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The Structure of Sodium Thymonucleate Fibres. III. The Three-Dimensional Patterson Function*

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The three-dimensional Patterson function of the crystalline form of sodium thymonucleate has been calculated, using the same intensity data as for the cylindrical Patterson function already described. Further evidence was thus obtained in support of the modified form of the Watson & Crick model previously deduced from the cylindrical function. In addition, the use of three-dimensional data made it possible to determine the orientation of the helical molecule in the unit cell.

It is shown that the size and shape of the unit cell is such as to reduce to a minimum the distance between certain phosphate groups in neighbouring molecules.

Introduction

The molecular structure of sodium thymonucleate (SDN)‡ fibres is very sensitive to the humidity of the surrounding atmosphere (Franklin & Gosling, 1953*a*). By varying the water content of highly orientated fibres we have shown, from the changes in the X-ray diagram, that SDN may exist in two different structural states, which we have called *A* and *B*. The change $A \rightleftharpoons B$ is, in general, readily reversible. *B* is the equilibrium form at high relative humidity and *A* is a crystalline form containing about 40% by weight of water, best obtained in fibres at a relative humidity of about 75%. At lower relative humidity the structure becomes (reversibly) disordered.

Watson & Crick (1953*a*) have proposed a structure for DNA in which the phosphate-desoxyribose backbone chains form two coaxial helical strands related by a diad axis. This structure must be considered in relation to our structure *B*, in which each helical molecule may be assumed to be shielded from the deforming influence of neighbouring molecules by a sheath of water. The molecule is then free to take up its least-energy configuration and the X-ray diagram represents the continuous transform of a single molecule. We have shown (Franklin & Gosling, 1953*b*; Gosling, 1954) that the X-ray diagram of structure *B*, taken in conjunction with measurements of density and water content, lends support to the general features of the Watson & Crick model, but indicates that the molecule is rather more compact than was suggested by these authors, the phosphorus atoms lying on coaxial helices of radius about 8·5 Å rather than 10 Å.

If a two-strand helical molecule exists in structure *B*, then it follows, from the ease of transition from one structure to the other, that the molecule in structure *A* must also be a two-strand helix; the structural modifications induced by the close proximity of neighbouring molecules in the crystalline form must be relatively minor ones. Therefore, although structure *B* is undoubtedly the more closely related to DNA in its natural state, a more detailed study of structure *A* is justified, both by the close relationship which it must bear to structure *B* and by the greater wealth and precision of the diffraction data available.

The measurement of intensities and *R*-space parameters for structure *A*, and the use of these to calculate the cylindrically symmetrical Patterson function, have already been described (Franklin & Gosling, 1953*c*). The existence of the two-strand helix in structure *A* was clearly revealed in the Patterson function, and it was shown that in changing from *B* to *A* the number of residues per turn of each strand increases from ten to eleven and the pitch of the helix decreases from 33·1 Å to 28·1 Å (Franklin & Gosling, 1953*d*). In structure *A* we have suggested that the phosphorus atoms lie equispaced on a two-strand helix of radius close to 9 Å, with a separation of $\frac{1}{2}c$ between the strands, the phosphorus atoms in one strand being directly above those in the other strand in the direction of the axis of the helix, with the sugar and base rings turned inwards towards that axis. This model is examined in this paper with the aid of the three-dimensional Patterson function of structure *A*.

Calculation of the three-dimensional Patterson function

The experimental data used for the calculation of the three-dimensional Patterson function were the same as those used to obtain the cylindrical function previously described (Franklin & Gosling, 1953*c*). We

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‡ In previous papers we have used the notation NaDNA, but SDN (as used by American authors) is clearly preferable.

Table 1. *Data used in calculation of the three-dimensional Patterson*

Layer	$(\xi/\lambda)\times10^2$ Obs.	Calc.	*hkl*	$I_{corr.}$
0	5·25	5·21	110	9
	8·80	8·81	130	104
	9·00	9·14	200	30
	10·00	10·04	040	3
	10·50	10·42	220	4
	13·25	13·35	150	13
	13·8	13·57	240	5·5
		13·94	310	5·5
	15·75	15·64	330	5
	17·55	17·62	260	9
	18·20	18·16	170	12
		18·28	400	9
	18·85	18·72	350	4
		18·96	420	8
	20·00	20·08	080	7
	20·80	20·86	440	5
	22·10	22·07	280	6
		22·29	370	6
	22·85	22·99	510	10·5
		23·04	190	10·5
	24·00	25·34	530	14
1	4·90	4·86	$11\bar{1}$	22
	5·50	5·57	111	11
	8·60	8·61	$13\bar{1}$	39
	9·05	9·02	131	23
	10·10	10·08	$22\bar{1}$	10
	13·10	13·22	$15\bar{1}$	16
	15·30	15·29	$33\bar{1}$	7
	16·10	16·00	331	7
	22·60	22·59	$51\bar{1}$	6
		22·54	371	4
	23·50	23·38	$46\bar{1}$	4
		23·67	$53\bar{1}$	5
2	4·55	4·53	$11\bar{2}$	52
	4·90	5·08	022	32
	5·90	5·93	122	29
	9·80	9·73	$22\bar{2}$	20
	11·15	11·14	222	19
	13·00	13·05	$24\bar{2}$	14
		13·14	$31\bar{2}$	14
		13·18	$15\bar{2}$	14
	17·20	17·21	$26\bar{2}$	31
	18·05	18·00	$35\bar{2}$	9
		17·97	$17\bar{2}$	9
		18·05	262	9
	20·00	20·10	$08\bar{2}$	2·5
		20·16	$44\bar{2}$	2·5
	21·9	21·80	$37\bar{2}$	6
		21·75	$28\bar{2}$	6
	22·90	22·79	372	5
		22·90	$19\bar{2}$	5
		23·08	$46\bar{2}$	5
	26·60	26·63	$48\bar{2}$	1
		26·86	552	1
		26·45	$2,10,\bar{2}$	1
		26·62	$60\bar{2}$	1
	27·90	27·63	$1,11,\bar{2}$	1·5
		28·20	572	1·5
3	12·80	12·80	$24\bar{3}$	3·5
		12·76	$31\bar{3}$	3·5
	14·55	14·41	243	4
		14·60	333	3
	14·95	15·11	063	4
		15·12	313	4

Table 1 (*cont.*)

Layer	$(\xi/\lambda)\times10^2$ Obs.	Calc.	*hkl*	$I_{corr.}$
	19·80	19·82	$44\bar{3}$	2
	21·50	21·60	$28\bar{3}$	2
4	12·35	12·56	$31\bar{4}$	1·5
		12·37	$24\bar{4}$	1·5
	14·10	14·16	154	10
		14·26	$33\bar{4}$	9
	14·95	14·70	244	13·5
		15·15	064	13·5
	16·90	16·84	$26\bar{4}$	14
		17·06	334	4
	21·45	21·34	$37\bar{4}$	2·5
		21·43	$28\bar{4}$	2·5
	22·75	22·77	284	2
		22·78	$19\bar{4}$	3
5	11·90	11·98	$31\bar{5}$	12
	12·40	12·32	$24\bar{5}$	6
		12·22	225	6
	14·0	12·34	155	3
6	8·40	8·40	$22\bar{6}$	12
	10·4	10·33	046	14
		10·26	136	14
	11·70	11·59	$31\bar{6}$	12
	12·75	12·93	$15\bar{6}$	12
	13·50	13·58	$33\bar{6}$	21
7	7·90	7·79	117	4
		7·74	$13\bar{7}$	5
	11·0	11·19	$31\bar{7}$	11
	12·1	11·94	207	5
		11·87	$24\bar{7}$	5
	13·2	13·26	$33\bar{7}$	4
		12·95	227	6
8	10·80	10·81	$31\bar{8}$	10
		10·82	138	6
	12·50	12·83	$15\bar{8}$	9
		12·34	208	6
	15·70	15·89	$42\bar{8}$	3
9	5·7	5·54	$20\bar{9}$	2

repeat here, for convenience, that the unit cell parameters are

$$a = 22{\cdot}0,\ b = 39{\cdot}8,\ c = 28{\cdot}1\ \text{Å};\ \beta = 96{\cdot}5°,$$

with c coincident with the fibre axis. The space group is $C2$ and an 'artificial temperature factor' of

$$\exp\left[-(4{\cdot}56)^2\times(2\sin\theta/\lambda)^2\right]$$

was applied to all intensities. The methods used to index the observed reflexions have already been described (Franklin & Gosling, 1953*c*).

In Table 1 we list the observed and calculated ξ values with their indices and observed (corrected) intensities for each layer line. It will be seen from the table that for the larger values of θ no reflexion could be indexed unambiguously. In cases where the possible indices included *hkl* and $hk\bar{l}$ the photograph showing double orientation (Franklin & Gosling, 1953*a*) was used as a guide in distributing the ob-

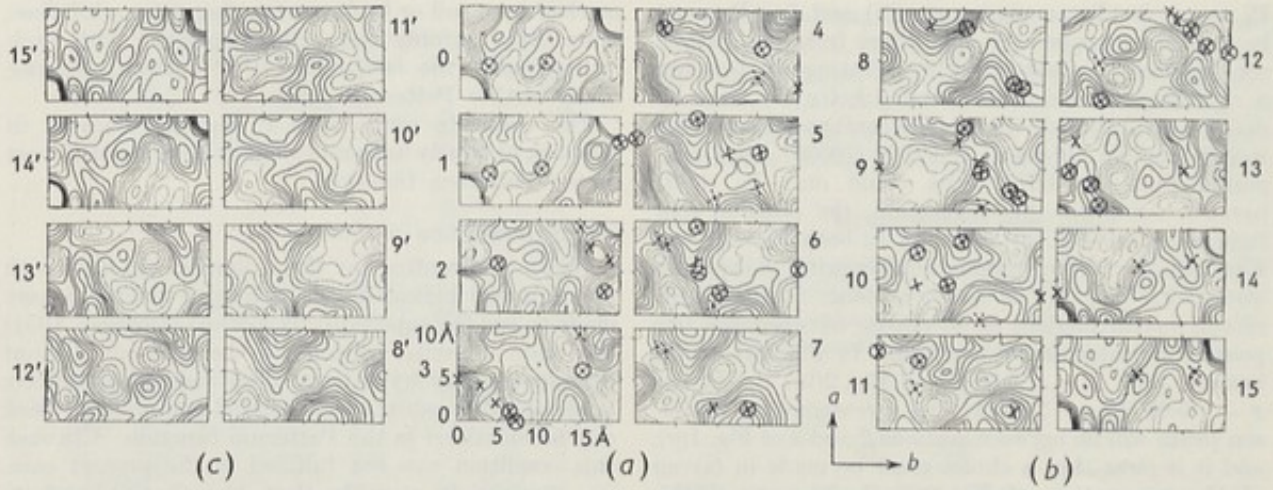


Fig. 1. (*a*), (*b*) The three-dimensional Patterson function of SDN, shown as 16 *a*–*b* quarter-cell sections from $z = 0$ to $z = \frac{1}{4}c$. ×: *Intra*-strand P–P vector; ⊗: *Inter*-strand P–P vector.

These are the *intra*-helical P–P vectors to be expected from the proposed two-strand helical model. A broken cross denotes a vector peak close to a half-section level in *Z*.

(*c*) The *a*–*b* sections of Fig. 1(*b*) reflected across a diad axis at the quarter cell height. The similarity between these sections and Fig. 1(*a*) suggests that part of the structure repeats in half the cell period.

served intensity. Where no external guide was available the intensity was equally divided among the possible reflexions.

The three-dimensional Patterson function (Fig. 1 (*a*, *b*)) was calculated at intervals of *a*/30, *b*/60 and *c*/30 in the form of 16 *a*–*b* sections.

