Jaque a la dama: Rosalind Franklin en King's College

Publicado por **Miguel Vicente** el **11 agosto, 2008**

La biografía de Rosalind Franklin atrae algún que otro comentario, no sé si porque su figura despierta reacciones controvertidas, o porque son los otros tres protagonistas de la historia de la doble hélice quienes las provocan.

Puede que Rosalind Franklin no fuese la persona más llevadera del mundo, pero hay motivos para pensar que las equivocaciones y los aciertos posiblemente debieran repartirse con menos parcialidad. Su estancia postdoctoral en París, a una edad en la que fácilmente se pudo sentir atraída por unas ideas más liberales que las que prevalecían en la clase media británica de posguerra, puede que no la hiciesen especialmente atractiva al estamento investigador del King´s, que en general la consideraban afrancesada.

Rosalind Franklin en una excursión por la Toscana en 1950.

Durante los años de postdoctoral en París adquirió un buen conocimiento del idioma y de la cocina francesa.

Foto por Vittorio Luzzati.

Se dice que Rosalind era bastante vehemente, algo por lo que me siento inclinado a comprenderla, e incluso agresiva, lo contrario que le ocurría a Maurice Wilkins, con fama de tímido, calculador y para quien el trabajo duro ocupaba más que la imaginación.

Traduzco en esta ampliación al artículo anterior un material que se encuentra en la página que recopila los documentos de Rosalind Franklin, doy titulo a diferentes secciones e incluyo algún comentario mío sobre todo ello. En color azul aparecerá la traducción de los textos, y en negro lo que comento o añado y que obviamente es mi interpretación, a veces no muy comedida y utilizando los recursos mas atrevidos que me permite el uso del castellano en vez del inglés, y escrita para que la entienda el profano.

Para ponernos en antecedentes tomamos la acción cuando Rosalind Franklin se traslada desde París a Londres para trabajar en el departamento del Profesor John Turton Randall.

Textos originales en inglés

In 1950 she was awarded a three-year Turner and Newall Fellowship to work in John T. Randall's Biophysics Unit at King's College London. Randall had originally planned to have Franklin build up a crystallography section and work on analyzing proteins. At the suggestion of the assistant lab chief, Maurice Wilkins, however, Randall asked Franklin to investigate DNA instead. Wilkins had just begun doing X-ray diffraction work on some unusually good DNA samples. He expected that he and Franklin would work together, but Randall's communication to Franklin did not convey this; it said that only she

and graduate student Raymond Gosling would do the DNA work. Her subsequent relations with Wilkins suffered from this misunderstanding (and perhaps from Franklin's unhappiness with the less collegial culture at King's). Within six months of her arrival at King's in early 1951, they were having very little to do with each other. Working with Gosling, Franklin took increasingly clear x-ray diffraction photos of DNA, and quickly discovered that there were two forms--wet and dry--which produced very different pictures. The wet form she realized was probably helical in structure, with the phosphates on the outside of the ribose chains. Her mathematical analyses of the dry form diffractions, however, did not indicate a helical structure, and she spent over a year trying to resolve the differences. By early 1953 she had concluded that both forms had two helices.

Watson and Crick had not stopped thinking about DNA, and they were in regular communication with Wilkins, eager to learn whatever they could of the progress at King's. In January 1953, spurred by Linus Pauling's publication of a 3-helix model (similar to the one they made in 1951), they resumed work on their DNA model, determined to get it right before Pauling or someone else did. Two pieces of evidence from Franklin's work were crucial to their correct model: first, a very clear photo of the B form taken in May 1952 labeled "51" which Gosling had given to Wilkins as part of his graduate research work, and which Wilkins showed to Watson without Franklin's knowledge; and second, the MRC report, given to Watson and Crick by Max Perutz, a member of the MRC committee that reviewed the work at Randall's lab. The report contained details of Franklin's work (as yet unpublished), including her identification of the unit cell as belonging to the crystal space group called face-centered monoclinic C2. The photo confirmed the helical pattern, and the unit cell type told Crick, a physicist with more theoretical crystallography expertise than Franklin, that the helices ran in opposite directions. By early March, they had their model.

Franklin, still unhappy at King's, had arranged to transfer to J. D. Bernal's lab at Birkbeck College, and was hurrying to finish writing up her work on the A form before leaving. She was unaware of the "race for the double helix" that was in process. In February 1953, however, she looked again at photo #51 and began analyzing it. Several days later she concluded that both A and B forms were two-chain helices, although she had not resolved the configuration of the bases inside. She and Gosling drafted an article on the likely molecular structure by mid-March. This appeared, in expanded and modified form, with Watson and Crick's announcement in *Nature* on April 25, but the draft was done before they had heard about the Watson-Crick model.

(véanse también los Anexos I y II)

Traducción y comentarios

¿Por qué Maurice Wilkins le hizo el vacío a Rosalind Franklin?

En 1950 le fue concedida (a Rosalind Franklin) la beca “Turner y Newall” para trabajar por tres años en la Unidad de Biofísica de Randall en el King’s College de Londres. En un principio Randall dispuso que Franklin iniciase una sección de cristalografía y trabajase en el análisis de proteínas. Sin embargo por indicación del adjunto jefe del laboratorio, Maurice Wilkins, Randall pidió a Franklin que en vez de ello investigase el ADN. Wilkins había comenzado a trabajar en la difracción de rayos X con unas muestras de ADN de excepcional calidad. Esperaba que Franklin y él trabajaran juntos, pero lo que le dijo Randall a Franklin no era así; le comunicó que el doctorando Raymond Gosling y ella harían el trabajo del ADN. Su relación posterior con Wilkins se resintió de este malentendido (y quizás del disgusto de Franklin con el ambiente de menos camaradería del King’s). A principio de 1951, a los seis meses de llegar a King’s, ya se relacionaban muy poco.

No me extraña que a Wilkins le sentase mal el que le metiesen a una recién llegada a trabajar en su tema. Conociendo el ambiente de los laboratorios y la peculiar idiosincrasia británica estoy seguro de que Rosalind se debió sentir como aterrizando en Marte. ¿Fue todo una metedura de pata de Randall o es que le tenía ganas a Wilkins por cualquier otro asunto? Es casi inconcebible que Wilkins hablara abiertamente con Franklin sobre ello.

Y a pesar de todo es helicoidal

Trabajando con Gosling, Franklin fue tomando fotos de difracción de rayos X del ADN cada vez más nítidas, y pronto descubrió que había dos formas- la seca y la hidratada- que producían imágenes muy

distintas. Se dio cuenta de que la forma hidratada era probablemente de estructura helicoidal, con los fosfatos por fuera de las cadenas de ribosa. Sin embargo su análisis matemático de la forma seca no indicaba una estructura helicoidal. Fue al principio de 1953 cuando concluyó que las dos formas tenían dos hélices.

Rosalind debía estar ya al tanto de la hostilidad de Wilkins, y le pagaba con la misma moneda. Cuando tuvo resultados que parecían contradecir que la forma seca de ADN no era fácil de asimilar a una hélice, una estructura que, sin tener pruebas, a Wilkins le atraía, anunció (no se sabe a ciencia cierta si con mucha difusión) un jocoso "party" para enterrar la difunta teoría; invitado a hacer el responso: Maurice Wilkins. Las pruebas pusieron a Rosalind en su sitio y quizás a su pesar tuvo que admitir que los resultados de la forma B eran una doble hélice.

¿Sirve de algo la confidencialidad?

Watson y Crick no habían parado de cavilar sobre el ADN, y, deseosos de averiguar cuanto pudiesen de los descubrimientos en el King's, se comunicaban frecuentemente con Wilkins. En enero de 1953, espoleados por la publicación por Pauling de un modelo de tres hélices (similar al que ellos habían hecho en 1951), decidieron que iban a resolverlo antes de que Pauling o cualquier otro lo hiciese. Dos pruebas del trabajo de Franklin fueron cruciales para su modelo correcto: la primera una nítida foto de la forma B tomada en mayo de 1952 y rotulada "51" que Gosling había dado a Wilkins como parte de su trabajo experimental de doctorado y que Wilkins enseño a Watson sin que Franklin lo supiese; y la segunda el informe al MRC que fue facilitado a Watson y a Crick por Max Perutz, miembro del comité del MRC que evaluaba el trabajo en el laboratorio de Randall. El informe tenía detalles del trabajo (no publicado) de Franklin, incluyendo su identificación de la celda unidad como perteneciente al grupo cristalográfico monoclínico C2 centrado en la cara. La foto confirmaba el patrón helicoidal, y el tipo de la celda unidad le indicaba a Crick, un físico con más conocimientos cristalográficos teóricos que Franklin, que las hélices iban en direcciones opuestas. Ya a primeros de marzo tenían ellos el modelo.

Para situarse imagine el lector que los fichajes del Barcelona los tuviese que aprobar el Real Madrid, algo así es la evaluación que se hace en la investigación científica. Todo resultado científico es en principio confidencial y, en buena ley, no debe salir de las cuatro paredes entre las que se ha obtenido. Hasta que no se publican, las tesis doctorales son asimismo confidenciales y quien supervisa o corrige una de ellas, en su todo o en parte, está obligado a no divulgar nada de lo que allí se dice. En este caso la confidencialidad fue claramente rota por Wilkins. Especialmente estrepitosa fue la acción de Perutz, pues rompió la confidencialidad de un informe sometido para evaluar el trabajo de un grupo, algo que para el grupo es prácticamente obligatorio si quiere sobrevivir. No es extraño que los comités de evaluación sean mirados con cierto recelo. En otro ambiente estas acciones serían objeto de una denuncia, pero no es así en la investigación, donde todo acaba siendo asimilado, "para el bien de la ciencia", en decisiones en las que cada científico se coloca según el poder que tiene. ¿Por qué? Porque los científicos son siempre evaluados y juzgados inicialmente por sus colegas, generalmente por los que tienen más poder que ellos. Es más el evaluador permanece anónimo para el evaluado, no al contrario. Lo más sorprendente es que nadie se haya rebelado ¿será que los investigadores no son tan inconformistas como ellos se pintan?

¿Qué es lo que le faltó a Rosalind Franklin?

Franklin, todavía descontenta en King's, había gestionado irse al laboratorio de J.D. Bernal en el Colegio Universitario Birkbek, y se apresuraba a terminar el trabajo sobre la forma A antes de irse. No estaba enterada de que se corría la "carrera por la doble hélice". No obstante en febrero de 1953 volvió a inspeccionar la foto nº 51 y comenzó a analizarla. Varios días después concluyó que tanto la forma A como la B eran hélices bicatenarias, aunque no tenía la solución para la disposición de las bases en su interior. Gosling y ella esbozaron hacia mediados de marzo un artículo sobre la estructura molecular más probable. Apareció en Nature el 25 de abril, corregido y aumentado, junto al anuncio de Watson y Crick, pero el borrador se había redactado antes de que se hubiesen enterado del modelo de Watson-Crick.

Se desprende de los cuadernos de Rosalind (véase Anexo I), y del borrador del artículo que escribió antes de tener idea del modelo que propusieron Watson y Crick (véase Anexo II), que llegó a deducir la estructura de doble hélice de manera independiente y puede que a la vez que la célebre pareja. Le faltó sin embargo un aspecto fundamental, que explica la propiedad del ADN de contener la información genética de manera que su replicación genera automáticamente dos copias que llevan exactamente la misma información. Es lo que se conoce como "apareamiento de las bases", el que frente a timina solo cabe que se coloque la adenina, y que la guanina se enfrente siempre a citosina. Si creemos a Watson a

él se le ocurrió haciendo recortables con la forma de los cuatro compuestos y jugando a casar unas con otras. Ya puestos a hacer amigos, Watson y Crick dejaron a Jerry Donohue en el campo de los que se sintieron menospreciados, era un químico que le hizo ver a Watson que la forma química de las bases en el ADN no era la que él pensaba sino otra (la forma correcta es la que los químicos llaman ceto y no la enol).

