 2—7. For example, the positions of relatively sharp bands at 1528 cm^{-1} (Fig. 2), at 1302 cm^{-1} (Fig.

12) J. Josse, A. D. Kaiser and A. Kornberg, *J. Biol. Chem.*, **236**, 864 (1961).

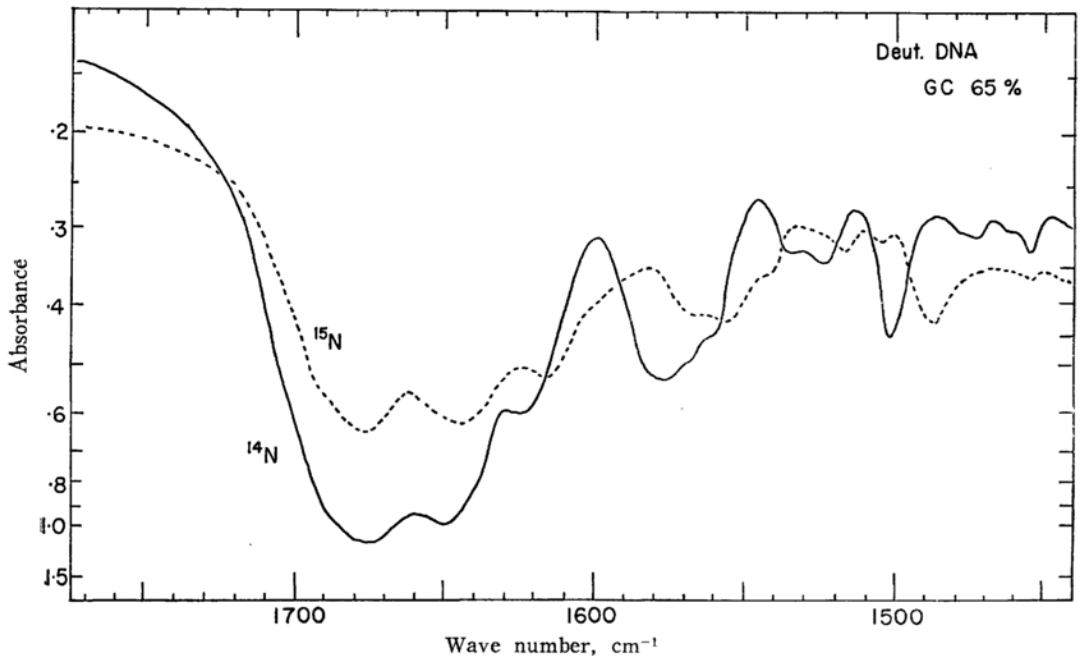

Fig. 7. An expanded recording of the infrared absorption spectra of deuterated films of DNA from *Bacille de Calmette-Guérin* (——) and of ^{15}N-DNA from *Pseudomonas aeruginosa* (·····). Observed at 92% relative humidity.

3), at 778 cm^{-1} (Fig. 4), at 1501 cm^{-1} (Fig. 5), and at 778 cm^{-1} (Fig. 6) are all found to remain constant within 2 cm^{-1} for ^{14}N-DNA's from Micrococcus, B. C. G., and calf thymus, while the corresponding bands are found at 3—17 cm^{-1} lower frequencies for the ^{15}N-DNA. This fact indicates that the frequencies of these bands are not sensitive to the origin of the DNA samples, and observed shifts are solely ascribed to the $^{14}N \rightarrow {}^{15}N$ substitution in the base residues.

The amount of the frequency shift was measured for each absorption band by repeating a recording of the ^{14}N and ^{15}N spectra alternately in a small spectral range with a low scan speed and with an expanded wave-number scale (see Fig. 7, for example). The results of such measurements are given in Tables 2 and 3. For sharp and isolated absorption peaks, the amounts of shift were determined almost as accurately as those for simpler samples,[13] within 0.5 cm^{-1} or so. Sometimes, however, the measurement can be made only less accurately because the band in question is too broad or because the shifted absorption maximum is obscured by another absorption band. Such a case is indicated by * in Tables 2 and 3. The data of ^{15}N-isotope shifts are in general useful for judging the nature of the normal vibrations by which the absorption bands are caused. The judgement is often made through the amount:

$$\frac{\Delta\lambda}{\lambda^\circ} = \frac{\lambda(^{15}\mathrm{N}) - \lambda(^{14}\mathrm{N})}{\lambda(^{14}\mathrm{N})} \qquad (1)$$

rather than through the frequency shift $\Delta\nu$ itself. Here, $\lambda = 4\pi^2c^2\nu^2$, c being the velocity of light and ν the observed frequency (in reciprocal centimeters). This amount $\Delta\lambda/\lambda^\circ$ directly reflects how much movements of nitrogen atoms take place in the normal vibration now in question. Thus, on the basis of what Miyazawa[14] showed,

$$\frac{\Delta\lambda_k}{\lambda_k^\circ} = -\sum_N (L_{Nk})^2 \Delta m \qquad (2)$$

where, L_{Nk} is the amount of the displacement of each nitrogen atom relative to the center of gravity of the molecule, which is caused by the unit displacement (a deformation of the whole molecule) along the kth normal coordinate of the molecule. If the normal coordinate is expressed by the unit Å (atomic mass unit)$^{1/2}$ the unit of $(L_{Nk})^2$ should be (atomic mass unit)$^{-1}$. The isotopic mass change Δm is practically +1 in the atomic