Interpretation of the Patterson function

We have shown previously from a study of both the X-ray diagram of structure *B* (Franklin & Gosling, 1953*b*) and the cylindrical Patterson function of structure *A* (Franklin & Gosling, 1953*d*) that the principal diffraction features are due to phosphate–phosphate vectors. This is to be expected since these are the heaviest groups in the structure. If z is the atomic number of any atom, the value of Σz^2 for the group PO_4Na is 602 and that for the remainder of the nucleotide is only 685 (cytidine) to 819 (guanine). Thus the PO_4Na group, which is highly compact, may be expected to act as a 'heavy atom'. Moreover, since the P atom lies at the centre of the $-PO_4$ group, the phosphate–phosphate vectors may be grossly identified with the P–P vectors. We shall therefore seek to interpret the three-dimensional Patterson function in terms of P–P vectors.

(i) *Orientation of the helix*

In order to determine the P–P vector peaks to be expected from the model outlined above it was first necessary to determine the orientation, with respect to the cell parameters, of the arrangement of eleven phosphorus atoms forming one turn of each strand of the helical molecule. The axis of the helix is known to coincide with the *c* axis of the unit cell and the pitch of the helix is equal to *c*. The density indicates that only one helical molecule traverses each primitive unit cell. Therefore, if the helix can be right-handed or left-handed, there are four possible arrangements of the eleven phosphorus atoms about a lattice point that could comply with the space-group symmetry. These are shown in Fig. 2 as projections of a turn of one strand of the helix on to a plane at right-angles to the *c* axis through the level $z = 0$. Since the phosphate–sugar backbone chain is non-centric, the symmetry axes along *b* cannot pass through a chain, but must relate one chain, *n*, to the other, *n*′. Thus in arrangements (i) and (ii) P_0 must be placed at $z = \frac{1}{4}c$ and so be related by the diad axis at $\frac{1}{2}c$ to the atom

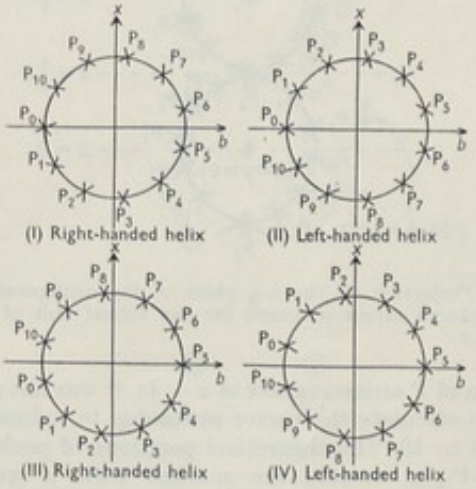


Fig. 2. Projections of the four possible helical arrangements of the eleven phosphorus atoms in one turn of one strand of structure *A*.

P'_0 at $z = \frac{3}{4}c$. In arrangements (iii) and (iv) P_5 must be considered to be at $z = \frac{1}{4}c$. Since from the Patterson function it is not possible to distinguish between a right-handed and a left-handed helix, we have to decide only between two possible configurations. From model-building, Watson & Crick (1953*b*) have suggested that the DNA helix could only be right-handed. Adopting this suggestion, the possible configurations are Fig. 2(i) and (iii). In both Fig. 2(i) and Fig. 2(iii) one vector joining neighbouring phosphorus atoms, P_n–P_{n+1}, lies in the a–c plane. Consideration of this vector enables us to choose between the two possible orientations of the helix. In Fig. 2(i), P_5–P_6 has $x = 5{\cdot}07$ Å, $z = 2{\cdot}55$ Å. In Fig. 2(iii) P_{10}–P_0 has $x = -5{\cdot}07$ Å, $z = 2{\cdot}55$ Å. The corresponding Patterson peaks will lie between sections 2 and 3 of Fig. 1(*a*), and it is clear that a choice must be made in favour of the orientation of Fig. 2(i). A diagram of this arrangement of phosphorus atoms projected on to the a–c plane is shown in Fig. 3.

(ii) *Intra-helical* P–P *vectors*: P_n–$P_{(n+m)}$ and P_n–$P_{(n'+m}$

The *intra*-helical phosphorus vectors for one strand P_n–$P_{(n+m)}$, and the vectors from one strand to the next, P_n–$P_{(n'+m)}$, were determined graphically for $n = 0$–10 and $m = 1$–5, using that configuration of P atoms shown in Figs. 2(i) and 3. Since the proposed

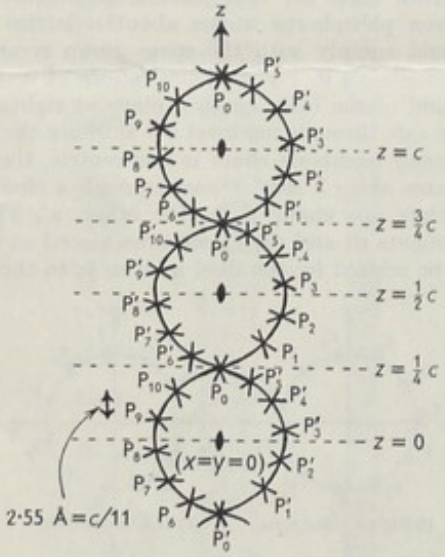


Fig. 3. Projection on the a–c plane of the configuration of phosphorus atoms proposed for one helical unit of structure *A*.

pattern of P atoms repeats at $z = \frac{1}{2}c$, it was not necessary to calculate the vector series due to values of m from 6 to 10. The theoretical positions of peaks due to P_n–$P_{(n+m)}$ vectors are marked with a cross in Fig. 1(*a*, *b*); where the position lies nearly half-way between two sections, it is indicated on both sections by a broken cross. The P_n–$P_{(n'+m}$ vectors are similarly marked by a full or broken cross ringed by a full line. Since there is only one helix associated with each lattice point, the *inter-helical* P–P vectors give rise to no further Patterson peaks.

The complete array of P–P vectors indicated in Fig. 1(*a*, *b*) clearly accounts well for a substantial part of the Patterson function.

(iii) *Superposition method*

Before confronting the three-dimensional data with the proposed helical model, extensive attempts were made to use the superposition method (Buerger, 1951) to obtain a partial solution of the problem without introducing any hypothesis. This method is strictly applicable only when a well-isolated non-multiple peak can be identified in the Patterson function. Although this condition was not fulfilled in the present case, we thought it possible that phosphate–phosphate vectors might predominate to such an extent that certain reasonably well defined peaks might be effectively entirely due to them, and that the use of such peaks in a series of three-dimensional superpositions might give some indication of the arrangement of these groups in the structure. In spite of rather numerous attempts, using a variety of possible peaks as displacement vectors, and searching for the common elements in the resulting superposition functions, no progress was made by this method before a model structure became available.

After the development of the two-strand helical model described above, we returned to the superposition method to look for confirmation of the model. For this purpose superposition functions were traced using displacement vectors corresponding to certain of the P–P vectors of the model, with the object of investigating whether or not the other P positions of the model appeared as prominent features of the resulting functions.

In the proposed model, if the phosphorus atoms only are considered, the fibre-axis repeat period is halved, pseudo-diad axes being present at heights $c = \frac{1}{4}$ and $c = \frac{3}{4}$ in the complete unit cell. It follows that that part of the Patterson function due to P–P vectors must also show this extra symmetry. That this pseudo-halving of c is a strong feature of the Patterson function may be seen from Fig. 1(*c*). This figure consists of sections 15–8 rotated about a diad axis parallel to b at height $\frac{1}{4}c$. There is clearly a strong resemblance between this set of reflected sections and the sections 0–7 of Fig. 1(*a*).

In order to remove those peaks not related by this additional symmetry, the two sets of sections shown in Fig. 1(*c*) and Fig. 1(*a*) were superimposed and the common positive regions were traced. Peaks in negative regions were also indicated. The eight Patterson sections resulting from this procedure should contain all the P–P vectors of the proposed model.

Using these sections, three superposition functions were constructed using the three displacement vectors

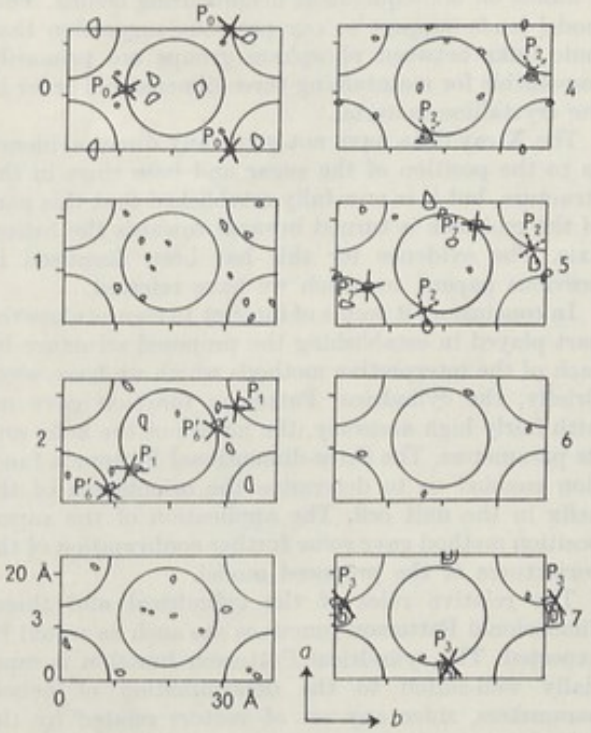


Fig. 4. Superposition function prepared from the Patterson sections, using some of the P–P distances given by the proposed helical structure.

P_0–P_3, P_0–P_4, P_3–P_4 obtained from the model (projections shown in Figs. 2(i) and 3). These three functions were then superimposed on one another using the spatial relationships of P_0, P_3 and P_4 given by the model, and the part common to all three superposition functions was traced.

The sections of this final superposition function are shown in Fig. 4. On each section there are also shown circular traces of 9 Å radius, on which the P atoms of the model structure would lie. (These traces should be elliptical owing to the slope of the a–b section, but the difference between the major and minor axes is only 0·06 Å and can therefore be neglected.) The section numbers at which the phosphorus atoms should occur are listed in Table 2 (P_3, P_4 and P_5 are related by a pseudo-diad axis to P_8', P_7' and P_6').

Table 2

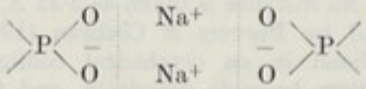
P_N	P_0	P_6'	P_1	P_2	P_7'	P_3	P_8'
Section	0	1·7	2·1	4·5	4·9	7·1	7·9

The expected position of these P atoms is marked on the appropriate sections with a full cross, except for P_2, the coordinates of which are indicated on both sections 4 and 5.

It seems that Fig. 4 provides substantial confirmation of the proposed helical array of P atoms. Only two of the atoms P_0, P_3 and P_4 were introduced into each of the three primary superposition functions, and the final superposition function (Fig. 4) contains not only peaks corresponding to all three of these atoms, but, in addition, the remaining P atoms, P_1, P_2 and P_5 (P_6'), all appear distinctly in their correct positions. Moreover, the cylinders of radius 9 Å, on which all the P atoms lie, are seen to be sufficiently free from extraneous peaks for these results to be significant.

Inter-helical bonding

We have suggested previously that neighbouring molecules of SDN in the crystalline structure would be most probably linked to one another by ionic bonds represented schematically by



This suggestion finds support in the structure which we have described here.