Según Frederick Dayton, que fue profesor de Rosalind en Cambridge, a ella le faltaba la capacidad de ayudar a que el receptor de sus ideas viese que los hechos hablaban por sí mismos. Esto mismo Crick lo interpretó como que carecía de capacidad especulativa, lo que ciertamente a él y a Watson no les faltaba. Lynne Osman Elkin opina que Rosalind no desarrolló esa capacidad especulativa porque no tenía nadie en King's con quien discutir.

Pero quizás a Rosalind Franklin le faltó sobre todo categoría en el escalafón, y sobre todo desparpajo, algo que Crick y Watson se prestaban mutuamente y que les permitía tener conexiones e informaciones que Rosalind puede que nunca tuviese.

El sutil ejercicio del poder

Muestro, y traduzco, a continuación la carta que Randall envió a Rosalind Franklin, conminándola a dejar de pensar sobre el ADN. He intentado conservar el sabor que subyace a la literalidad de las palabras, pero recomiendo que quien se maneje en inglés intente leer el original, también es preciso estar un poco al tanto de los significados sutiles de la "cortesía" inglesa.

UNIVERSITY OF LONDON KING'S COLLEGE.

From The Wheatstone Professor of Physics,
J. T. RANDALL, F.R.S.

TEMPLE BAR 5653. STRAND, W.C.2.

Miss R.E. Franklin,
Birkbeck College Research Laboratory,
21 Torrington Square,
London, W.C.1

17th April 1953

Dear Miss Franklin,

You will no doubt remember that when we discussed the question of your leaving my laboratory you agreed that it would be better for you to cease to work on the nucleic acid problem and take up something else. I appreciate that it is difficult to stop thinking immediately about a subject on which you have been so deeply engaged, but I should be grateful if you could now clear up, or write up, the work to the appropriate stage. A very real point about which I am a little troubled is that it is obviously not right that Gosling should be supervised by someone not specifically resident in this laboratory. You will realise that the necessary reorganisation for this purpose which arises from your departure cannot really proceed while you remain, in an intellectual sense, a member of the laboratory.

Yours sincerely,

J T Randall

KING'S COLLEGE, UNIVERSIDAD DE LONDRES
De J. T, Randall, profesor "Wheatstone" de Física F.R.S.
(F.R.S.: Abreviatura de "Miembro de la Real Sociedad")
TEMPLE BAR 5653
STRAND, W.C. 2

Todo lo anterior está impreso en el membrete, y ya es de por sí una exhibición de poder, las cartas de Rosalind Franklin, mas o menos con la categoría de postdoctoral, se escriben siempre en papel con membrete genérico del King's. También hay que advertir que la carta de Randall está mecanografiada, algo que Rosalind Franklin hace en los borradores de sus trabajos pero no en su correspondencia. No puedo saber exactamente las facilidades respectivas que tenían un catedrático y un postdoctroal en King's pero creo que mecanografiar una carta denota dos cosas, un distanciamiento en la comunicación de una decisión desde una posición oficial, y la intervención de una mecanógrafa o al menos la disponibilidad de una máquina de escribir personal, las dos cosas símbolo en los cincuenta del estatus del catedrático.

Señorita R.E. Franklin
Laboratorio de Investigación del Colegio Birkbeck
Plaza Torrington 21
Londres W.C. 1
17 de abril de 1953

Querida señorita Franklin,

Sin duda recordará que, cuando hablamos sobre su marcha de mi laboratorio, usted aceptó que sería lo mejor para usted dejar de trabajar en el tema del ácido nucleico y hacerlo en otra cosa. Entiendo que es difícil dejar de pensar de repente en un tema en el que usted ha estado tan profundamente involucrada, pero le agradeceré si en estos momentos pudiera recoger, o escribir, el trabajo de manera adecuada. Un asunto muy concreto sobre el que estoy algo preocupado es que obviamente no es bueno que Gosling sea dirigido por alguien que no esté específicamente en este laboratorio. Se dará usted cuenta de que la reorganización imprescindible para ello, que surge de su marcha, no puede efectuarse mientras permanezca usted, de forma intelectual, como miembro del laboratorio.

Atentamente,
JT Randall

(firma autógrafa)

Comentaré dos puntos, el primero la frase "usted aceptó que sería lo mejor para usted". Es una frase hipócrita en la que una imposición se hace no solo contra la voluntad de la persona a la que se le impone sino que, para más sarcasmo, se le intenta hacer aceptar que es por su bien. El segundo es que prácticamente se prohíbe a Rosalind Franklin que siga pensando sobre el ADN, y aún más se le prohíbe que se comunique científicamente con su anterior estudiante. Creo que no se precisa más comentario.

ANEXO I

(Cuaderno de laboratorio de Rosalind Franklin, 1953)

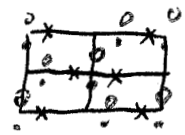
Results of superposition of 3-dimensional Patterson of crystalline Na DNA

Space group C 2

- axes found in superposition functions
- mirror planes impossible ∴ asymmetric C

Axial sections

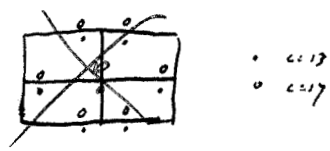
one axial section contains principal maxima (c=0)



the other no important maxima ($c=\frac{1}{2}$)

Peaks at heights 13/30 & 17/30

v strong, also in pseudo-hexagonal array



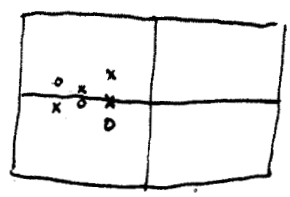
[N.B. hexagons appear canted, ∴ superposition method is not capable of eliminating central peak. But I can't reconcile centring w density ½ would have pseudo-cell of side a ~ b ~ 13 Å]

Possible structure having chains along a-c diagonal

but fibres can't be built this way – unreasonable

∴ search for alternative – gives:–

Figure of 8 structure (in projection) } peaks on chain in one cell.



2 pairs of chains / primitive cell (½ pairs / full cell)

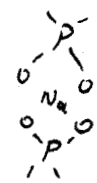
i.e. 56 nucleotides / full cell.

Consistent w density data if

I ~25% H_2O

II 2 P atoms per peak

i.e. 2 chains "back to back"



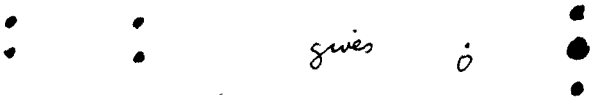
AK

See later, p46, for rejection of this "double helix" with P atoms in fibre period

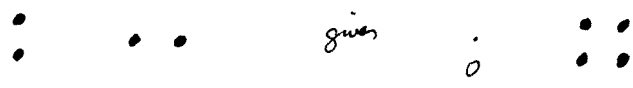
i.e. chains are ⫫ pairs, one upside-down w.r.t the other

Patterson peaks representing Phosphates

If pairs are all ⫫ // orient^{n} & resol^{n} is poor, maxima will give distances between mid-pt of pairs

i.e.  gives

If pairs ⊥ one another, no central overlap

i.e.  gives

does this indicate that pairs at $c=\frac{1}{8}$ are inclined to other pairs at $\frac{1}{4}$ ∴ give weaker peaks?

Peak at 4·3 Å on section $c=\frac{1}{2}$ would then be P–P distance with pair?

but in that case Na is not directly between P–P, or resolution would be destroyed by 2 Na–P peaks

Peak at height 0 lies on axis

∴ 2 P atoms attached to similar nucleotides

- apart from this, symmetry does not control nucleotide sequence except that ∴ a "back-to-back" pair of chains top half of one is similar to bottom half of other

Each chain only) peaks in unit cell

∴ can't reconcile nucleotide sequence with Chargaff's analysis

~~if all chains are same, sequence must be~~ ~~A B C D D C B~~

N.B. Symmetry axis does not affect sequence within one chain

Distance between neighbouring peaks 5.7Å (∴ 3 dimensions)

Nearest to agreement with Chargaff analysis would be

4 purines, 3 pyrimidines, with 2 purines & 2 pyrimidines occupying equivalent positions

- c.f. Broomhead, & similar XRd structures of adenine & guanine

Construction of models

Scale $\frac{1}{2}$" : 1 Å (as for Patterson diagrams)

Backbone chain

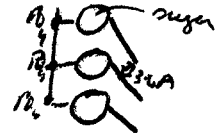
PO_4 tetrahedra – wooden balls, tetrahedrally pierced (France) planes in centre.
Sugar ring constructed (wire) on Furberg model

Coordinate models using only phosphates and sugar rings

show that it is possible to construct straight-chain model with P–P = 5·7 Å, all sugar rings in identical pos"n

Pyrimidines for pos'n of base chain, are then inclined at 25–30° to backbone
- this would leave inter-base plane spacing ~ 5·0 Å

Base-planes can be tilted on this model to give inter-plane spacing 3·4 Å but there is then very little overlap, i.e. very little van der Waals attraction



∴ if P–P distance 5·7 Å, not straight chain

– this (among other things) **eliminates a-c diagonal structure**

Putting **pyrimidine rings ⊥ and in full contact** we can have 3 phosphates in straight line, at P–P distance ~ 5·7 Å

but 4th must lie well off this line to bring 4th pyrimidine into contact

(system involves some distortion of N_3 bonds)

[linked to sugar]

PTO

If next base is a purine then next P out-of-line contact ~~P~~ brings 6-ring of purine into contact with pyrimidine

This arrangement for 1 or 3 P has sugar ring nearly || to P chain

⇒ 13 base rings either ⊥ or inclined to chain

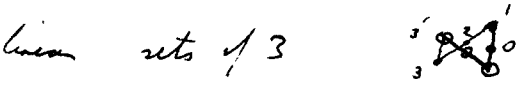
Backbone chain and symmetry at $c = 0$

Consider 2 pairs of chains 1, 1′ and 2, 2′, separated by $b = \frac{1}{3}$

1 is related to 1′ and 2 to 2′ by diad axis

1 —— 2 and 1′ to 2′ by rotation through 180°

Construct wire model of chain corresponding to Patterson peaks

i.e. 7 steps of each 5·7 Å, with 3 successive sets of 3  + reasonable angles

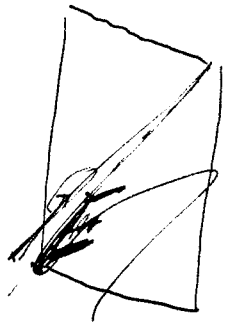
⇒ angle 0–1–2 ~ 100°

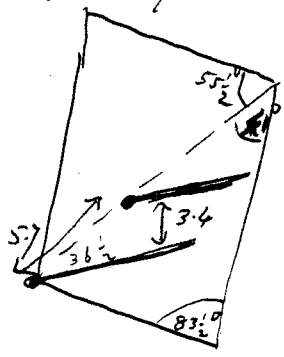
angle 2–3–3′ ~ 110°

For accurate model these angles would be equal, since 3-3′ ∥ 1-0′

∴ angles 100 – 110°

Suppose length of pyrimidine nucleotide is in a-c plane
Then for a-c diagonal peak of structure (i.e. peaks 1'-0-1)



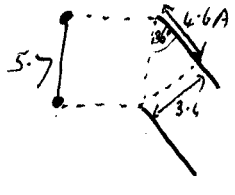


$\sin^{-1}\frac{3.4}{5.7} = 36\frac{1}{2}^\circ$

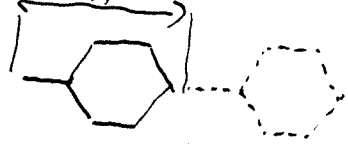
∴ if ~~ba~~ plane of pyrimidine is ⊥ a-c plane, it makes
∠ $36\frac{1}{2}^\circ$ with phosphate chain
and $36\frac{1}{2} + 41 = 77\frac{1}{2}$ with fibre axis

Unlikely the ∠ between base & backbone is so small
- more probable base is also inclined to a-c plane
- this would make its height more nearly ⊥ to both backbone chain and fibre axis