13) M. Tsuboi, *Spectrochim. Acta*, **16**, 505 (1960); M. Tsuboi, T. Takenishi and A. Nakamura, *Spectrochim. Acta*, **17**, 634 (1961); M. Tsuboi, T. Takenishi and A. Nakamura, *ibid.*, **19**, 271 (1963).

14) T. Miyazawa, *J. Mol. Spectroscopy*, **13**, 321 (1964).

mass unit in the present case, and therefore this factor Δm can be ignored in the numerical calculation on Eq. (2). The summation is to be conducted for all the nitrogen atoms, on which the isotopic replacement has taken place.

In a less strict discussion of an infrared spectrum, the vibrational mode for each absorption band is often expressed in terms of the internal symmetry coordinate, such as NH_2 scissoring or NH out-of-plane deformation. Even in a complicated large molecule, a normal coordinate can sometimes be expressed approximately by an internal symmetry coordinate or by a linear combination of only a few internal symmetry coordinates. Therefore, it would be convenient to know the ^{15}N isotopic frequency shift for a hypothetical vibration along an internal symmetry coordinate t. This can be obtained by the formula,[13]

$$\frac{\Delta\lambda}{\lambda^\circ} = \frac{\Delta G_{tt}}{G_{tt}^\circ} \qquad (3)$$

where $\Delta \boldsymbol{G} = \boldsymbol{G} - \boldsymbol{G}^\circ$, $\boldsymbol{G}$ and $\boldsymbol{G}^\circ$ being the inverse kinetic energy matrices for the ^{15}N and ^{14}N species, respectively.

The $\Delta\lambda/\lambda^\circ$ values calculated by Eq. (1) from the observed frequencies are also given in Tables 2 and 3.

Discussion

The assignments of the infrared absorption bands of DNA were previously made on the basis of the deuteration effects, humidity effects, anisotropy of the absorptions, and the knowledge on the molecular vibrations of hypophosphites and other simple compounds. The effects of the base composition and of a ^{15}N-substitution are now obtained as new data. Based upon these new date, a few additional aspects of the nature of some of the absorption bands listed in Tables 2 and 3 are given below.

A strong band at 1705 cm^{-1} of undeuterated calf thymus DNA was found to be characteristic of its secondary structure.[15,16] It has now be shown that both of the GC and AT base pairs contribute to this band. The dipole oscillation takes place in the base plane in both base pairs. On the basis of the frequency and on the basis of the observed deuteration effect (see Fig. 1, for example), an NH_2 or NH in-plane deformation vibration is considered to take part in the normal vibration now in question. The observed amount of ^{15}N isotopic shift ($\Delta\lambda/\lambda_\circ = -0.006$) of the 1705 cm^{-1} band of the GC pair is almost equal to the calculated value ($\Delta\lambda/\lambda^\circ = -0.007$) for a pure (C-)$NH_2$ scissoring vibration and also to the calculated value ($\Delta\lambda/\lambda^\circ = -0.007$) for a pure NH in plane deformation vibration (on Eq. (3)). This, however, does not necessarily mean that the 1705 cm^{-1} band should be assigned to either an almost pure NH_2 scissoring or an almost pure NH in-plane deformation. It is possible, for example, that the actual mode of vibration is a combination of the NH_2 scissoring, NH deformation, C-N stretching and C=O stretching.

No normal coordinate treatment has yet been made of adenine, thymine, guanine, or cytosine. For cyanuric acid, however, which is somewhat similar structure to thymine, a normal coordinate treatment was made by Dr. Issei Harada (Master Thesis, 1963, and Doctor Thesis, 1966, University of Tokyo). His calculation was made of a hydrogen-bonded sheet in the cyanuric acid crystal. According to his calculation, there should be hree infrared-active vibrations at 1752, 1745, and 1696 cm^{-1} (There are actually three or four strong bands observed in the 1780—1715 cm^{-1} region). In all of these vibrations the C=O stretching motions are predominant but there are appreciable contributions from C-N stretching and NH in plane deformation vibrations. By applying Eq. (2) on the result of his calculation, it has been found that the three frequencies 1752, 1745, and 1696 cm^{-1} should give ^{15}N-shifts, $\Delta\lambda/\lambda^\circ = -0.015$, -0.013, and -0.003, respectively.