From the sections of the helices shown in Fig. 4, strong inter-helical phosphate–phosphate bonds, of the type suggested above, might be expected between P_1 and P_3 in one helix and P_6' and P_8' respectively, in adjoining helices. The inter-helical P_1–P_6' distance is 4·9 Å and the P_3–P_8' distance is 4·4 Å. For any one helix there are *eight* positions of the P_1–P_6' type and *four* positions of the P_3–P_8' type in each complete period of the structure. Now the size and shape of the monoclinic face-centred unit cell are such that the c-axis displacement of the face-centring helix with respect to those at the corners of the cell is $\pm c/22$, i.e. this c-axis displacement is exactly half that between neighbouring P atoms on any given helical strand. With the two strands of the helical molecules equally spaced on the helical axis, and orientated as described above, this has the effect of bringing two P atoms of neighbouring helices into the closest possible proximity for the given values of a and b. The two P

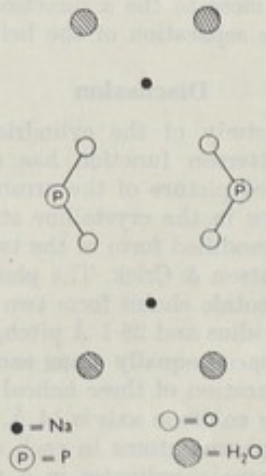


Fig. 5. Schematic representation of phosphate–phosphate inter-helical bonding involving octahedrally co-ordinated sodium ions as intermediaries. (Other water molecules lie above and below the plane of the diagram.)

atoms which are brought close together in this way lie on non-equivalent helical strands; that is, they lie on strands of the type which are related to one another by a true diad in the full structure.

These P–P distances are at first sight surprisingly small. They are too small to permit the Na^+ ions to be situated directly between the two PO_4^- groups. It seems reasonable to suppose, however, that the bonding would be affected by the two Na^+ ions situated just outside the phosphate groups, their coordination (probably 5- or 6-fold) being completed by the water molecules known to be present. This is shown schematically in Fig. 5.

If the O–Na distance is taken as 2·32 Å, the lowest value found by Beevers & Cochran (1947) for an Na–(OH) bond in an octahedral configuration in sucrose sodium bromide dihydrate, and the O–P–O angle is taken as 116° with the P–O bond of length 1·5 Å, then the P–P distance for the planar arrangement shown in Fig. 5 is 4·87 Å. This value agrees well with the estimated P_1–P_6' distance of 4·9 Å. That the P_3–P_8' distance is apparently as low as 4·4 Å (Fig. 4, section 7) suggests that either the helices may be slightly flattened in the *a* direction, or that in this case the two $\rangle PO_2^-$ groups are not coplanar.

If the Na^+ are 6-coordinated this type of linkage would determine, for half the nucleotides, the position of four of the average eight oxygen atoms that the water-content measurements indicate are associated with each nucleotide in structure *A*.

Inspection of sections 4 and 5 of Fig. 4 shows that the phosphorus atom P_2 lies very close to the adjoining helix. The nearest phosphorus atom on the neighbouring helix is P_8' at a distance of 7·8 Å. A phosphate–phosphate linkage between these groups would therefore seem unlikely. It seems possible, therefore, that in this case there may be a base-phosphate bridge between neighbouring helices. Such additional *inter*-helical bonding close to the *a* direction may in part explain why the separation of the helices is least in that direction.

Discussion

The combined study of the cylindrical and three-dimensional Patterson function has enabled us to suggest a detailed picture of the arrangement of the phosphate groups in the crystalline structure (*A*) of SDN. This is a modified form of the two-strand helix proposed by Watson & Crick. The phosphorus atoms of two polynucleotide chains form two coaxial helical strands of 9 Å radius and 28·1 Å pitch, with 11 phosphorus atoms spaced equally along each turn of each strand. The separation of these helical strands in the direction of their common axis is 14 Å. Corresponding successive phosphorus atoms in each strand, P_n and P_n', have the same coordinates in a plane at right-angles to the axis of the helix. The orientation of the helix and the shape of the unit cell are such as to reduce to a minimum the distance between certain pairs of P atoms on non-equivalent neighbouring chains. This model lends support to our previous suggestion that ionic links between phosphate groups are primarily responsible for maintaining three-dimensional order in the crystalline material.

The X-ray data have not given any direct evidence as to the position of the sugar and base rings in the structure, but it is now fully established that this part of the molecule is turned inwards towards the helical axis. The evidence for this has been discussed in previous papers, to which we have referred.

In conclusion, it seems of interest to recapitulate the part played in establishing the proposed structure by each of the interpretive methods which we have used. Briefly, the cylindrical Patterson function gave us, with fairly high accuracy, the nature of the helix and its parameters. The three-dimensional Patterson function enabled us to determine the orientation of the helix in the unit cell. The application of the superposition method gave some further confirmation of the correctness of the proposed model.

The relative roles of the cylindrical and three-dimensional Patterson functions are such as would be expected. The cylindrical Patterson function is especially well-suited to the determination of helical parameters, since any set of vectors related by the helical axis in the structure appears as a single vector on the cylindrical Patterson function. The cylindrical Patterson function is therefore a more powerful tool than the three-dimensional for detecting helical features of the structure. But for determining the orientation of the helix in the unit cell it is obviously necessary to use three-dimensional diffraction data.

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24 November 1975

Fellowship Rep
Jan 1952
Colloq 1951

no 34A° in 1951!

Professor M. H. F. Wilkins, FRS,
Department of Biophysics,
King's College,
26-29 Drury Lane,
London, WC2B 5RL.

Dear Maurice,

I enclose xerox copies of Rosalind's notes for her November 1951 Colloquium and some other notes which I found with those for the Colloquium, and which I infer to be a preliminary draft plus associated calculations. I have annotated certain places in pencil on the original for Olbey. These are signed.

I also enclose copies of the Fellowship Reports, again with my annotations in the margin.

Yours sincerely,

Aaron

A. Klug

Encs.

Points marked PF are pre Franklin. Says she have assumed everything in these notes was new work + ideas of R.F. MHFW.

Collegium
Nov. 1951

Experimental

Better resolution camera shows more spots

I/ too wet, complete change in picture → much sharper, suggesting loss of 3-dimensional order

∴ Systematic study of effect of moisture on structure. Revealed 3 types

2d3

✓ ① wet PF ② Xtalline PF ③ dry

Other humidities give mixtures

i.e 3 more or less well-defined states

[Slow photos]

if it were only oblique scans? we had such in July 51. In fact 3 layer lines were visible, but not very clear at all

① Meridian arc at 3.4

✓ 2 oblique arcs at ~ 40° to meridian

Sharp intense spot on equator

i.e. only equator shows high order ~~projection of structure~~

along period v weak

② Xtalline order. Amount seen or present ltd. by expt difficulties - better resol" camera reveals more spots. Also better specimen - difficult to get good fibres and small

③ hk̄L and hkO gradually disappear

PF leaving only 3.4 on meridian (+ 2 side ones)

Photograph depends not only on RH but on history

① Hysteresis at wet end - difficult to wet after long drying

② Xtalline photograph greatly improved if strongly dried before wetting. This effect is v marked - better resolution given by ragged bundle after strong drying (then wetting) than by single fibre wet

PF

Hysteresis at wet end

Measurements of adsⁿ of H_2O on 4 specimens at diff. RH, starting from strongly dried over P_2O_5 - vacuo.

→ nearly constant values btwn 70% - 80% RH, which was RH found to give best Xtalline photo

∴ this gives measure of H_2O in Xtal structure ~ 42%

(these measurements not made on pulled fibres, but unpulled fibres give same X-ray diagram)

Large uptake > 80%

Measurements complete - about 2 measurements without previous strong drying

probably R.G.'s cell

Unit cell

$a = 25.7$ $b = 42.2$ $c = 27.4$

$\beta = 118° 30'$

Can take monoclinic
Face-centred
This is nearly but not quite hexagonal in projection
- in projection each lattice point has 2 neighbours at 22.6 Å
4 " 23.9 Å

Density

Using Astbury's value, 1.63 for density dry DNA,
Measured value 42% for H_2O content of X-ethidium
and 330 for mean M.W. nucleotide
gives 4 nucleotides / unit cell (face-centred)
i.e. 2 / primitive cell
= prob" 2 / primitive cell

I Interpretation

General hypotheses

~~change~~ in

Chain groups

Structure is v nearly hexagonal — section ⊥ fibre axis

P.F ~~Molecular~~ chains presumably run ∥ fibre axis A.

Suggests that structure is only slightly distorted cylindrical units in nearly hexagonal close packing

Evidence for spiral structure

27 Å period — strong

1. Straight chain, ~~not~~ twisted, highly improbable — unbalanced forces

2. Absence of reflections on meridian — X-Talbot

PF form suggests spiral structure, in which electron density projected onto fibre axis is nearly uniform

3. Strong 27 Å period. This is most too marked to result from diff. bases diff. nucleotides, & must mean nucleotides in equivalent positions occur only at intervals of 27 Å. Suggests 27 Å is length of turn of spiral

PF Near-hexagonal packing suggests that there is only one helix (containing possibly >1 chain) per lattice point

Density measurements (24 residues/27 Å) suggest >1 chain

Change crystalline ⇄ wet

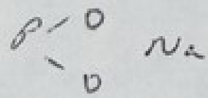
In wet diagram, only equator shows sharp reflection
suggests ofilaments units randomly displaced ⊥ fibre axis
Design (other than equator) this represents form factor of single
~~Retained~~ lattice gone
Equator not ~~shift~~ only ~ 10%

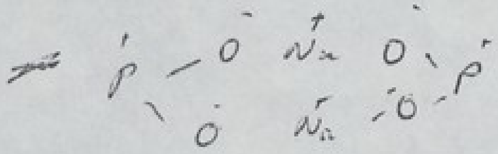
~~equator~~ of . suggests that group of ~~lattice~~ chains associated
with a single lattice point remains intact, i.e. confirms
that the (2, 3 or 4 ?) chains are bonded together more strongly
than chains - diff. groups, and are not separated by action of
water
It is - this change that large length change occurs. isolated helix
has not got - same structure as - xtal - xtal form unless some strain ~~helix~~, if bonds

Crystalline structure

Here inter-unit bonds are as important as
intra, & must be considered. They might be
1/ Base - base (NH - CO bonds ?)
2/ Base - phosphate
3/ Phosphate - phosphate

They are the bonds which are destroyed by H_2O or RH > 80%.
1/ certainly ruled out. 2 doubtful
3 Phosphate - phosphate bonds highly probable

H_2O not make for 

 (and variants) as inter-unit

links not account well for sensitivity of structure to water content, and for large amount of water in Xtalline structure (~~prop~~ prob. in neighbourhood of P OOR)

~ 8 molecules H_2O / nucleotide in Xtalline structure

Change Xtalline ⇌ dry

Thorough drying stabilises Xtalline structure subsequently formed. i.e. although, on drying, Xtalline order disappears, the inter-unit bonds responsible for it are preserved and strengthened in the dry state

On drying, hkl and hk0 reflections gradually fade

PF out - decreasing → 0 in intensity while getting only slightly more diffuse.

Suggests that 3-dimensional ~~skeletal~~ skeleton remains but becomes

PF strained and buckled owing to decrease of water and presumably makes holes in inter-unit spaces

- confirmed by LAS in v dry photo

Intensities

Conclusion

Big helix w several chains, phosphates on outside, phosphate . phosphate inter bond, disrupted by water

. Phosphate binds available to proteins

Difficulties

24 / u. cell

How homogeneous?