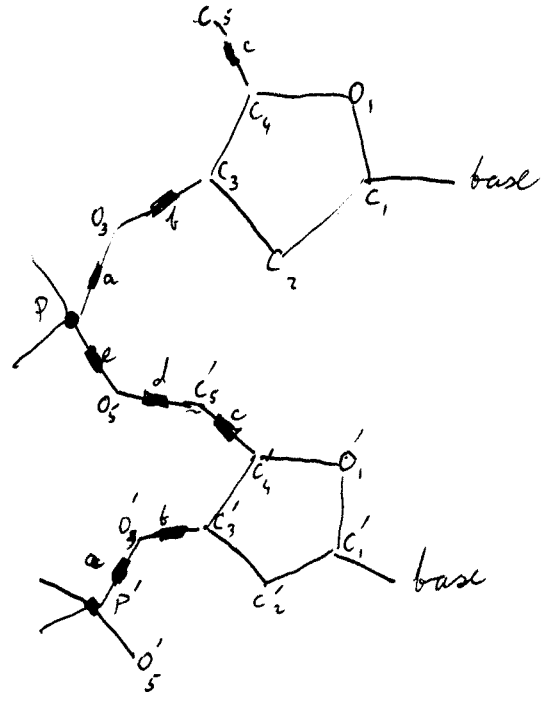
In general, if all P-sugar are equivalent, then $C_1 - C_1' = 5.7$ Å



i.e. no overlap of // 4·6Å pyrimidines



∴ sugar rings must be skew to one another,
with ⊥ distance between bonds C_1N and $C_1'N'$ ~ 3·4 Å
- bases then // one another but differently oriented



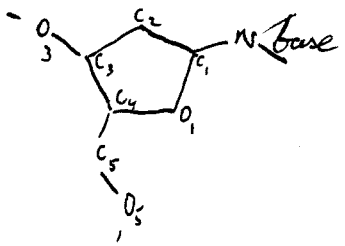
Free rotation of a, b, c, d, e

Wire model of backbone chain and sugar rings

Scale 1" = 1Å

Sugar ring constructed as - Furberg and Beevers + Cochran

i.e. 4 atoms coplanar and C_3' ½Å out of plane, bringing O_3 on to plane



All free rotation obtained by making joints with curtain ring

PO_4 tetrahedra

Requirements

1. Neighboring P on chain 5.7" apart
2. 2 un-bound O of PO_4 facing away from sugar
3. $P\begin{smallmatrix}O\\-O\end{smallmatrix}-$ near vertical plane (Fraser, but this was not for structure A)
4. Possibility of placing neighboring bases parallel at ~ 3.4 Å between planes

Observations

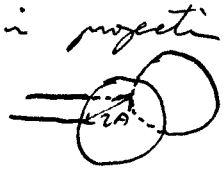
Distance apart of $P\,P'$ determined by rotations b, c, d, eg. c

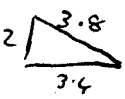
Distance apart of $C_2\,C_5'$ —————— a, b, e

(closest approach ~ 1.2")

Fully extended chain gives $P-P$ ~ 6.8 Å

If vdw contact d ~ 3.4 Å between bases, nucleic pyrimidines are ~ 3Å in diameter abt C_1N bonds, seen in projection ⊥ plane of rings, must ~~be~~ be > ~ 2Å apart

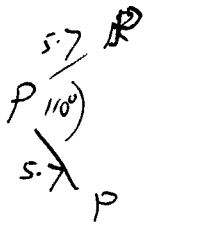




∴ ~~separation~~ distance between N and N' > 3.8 Å

It doesn't seem to be possible to put 3 P groups in a line running 5.7 Å and have C, N bonds in pairs for 11 bases at 3.4 Å separation. Is it possible for 3 P making bend of 110°?

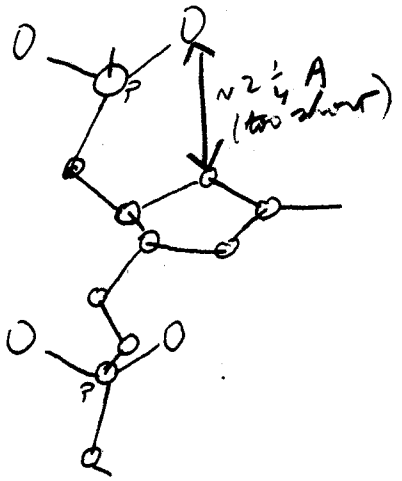
If so, perhaps structure has groups of 2 or 3 bases in 11 contact, and break of contact when chain turns a corner



= this config it is easy to avoid steric hindrance of sugar rings. Not easy for P 5.7 P 5.7 P

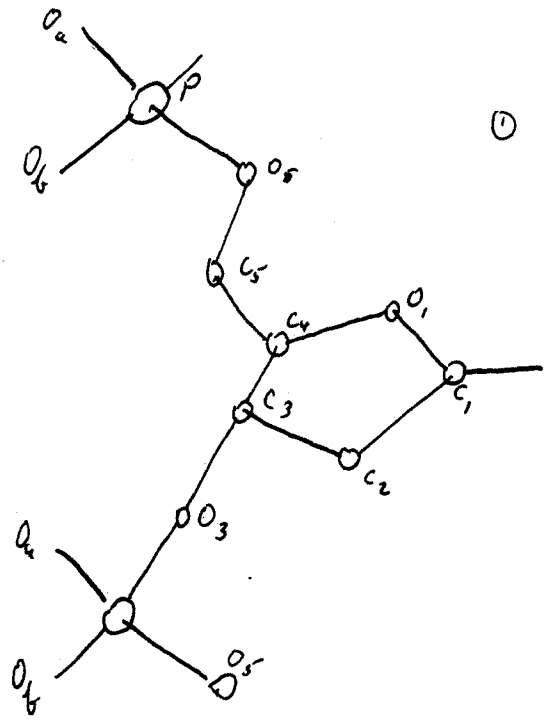
Arrangements of backbone chain with P-P:5:7

1. Sugar rings ~ ⊥ P-P makes it easier to avoid steric hindrance



This leaves $P\langle^{O}_{O}$ directed to one side of sugar ring

2. Sugar rings ~ // P-P — again $P\langle^{O}_{O}$ to one side



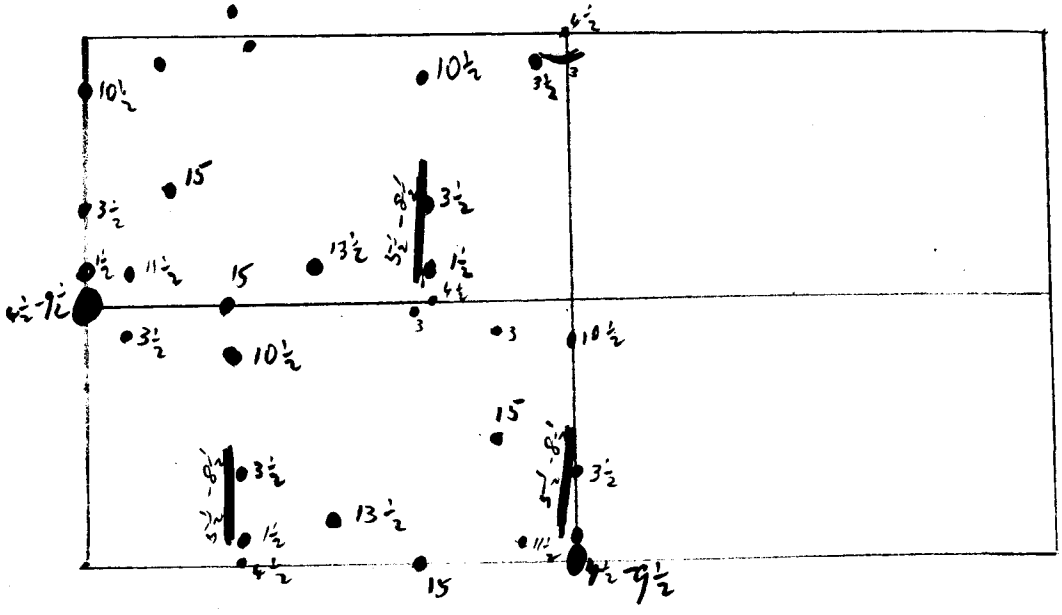
Steric factors

① Make C_5 equidistant from O_3 and O_b

② C_3 tilted away from O_a, but not equidistant betw. O_a and O_5 ∴ this would make C_2 approach $O_{5'}$ ∴ compromise

③ $P-O_{5'}-C_5-C_4$ co-planar
This keeps $O_{5'}$ at max. (& equal) distance from O_1 and C_3

M 9.B



/ figure of eight A.K.

Objection to α structure

Part of chain -1, 0 - 1 lies along a - c diagonal
P-P of pair at 0 must lie in a - c plane, ∴
Patterson shows that this must be ∥ along a - c diagonal
i.e. ∥ same direction as chain
∴ impossible

Superposition

To obtain function which gives members of atom-pairs
and not their centres, must take account of fact that
V_g is not strictly an axial section but on either side of it
Suppose P-P distance in pair = 4.5 Å
and corresponds to peak on section $\frac{3}{c}$ a = 5 cms
the axes at c = $1\frac{1}{2}$, a = $2\frac{1}{2}$ cms

This is M g B

3.2.53

M g B doesn't help solve the problem of how
the a - c diagonal streak is related to the $\frac{1}{4}$ - cell
heavy peak, nor does it show pos. alternative for
for chain

M2g

Peak at height 13 repeats at $\frac{1}{3}$ of away ∴ this ~~done~~ combining M2 and Mg by placing V_g not at height 0 but at height 13

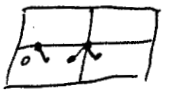
i.e. put OMg on 0/13 M2, and giving Mg displacement ÷ 8.3 cms, and M_2 $\frac{1}{2}$ shift where necessary axes are then at height −13 ∠ (2.7) i Mg (and a = 0)

∴ draw Mg c derived fm MG with axes at height 17

and M2 B ∴ ∵ M_2 with axis at 0/17

Principal positions of $c=2$ vectors : Patterson

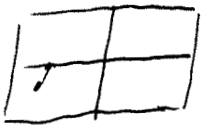
Sections 0 – 2 (twice)



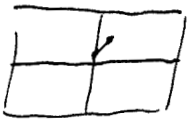
N.B. not at 0

∴ not 11 pairs

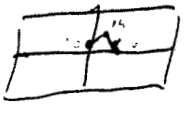
Sections 10 – 12



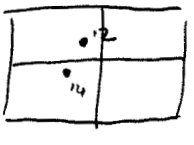
Sections 3 – 5
(or 2 – 4)



Sections 14 – 16



Sections 12 – 14



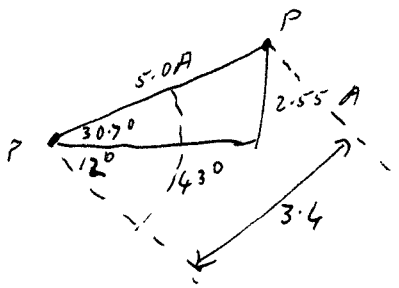
If there are 11 nucleotides per chain

mean inter-P spacing along C-axis is 2.55 Å

If P.P : 5.0 Å

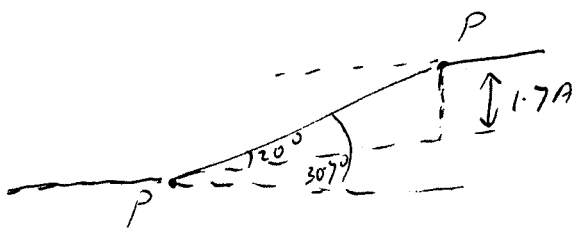
and inter-nucleotide base spacing 3.4 Å

this makes bases inclined at ~ 12° to ⊥ to fibre axis



but then no contact ∴ bases too small

or if chain is ≠



bases inclined ~ 11° to fibre axis

but only 20° to P-P chain

∴ impossible

Possible steps in chain

Predominant near-origin peaks in P are ~ 5Å

① within 2 peak 122 - strongest of all but c=15
(∴ in general pos')

∴ suppose this is important step in chain

also ② step along a-c diagonal (within 3

or ③ within 2, peak 7.5 (minus ① with a-component reversed)

If these are the 3 poss. chain steps, then total number of ②+③ is equal to no. of ①

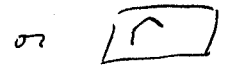
∴ total translation in 'a' = 0

There is no horizontal translation of ~5Å

2 consecutive ① can occur (within 2½ of 5)

or 3 " ①

or ① followed by ③ i.e. a  possible

but not ① followed by ② but not  or 

N.B. angle of ① in a-b plane is somewhat variable

- probably some peaks lie nearer 'b' axis, to account for resolution power on 'b' axis than 'a' although 'b' axis is further from main maxima

∴ by

② can be followed by ③ but not by ①

and ③ can be followed by ① (~~surely~~) but not by ②

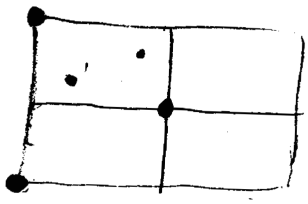
but ~~③~~ ③ following ① giving peak c~5, b~½ which falls in hole

Possible sequences of peaks ① ② or ③

① - ① - ① ② - ② - ② ③ - ③ - ③

① - ① - ③

M12

0


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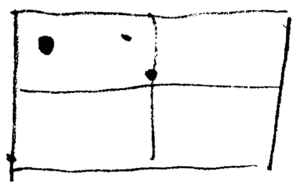

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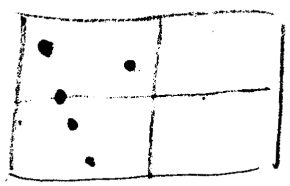

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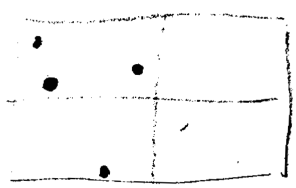


(cont^d from opp.)