It is noteworthy that the band at 1705 cm^{-1} of base residues shifted towards lower frequency (but not towards higher) on breaking the base pairing. A possible explanation has been given elsewhere[15] on the basis of an assumption that the vibration in question involves the C=O and C=N stretching modes.

A strong absorption at 1680 cm^{-1} of deuterated calf thymus DNA was also found to be characteristic of its secondary structure.[15,16] This absorption is now found to be a superposition of the two bands: the 1676 cm^{-1} band of the GC pair and the 1687 cm^{-1} band of the AT pair (Fig. 5). The 1676 cm^{-1} band does not shift at all on ^{15}N substitution (Fig. 7). Therefore, this band is assignable to a vibration in which the main contribution comes from the carbonyl stretching motions in the GC base pair.

The 1576 cm^{-1} band of the deuterated DNA is due to the GC pair (Fig. 5). This is further assigned to the guanine moiety of the GC

15) Y. Kyogoku, M. Tsuboi, T. Shimanouchi and I. Watanabe, *Nature*, **189**, 120 (1961).
16) Y. Kyogoku, M. Tsuboi, T. Shimanouchi and I. Watanabe, *J. Mol. Biol.*, **3**, 741 (1961).

pair, because deuterated guanosine gives a very strong absorption band at 1570 cm^{-1} while deuterated cytidine does not.[17] Also, deuterated poly (G+C) gives a strong absorption band at 1577 cm^{-1} [18] while deuterated poly (I+C) does not.[19] On the basis of its frequency and on the basis of the observed amount of ^{15}N isotope shift ($\Delta\lambda/\lambda = -0.021$), this band is associated to a C-N stretching vibration in the guanine residue. According to the calculation made by Dr. Harada (mentioned above), cyanuric acid (undeuterated) should have vibrations at 1542, 1473, and 1459 cm^{-1}. In each of these vibrations, C-N stretching motions are predominant. If Eq. (2) is applied to the results of his calculation, it is concluded that the three frequencies should give ^{15}N-shift of the amounts $\Delta\lambda/\lambda^\circ = -0.040$, -0.021, and -0.021, respectively.

The 1501 cm^{-1} band of the deuterated DNA, which has been assigned to the GC pair, may further be assigned to the cytosine moiety. A strong absorption is found here in the spectra of deuterated cytidine,[17] and deuterated polyribocytidylic acid,[20] and deuterated poly (I+C)[19] whereas no strong absorption is found in the spectum of deuterated guanosine.[17] Because it is shifted greatly ($\Delta\lambda/\lambda^\circ = -0.019$) on ^{15}N-substitution, it is assigned to a C-N stretching vibration in the cytosine residue.

As was mentioned earlier,[8,20,21] the absorptions around 780 cm^{-1} of deuterated polynucleotides are noticeable ones. The fairly strong and sharp absorption peaks in this region are assigned to some out-of-plane vibrations in the base residues involved in wet polynuclotides. As has been mentioned above, it is now clear that the absorption peak at 773 cm^{-1} is due to the A-T base pair and that at 778 cm^{-1} to the G-C base pair. These absorption peaks appear only for deuterated DNA's and not for undeuterated ones. Therefore, the absorptions may be associated with the N-D and/or ND_2 groups. If the vibration for each of these bands were a pure N-D out-of-plane deformation vibration, however, the ^{15}N-isotope shift should be as high as $\Delta\lambda/\lambda^\circ = -0.028$ (Eq. (3)). If it were a pure ND_2 out-of-plane deformation vibration, the ^{15}N-isotope shift should be as high as $\Delta\lambda/\lambda^\circ = -0.023$. Actually observed ^{15}N-shift is only $\Delta\lambda/\lambda^\circ = -0.008$ for the 778 cm^{-1} band of the G-C base pair, and hence the normal vibration at 778 cm^{-1} would be a mixture of an N-D and/or ND_2 out-of-plane motion with some other out-of-plane modes of the G-C base-pair structure.

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Note : After the completion of the manuscript of this paper, a paper by H. Fritzsche (*Biopolymers*, **5**, 863 (1967)), which reports the infrared spectra of DNA's with different base compositions, has appeared. His assignments of the 1505 and 1573 cm^{-1} bands respectively to C and G are in agreement with ours.

17) M. Tsuboi, Y. Kyogoku and T. Shimanouchi, *Biochim. Biophys. Acta*, **55**, 1 (1962).

18) Poly (G+C) is a double helical complex of polyriboguanylic acid and polyribocytidylic acid. Its infrared spectrum was observed by S. Higuchi and M. Tsuboi (to be published).

19) Poly (I+C) is a double-helical complex of polyriboinosinic acid and polyribocytidylic acid. Its infrared spectrum was observed by M. Tsuboi, K. Matsuo, and S. Higuchi (*Biopolymers*, **6**, 123 (1968).

20) M. Tsuboi, *J. Poly. Sci.*, Part C, No. 7, 125 (1964).

21) M. Tsuboi, *Biopolymers*, Symposia No. 1, 527 (1964).