Plateau & xtallinity coincide

No amorphous rings

Can't measure ∴ disorder background

~~Description of lab, photos & fibre pulling~~

3 states ① dry ② Xtalline ③ wet

1/ Strong drying converts Xtalline structure

- wet ⇌ dry structure reversible

between 75% and 92% humidity ~~exist~~ when strongly dried before taking to 75%

i.e. inter-unit bonds in Xtalline state ② are strengthened in dry state ①

& state ③ then obtained w difficulty

- hysteresis

2/ This confirms that ~~adsorption~~ water ~~dissolves~~ disrupts inter-unit bonds & ∴ water is ~~soaked~~ outside rather than inside cylindrical units. Anyway the stuff ultimately dissolves, i.e. chains are separated from one another by water

3/ $F^2_{110} \approx 4 F^2_{100}$

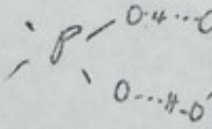
- suggests units don't have v heavy core

- ~~and~~ lead to $F_{100} > F_{110}$ for small heavy core

4/ Addition of water → F_{100} gets reversed, (F_{110} decreased how ? disorder)

Since water is presumed to go between units, this suggests that F_{100} in state ② is -ve

i.e. again units do not have heavy core

5/  links seem highly probable - either with helix or inter-helical.

6. Since not heavy ions, suggest  etc near outside & responsible for inter-helical links

7. Water will go for  etc (proteins etc don't say anything about water) & this explains why action of water is to weaken the inter-helical bonds. Introduction of layer of adsorbed water between helices

8. Density considerations suggest 2 units per 3 Å height - pursuing all. If these 2 units are equivalent they are associated w same lattice point & are presumably combined in same helix

9. Increased spacing corresponds w to 1 layer adsorbed water fitting into existing close-packed P-O-H system

10. ~~Structure~~ Helical structure in (3) can't be same as in (2) ∴ large increase in length

assumes that 3·4 remains in A structure
hence 2 chain idea is messed completely.

11. Action of water among phosphate links would be to make nucleotide less polar, reducing conc^{n} of charge on P

12. Change (2) A ⇌ B (3) is discontinuous (though strands long chains might be partly each)

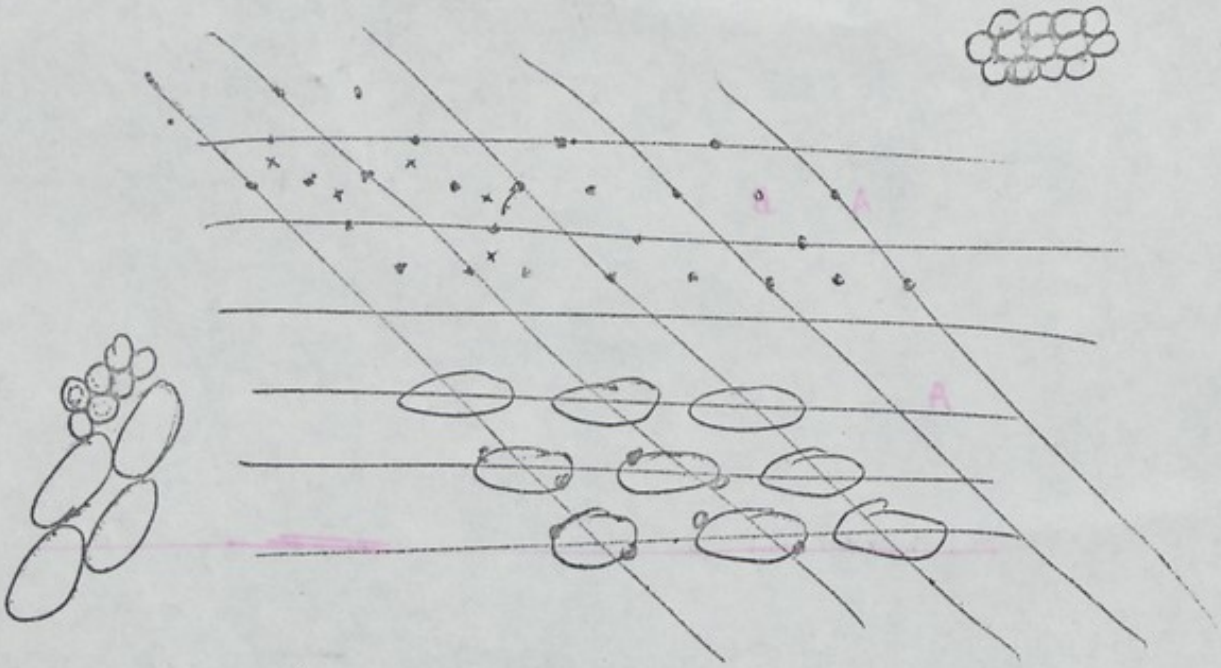
13. Change (2) A ⇌ (1) dry.

3·4 Å period along axis is preserved (but diffuse)
2 inter-helical links preserved (cf water experiments)
but all strong spots, including those on equator disappear
(and dichroism etc disappears)

∴ chains probably bubbled or distorted ∴
strain due to holes in structure between strands. Cf spots
c.f. photo of dry specimen shows LAS,
showing holes (others don't)

14. 'c' period (27 Å) probably contains 6 nucleotides per chain - symmetry considerations make 8 unlikely
8 - unresp. 3·4 Å only specially strong in strata (3)

15. Density



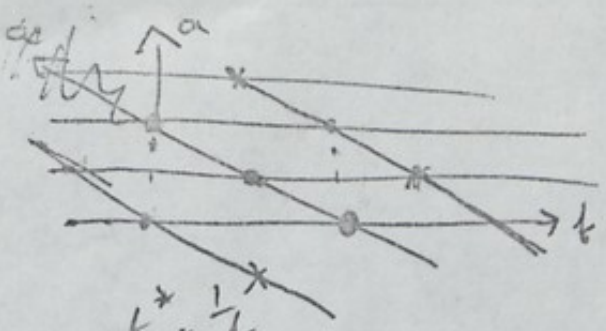
$F^2_{110} > F^2_{100}$ by ~ 4:1

Uniform density cylinder w holes between would give

this
but holes are almost certainly filled w water (40%)
& water nearly as high electron density as the rest

Uniform density molecule w heavy core of finite size would give $F_{100} > F_{110}$

State (2)



$b^* = \frac{1}{b}$

$b = 42.2$
$a\sin\beta = 22.6$
$a^* = \frac{1}{a\sin\beta}$
$\beta^* = 61°30'$ $\beta = 118°30'$ $c = \frac{24.1}{\sin\beta} = 27.4A$

$b/a\sin\beta > \sqrt{3}$

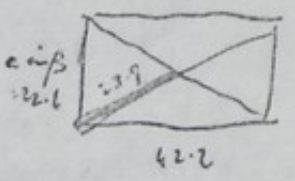
$\sin\beta = .8788$
$a = 25.7$
$\cos\beta = .4772$

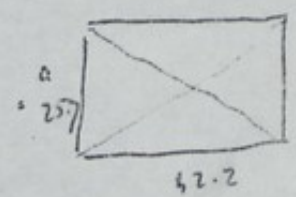
Each has 4 equivalent and 2 non-equivalent neighbours
4 equivalent are further than 2 non-equivalent

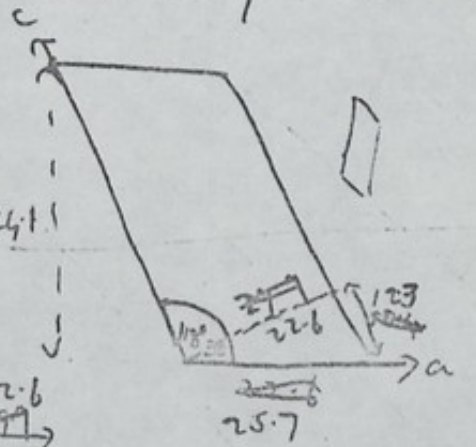
Equivalent pairs neighbours $\left\{\begin{array}{l} 2 \text{ @ } 22.6A \\ 4 \text{ @ } \frac{1}{2}\sqrt{22.6^2+42.2^2} = \frac{1}{2}\sqrt{511+1781} = \frac{1}{2}\sqrt{2292} = 23.9 \end{array}\right.$

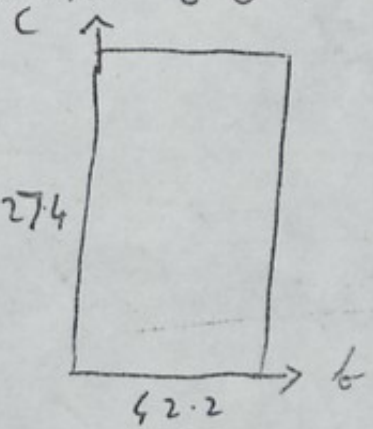
Unit neighbours $\left\{\begin{array}{l} 2 \text{ @ } 19.9 \\ 2 \text{ @ } 23.3 \end{array}\right.$

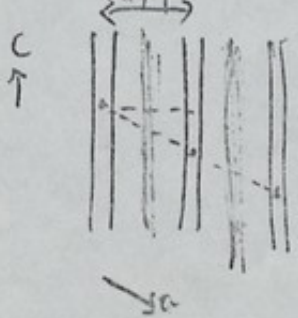
does 22.6A correspond. sheet G
and 23.9 .. inter-sheet G @ ?











$\frac{1}{2}\sqrt{25.7^2+42.2^2} = \frac{1}{2}\sqrt{660+1781} = \frac{1}{2}\sqrt{2421} = 24.6$

∴ Equivalent pairs neighbours 2 @ 25.7A
and 4 @ 24.6A

A thins dry $d = 1.65$

Mean nucleotide weight $330 / 6 \times 10^{23}$ g

Suppose density of nucleotide pair of structure $= 1.66$ g/cm^3

1 nucleotide occupies $\frac{330 \times 10^{24}}{6 \times 1.63 \times 10^{23}} = 336$ A^3

Suppose structure is 60% water having density 1.00 g/cm^3

1 nucleotide is associated with $\frac{42}{60} \times \frac{356}{6 \times 1.00 \times 10^{23}} \times 330 \times 10^{24} = 249$ A^3 water

∴ Volume of 1 nucleotide + associated water ~ 585 A^3

Suppose thickness associate w. nucleotide is 3.4 A

surface area per nucleotide + associated water $= \frac{1170}{3.00} = 390$ A^2

Surface area of hexagonal cell of side 22.6 A $= \frac{\sqrt{3}}{2} \times 22.6^2 = 443$ A^2

Vol. unit cell $27.45 \times 42.2 \times 22.6 = 26200 = 27.9$ nucleotides/u. cell

Remaining cell, 22 nucleotides

Presumably written Jan 52 ~~Dec 51~~

N.B no mention of 34 A° period
I had pattern then & it looks as tho' none of us bothered to measure it!

4 mol water per nucleo = 55 mole

20% wt water for crystalline
helix not identical in cryst & wet

Interim Annual Report: January 1st 1951 - January 1st 1952

see end → 7 Feb 52 RF Franklin

During 1950 M.H.F. Wilkins succeeded in obtaining well-oriented fine fibres from a specimen of desoxyribose nucleic acid prepared by Professor Signer (Berne), and with R.G. Gosling showed that a bundle of these fibres gave X-ray diagrams of exceptional quality. In January 1951 it was agreed that I should undertake, in collaboration with Gosling, a systematic X-ray investigation of these fibres.

Apparatus

3 months visiting carbon work!

The greater part of the first eight months was taken up with the assembling of the necessary apparatus. Gosling's original X-ray photographs were taken with standard apparatus (Raymax tube and Unicam camera) not well suited to this type of work. The diffraction pattern of DNA, like that of proteins, is confined to rather small angles ($2 \sin \theta/\lambda < \sim 0.3$), and the amount of information that can be obtained from a fibre diagram is, therefore, to a large extent determined by the X-ray-optical resolving power of the system used. An X-ray tube having a small focus with high intrinsic brilliance is required, together with a fine collimator and small specimen.