~~This suggests Harker peak c+b a = 8.0~~

M 11

Peak on section 2, $a = -4.8$ cms $b = 3.3$ cms

Possible pos^{n}s of peaks in -ve region of Patterson marked in, and ~~mark~~ when they coincide with +ve regions of 2nd sheet, these were marked with dotted lines on M11

M12

Suppose M11 vector occurs twice consecutively. Then M11 is multiple, 2 structures being related by V_{11}

∴ M12 is M11 displaced V_{11} on itself

$a = -5^{-}.5$ $b = .$ ~~3.3~~ 3.5^{-}

M12 Axes on section 7, with a ~ -2.0 cms?

7.2.53

Try M13, using peak at height 4, a ~ 3.0 cms b = 0 in M11

distance of this peak from peak on section 1, ?

9.2.53

Possible pos^{n}s of axes in M11

c = 4	a = 3.5^{-} cms	c = 11	a = -3.5^{-} cms
c = 7	a = -0.5^{-}	c = 8	a = $+0.5^{-}$
c = 1	a = -2.5^{-}	c = 14	a = $+2.5^{-}$ — no ∴ gives peak on wrong a-c diag. or section 4
c = $4\frac{1}{2}$	a = 0	gives Harker peaks	c = 7 a = 4.8 and c = 11 a = -4.8 } v good

V_{11} gives 2 Harker peaks differing by c = 4, a = 10 cms

∴ superposition of a-c section on itself, with displacement c = 4, a = 10 gives pos^{n}s. Harker peaks & hence pos^{n}s. pos^{n}s of axes

10.2.53

Structure B

Evidence for 2-chain (or 1-chain helix) ?

49c - general trend is as for right continuous helix

(which differs from right discontinuous chain in integral no. residues/turn only in contrib^{n} of high order $\bar{J}$'s & the latter)

and this is indistinguishable from double helix with residues on each having same z-value, since 2nd chain has opp. signs in ξ & η terms (eq 1-2), & eq^{n} 2 contains only $R^2 = \xi^2 + \eta^2$ & $\tan\psi = \eta/\xi$

13.3.53.

Axes derived from Other possible positions of VII' derived from VII

$\frac{1}{2}(a-5$	$\frac{1}{2}(c+2)$	$a-10$	$c+4$	Harker peaks
-4·7	6	-14·4	14	~~✓~~ ✓
-4·0	6½	-13·0	15	~~✓~~ ✓
7·0	6½	9·0	15	~~✓~~ ?
4·8	7	4·6	16	~~✓~~ ?
6·0	3	7·0	8	×
0	3	-5	8	×

Possible pos'ns of Harker peaks of V_{11} and axes in M_{11}

Harker peaks are a , c

at a-10 , c+4

then axes is at $\frac{1}{2}(a-5)$ and $\frac{1}{2}(c+2)$

Observed	a - 10 (obs)	c+4	$\frac{1}{2}(a-5)$	$\frac{1}{2}(c+2)$	
V' good (weighted)	1.4	$4\frac{1}{2}$ +	3.2	$(1\frac{1}{4})$ $1\frac{1}{2}$	~~a-5 ; 5 ?~~
no V' X	21.0	$6\frac{1}{2}$ -	13.0	$(2\frac{1}{4})$ 2	
	11.6	$9\frac{1}{2}$ +	8.3	$(3\frac{3}{4})$ 4	IID likis a = 8.0, c = 4
X	23.0	$10\frac{1}{2}$ -	14.0	$(4\frac{1}{4})$ 4	IIC ~~tiny $4\frac{1}{2}$?~~
V' good but axis better @ 6 ✓	12.8	$13\frac{1}{2}$ -	8.9	$(5\frac{3}{4})$ $5\frac{1}{2}$	MIIE ~ no good : origin peaks are lost
V v good	~~23.0~~ 19.8	$13\frac{1}{2}$ +	~~15.5~~	$(5\frac{3}{4})$ 6	
		20	~~14~~ 12.4	9 ✓	II B
		~~5½~~	15.6	$5\frac{1}{2}$ -	

M911

M9 shows a vector V11 having one end in common with end of V9

∴ put M9 on M11 with origin at a : 2.4 as b . 6 . 8

and OM911 is OM9 on 29M11

i.e. axes on M11 at a : 2.4 c : 1

if true axes are M9 axes

M911 like for axes on M9 axes

M. Wt

Mol. monoclinic unit cell 11.2×10^3

Vol. " " " $12230\ \text{Å}^3$

Suppose partial sp. vol. DNA is 0.55 (as did Riley)

Vol. of 22 nucleotides is $\dfrac{0.55 \times 330 \times 22 \times 10^{24}}{6.03 \times 10^{23}} = 6620\ \text{Å}^3$

$\therefore$ vol. assoc. water $= 12230 - 6620 = 5610\ \text{Å}^3$

$\simeq 45.8\%$ of volume

$\therefore$ Wt % of water is $\dfrac{45.8}{45.8 + 54.2 \times \frac{1}{0.55}} = \dfrac{45.8}{45.8 + 98.7} = \dfrac{45.8}{144.5} = 31\%$

Density of water in Na DNA

Dry density 1.63 sp. vol. .615

Density + 35% (wgt) of water = 1.52 (wgt) i.e. sp. vol. .658

vol. 1.35 g of ~~mixture~~ wet DNA : .889 cc

∴ vol. of .35 g water : .274 cc if vol. DNA is unit.

i.e. density of water : 1.28 g/cc

Suppose density of water : 1.00 g/cm³

Vol of 1.35 g wet Na DNA: .615 + 0.35 = .965 cc

density : $\frac{1.35}{.965}$ = 1.40 g/cc

Suppose density of water 1.00 ~~d~~ of mixture 1.52 i.e. vol 1.35 g : $\frac{1.35}{1.52}$ = 0.89 cc

sp. vol. DNA : 0.89 − 0.35 = 0.54

Suppose partial sp. vol. DNA 0.55

d ——— H_2O 1.00

Then for 40% H_2O, 1.4 g ~~occupies~~ occupies 0.55 + 0.40 : 0.95 cc

$d = \frac{1.4}{.95} = 1.475$

35% H_2O $d = \frac{1.35}{0.90} = 1.50$

30% H_2O $d = \frac{1.30}{0.85} = 1.53$

a - c diagonal

All Patterson peaks streak along forward a - c diagonal

- this suggest possibility of disorder in direction of diagonal

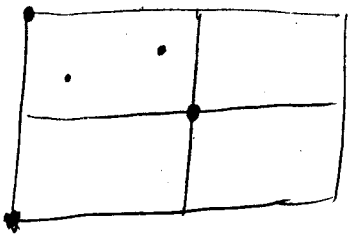
M II C

No good

- Lots 1 – 4 almost nothing

M12 (first 15 sheets only)

0



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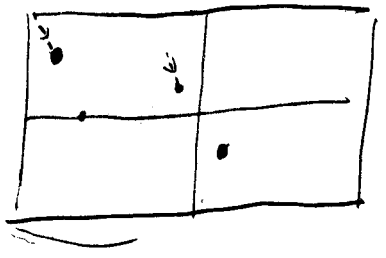

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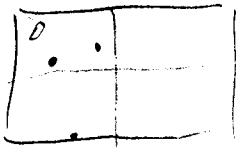

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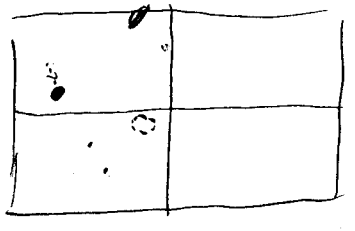

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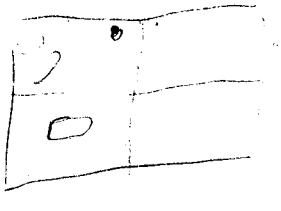

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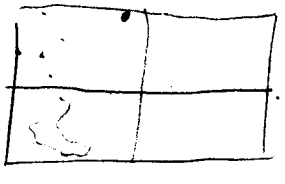

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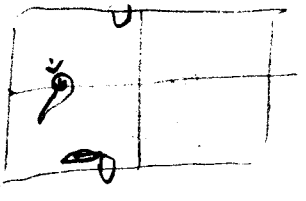

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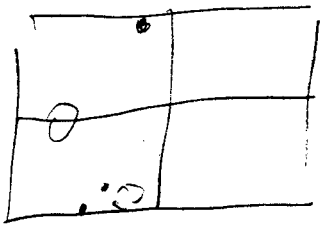

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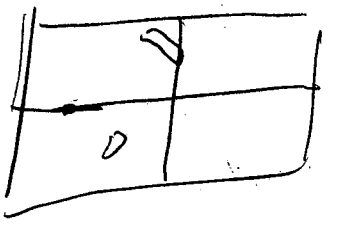

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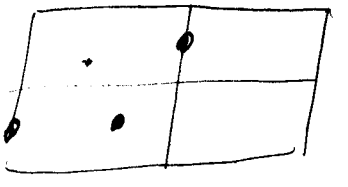

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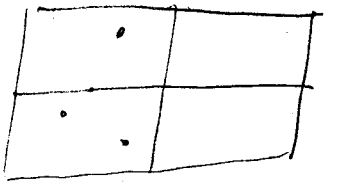

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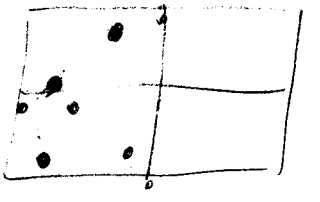

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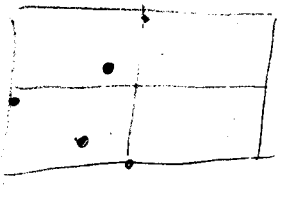

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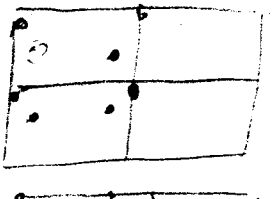
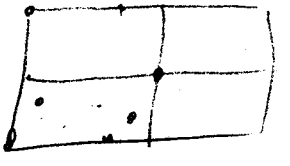


15

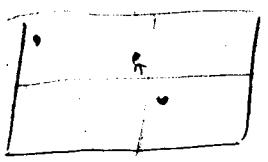


M 4. 13. 11

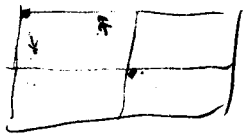
O M 13 on 1911, with MB reflected across ~~[illegible]~~ B plane
(i.e. turned over about axis

peaks used are 2 on section 0
and 1 on section 28
(marked with red crosses)

0



2



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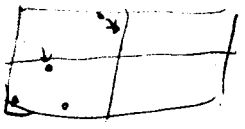
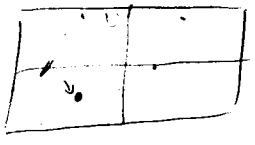
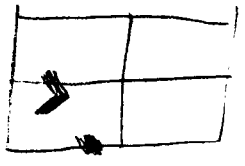


7



9



M 13.11

Possible positions of axes

Search for V_{13}', with onset & position. The axis lies ½ way between this and sheet 0

Section 16 — no : 7 & 8 not axial.

