Notes for talk on Brooklyn work

for Thursday Sept. 30th 1954.

Introduction

{ limited to ribonuclease – not collagen, plane viruses or gossip.
" " my work on ribonuclease : brief mention of other work (no other x-ray work)

general picture 1 3D Patterson

2 Isomorphous replacement studies.

3D Patterson

Mag. Lab. & Crich.

data collected by others – moderately accurate. (errors increase with $\frac{1}{d}$)

computed by Mayfield on I.B.M. machines. (origin removed)

Average intensity curve : shows low to Å peak.

Plotting of the Patterson – Vittorio's method.

Each contour (assuming Fourier value) ≐ 100 elec.^{2}/Å^3.

5 Å not shell nor complete isotropic.

Mirror plane effect : show sections : give explanation.
not enhanced peakiness is due to coherence of the vectors
not their number.

Harker section suggests second molecule is not near $x = z = 0$.

Molecular location : plotting lumpiness.
(can be done by folding the intensities).

Rods no rods anywhere.

sequence of lumps in the c direction.
but ~~other seq~~ (1) not very high (≃ 1 length (35Å) of α helix)
(2) others in other directions. (remember relative weighting)

mean "rod direction" = Elliotts Infra-red direction.

(also shown in asymmetry of 10Å - 5Å reflections).

Ribonuclease VI (Magdoff + Crick)

Grown using dye ~~id~~ iodo-phenol blue
(tertiary butyl).

Coloured deep blue : highly pleochroic when view along a^*
strong absorption when electric vector ∥ to be
∴ ~~do~~ plane of dye roughly ⊥ to c.

		Space group	a	b	c	β	vol.
Cell dimensions.	VI	C2	70.60	38.99	51.65	103.96°	138,000
	II	$P2_1$	30.28	38.39	53.16	105.83°	59,400

Patterson of hol's : superposition. (show slide)
(omit discussion of origin region) & comparison of oko's
(copy from paper).

assume screw did remain the same.

layering of ribonuclease VI : due to ~~a~~ v. strong 200

~~Thus 200~~ Also 001 ≠ 0 Thus we can roughly locate molecules in VI

∴ — — — — — II

Accuracy and Shrinkage (Magdoff + Crick)

Background : variability of ribonuclease lattices.

how large a heavy atom? - depends on accuracy.

Adding dyes - not described here : Accuracy investigation.

found fluctuations in intensity.

2 (Studies limited to the hol's)

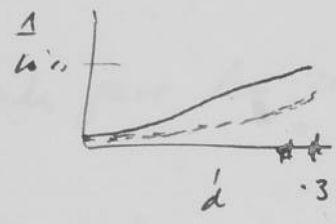
Spectrometer : - check on line profile.

- absorption correction.

General performance appears to be satisfactory.

Two similar crystals (50 is tertiary butyl)

(checked temp. factor the same)



plotted $\Delta = \frac{\Sigma |I - I'|}{\Sigma I}$ for groups.

calculated $\sqrt{\frac{4C}{\pi}}$, allowing for background.

Changes due to solvent : cell dimensions.

Fig 3. tertiabutyl alcohol v ethyl alcohol.

Fig 4. - - - v monoacetin.

Fig 5. - - — v dye.

Shrinkage effect

Study of two adjacent reflections $80\bar{9}$ and $80\bar{10}$

	$80\bar{9}$	$80\bar{10}$
initially	4300	3700
milk cold jet a top of capillary.	5300	1600

5° drop

time-constant of return ~ 2 minutes.

Small 1°C jet near capillary : depended upon aspect.

in one case could be done by the sustained hear of the hand.

"natural" variation. 2300 3600 3500 2900 3600 2700 counts.

shown for all crystals of n.b.o. II studied.

for $80\bar{6}$ ... $80\bar{10}$: showed that 16°C steady gave less change than 1° local.

Quantitative work : wet v damp. - - Fig 6.

Large jets

		a	c	β
all dimensions..	wet	30.49	53.20	105.94°
	Damp	30.17	52.96	106.03°
	Δ	·32	·24	·09°

Breadth of reflection

	peak width.	2θ for $30\bar{4}$	Count for $80\bar{10}$
normal	·05°	9.680	12,500
slightly shrunk	·06°	9.75°	8,300
shrunk further.	·16°	9.82°	3,500

Patterson : very small near origin.

- changes appear somewhat greater than a "breathing" change.
- mols. may rotate slightly about an axis not far from c.

3-D data : comparison — fig. 7.

Photographic data : give an average.

Lessons ① measure cell dimensions very accurately.

② mount crystal wet and/or use large jets.

③ correct for ~~[illegible]~~ capillary absorption.

Implications : "damp" corresponds to ~ 30 H_2O or to alcohol } per protein mol.

β-lactoglobulin

Theory (Crick & Magdoff)

Preliminary results only:

- 3D v. 2D. ?
- How heavy an atom ? (define N and n)

Results: 1

P2,

$$\frac{\overset{\sim\sim}{\Delta I}}{\overline{I}_p} \simeq 2\sqrt{\frac{n}{N}}\,\frac{f_H}{f_p} \quad \text{centrosymmetric.}$$

$$\simeq \sqrt{2}\sqrt{\frac{n}{N}}\,\frac{f_H}{f_p} \quad \text{non-- - - - - -}$$

2 Heavy atom peak

General RMS background

- for near 3D Patt. $\simeq \dfrac{\sqrt{m_3}\, f_H^2}{(2Nf_p^2 + 2n f_H^2)} \simeq \sqrt{m_3}\,\dfrac{f_H^2}{2Nf_p^2}$

peak height / RMS background

- for difference 3D Patt. without errors $\simeq \dfrac{\sqrt{m_3}}{\sqrt{8}\,\sqrt{Nn}}\,\dfrac{f_H}{f_p}$

- for 2D $(\Delta F)^2$ Patt. $\simeq \dfrac{\sqrt{m_2}}{2}\,\dfrac{1}{\sqrt{Nn}}\,\dfrac{f_H}{f_p}\cdot\dfrac{1}{p}$

($\overline{I}\,p = \overline{\text{error}}$)

Ratio of last two cases. $\dfrac{\text{3D contrast}}{\text{2D contrast.}} = \sqrt{\dfrac{m_3}{2m_2}}\cdot p.$