An Ehrenberg fine-focus tube, made in the workshop of Birkbeck College, was assembled. This is now in use with a North-American Phillips micro-camera for taking high-resolution photographs of single fibres of DNA.

by Stokes

A Beaudouin tube (French make) is also in use with the Unicam camera, for taking photographs where larger specimens are available

or less high resolution is required. The tube has a focus which is intermediate in size between those of the Raymax and the Ehrenberg tubes, and has the advantage of being very adaptable and giving a larger total intensity than the Ehrenberg tube. The focal spot is circular and its diameter can be varied from about 0.1 to 1.0 mm.

Preliminary Results

It is proposed to attempt a quantitative interpretation of the fibre diagram (which shows a high degree of crystallinity in the DNA fibres) by means of Patterson functions. As a first step a cylindrical Patterson function will be calculated. With the aid of this it is hoped that it will be possible to index the diagram with reasonable certainty, thus enabling the Patterson function to be calculated subsequently in three dimensions.

Before embarking on these calculations it seemed desirable to ascertain that the photographs used were the best which could be obtained. A systematic search for the best conditions, especially with respect to relative humidity, yielded some preliminary results of considerable interest. These may be summarised as follows:

The Crystalline State

The highly crystalline fibre diagram given by DNA fibres is obtained only in a certain humidity range, about 70% to 80%. The general characteristics of the diagram suggest that the DNA chains are in a helical form.

Higher humidity gives a diagram showing ~~only~~ the following principal features

(1) A sharp spot at ~22A on the equator;

(2) Diffuse meridional arc at ~3.4A

(3) Two diffuse spots at about 40° to the meridian.

This diagram appears to correspond to scattering by individual helical units; i.e. it shows the form factor of the helical units (except for the sharp equatorial spot which is related to an inter-helical distance). That is, at high humidity a water sheath disrupts the spatial relationships between neighbouring helices, and only the parallelism of their axes is preserved.

During the change "crystalline → wet" a considerable increase in length of the fibres occurs. The helix in the wet state is therefore presumably not identical with that of the crystalline state.

Lower humidity With decreasing humidity the crystalline diagram gradually fades away without decreasing appreciably in sharpness. This means that the basic lattice is preserved while disorder about the lattice-points increases, more and more scattered radiation passing from the sharp spots into the diffuse background.

Hysteresis effects

1. The crystalline state is associated with ~20% weight water (on dry DNA). But both the water content and the structural state of the DNA fibres are highly dependent on history as well as on relative humidity; i.e. there is a strong hysteresis in water uptake.

2. The effect of strong drying is to make the crystalline state obtained on subsequent wetting both more stable and more perfect. After strong drying and re-wetting the crystalline form

can only be destroyed at very high humidities. The spots on the "crystalline" diagram are much sharper when the substance was previously strongly dried.

The crystalline form

The fibre diagram of the crystalline form can be indexed tentatively on the basis of a face-centred monoclinic unit cell,

$a = 25.7$ A $b = 42.2$ A $c = 27.4$ A

$\beta = 118° 30'$

This is nearly hexagonal in projection, each lattice point having two neighbours at 22.6A and four neighbours at 23.8A. It therefore suggests that the structure is built up of near-cylindrical units.

The 27A layer-line spacing is very strong, which suggests that it corresponds to one turn of a helix.

Astbury's density measurement (1.63 gm/cm^3 for dry DNA), together with our water-content measurements, indicates

24 nucleotides per primitive unit cell

and 4 molecules of water associated with each nucleotide.

Interpretation

The results suggest a helical structure (which must be very closely packed) containing probably 2, 3 or 4 co-axial nucleic acid chains per helical unit, and having the phosphate groups near the outside. It is the phosphate groups which would be capable of absorbing water in large quantities and of forming strong inter-helical bonds in the presences of considerable quantities of water,

thus giving the substance a 3-dimensional crystalline structure. These bonds would be disrupted in the presence of excessive quantities of water (leading first to the "wet" structure of independent helices with parallel axes, and ultimately to solution of the DNA in the water) and would remain strong in the absence of water, thus explaining the cementing effect of strong drying. The dry structure is distorted and stained due to the holes left by removal of water, but contains intact the skeleton of the crystalline structure.

Wilkins

Rosalind E. Franklin

Wheatstone Physics Laboratory,
King's College

7th February 1952

includes Nature pull"
hence presumably post Watson Crick
Klug agrees it is post-WC.

2 3 A/R

INTERIM ANNUAL REPORT JANUARY 1951 - JANUARY 1953

ROSALIND E. FRANKLIN,
Crystallographic Laboratory,
Birkbeck College.

X-RAY STUDIES OF SODIUM DESOXYRIBONUCLEATE FIBRES

The first Annual Report (January 1950-January 1951) was concerned with the construction of apparatus, followed by a qualitative survey of the types of X-ray fibre-diagram obtained from sodium thymonucleate (NaDNA). Particular attention was paid to the influence of water content and, from the results, certain deductions were made concerning the role of water in the structure. It was shown that from well-oriented fibres two distinct structures can be obtained. Structure A, a crystalline form, is obtained at about 75% relative humidity. Structure B is a less ordered form obtained at humidities above about 90%. The second year's work has been almost entirely concerned with a quantitative study of Structure A.

Measurements were made on photographs taken with a specimen-film distance of 15 mm using the Phillips micro-camera and Ehrenberg-Spear fine-focus tube as described in the previous report. In order to search for further reflections on or near the equator, photographs were also taken with the fibre inclined to the X-ray beam at a series of angles in the range ~~85°~~ 70°

85° to 70°. A special micro-camera was designed and constructed for this purpose. The specimen-film distance and collimator dimensions were the same as those of the Phillips camera. These photographs revealed only one reflection not observed with the fibre perpendicular to the X-ray beam. This reflection lies on the 11th layer-line on, or close to, the fibre-axis direction. Although its intensity is negligibly small in the quantitative treatment of the diagram, its existence is nevertheless of importance in suggesting that they may be 11 nucleotides in one repeat period of the nucleic acid chain in structure A.

MEASUREMENT OF THE PHOTOGRAPHS.

For the measurement of the R-space co-ordinates and the intensities of the 66 independent reflections observed, standard methods could not be applied owing to the small size of the photographs and to the variety of shapes and sizes of the photographic spots. The micro-photographs were therefore projected on to a white cardboard screen, using a magnification of about x 10. The centres of the reflections were then marked on the card, and their x- and y- co-ordinates measured. The use of the projection rather than a travelling microscope was found not only to be much less fatiguing, but also to provide a more reliable estimate of the positions of weak reflections and a more convenient method of making measurements on curved layer-lines.

From the measured x and y values, the reciprocal-space ξ and ζ co-ordinates were calculated. It was then necessary to apply a correction to the observed ξ and ζ values owing

to the fact that it was not found possible to place the NaDNA fibre exactly perpendicular to the X-ray beam. It can be shown that, if the fibre deviates from the ideal position by an angle γ, then

$$\tan \gamma = (\zeta_1 + \zeta_2)/\rho^2 \qquad (1)$$

where ζ_1 and ζ_2 are the apparent ζ-values of the same reflection above and below the equator,

and $\rho^2 = \zeta^2 + \xi^2$ (2)

Since ρ is the most accurately measurable parameter for each reflection, the procedure adopted was to use a series of measurements of ζ_1, ζ_2 and ρ to determine γ for the photograph, and hence the true ζ-value for each layer-line. All ξ-values were then obtained from measurements of ρ and the use of equation (2).

For the measurement of <u>intensities</u> it was necessary either to explore each spot photometrically, or to estimate its maximum intensity and consider separately the question of spot shape and size. The latter alternative was adopted. The maximum intensity of each spot was estimated visually by comparison with a standard scale. The scale was prepared with the aid of the ultra-fine collimating system of a low-angle camera, used to obtain a set of photographic streaks of width comparable with that of the spots on the NaDNA micro-photographs. The NaDNA photographs were projected, as for the measurement of the positions of reflections, and the scale displaced by hand across the photograph in the projection.

CORRECTIONS TO OBSERVED INTENSITIES

To obtain true integrated from observed maximum intensities it was necessary to apply, in addition to the usual corrections for polarisation and camera geometry, a number of experimentally and theoretically determined correction factors designed to take account of the shape and size of the photographic spots.

The resulting integrated intensities are inevitably less accurate than those which can be measured from photographs of single crystals, but were considered to be sufficiently accurate to justify their use in the calculation of Patterson functions.

ARTIFICIAL TEMPERATURE FACTOR

wrong: above 3A it decreases

Since the corrected intensities showed little, if any, tendency to decrease with increasing θ an "artificial temperature factor" was applied. Intensities were multiplied by $e^{-a^2 s^2}$ where $a = 4.56$, a value chosen to reduce to 0.3 of its value the intensity of the furthest equatorial reflection observed.

THE CYLINDRICALLY AVERAGED PATTERSON FUNCTION

The fibre diagram of structure A shows 66 reflections distributed on 9 well-defined layer-lines. During the course of attempts to index the reflections it became fairly clear that the unit cell was monoclinic c-face-centred, with the c-axis parallel to the fibre axis. However, owing to the inevitable errors of measurement, and to the ambiguities of indexing reflections at large angles of diffraction, it was not found possible, by direct inspection, to establish all the cell parameters with

certainty. It was therefore decided to calculate the cylindrically symmetrical Patterson function described by MacGillavry and Bruins (1948). This function contains all the information which can be obtained from the fibre diagram without allotting to the reflections any indices other than their layer-line numbers, and is therefore periodic in the c-direction only. It is the function which would result from taking the true three-dimensional Patterson function, giving it cylindrical symmetry by rotating it about an axis through the origin and parallel to the fibre axis, and then taking a section through the axis of rotation.

The principal periodicities, or lattice translations, other than that corresponding to the layer-plane spacing, will be revealed as important peaks in this aperiodic Patterson function. They will, in general, be distinguished from other Patterson peaks in that their second (and higher) orders will be observed.

The fibre-axis repeat period was found to be 28.1 A. The cylindrical Patterson function was calculated at intervals of 28.1/30 A in z and of 1A in x in the range x = 0 to 50 A.

The Patterson function shows a number of strong, well-defined peaks, and among these we must look for the lattice translations.

The first important region of high density occurs at x = 12 - 14 A and z = 5 - 9 A. However, it can readily be shown that it is impossible to index the equatorial reflections on the basis of a unit cell in which one parameter has an x-component of about 13 A.

The peaks around x = 22 A, z = 2 A, and x = 40 A, z = 0 were next selected as possibly containing lattice vectors. These agreed well with the b/a. sinβ ratio of 1.82 indicated by the application of a Bunn chart to the equatorial reflections, and led to the satisfactory indexing of all the 66 observed reflections on the basis of a face-centred monoclinic unit cell having the following parameters

a = 22.0 A

b = 39.8 A

c = 28.1 A

and β = 96.5

Agreement between calculated and observed values of $\frac{1}{d}$ was generally better than 1%, and in no case worse than 2%.

It was found that for the larger values of $\frac{1}{d}$ on the equator and the first and second layer-lines no reflection could be indexed unambiguously; reflections which should have been well-resolved and single were absent. This result is clearly not fortuitous. It seems to imply that the presence or absence of observable reflections in this region is not of great significance; single reflections are not strong enough to be distinguished from the rather strong diffuse background, and only where the geometry of the reciprocal lattice is such that two or more reflections reinforce one another can a photographic effect be observed. On this account the introduction of an "artificial temperature factor" mentioned above is more than usually important.

When all possible indices of the observed reflections are

taken into account the total number of reflections is increased from 66 to 92.