Section 13 → bad axial sections

25 "

26 "

5 less bad · axial sects 2/3, 17/18

See preliminary mention of Structure B on 15-2-53 p. 28

23.2.53

Structure B

A.K.

Photograph 51 C

3·4 A are − 158·5 mm on projection

∴ 158·5 = 2R tan 2θ where R is effective specimen-film distance for projection

For $d = 3.40$ A, $\theta_{CuK\alpha} = 13^\circ 4'$ $\tan 2\theta = 0.491$

$$R = \frac{158.5}{2 \times 0.491} = 161.4 \text{ mm} \qquad 2R = 322.8$$

Equator mm	tan 2θ	θ	d (Å)	1/d	λ/d	mm on Bernal chart	Conversion factor
20·2	·0625	1° 48′	24·5	·041	·0629	12·6	(·624)
54·7	·1695	4° 49′	9·16	·109	·168	33·6	·614
82	·254	7° 8′	6·19	·162	·249	50·7	·618
to 100	to ·310	8° 37′	to 5·13	·195	·300	61·7	·617
						mean	·616
120	·372	10° 12′	4·34	·230	·355		
to 152	to ·470	12° 36′	3·52	·284	·438		

These equatorial maxima do not correspond to maxima in $J_0^2(x)$,

which are approximately at 0, a, 2a, 3a

min. $J_0(x)$ for x	3·83 10·18
max. $J_0(x)$ for x	7·02 13·32

Best fit, assume 1st max. is missing (9·16 A reflexion is v weak)

The max. at $\frac{\lambda}{d}$: ·27, ~~·405~~ ·391, and ·149 ·27

gives $2\pi R r \simeq$ ~~5·24~~ 7·0 for $R = \frac{2\pi\theta}{\lambda} = \frac{1}{d} = \frac{\lambda}{2d}$ ~~·27~~ ·175

$\therefore r \simeq$ ~~13·4~~ $\frac{7.0}{2\pi \times 175}$: ~~12·2~~ 6·36

5th layer-line

1st diffuse spot

$2l$: 88 to 100 mm $\tan 2\theta$ .2725 to .310 θ 7°37′ to 8°37′

d: 5.80 to 5.10 $\frac{1}{d^2}$: .0298 to .0385

$\left(\frac{c^*}{5}\right)^2 : \left(\frac{3}{34}\right)^2$: .02165

$\therefore \left(\frac{\xi}{\lambda}\right)^2$: .0082 to .0169 $\frac{\xi}{\lambda}$: .0905 to .130

ξ : .139 to .200

1st max. for $J_5^2(x)$ has $x \sim 6.5$

$\therefore$ if r: 6.36A, $R = \frac{\xi}{\lambda} = \frac{6.5}{2\pi \times 6.36}$: .163

- fits better with max. $\doteq J_3(x)^2$

or J_5, $\therefore r$: 9.4A, giving ξ/λ : .113

3rd layer line

1st streak of spots

$2l$: 49 to 65 mm $\tan 2\theta$: .152 to .201 θ: 4°19′ to 5°41′

d: 10.24 to 7.76 $\frac{1}{d^2}$: .00953 to .01665

$\frac{3}{c^*} : \left(\frac{3}{34}\right)^2$: .00780

$\therefore \left(\frac{\xi}{\lambda}\right)^2$: .00153 to .00885, $\frac{\xi}{\lambda}$: .0391 to .0940

1st max. for $J_3^2(x)$ has $x \sim 4.2$

$\therefore R = \frac{\xi}{\lambda} = \frac{4.2}{2\pi \times 6.36}$: .105

for r: 9.4A $R = \xi/\lambda$: .071

but equator, taking r: 9.4A

should have maxima at $\frac{1}{9.4 \times 2\pi}(3.83, 7.02, 10.18, 13.32) = \xi/\lambda$
i.e. at .065, .119, .172, .226 and zero at 2.40 ″ and zero at .0406

or ξ : .100, .183, .265, .348
and zero at ξ: .0625 i.e. exactly on observed strong peak

2 helices of different radius for simple case of whole number of residues per turn.

Following Cochran Crick & Vand (Acta Cryst. 5 581, 1952)

term $J_n(2\pi Rr)e^{in(\psi+\frac{1}{2}\pi)}$ becomes $J_n(2\pi Rr)e^{i(n\psi - n\phi + \frac{n}{2}\pi + 2\pi l z/c)}$

(in present case, $l = n$)

For equator $I = \left[J_0(2\pi Rr_1) + J_0(2\pi Rr_2)\right]^2 = J_0^2(2\pi Rr_1) + J_0^2(2\pi Rr_2) + 2J_0(2\pi Rr_1)J_0(2\pi Rr_2)$

For n^{th} layer line, $I = FF^* = J_n^2(2\pi Rr_1) + J_n^2(2\pi Rr_2) + J_n(2\pi Rr_1)J_n(2\pi Rr_2)\left(e^{in(\phi - 2\pi z^*/c)} + e^{-in(\phi - 2\pi z/c)}\right)$

$= J_n^2(2\pi Rr_1) + J_n^2(2\pi Rr_2) + 2J_n(2\pi Rr_1)J_n(2\pi Rr_2)\cos\left[n(\phi - 2\pi z/c)\right]$

Here terms in $J_n J_n'$ may give <u>negative contribution to intensity</u>

but on any layer-line, moving out from the meridian, when the first maxim appears in a J_n contribution (i.e. for the J_n term corresponding to largest value of r) there is no negative term in I, since all other J_n terms are small and +ve

∴ even for complex helical structures, first maxima should give maxim diameter.

On equator, first max. (in J_0^2), excluding central max, involves -ve J_0, ∴ max. diameter can't necessarily be got from equator

In general

$$F_n = e^{in(\psi + \frac{\pi}{2})} \left[J_n(x_1) e^{in\alpha_1} + J_n(x_2) e^{in\alpha_2} + \cdots + J_n(x_\rho) e^{in\alpha_\rho} \right]$$

where $\alpha_q = \phi_q - 2\pi z_q / c$

and $x_q = 2\pi R r_q$

and $I = FF^* = \sum_{\rho}^{\rho} J_n^2(x_\rho) + \sum_{\rho \neq q}^{\rho q} J_n(x_\rho) J_n(x_q) \cos[n(\alpha_\rho - \alpha_q)]$

and same argument applies

i.e. first max. give outside radius

but later max. and equatorial non-central max. complicated

2-strand helix with pairs of groups at opp. ends of diameter

$x_1 = x_2$, $\alpha_1 - \alpha_2 = n\pi$

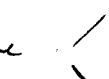
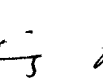
$\therefore$ layer-lines absent for n odd

and $I = 4 J_n^2(x)$ for n even

Conclusion

Structure B does not fit right helical theory, even for low layer-lines. ξ-values of first maxima are too small for right-stranded helix, & even more so for multi-stranded.

Closing radius of helix so that layer-line maxima fit for right-stranded (i.e. $r = 9.4\,A$) gives a bad fit for equator

Outer layer  emanating from 3.4 reflection indicates repeat at 3.4 A is a major — i.e. riser  represents structure factor of motif figure, which exists only for $c = n \times 3.4$, & figure repeats for 3.4 A not as for helix

ie J_5 on 5th l.l.
(In R.E.F's terminology, there could still be two chains, unrelated by the symmetry)
See P. 46
A.K.

Structure B if helix is triple-strand helix

nic 2-strand, fitting 5th layer-line max. to $J_{10}(x)$, not require $r \sim 17A$ which is much too big

Suppose ~~diameter~~ radius of outer helix 8·5 A

This gives 3rd layer-line max. at ξ/λ = ~~·122~~ ·0805
at 5th ——————— ξ/λ = ·122

– these values are reasonable, because max $\doteq J_3$ — J_5 is near outer edge of peak

For equator, we then have max. at ξ/λ = ·072, ·132, ·190, ·250
and 1 zero for ξ/λ = ·045

– this still has zero rather near the 2~~·5~~ A equator spot

Length of helix of ~~diameter~~ radius 8·5A pitch 34A

$l^2 = 34^2 + (8{\cdot}5 \times 2\pi)^2 = 1156 + 2850 = 4006$

$l = 63{\cdot}3$

∴ distance between atoms on 8·5A radius helix = $\frac{63{\cdot}3}{10}$

$= 6{\cdot}3A$

Intensity of inner \ / / \ compared with rest of photograph suggests that outer helix (~8·5A) is heavy part of structure, i.e. P

For radius 10A

$l^2 = 1156 + 3950 = 5106$ $\quad l = 7{\cdot}15A$

For radius 9·4A $l^2 = 1156 + 3490 = 4646$ $\quad l = 6{\cdot}82A$

[1] right-hand helix as above = basis of structure B,
the structure A is probably similar, with P-P distance
along fibre axis < 3.4 A, probably 2-2.5A
(c.f. 2A indicated by pos^{n} of P-P peaks = Pattern
d 2.5A " " 11th layer line reflection)

Many kind a.k.

Single-chain helix for structure B rules out atom-pairings for structure A (with 7 pairs in fibre period)

∴ single chain has 3.4 Å in c between neighbouring P

∴ structure A has less

There is correct a.K.

Suppose important translation in Patterson is

$c = \frac{1}{11}$ $b = \pm\frac{1}{11}$ $a = -\frac{2}{11}$ (corresp. approx. to Patterson peak)

Then important reflections are those for which

$$h\left(-\frac{2}{11}\right) + k\left(\pm\frac{1}{11}\right) + l\,\frac{1}{11} = 1$$

$$-2h \pm k + l = 11$$

$$-2h \pm k = 11 - l$$

i.e. on 11th layer-line and expect only 0,0,11

and mass of reflections on layer-lines 6, 7, 8, without (00l) =

does not indicate steps of $\sim\frac{1}{7}$ in c

These reflections due to the bases. R.E.F. missed this. a.K.

Structure B

7th layer-line

2ℓ : 122 – 138 mm to 2θ : 378 to 427

θ : 10° 21′ to 11° 34′ d : 4·28 to 3·84 Å

$\frac{1}{d^2}$: ·0546 to ~~·0377~~ ·0678

$\left(\frac{H}{C}r^2\right)$: $\left(\frac{7}{64}\right)^2$: ·0424 ∴ $(\xi/\lambda)^2$: ·0122 to ·0254

ξ/λ : ·110 to ·159

I.r max. for $J_7(x)$ has $x \sim 9.0$

∴ for $r = 8.5$,

$R = \xi/\lambda = \frac{9}{2\pi \times 8.5}$: ·168

for r : 9·4 ξ/λ : ·152

N.B. Here we expect observed max. to be displaced inwards : Lorentz etc

9

9th layer-line should also have max. of same value as 3rd

i.e. ξ/λ : ·039 to ·094

– this is apparently absent

3-chain ~ 2-chain helix?

Chains are not equally spaced, ∴ this would mean n^{th} layer-line contains 3Zs

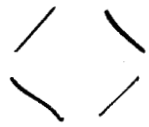
∴ 3-chain helix is highly improbable

∴ chains would be non-equivalent (2 chains ~~change~~ would be equivalent)

Also ∵ structure A believed to have 2 chains / unit cell

R.E.F. is at last making the correct correlation between the A and B. A.K.