SPACE GROUP

Owing to the relatively small number of reflections observed, and to the ambiguity of indexing the reflections at large θ, systematic absences cannot be detected with certainty. However, since the asymmetric carbon atoms of the sugar rings preclude the existence of a plane of symmetry, C2 is the only space-group possible.

DOUBLE ORIENTATION

A fortunate accident provided a rather satisfactory confirmation of the correctness of the indexing scheme. One fibre, of diameter about 40 μ was found to give a photograph showing strong double orientation. That is, the crystallites were not in random orientation about the fibre axis, and gave, as a result, something intermediate between a rotation and an oscillation photograph.

A list was drawn up in which those reflections which were strongest in the top left-hand quadrant of this photograph were labelled L and those strongest in the top right-hand quadrant were labelled R. It was then found that all reflections labelled L had been allotted indices $hk\bar{l}$ whereas all R reflections had indices hkl. The distribution of the observed reflections among the different quadrants in R-space as determined independently by the process of allotting indices is thus directly confirmed by comparison with the distribution revealed in this

photograph.

It was thought that the double orientation shown in this exceptional photograph might be due either to a mechanical accident to the fibre or to preferential orientation of the crystallites near the surface. However, attempts to reproduce the effect failed, and neither of these suggestions has been confirmed.

DENSITY DETERMINATION

The density of NaDNA at various humidities was measured by the following method.

Homogeneous lumps of dry Na DNA were prepared by allowing pieces of swollen gel to dry slowly, stirring them gently at first to allow trapped air to escape. The lumps were then dried over P_2O_5 at room temperature for several weeks. To measure the density of the dry substance, each lump was placed in CCl_4 in a test-tube, and the temperature allowed to rise slowly from below - 10°C until the lump just sank. In this way a density range of about 1.65 to 1.58 g/cc, corresponding to temperatures for -10 C to 25°C, can be satisfactorily covered. The density of dry NaDNA was found to be 1.625 ± 0.002 g/cc at 4 ± 1°C, in good agreement with Astbury's value of 1.63 (Astbury 1947).

By using, in the same temperature range, $CHCl_3$ in place of CCl_4 densities between about 1.54 and 1.48 g/cc can be measured. Lumps of NaDNA were maintained at the required relative humidity until equilibrium was reached, then immersed in the appropriate

liquid (CCl_4 or $CHCl_3$) the temperature of which was rapidly adjusted to give a density measurement. In this way the density of NaDNA at 75% R.H. was found to be 1.521 ± 0.002 g/cc (corresponding to a temperature of 2°C to 4°C in $CHCl_3$). The water uptake of the lumps at 75% R.H. was 32 - 42%.

The number of nucleotides per unit cell cannot be deduced directly from the measured density owing to uncertainty as to the quantity of water in the crystallites. The total water content of a micro-crystalline mass of NaDNA at 75% R.H. was found to be about 40%, but it has not been possible to determine directly how much of this is in the crystallites. However, calculation shows that if the water content of the crystallite is assumed to be between 22% and 50% of the dry weight, then the number of nucleotides per face-centred unit cell lies between 56 and 44.

It was mentioned above that the occurrence of a near-meridional reflection on the 11th layer line suggests that there are 11 nucleotides per chain in the unit cell. The density measurements therefore suggest that there are 4 chains passing through the face-centred unit cell (or two chains associated with each lattice pair).

THE THREE-DIMENSIONAL PATTERSON FUNCTION

Having indexed the reflections in the manner described above, the complete three-dimensional Patterson function was calculated.

The three-dimensional Patterson function contains a rather small number of peaks, and these are very strong in relation to

the origin peak. This apparent simplicity we believe to result from the small number of terms experimentally obtainable for use in the transform rather than to a real simplicity of the structure. The fewer the terms the greater will be the relative height of the strongest peaks.

Nevertheless, it seemed probable that the principal features of the Patterson would be due to phosphate-phosphate vectors, the phosphate group acting to some extent as a "heavy atom". Extensive efforts were therefore made to achieve a direct partial interpretation of the Patterson function by means of superposition methods. Although it was possible to obtain in this way direct evidence for the existence of a symmetry axis and thus to confirm the space group (C2), no significant progress was made towards the solution of the structure and the method has been provisionally abandoned.

There are two striking features of the three-dimensional Patterson function which give some direct information concerning the structure. Peaks occurring at about 5.3 A from the origin and with c-coordinates of about 2.5 A are very strong and well-resolved. These can only be phosphate-phosphate peaks representing a single step in the back-bone chain and are consistent with the suggestion (made above) that there are 11 nucleotides in a repeat period of 28.1 A. Further, a very strong peak occurs at approximately a = b = 0, c = 14 A. While this peak on the a-b section at half the cell-height is the strongest feature of the Patterson function, suggesting a pseudo-halving of the cell, the other features of this section are almost exactly the inverse

of the a-b section having c = O. This suggests that only a part of the structure - the phosphate groups, for example - repeats at half the cell height, while the rest of the structure does not.

STRUCTURE B

The general form of the X-ray fibre-diagram of structure B is typical of that shown by Cochran, Crick and Vand to be given by helical structures. Using the theory developed by these authors it has been shown that there are strong indications that structure B contains a two-chain helix. The phosphate groups lie on the outside of the helical structure, and the phosphate groups of the two chains of the helix are separated from one another by about 3/8 of the fibre-axis repeat period.

PUBLICATIONS

Two papers entitled:

"Fibre Diagrams of Sodium Thymonucleate:

I The Influence of Water Content

II The Cylindrically Symmetrical Patterson Function"

written in collaboration with R.G. Gosling have been accepted for publication in Acta Crystallographica.

A note on "Molecular Configuration in Sodium Thymonucleate" was published in Nature, 171, 740 (1953) in collaboration with R.G. Gosling.

from DNA history file

Why did Randall not discuss this with me?

According to JTR this was written by RF for Randall.

"Helix not of the same form"

3.12.51

40% water in A structure

Franklin is author not Franklin + Gosling

The only type of structure discussed is Helical — all α helices

Notes on aspects of the structure of Calf Thymus DNA as revealed by its interaction with water

3.12.51

The highly crystalline fibre diagram given by DNA fibres is obtained only in a certain humidity range, about 70% to 80%

Higher humidity gives a diagram showing only

(1) A sharp spot at ~22A on the equator

(2) Diffuse meridional arc at ~3.4A

(3) Two diffuse spots at about 40° to the meridian.

This diagram probably corresponds to scattering by individual helical units; i.e. it shows the form factor of the helical units (except for the sharp equatorial spot which is related to an inter-helical distance). That is, at high humidity a water sheath disrupts the spatial relationships between neighbouring helices, and only the parallelism of their axes is preserved.

N.B. It is during the change "crystalline → wet" that the greatest length increase of the fibres occurs. The helix is therefore presumably not of the same form in the two states.

Lower humidity. With decreasing humidity the crystalline diagram gradually fades over without decreasing appreciably in sharpness. This means that the basic lattice is preserved while disorder about the lattice-points increases, more and more scattered radiation passing from the sharp spots into the diffuse background.

Hysteresis effects

1. The crystalline state is associated with ~ 40% weight

water (on dry DNA). But both the water content and the structural state of the DNA fibres are highly dependent on history as well as on RH; i.e. there is a strong hysteresis in water ~~xxx~~ uptake.

2. The effect of strong drying is to make the crystalline state obtained on subsequent wetting both more stable and more perfect. After strong drying and re-wetting the crystalline form can only be destroyed at very high humidities. The spots on the "crystalline" diagram are much sharper when the substance was previously strongly dried. Wilkins & Gosling

The crystalline form

Indexed tentatively on the basis of a face-centred monoclinic unit cell, ~~xxxxxxxxx~~

a = 25.7 A b = 42.2 A c = 27.4 A

β = 118°30' β = 96·5° in 1952 Report Probably Gosling? pre Franklin?

This is nearly hexagonal in projection, each lattice point having two neighbours at 22.6A and four neighbours at 23.9A, and therefore suggests that the structure is built up of near-cylindrical units.

The 27A layer-line spacing is very strong, which suggests that it corresponds to one turn of a helix.

Astbury's density measurement (1.63 gm/cm^3 for dry DNA) together with our water-content measurements indicates

24 nucleotides per primitive unit cell

and 8 molecules of water associated with each nucleotide.

Interpretation

Suggest helical structure (which must be very closely packed) containing probably 2,3 or 4 nucleic acid chains per helical unit, and having the phosphate groups near the outside. It is the phosphate groups which would be capable of absorbing water in large quantities and of forming strong inter-helical bonds in the presences of considerable quantities of water, thus giving the substance a 3-dimensional crystalline structure. These bonds would be disrupted in the presence of excessive quantities of water (leading first to the "wet" structure of independent helices with parallel axes, and ultimately to solution of the DNA in the water) and would remain strong in the absence of water, thus explaining the cementing effect of strong drying. The dry structure is distorted and strained due to the holes left by removal of water, but contains intact the skeleton of the crystalline structure.

why no choice?

?

?

cementing

R.E. FRANKLIN

3.12.51

at end. dated 3 Sept 52

"corresponding" length change

presumably prepared several months before DEC 15 visit to MRC?

X-RAY STUDIES OF CALF-THYMUS D.N.A.

R.E. FRANKLIN and R.G. GOSLING

This is the MRC Report as presented in MRC 52/815

A. The Role of Water

The crystalline form of calf thymus D.N.A. is obtained at about 75% RH and contains about 20% by weight of water.

Increasing the water content leads to the formation of a different structural modification which is less highly ordered. The water content of this form is ill-defined.

The change from the first to the second structure is accompanied by a change in the fibre-axis repeat period of 28A to 34A and a corresponding macroscopic length-change of the fibre of about 20%

Decreasing the water-content below 20% leads to a gradual fading out of the crystalline X-ray pattern and a corresponding increase in the diffuse background scattering. After strong drying only diffuse scattering is observed.

All these changes are readily reversible. The following explanation is suggested:

The phosphate groups, being the most polar part of the structure, would be expected to associate with one another and also with the water molecules. Phosphate-phosphate bonds are considered to be responsible for inter-molecular linking in the crystalline structure. The water molecules are grouped around these bonds (four water molecules per phosphorus atom). Increased water content weakens these bonds and leads first to a less highl

ordered structure and, ultimately, to gel formation and solution. Drying leaves the phosphate-phosphate links intact but leads to the formation of holes in the structure with resulting strain and deformation. The three-dimensional skeleton is preserved in distorted form and crystalline order is restored when the humidity is again increased.

B. The Cylindrically Symmetrical Patterson Function

It was apparent that the crystalline form was based on a face-centred monoclinic unit cell with the C-axis parallel to the fibre axis. But it was not found possible, by direct inspection, to allot all the lattice parameters accurately and unambiguously. To obtain the unit cell with certainty the cylindrically symmetrical Patterson function was calculated. This function is periodic in the fibre-axis direction only.

Special techniques were developed for the measurement of the positions and intensities of the reflections. This was necessary, firstly because all measurements had to be made on micro-photographs, and secondly because the observed reflections were of a variety of shapes and sizes so that integrated intensities could not be directly measured.

On the Patterson function obtained, the lattice translations could be readily identified. On the basis of a unit cell defined by

a = 22.0 A

b = 20.2 A

c = 28.1 A

β = 96.5°

the 66 independent reflections observed could all be indexed with an error less than 1%.

B 96-5

A very satisfactory confirmation of the correctness of the unit cell and the indexing was provided by a fortunate accident which it has so far not been found possible to reproduce. One fibre was obtained which gave a photograph showing strong double orientation. It was found that in this photograph those spots which had been indexed $\bar{h}kl$ were strongest in one pair of quadrants while those indexed hkl were strongest in the other pair.