N. B.

Outer Pattern  emanating from 3·4 spot is not present

of pattern  from origin. If it were they would

cross on 5th layer-line

Inner  meets  on 5th layer-line

$J_{10}(x)$ has first max. for $x \sim 11.8 = 2\pi R r$

Observed max. on equator, $R = .162$ & $.195$

$\therefore r = 11.6$ & 9.6

Here observed max. is displaced inwards $\therefore$ 3rd max. of J_0 for $r = 9.4$A has $R = .172$

Density

Volume of cylinder radius 10A, height 34 A $= \pi \times 100 \times 34 = \frac{10700}{3400}$ A^3

Vol. dry nucleotide (density 1·63) = 336 A^3

$\therefore$ no. nucleotides / cylinder $= \frac{10700}{336} = 32$

Since some fairly dry fibres give structure A, density of cylinder is prob. near density of dry DNA, water lying mainly outside cylinder.

$\therefore$ this suggests 3 chains

but not easy to reconcile w structure A

For helix of radius 8·5A Vol. cylinder $= \pi \times 8.5^2 \times 34 \simeq 7700$ A^3

no. nucleotides $= \frac{7700}{336} = 23$

A form
Superposition map

5. 3. 53

M 11

Uses peaks on section 2 unit, it seems, must be a P-P peak. Other superpositions may be fruitless : used part of Patterson not due to P-P

V 11 is clearly multiple — or, more probably, peak is due to agglomeration of near-equal vectors. ∴ should be several valid positions for axes (as described q. 2. 53)

∴ take M11B and M11D and look for possible chain common to each — allowing shift & b-direction of one relative to other

To find correct pos^n of M11B w.r.t M11D, look for the 4 origin peaks in each

— these are at height 8 — 110 in M11B — & 3, 5 in M11D

(M11C no good, as shown by fact that origin peaks are lost)

M11B can be put on M11D in 4 ways

1/ axes of 0 on 11B may correspond height 15 on 11D

∴ ~~rotate through 180° and~~ put 0-7 on 15-8

i.e. on 15-8 upside-down

2/ 11B and 11D may give different enantiomorphs

— this corresponds to 0-7 — 0-7 rotated and upside-down

i.e. poss^ns are
i) 0-7 on 0-7
ii) 0-7 — 0-7 rotated and upside-down
iii) 0-7 on 15-8 upside-down
iv) 0-7 — 15-8 rotated

M 11 D 0-7 on M11 B 15-8 upside-down

11 D 0-7 at ~~~ -11 cms gives good fit for 3-5, quite good for 10~~
- 16.3   3, 5, 8, 10 all fit, and ~~but bad for 8~~ general fit good

∴ Impossible ∵ peaks at 5 not consistent w peaks at 0

M11 D 0-7 on M11 B 15-8 ~~rotated~~ rotated

11 D 0-7 at ~ +3.5 cms - gives 5-10 nearly coincident, 3 & 8 quite good
(2.7 cms given)

11 D 8-15 on 11 B 0-7 rot. , general fit good

M 11 D 0-7 on M 11 B 0-7

M 11 D at - 15.7 cms → good fit for 3, 5, 8, 10 but using v small peaks
general fit good on 0-7, bad on 15-8

M 11 D at - 8.7 cms.

M 11 D 0-7 on M 11 B 0-7 rotated and upside-down

~~11 D 0-7 at ~ 21.5 cms gives 3 & 5 good~~
~~general fit poor~~

Presumably during the [illegible] REF was
[illegible] no. ~~[illegible]~~ on the B form. "Rough draft"
dated 17 March 1953

MII superpositions 18.3.53

~~MII B and MII E both have origin peaks of heights 8 & 10~~
~~These have different 'a' values, ∴ must relate to different chains~~

~~How can MII E, 8-15 be fitted to MII B?~~

MII B has origin peaks at 8 & 10, MII E at 5, 7
These have different 'a' values, ∴ either belong to diff. chains
or to diff parts of same chain

A. Diff. chains, same heights

~~MII E 0-7 on MII B 8-15 upside-down~~

a: 0 b: +7.9 cms
general fit on other peaks less good

MII E 0-7 on MII B 8-15 rotated

a: b/2, b: 11.8 cms good on both sets

MII E 0-7 on MII B 0-7

a: ½ b: −1.0 cms

a: 0 b: −8.2 cms ~~origin~~ 0 peaks good
a: 0 b: +8.8

MII E 0-7 on MII B 0-7 rotated and upside-down

Is structure A triclinic?

2-axis can't pass through DNA chain ∵ asymmetric sequence

__but__ superpositions always show peak on axes

If peaks are P, axis can ∴ only be pseudo-axis

i.e. array of P monoclinic, but true symmetry triclinic

In this case, whole Patterson is wrong

∵ each reflection is unresolved pair, (hkl) and $(h\bar{k}l)$

[If structure is triclinic and P's ~ monoclinic will Patterson give ~ correct P-P vectors?]

__If__ structure truly monoclinic

__either__ chains don't go through axes

i.e. no peaks on axes

__or__ sequence of phospho-ester links is 3 3 5 5 3 3 . etc

– this is symmetric about phosphorus atom

but improbable a) ∵ evidence from enzymatic degradation

b) ∵ prob. impossible to get equally spaced bases, distance 3.4A

21. 4. 53

Intensities of structure A suggest equally spaced chains (pseudo-halving of cell)

Then unit has true diad $\frac{1}{2}$-way between planes

+ pseudo-diad through chain, relating phosphates only

- can we arrange units so that pseudo-diad applies to whole structure and true diad does not?

Pseudo-diad passing through P is only possible if chains are equally spaced.

Vertical distance between 2 chains would then be ~ same as shorter vertical distance in Crick model.

Shapes of Patterson peaks

All peaks are stretched along a-c axis

- can this be due to P···Na ≈ that direction ?

- well-resolved ~~beak~~ peak on C:2 suggests that Na does not lie half-way between 2 P's

Shapes of P-P, Na-Na, and P-Na peaks were calculated by approximating f^2 curve (or f_P, f_{Na}) by $e^{-a^2 s^2}$ and taking account of artificial temperature factor used

Results

P-P	r(A)	0	0.5	1.0	1.5	2.0	2.5	3.0
P-P	P(r)	115.2	108.0	88.8	64.0	40.4	22.6	10.9
Na-Na		66.3	59.4	42.6	24.1	11.4	4.3	1.3
P-Na		85.2	78.5	61.8	41.3	23.5	11.4	4.7
P-P + Na-Na		181.5	167.4	131.4	88.1	51.8	26.9	12.2
2 (P-Na)		170.4	157.0	123.6	82.6	47.0	22.8	9.4
Shape of peak for P-Na distance 3.0A		190.9	190.2	178.4	170.7	175.4	183.9	182.6
Shape of peak for P-Na 2.5A		204.3	214.4	213.0	211.7	208.8	197.3	169.2
Shape of peak for P-Na 2.0A		228.5	250.0	255.0	245.1	222.2	183.9	135.8

Isolated Patterson peaks mostly have max. dimension approx. twice min. dimension

½-peak width of P-P + Na-Na (Patterson) ~ 1.5 × 2 Å

∴ this is minim dimension.

∴ ∴ ½-peak width of P—Na combination must be ~ 3.0 × 2 Å

∴ this requires P - Na distance ~ 2.0 Å

7.5.53

Attempts to adapt Watson and Crick model to structure A

Modifications introduced

1/ P–P distance decreased from 7·0 A to 5·3 A
(as suggested by peaks on sections 2-3)

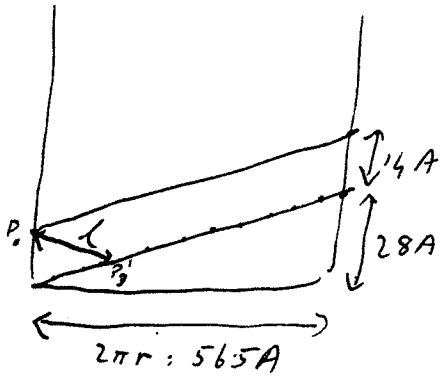
2/ 11 nucleotides per turn instead of 10
(as suggested by meridional peak on 11th layer-line)

3/ 2 chains of helix are equally spaced
(as suggested by pseudo-halving of cell
& indication on Patterson that only part of structure
repeats at half-cell height

4/ P on radius 9A instead of 10A
(as indicated by cylindrical Patterson)

Suppose chains equally spaced
and P's vertically above one another on chain

Draw schematically un-wrapped helix



Suppose P_0 P_3' are linked through
atom C_3' of same purine-pyrimidine
complex. Then on W & C model
$P_0 - P_3' = 15{\cdot}2A$

To obtain length of arc on un-wrapped helix, consider helix of
same radius, of which P_3' P_0 are is a part
Then this helix makes 3/11 of a turn ∴ length $\left(\frac{1}{2} - \frac{3}{11}\right) \times 28A = \frac{5}{22} \times 28A$
i.e. whole turn ∴ length $\frac{5}{22} \times \frac{11}{3} \times 28 = \frac{5}{6} \times 28 = 23{\cdot}4A$
∴ length of whole turn $= \sqrt{56{\cdot}5^2 + 23{\cdot}4^2} = \sqrt{3192 + 548} = \sqrt{3740} = 61{\cdot}2A$
∴ length of arc $l = \frac{3}{11} \times 61{\cdot}2 = 16{\cdot}8A$

8.5.53

Length of P-P' bracket through C_3's to same P_4-P_5 complex

Crick says 15.2 A

but this is inconsistent w other data of his model - too small

$C_1 - C_1' = 11A$

$C_1 - P \sim 3.5A$ (from wire model)

and these are fairly nearly co-linear

$\therefore PP' \sim 18A$

- this is consistent with other W & C data

When chain contracts & form structure A, $P - C_3$ can't decrease much - contraction must occur between C_3 — P on other side

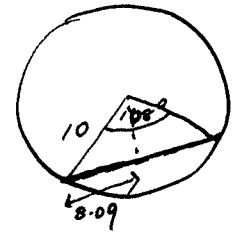
$\therefore PP'$ still $\sim 18A$

If $P_0 P_0'$ are vertically above one another, distance 15A

x-y sep of $P_0 P_3'$ is 16.18

z sep $(15.0 - 3 \times 3.4) = 4.8A$

$\therefore P_0 P_3' = \sqrt{16.18^2 + 4.8^2} = \sqrt{261.8 + 23.0} = 16.8A$



If P_0 rotated $\sim 1A$ behind P_0' on helix

$P_0 P_3' \sim 17.6A$

11-residue helix : structure A

Difficulties

Can they have one helix / primitive cell

∴ how account for 13 Å vector ⊥ C plane?

↳ how account for pseudo-beauty

Density

Vol. dry nucleotide, density 1·63 g/cc, = 336 A^3

Partial sp. vol. 0·55 (Kahler, J. Phys. Coll. Chem. 52 1948 676)

Suppose partial sp. vol. in structure A is 0·55

Then 330 g occupy $0·55 \times 330 = 181·5$ cc

1 nucleotide occupies $\frac{181·5 \times 10^{24}}{6·03 \times 10^{23}}$ $Å^3$ $= 296$ $Å^3$

1 molecule H_2O occupies $\frac{18 \times 10^{24}}{6·03 \times 10^{23}} = 29·85$ $Å^3$

Vol. of complete unit cell 2465 $Å^3$

1 nucleotide + 4 H_2O ∴ 415 $Å^3$ giving 59 nucleotides / unit cell

∴ 5 " " 445 55

6 475 51·5

7 505 48·5

8 535 45·7

9 565 43·3

Structure B. Suppose 24·5A reflection is (100) of hexag. close-packed rods

Then vol. unit cell: $\frac{2}{\sqrt{3}} \times 24·5^2 = 694$ $Å^3$ $\times 34 = 694 \times 34$ $Å^3$

Suppose this contains 20 nucleotides, i.e. M.Wt $20 \times 330 = 6600$

then density due to nucleotides only is

$$\frac{6600}{6·03 \times 10^{23} \times 694 \times 10^{-24} \times 34} = 0·465 \text{ g/cc}$$

∴ if true density is 1·52 g/cc

water content must be $\frac{1·52 - 0·465}{0·465} \times 100 = 227\%$ of dry weight!

If 40 nucleotides = unit cell

water content is $\frac{1·52 - 0·93}{0·93} = 63·5\%$ of dry weight

still high, but more reasonable

11. 5. 53

Equatorial reflections in structure B

Main reflection ~ 24·5 Å

If this is inter-helical distance, density is much too low

Some inter-penetration of helices in one direction must be possible, the spacing in other direction being 24·5 Å

but nucleo-protein gives same spacing, 24·5 Å,

and in this case, on W & C model, inter-penetration quite impossible

spacing measured on R & W's published photo

Distances in Å

	① R & W	② Structure B	Ratio ①/②
equatorial	15·4	7·5	2·05
2nd equatorial	21·7	10·3	2·1
3·4 Å reflection	111	57	1·95

Density calculations (see above) suggest packing is such that there are 2 helices per unit cell

(i.e. not hexag. close-packing

- reasonable ∵ helix hasn't got hexag. symmetry

M. Wt. of 20 nucleotides 6600

+50% H_2O 9900

∴ X-section of unit cell, for density 1.50 is

$$\frac{9900 \times 10^{16}}{6.03 \times 10^{23} \times 1.50 \times 34 \times 10^{-8}} \text{ Å}^2 = 322 \text{ Å}^2$$

If hexag. close-packing, $\frac{\sqrt{3}}{2} a^2 = 322$ $a^2 = 372$

$a = 19.3$

If distorted, $a\,b \sin\gamma = 322$

and $b \sin\gamma = 24.5$ (∵ strong reflection)

∴ $a = 13.1$ Å - impossible for W & C model

or Unit cell contains 2 helices

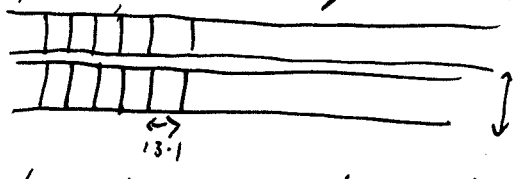
and $a = 26.2$ Å.

This is only slightly distorted from graphite-like structure,

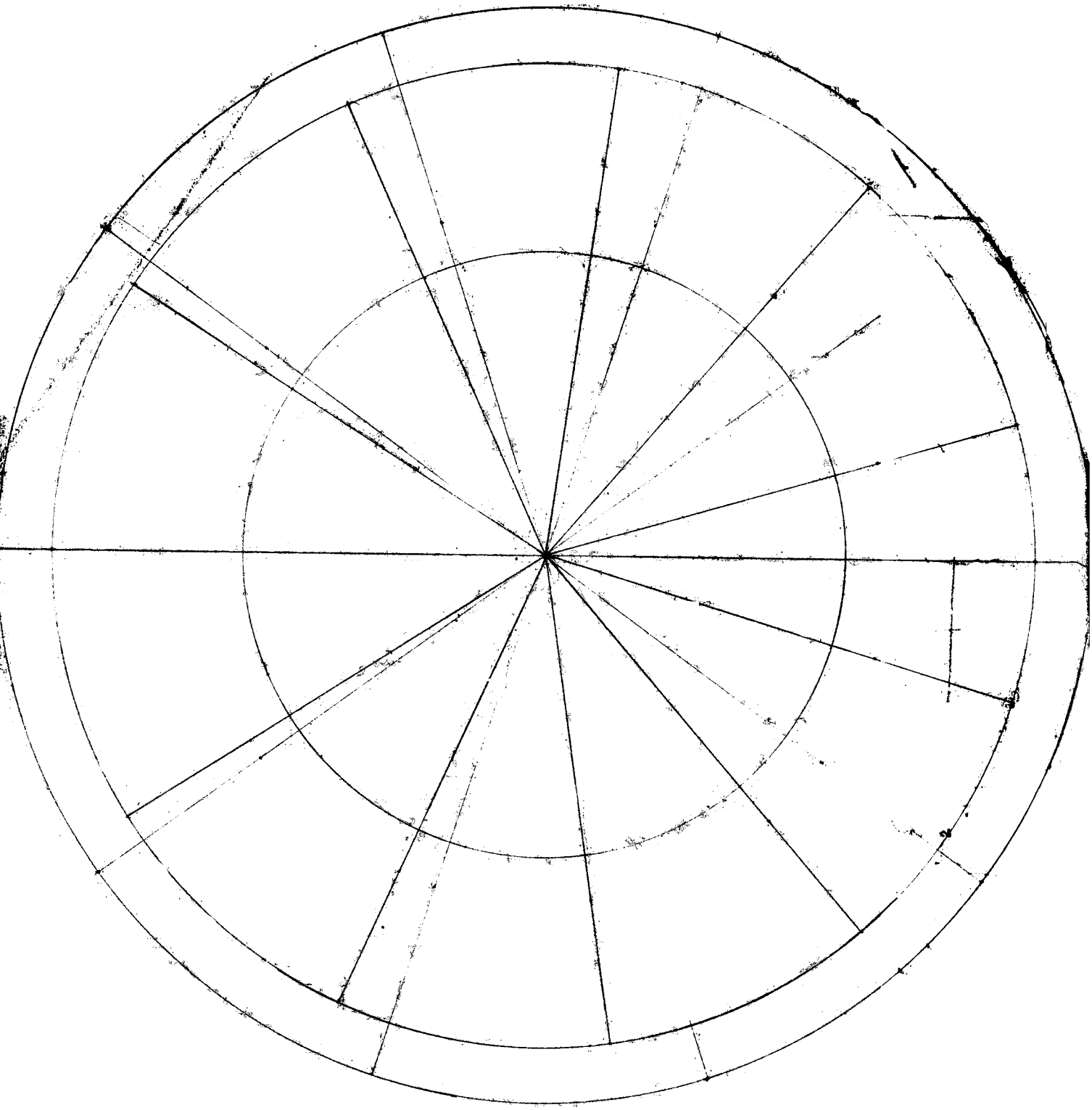
giving inter-helical distance ~ 16 Å

∴ Helices in sheets, with inter-sheet sep^n 24.5 Å and inter-penetrating

giving other dimension $\frac{322}{24.5} = 13.1$ Å



In nucleo-protein, then 13.1 Å must have to expand, and 24.5 Å stays const.



1cm = 1Å

W & C modified for structure A

Cylindrical Patterson suggests helix of diameter 18A

∴ suppose P_1' on helix of radius 9.0A

and 11 residues per chain (∵ 11th layer-line reflection)

Horizontal spt of P-P on 10A, 10-residue helix : $20 \sin 18° = 6.18$A

9A 11-residue helix : $18 \sin \frac{32.73°}{2} = 5.07$A

P-P for B : $\sqrt{6.18^2 + 3.4^2} = \sqrt{38.19 + 11.56} = \sqrt{49.65} = 7.05$A

A : $\sqrt{5.07^2 + 2.55^2} = \sqrt{25.70 + 6.53} = \sqrt{32.23} = 5.68$A

∵ near inter-base distance is const. ∠ of tilt ~ 40°.

Suppose ∠ of tilt 40° and $C_1' - C_1'$ const. at 11 Å

then horiz. spt of $C_1' - C_1' = 11 \cos 40° = 8.4$A

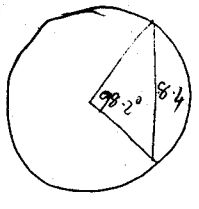
~~∴ C_1' lies on ⊙ of radius 4.2A~~

Suppose P_n and P_n' lie vertically above one another

(∵ 2-height peak suggests this ∴ approx. true)

Then on ⊙ containing all C_1', chord subtending $\frac{3 \times 360°}{11}$

at centre is of length 8.4A



radius : $\frac{4.2}{\sin 49.1°} = 5.55$A $\frac{4.29}{\sin 49.1} : 5.67$A

[for structure B, equivalent radius is $\frac{5.5}{\sin 54°} = 6.8$A

∴ radial extension of sugar + phosphate ~ same in both cases]

Horizontal spt of $C_1' - C_1'$ is then $5.55 \times 2 \sin \frac{360}{2 \times 11} = 3.12$A

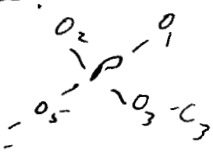
Total $C_1' - C_1'$: $\sqrt{3.12^2 + 2.55^2} = \sqrt{9.73 + 6.53} = \sqrt{16.26} = 4.03$A

Model giving approx right distances & reasonable angles

C_2 equidistant from O_1, O_5-

~~P - O_3 - C_3 - C_2 coplanar, ~ in vertical plane.~~

~~Also coplanar with O~~



giving max. r d'W distances for $C_2 O_2$ & $C_2 O_5$-

~ planar

P - O_5 - C_5 - C_4

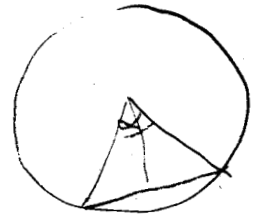
P - P 5·7 Å $C_1' - C_1'$ 4·04°

C_1' N points ~ 40° upwards (when $O_3 - C_3$ is below P)

Helix of radius 9.0 ~~Å~~ Pitch 28.1 Å

Length of one turn $= \sqrt{(2\times 9\pi)^2 + 28.1^2} = \sqrt{56.55^2 + 28.1^2} = \sqrt{3198 + 790}$

$= \sqrt{3988} = 63.15$ Å



$\alpha = \frac{360}{11} \times n$

x-cpt of vector on cylindrical Pattern

$= 2 \times 9 \sin \frac{360 \times n}{11 \times 2} = 18 \sin 16.35\, n$

$x_1 = 18 \times .2815 = 5.07$

$x_2 = 18 \times .5407 = 9.74$

$x_3 = 18 \times .7559 = 13.60$

$x_4 = 18 \times .9098 = 16.37$

$x_5 = 18 \times .9898 = 17.80$

Distances of atoms from axis in projection

with base tilt of 0°

① r	② $2\pi r$	③ $2\pi r \times .088$	$J_0(3)$
0.78	4.9	0.43	.95
1.31	8.2	0.72	.87
1.88	11.8	1.04	.75
2.28	14.3	1.26	.65
2.63	16.5	1.45	.55
2.73	17.2	1.51	.51
2.88	18.1	1.59	.46
3.06	19.2	1.69	.40
3.11	19.5	1.72	.39
(½) 3.35	21.0	1.85	(.31) .16
3.86	24.3	2.14	.14
3.92	24.6	2.16	.13
4.09	25.7	2.26	.08
4.10	25.8	2.27	.07
4.12	25.8	2.27	.07
4.44	27.9	2.45	.02
4.54	28.5	2.51	$\overline{.05}$
4.55	28.6	2.51	$\overline{.05}$
(½) 4.90	30.8	2.71	$(\overline{.15})\ \overline{.08}$
5.10	32.1	2.82	$\overline{.19}$
5.67	35.6	3.13	$\overline{.21}$
5.67	35.6	3.13	$\overline{.21}$

Total 21

Intensities in structure A

If one helix per lattice point then ~~equatorial~~ intensities depend only on structure factor of helix

∴ can't explain large diff. I for (130) and (200)

is this wrong, → large error in Patterson?

To calculate equatorial intensities, only require radial distance of each atom.

Suppose base complex tilted 40°.

Drawing (1" : 1Å) gives $C_1' - C_1'$: 11·2 Å

∴ (see 2 pages back) these lie on helix of radius 5·67 Å

Suppose tilt is about "diad" of complex. Then draw foreshortened complex by reducing distance of each atom from diad by factor ~~cos~~ 40° = 0·766. This gives projection

Then measure distance of each atom from axis

Intensities & positions of 1st equatorial reflection

Continuous transition from ~17 Å in near-dry DNA through 19 Å for structure A → >24 Å

This suggests that reflection has same significance in 2 phases
i.e. related to inter-unit distance in both cases

Intensity appears to have min. at ~19 Å

- c/ dry rather dry photo 34 in which reflect ~17-18 Å is stronger than round 19 Å in structure A

[Dry photo 34 has meridional arc of larger spacing than structure B (of ~36 Å) ?]