C. The Three-Dimensional Patterson Function

Having established the unit cell with certainty, it is now possible to calculate Patterson sections in the normal way. Work on these is in progress.

3rd September 1952

ROUGH DRAFT

This could have been written after WC news reached Kings which I estimate is ~ March 14 (or 13?)

If so however it was written rather rapidly

A NOTE ON MOLECULAR CONFIGURATION IN SODIUM THYMONUCLEATE

MHFW

Rosalind E. Franklin and R. G. Gosling

When they gave me the Nature mss I was struck by how rapid they had been.

does not claim to show 2 chains (but does give 3/8 argument for 2)

Nothing on inter-base H bonds. Why? That is strange

Date 17/3/53.

Sodium thymonucleate fibres give two distinct types of X-ray diagram. The first, corresponding to a crystalline form obtained at about 75% relative humidity, has been described in detail elsewhere( ). At high humidities a new structure, showing a lower degree of order appears, and persists over a wide range of ambient humidity and water content. The water content of the fibres, which are crystalline at lower humidities, may vary from about 50% to several hundred per cent. of the dry weight in this structure. Other fibres which do not give crystalline structure at all, show this less ordered structure at much lower humidities. The diagram of this structure, which we have called structure B, shows in striking manner the features characteristic of helical structures( ). Although this cannot be taken as proof that the structure is helical, other considerations make the existence of a helical structure highly probable.

Structure B is derived from the crystalline structure A when the sodium thymonucleate (NaDNA) fibres take up quantities of water in excess of about 40% of their weight. The change is accompanied by an increase of about 30% in the length of the fibre, and by a substantial re-arrangement of the molecule. It therefore seems reasonable to assume that in structure B the structural units of NaDNA (molecules or groups of molecules) are relatively free from the influence of neighbouring groups, each unit being shielded by a sheath of water. Each unit is therefore free to take up its least-energy configuration independently of its neighbours and, in view of the nature of the long-chain molecules involved, it is highly likely that the

general form will be helical ( ). If we adopt the hypothesis of a helical structure, it is immediately possible, from the X-ray diagram of structure B, to make certain deductions as to the nature and dimensions of the helix.

From the angle between the straight lines which can be drawn through the origin and the innermost maxima of the 1st, 2nd, 3rd and 5th layer-lines, the diameter of the helix can be calculated. It is found to be about 20A. Since this linear array of maxima is one of the strongest features of the diagram, we must conclude that a (crystallographically) very important part of the molecule lies on a helix of this diameter. This can only be the phosphate groups (or, perhaps, the phosphorus atoms). Thus, if the structure is helical we find that the phosphate groups lie on a helix of diameter about 20A, and the sugar and base groups must accordingly be turned inwards towards the helical axis.

This is in agreement with the conclusion which we reached previously by quite other reasoning ( ), namely that, whatever the structural unit, the phosphate groups must be on the outside. There were two principal reasons for believing this. The first derives from the work of Gulland and his collaborators who showed that even in aqueous solution the -CO and $-NH_2$ groups of the bases are inaccessible and cannot be titrated, whereas the phosphate groups are fully accessible. The second is our own observations on the way in which the structural units in the crystalline structure A are floated apart by an excess of water, the process being a continuous one which leads to the formation first of a gel and ultimately to a solution. The hygroscopic part of the molecule may be presumed to lie in the phosphate groups; ($(C_2H_5O)_2PO_2Na$ and $(C_3H_7O)_2PO_2Na$ are highly hygroscopic) and the simplest explanation of the above process is that these groups lie on the outside of the structural units. Furthermore the ready availability of the phosphate groups for interaction with proteins can also be explained this way.

The above estimate of 20A diameter was based on the assumption of a single strand helix. That is, the first maximum on the n^{th} layer-line corresponds to the first maximum in $J_n(2\pi rR)$. Where $J_n(u)$ is the n^{th} order Bessel Function of u, r is the radius of the helix and R the distance from the fibre-axis direction in reciprocal space.

The strong meridional maximum at 3.4 A° lies accurately on the 10th layer-line. From this new lines of maxima eminate, as from the origin, crossing the origin series on the 5th layer-line, corresponding to a $J_5(u)$ for each series, confirming that the second origin does lie on the 10th layer-line. This then, indicates that there are 10 structural units in one turn of the single-strand helix. For a helix of diameter 20 A° this gives a distance of 6A between neighbouring units in one molecule, which is a reasonable distance for the P-P value in NaDNA. (this distance in a fully extended chain is 6.8 A°).

If, instead of a single-strand helix we propose 2 equally spaced co-axial helical molecules, the first maximum on the n^{th} layer-line corresponds to the first maximum in $J_{2n}(2\pi rR)$. Since our value of R is fixed and the first maximum in $J_2(x)$ occurs at very nearly twice the value in x of the first maximum in $J_1(x)$ (which gave us $2r \simeq 20A°$) the value of 2r for a 2-strand helix must be $\simeq$ 40 A°. The cross-section of the helix would then be considerably greater than that of the primitive cell in the crystalline structure A, and this would seem highly improbable. The same argument, with even more force, eliminates the possibility of 3 equally spaced co-axial helical molecules.

On the theory of a single-strand helix, the series of equatorial maxima should correspond to the maxima of $J_0(4\pi r \sin\theta)$. The maxima on our photograph do not, however, fit this function. This is rather to be expected. For we know that the helix so far considered is only the most important member of a series of co-axial helicies of different radii, the non-phosphates parts

of the molecule must lie on a series of co-axial helicies of smaller radii. Following Crick, Cochran and Vand, the structure factor on the n^{th} layer-line for a series of co-axial helicies

$$F_n = \sum_j f_j J_n(2\pi R r_j) e^{i\left[n(\psi - \phi_j + \frac{1}{2}\pi) + 2\pi \frac{l}{c} z_j\right]}$$

(here give definitions)

Simplifying this, for the case of a whole number of residues per turn of the helix, we readily obtain

$$F_n = e^{in(\psi + \pi/2)} \left[f_1 J_n(x_1) e^{in\alpha_1} + f_2 J_n(x_2) e^{in\alpha_2} + \ldots\ldots f_j J_n(x_j) e^{in\alpha_j} \right]$$

where $\alpha_K = \phi_K - 2\pi z_K/c$

and $x_K = 2\pi R r_K$.

It follows that

$$I = \sum_0^j f_j^2 \left[J_j(x_j)\right]^2 + \sum_{j/K}^{jk} f_j f_K J_n(x_j) J_n(x_k) \cos\left[n(\alpha_j - \alpha_k)\right]$$

From this it is evident that the innermost maxima on the layer-lines will always be given by the helix of the longest diameter, the terms containing $J_n(x_j)\, J_n(x_k)$ for values of x_j and x_k smaller than the maximum being, in the region of the first maxima, very small. Later maxima, however, may be obliterated or shifted owing to the appearance of important negative terms in the expression for I.

Thus, while we do not attempt to offer a complete interpre-

tation of the fibre-diagram of structure B, we may state the following conclusions. The structure is probably helical. The phosphate groups lie on the outside of the structural unit, on a helix of diameter about 20 A. There are 10 phosphate groups per chain in one turn of the helix. The structure does not contain more than one equivalent co-axial chain, but the possibility of non-equivalent co-axial chains is not eliminated.

The total absence of an inner maximum on the fourth layer-line suggests that if there are 2 non-equivalent co-axial chains these are separated by $\frac{3}{8}$ of the fibre-axis period, that is by $4\frac{1}{2}$ A in the fibre-axis direction.
$\simeq 13A$.

+ intensities on offset e.l.

The 3/8 argument I think only applies if they are equivalent

That is the only helical bit which is non Stokes + Wilkins + goes back to Dec 51.

Oct 92.
Conclusion. 1. She is moving towards W C
2. she seems inexplicably muddled on several straightforward points
3. quite a lot is simply Stokes Frazer etc ideas of 1951.

$$8\overline{)28}\quad 3\cdot5$$
$$24$$
$$40$$
$3\cdot4\times8=27\cdot2$

16 June 1969

Notes by A.K. on Franklin and Gossling's draft of March 17, 1953

This is a precursor of the paper published in Nature in April 1953. It was written before R.E.F. had heard of the Watson-Crick model and the text is based very closely on her notebook entries for the period January - March 1953.

Page 3, paragraph 3

What is being done here is ruling out the possibility of a two-fold axis parallel to the axis of the molecule contained in a structure of twice the diameter. Note the terminology is rather different to that which would be used nowadays. Franklin used "strand" where we would now say "helix" in an abstract geometrical sense and the word "chain" is used in the current sense, i.e. as a physically connected structure.

Page 5

Since R.E.F. had not spotted the possibility of a perpendicular diad and she had already ruled out an axial diad, the formal conclusion was that if there were two chains, as was likely, they would not be exactly equivalent.

Oct 92. MHFW. Some peculiar confusions in this & points missed
e.g. H bonding base!! (she had seen Fraser)
but all that supports Klug's belief that it was
one W & C But she seem a long way off
the a double helix ?

Extracts from MRC 52/815 15 Dec 52 Biophysics Research Committee

either material is a precursor of native collagen. These materials are being compared with native collagen in various ways: electron microscopy, infra-red spectra, biochemical analysis, molecular weight, and light scattering techniques are being employed. Procollagens have a much lower molecular weight (~70,000) than native collagen appears to have when dissolved in organic acid. And yet the procollagens when precipitated give rise to fibrils similar in structure to native collagen. The high-angle X-ray diffraction pattern appears to be identical with that of native collagen.

(7) Banded and Unbanded Collagen

Long-Spacing Fibrils: When acid polysaccharide, such as chondroitin sulphate or gum arabic, is added to an acetic acid solution of collagen under appropriate conditions of pH, fibrils with spacings much greater than normal are observed when the precipitates are examined in the electron microscope. The spacings range from 1200-2400Å instead of the usual 640Å. The interest of such fibrils lies not in their similarity to those found in vivo, but in the ability of polysaccharide to modify the structure. A small amount of polysaccharide has long been observed in collagen analyses but it has never been proved that such material is a structural component.

It is legitimate to regard this phenomenon as part of the wider problem of the chemical and structural characteristics of banded and unbanded collagens.

It seems fairly certain from work in this laboratory and elsewhere that almost all collagens give similar, if not identical, high-angle X-ray diffraction patterns. In fact, at the present time there seem to be few other common links between the collagenous materials of different origin. Such an identification relates, of course, to distances of no more than a few Angstroms. At a coarser level of structure, however, (~100-700Å) collagens may show either the familiar bandings of mammalian material or the structureless features of, for example, earthworm cuticle. More detailed amino acid and sugar analyses are required in conjunction with structural investigations before this puzzle can be solved. It is a feature of collagen structure in which the laboratory is greatly interested.

NUCLEIC ACID RESEARCH

The research on nucleic acids, like that on collagen, has both a structural and a biological interest. Some time ago Wilkins found that fibres from sodium desoxyribonucleate gave remarkably good X-ray fibre diagrams. He also examined the optical properties of the fibres in relation to their molecular structure. The detailed examination of the structure has been continued by Miss Franklin and R.G. Gosling, and Wilkins has concentrated on a study of the oriented nucleoprotein of sperm heads. The biological implications of this work are indicated later in this section.

The study of nucleic acids in living cells has been continued by Walker (tissue cultures) and by Chayen (plant root meristem cells); and lately Wilkins and Davies have been measuring the dry weight of material in Tradescantia pollen grains during the course of cell division by means of

interference microscopy. Thus, while the work of Walker on nucleic acid content of nuclei relates only to part of the cell contents, the interference microscope enables the total content of the cell, other than water, to be measured.