Spacing on photos showing A+B (e.g. 75, 5,) ~20 Å - intensity lit

52 & 53 are same specimen as 51, at higher RH (92%)
- they have same equatorial spacing

Tilt of bases in structure A

6, 7, and 8 layer-lines suggest $\sim 25°$

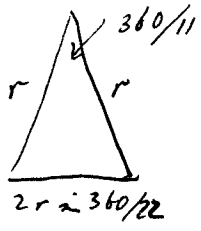
$\therefore$ ~~3~~ 5/3 mean values are 0·56, 0·48, ·44 for these layer-lines

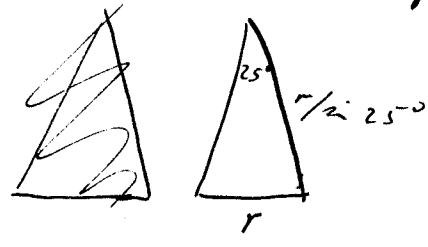
i.e. $\tan^{-1}$ 29°, 26°, 24°

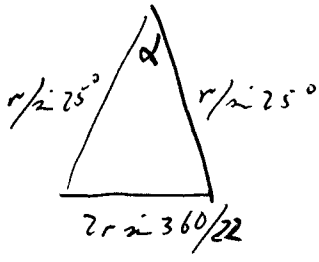
Suppose tilt 25°, find ∠ between planes of neighbouring bases

i.e. ∠ between ⊥'s to planes of bases

These lie on cone of semi-vertical angle 25°





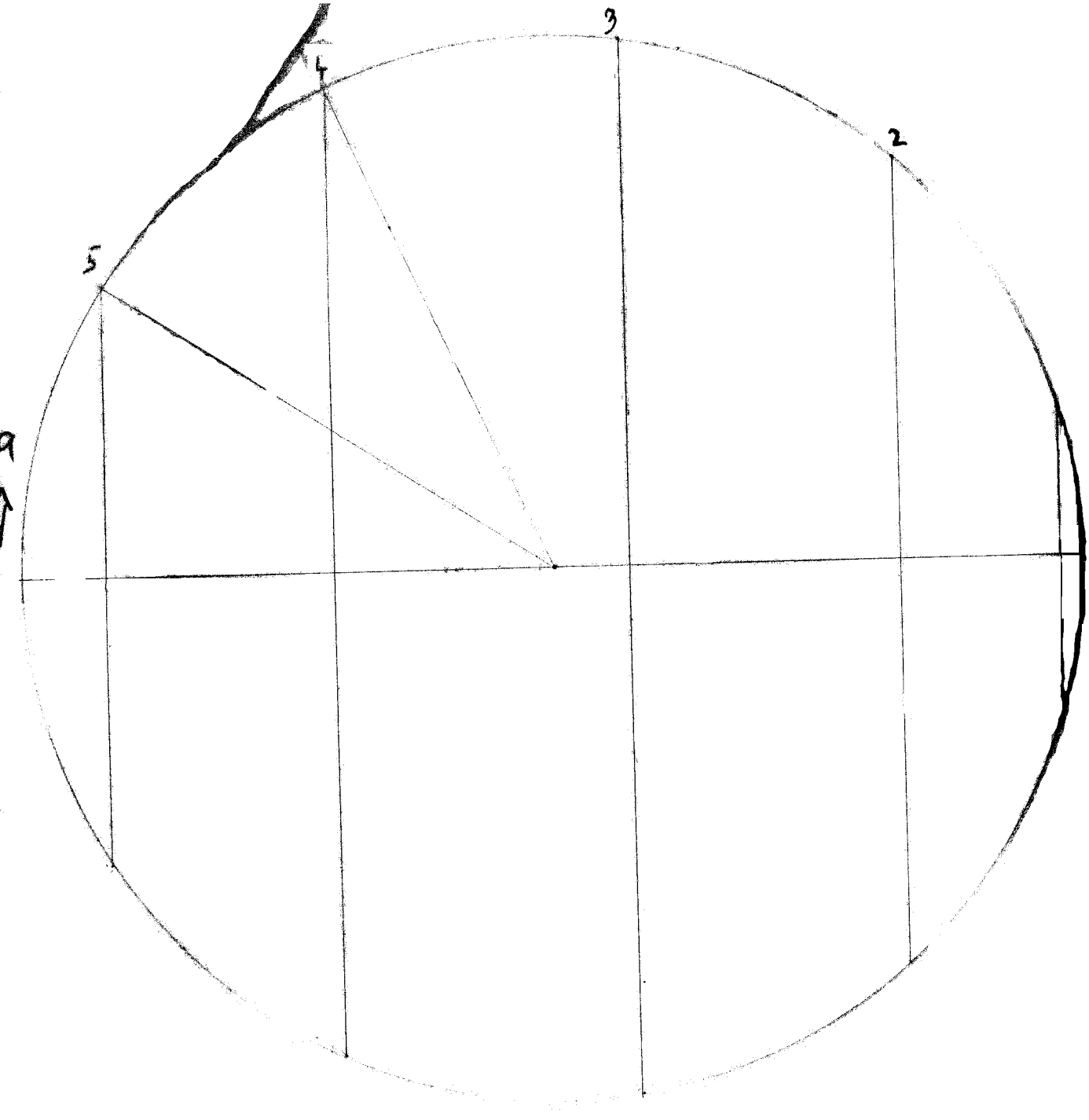


$$\alpha = 2\sin^{-1}\left(\sin\tfrac{360}{22}\sin 25\right)$$

$$= 2\sin^{-1}\cdot 2818 \times \cdot 4226 = 2\sin^{-1}\cdot 11.9$$

$$= 2 \times 6°\,50'$$

$$\simeq 13\tfrac{1}{2}°$$


3
4
2
5

Harker peaks for helix of radius 9Å (structure A)

	'a'	'c'	δ = $\frac{1a'}{22} \times 254$	'c' corrected Å	'c' section
1.	$18 \sin \frac{360}{22} = 18 \sin 16.36° : 18 \times .2818 : 5.07$	$\frac{c}{11}$	0.6	3.2, 17.2	
2.	$18 \sin 360 \times \frac{3}{22} : 18 \sin 49.1° : 18 \times .7559 : 13.6$	$\frac{3c}{11}$	1.6	9.3, 23.2	
3.	$18 \sin 360 \times \frac{5}{22} : 18 \sin 81.85° : 18 \times .9899 : 17.8$	$\frac{5c}{11}$	2.1	14.9, 0.8	
4.	$18 \sin 360 \times \frac{2}{11} : 18 \sin 65.5° : 18 \times .9100 : 16.4$	$\frac{7c}{11}$	1.9	19.8, 5.8	
5.	$18 \sin 360 \times \frac{1}{11} : 18 \sin 32.72° : 18 \times .5404 : 9.74$	$\frac{9c}{11}$	1.2	24.2, 10.1	

δ is : a-b sections are not ⊥ to fibre axis.

± δ depending on orientation (or hand) of helix

Examination of intra-helical vectors on 3-dimensional chart orientation hand to be as indicated opposite, & this gives + δ

Suppose the 2 helices on which P atoms lie are related by vector V_1

and suppose a vector v on Patterson is a P – X interaction, where X is any atom ~~other than P~~ (e.g. N_a)

then there is a similar vector $V_1 - v$

Suppose V_1 has $x = -1$ Å
$y = 0$
$z = 13$ Å (= section 14)

then find which Patterson peaks, un-accounted for by P-P vectors, occur in pairs v and $V_1 - v$. These will be P – X vectors

e.g. ~~xyz~~ $\underline{v}$ ~~section 10, $x = 6$ Å $y = 0$~~

$\underline{V_1 - v}$ ~~section 4~~

v section 12 $x \sim 8$ Å, $y \sim 7$ Å

gives section 2 $x \sim -9$ Å $y \sim -7$

ANEXO II

(Informe anual de Rosalind Franklin, 1953)

ANNUAL REPORT

1st JANUARY, 1953 - 1st JANUARY, 1954

ROSALIND E. FRANKLIN,

Birkbeck College Crystallographic Laboratory,

21 Torrington Square, W.C.1.

The work carried out during the past year may be divided into three periods:

1. 1st January to 16th March. King's College.

During this period I continued to work in King's College on the structure desoxyribonucleic acid. Two papers entitled:

"Fibre Diagrams of Sodium Thymonucleate:
I. The Influence of Water Content
II. The Cylindrically Symmetrical Patterson Function."

were written (in collaboration with R.G. Gosling) and sent to Acta Crystallographica in March 1953. These have now been published, and copies are attached to this report.

Further work on the 3-dimensional Patterson function was carried out, but no quantitative results were obtained in this way.

Measurements on the X-ray fibre-diagrams of Structure B (the less ordered form of sodium desoxyribonucleate, and that which we believe to exist more or less unmodified both in solution and in natural nucleo-protein) yielded a considerable amount of information. This is summarised in a note to Nature written in collabora-

-tion with R.G. Gosling (25th April, 1953) and entitled: "Molecular Configuration in Sodium Thymonucleate". A copy is enclosed with this report.

It is shown that the molecule of sodium thymonucleate in Structure B must consist of a two-strand helix, rather similar to that proposed by Watson and Crick (Nature, 25th April, 1953) but of smaller radius.

Since Structure B (NaDNA) has a 2-strand helical molecule, and since the change A $\rightleftharpoons$ B is, in general, readily reversible, it follows that a 2-strand helical molecule must also exist in Structure A. Evidence for a 2-strand helix in structure A was obtained from a study of the cylindrically averaged Patterson function.

2. March 1953 - November 1953. Birkbeck College.

Owing to unexpected delays in obtaining the necessary apparatus for carrying out a programme of X-ray crystallographic research on viruses, a substantial part of this period was spent in continuing the interpretation of the X-ray diagrams of nucleic acid and their Patterson functions. At the same time, a literature survey was carried out of previous work on the molecular structure of viruses.

The evidence for a two-strand helical molecule of the Structure A form of DNA was presented in a note to Nature, 25th July, 1953, written in collaboration with R.G. Gosling. A copy is enclosed. The helix is of radius 9A and has 11 residues per turn. The evidence is based mainly on a study of the cylindrically symmetrical Patterson function of Structure A. It has also been

shown that the proposed structure accounts for many of the strongest features of the 3-dimensional Patterson function.

3. November - December 1953. Birkbeck College.

During this period X-ray diffraction studies of tobacco mosaic virus were started. For this purpose an Ehrenberg-Spear fine-focus X-ray tube is used, with nickel-filtered copper Kα radiation. The X-ray camera is the Phillips micro-camera modified to take a specimen-film distance of 30 mm. or 60 mm. as well as the usual distances of 10 mm. and 15 mm. It is filled with hydrogen during all exposures.

The virus solution was kindly given to this laboratory by Dr. R. Markham.

The research is a continuation of the earlier studies of Bernal and Fankuchen (1942) and of Watson (1953).

Highly detailed diffraction diagrams of orientated virus specimens (prepared by the method of Bernal and Fankuchen) containing varying amounts of water have already been obtained. While the greater part of the high-angle pattern is substantially independent of water content, the reflections corresponding to distances of about 20 A vary strikingly. This suggests that the water most closely associated with the virus may lie on either side of some structural component having at least one dimension of about 20 A.

A detailed study of the small differences in the intra-particle pattern for wet and dry viruses should make it possible to calculate the Patterson function of the difference, and hence to locate the water.

Further, intensity measurements of the equatorial reflections,

which are related to inter-particle, should make it possible to decide whether or not the ribonucleic acid forms a central core in the rod-like particle, as has been suggested by several authors. Preliminary measurements indicate the presence of a heavy core (presumably RNA) in the rod.

4. Miscellaneous.

(a) In April 1954 I was invited to the "Steinkohlentagung" at Aachen, Germany. There I read a paper on "The Mechanism of Crystallite Growth in Carbons" which is to be published (in German) in Brennstoff-Chemie in December 1954. Reprints are not yet available.

The new part this work consisted in a kinetic explanation of the sharpness of the separation of carbonaceous solids into two classes, the graphitising and non-graphitising, and an explanation of the apparent elongated shape of the crystallites in graphitising carbons.

(b) In June 1953 I read a short paper on "Le rôle de l'eau dans l'acide graphitique" to an international colloquium in Paris on "Water in Solids". In this paper a new type of structure for graphitic acid is proposed. A reprint is enclosed.

(5 reprints enclosed)