Desoxyribose Nucleic Acid and Nucleoprotein Structure (M.H.F. Wilkins)

A molecular structure approach has been made to the question of the function of nucleic acid in cells.

First, X-ray evidence shows that D.N.A. from all kinds of source has the same basic molecular configuration which is little (if at all) dependent on the nucleotide ratio. Some grouping of polynucleotide chains takes place to give ~20A diameter rod-shaped units, and the internal chemical binding which holds each unit together is not affected much by the normal extraction procedure. The basic point is to find the general nature of this structure and the hydrogen bonding etc. in it. Using two dimensional data, the most reasonable interpretation was in terms of a helical structure and the experimental evidence for such helices was much clearer than that obtained for any protein. The crystalline material gives an X-ray picture with considerable elements of simplicity which could be accounted for by the helical ideas, but three dimensional data shows apparently that the basic physical explanation of the simplicity of the picture lies in some quite different and, a priori, much less likely structural characteristic. The 20A units, while roughly round in cross-section, appear to have highly asymmetric internal structure.

The same general configuration appears to exist in intact sperm heads and synthetic or extracted nucleoprotein, and in bacteriophage (and not in insect virus where the protein is different). It appears that the protein is probably bound electrostatically on the outside of the nucleic acid units and does not alter their structure. In some sperm the whole head has a crystalline (but somewhat imperfect) structure. In these sperm, the protein has very low molecular weight and it will be especially interesting to find if any high molecular weight protein exists in such sperm heads. If not, all the genetical characteristics may be supposed to lie in the D.N.A. (as in bacteriophage). Biochemical study of the composition of the protein is planned. In other kinds of cell nucleus with different biological function the proteins are quite different. The main idea is to find the structure of the D.N.A. first, then how it is linked to protein in the crystalline sperm heads, and then attempt to elucidate the more complex structure of the other kinds of cell nucleus. It may be that the characteristic X-ray picture of D.N.A. is especially related to a particular function of the nuclear nucleoprotein. In this way molecular structure and cytochemical studies begin to overlap.

X-ray Studies of Calf Thymus D.N.A. (R.E. Franklin and R.G. Gosling)

(a) The Role of Water: The crystalline form of calf thymus D.N.A. is obtained at about 75% RH and contains about 20% by weight of water.

Increasing the water content leads to the formation of a different structural modification which is less highly

ordered. The water content of this form is ill-defined.

The change from the first to the second structure is accompanied by a change in the fibre-axis repeat period of 28A to 34A and a corresponding microscopic length-change of the fibre of about 20%.

Decreasing the water-content below 20% leads to a gradual fading out of the crystalline X-ray pattern and a corresponding increase in the diffuse background scattering. After strong drying only diffuse scattering is observed.

All these changes are readily reversible. The following explanation is suggested:

The phosphate groups, being the most polar part of the structure, would be expected to associate with one another and also with the water molecules. Phosphate-phosphate bonds are considered to be responsible for intermolecular linking in the crystalline structure. The water molecules are grouped around these bonds (approximately four water molecules per phosphorus atom). Increased water content weakens these bonds and leads, first, to a less highly ordered structure and, ultimately, to gel formation and solution. Drying leaves the phosphate-phosphate links intact but leads to the formation of holes in the structure with resulting strain and deformation. The three-dimensional skeleton is preserved in distorted form and crystalline order is restored when the humidity is again increased.

(b) The Cylindrically Symmetrical Patterson Function: It was apparent that the crystalline form was based on a face-centred monoclinic unit cell with the C-axis parallel to the fibre axis. But it was not found possible, by direct inspection, to allot all the lattice parameters accurately and unambiguously. To obtain the unit cell with certainty the cylindrically symmetrical Patterson function was calculated. This function is periodic in the fibre-axis direction only.

Special techniques were developed for the measurement of the positions and intensities of the reflections. This was necessary, firstly because all measurements had to be made on micro-photographs, and secondly because the observed reflections were of a variety of shapes and sizes so that integrated intensities could not be directly measured.

On the Patterson function obtained, the lattice translations could be readily identified. On the basis of a unit cell defined by

$$a = 22.0 \text{ A}$$
$$b = 39.8 \text{ A}$$
$$c = 28.1 \text{ A}$$
$$\beta = 96.5^\circ$$

the 66 independent reflections observed could all be indexed with an error of less than 1%.

A very satisfactory confirmation of the correctness of the unit cell and the indexing was provided by a fortunate accident which it has so far not been possible to reproduce. One fibre was obtained which gave a photograph showing strong double orientation. It was found that in this photograph those spots which had been indexed $\bar{h}kl$ were strongest in one pair of quadrants while those indexed hkl were strongest in the other pair.

(c) The Three-Dimensional Patterson Function: Having established the unit cell with certainty, it is now possible to calculate Patterson sections in the normal way. Work on these is in progress.

Ultraviolet Absorption Measurements of the Contents of Living and Fixed Cell Nuclei (P.M.B. Walker)

The earlier measurements of ultraviolet absorption in chick fibroblasts and Feulgen staining in the same material are being extended to include the uptake of P32 into the D.N.A. molecule. Measurements can thus be made of the quantity of material and of its rate of increase. This work is being done in collaboration with the M.R.C. Radiotherapeutic Unit at Hammersmith.

The original field of work on avian fibroblasts is also being extended to include mammalian cells; it is intended also to investigate any possible differences between normal and neoplastic tissues.

In the course of his work on ultraviolet absorption of cells Mr. Walker has developed a high-speed recording microdensitometer which is an improvement on his earlier instrument. Photographic density is recorded directly on to paper and the instrument should have considerable use in the analysis of electron micrographs and X-ray diffraction photographs.

Electronic Techniques as aids to Biological Research (E.M. Deeley)

Electronic methods for the rapid measurement of the total amount of absorbing material in given biological specimens are being developed. The circuitry for a slow-speed microdensitometer which will employ a mechanical scanning device has been completed. This unit will cover the area of the specimen in a time of the order of 4 seconds. The possibility of using high-speed scanning methods is also being examined.

Circuits are also being developed for an ultraviolet spectrometer in which it is necessary to arrange for constant sensitivity to changes in density over a wide range of light intensity.

Localization of Nucleic Acids in Cells (J. Chayen)

Ribose nucleic acid (RNA) is associated with protein synthesis; desoxyribose nucleic acid (DNA) has been claimed to be 'gene material', being found invariably on the chromosomes. Dr. Chayen has shown that in embryonic plant cells DNA is present in the cytoplasm, its apparent localisation on the interphase chromosomes being a diffusion artefact produced by such treatment as the hydrolysis in the Feulgen reaction or paraffin embedding. In mitotic cells which are not actively synthesising, however, DNA is found in the interphase nucleus. The localisation of RNA in microsomes has also been studied by ultraviolet and electron microscopy.

The Effects of Fixatives on the Ultraviolet Absorbing Constituents of Chick Fibroblasts (H.G. Davies)

The experiments carried out by Mr. Davies on this

of intimation found, at last, among my archives.
Many thanks for loan of Sayre

A.S.

Stokes says this reads 1952

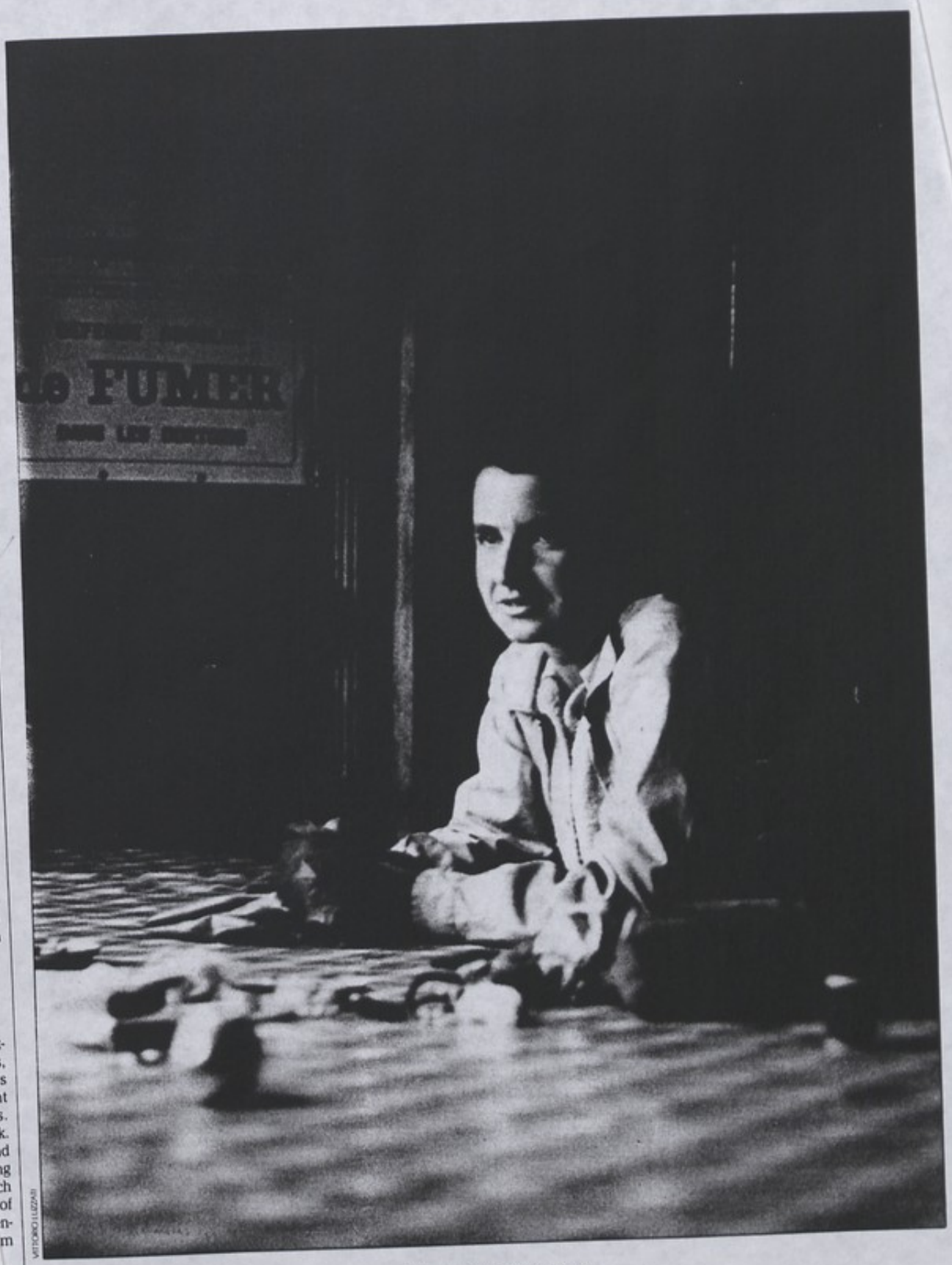
IT IS WITH GREAT REGRET THAT WE HAVE TO ANNOUNCE THE DEATH, ON FRIDAY 18TH JULY, 1952, OF D.N.A. HELIX (CRYSTALLINE).

DEATH FOLLOWED A PROTRACTED ILLNESS, WHICH AN INTENSIVE COURSE OF BESSELMAN INJECTIONS HAD FAILED TO RELIEVE.

A MEMORIAL SERVICE WILL BE HELD NEXT MONDAY OR TUESDAY.

IT IS HOPED THAT DR. M.H.F. WILKINS WILL SPEAK IN MEMORY OF THE LATE HELIX.

R.E. Franklin



VITTORIO LUZZATI

Rosalind Franklin while on a trip in France in 1950 or 1951, before she began working on DNA.