O.D.

assume M solution (in nucleotides) will have OD_{261} ~ 10,000

Thus 330 ~~mg~~ gm / litre ~ 10,000 OD

∴ 330 mg / ml ~ 10,000 OD

our undiluted OD is $.7 \times \frac{4}{.03} = 93$

∴ our conc = $\frac{330}{10^4} \times 93$ mg / ml ≈ 3.1 mg

3|28|
93

AMP

1 mg in 0.5 ml
2 mg in 1 ml.
2 gm in 1 litre
∴ ~ 6×10^{-3} M

3366p

2½ N NaOH is 100 gm / 1000 ml.
∴ 5 gm in 50 ml.

~~32~~

assume RNA 8 will be, say, hydrolysed at $\frac{1}{10}$th the rate.

assume chain length to be ~ 10 then M conc of ends is ~ 10^{-3} M of 3 mg / ml

i.e. less than Michaelis constant.

Thus probably a factor of 5 due to this?

$\frac{1}{30}$th
6

∴ first point should be at ~ 4 minutes. but conc. of enzyme has ∴ 10 minutes

∴ Try times.

	1	2	3	4	5	6	7
	~~0~~	~~4~~	~~8~~	~~15~~	~~30~~	~~60~~	~~120~~ mins.
	0	10	20	40	80	~~160~~ 175	(320 (5 hours))
Time out.	(1.55)	1.55	2.5	2.25	3.5	4.40	6.50
						(f 6.50)	(f 6.50)
OD_{625}	(no enzyme) ~~.2~~ .215	.367	.525	.680	1.06	4.55 (5.45)	abandoned

at 37°C

Thursday 6 March 58

Rate of hydrolysis of "yeast RNA" by prostatic acid phosphatase

The "yeast RNA" was commercial yeast RNA, ~~prepared~~ treated by Muriel 30 Jan 58: ~~[illegible]~~ (phenol treatment, alcohol precip, dialysed v H_2O)

optical density. λ_{260} λ_{280}

0.03 ml → 4 ml .698 .299

thus prob about 3 mg/ml

~~[illegible]~~ Prostatic phosphatase new prep by J.D.S.

conc. enzyme in .05M acetate buffer.

dilute enzyme is conc enzyme diluted to $\frac{1}{50}$th in H_2O.

assay by J.D.S.

	$A_{2'3'}$MP (10mg/ml)	0.1 ml	[i.e. 1mg AMP]
	M acetate pH5	0.1 ml	
37°C	enzyme ($\frac{1}{50}$th)	0.1 ml.	
	H_2O	0.2 ml	
		0.5 ml.	

Conc of AMP is thus ≈ 6×10^{-3} M

Michael contract suggests to be 2×10^{-3} M

This gave ½ digestion in about 2 minutes.

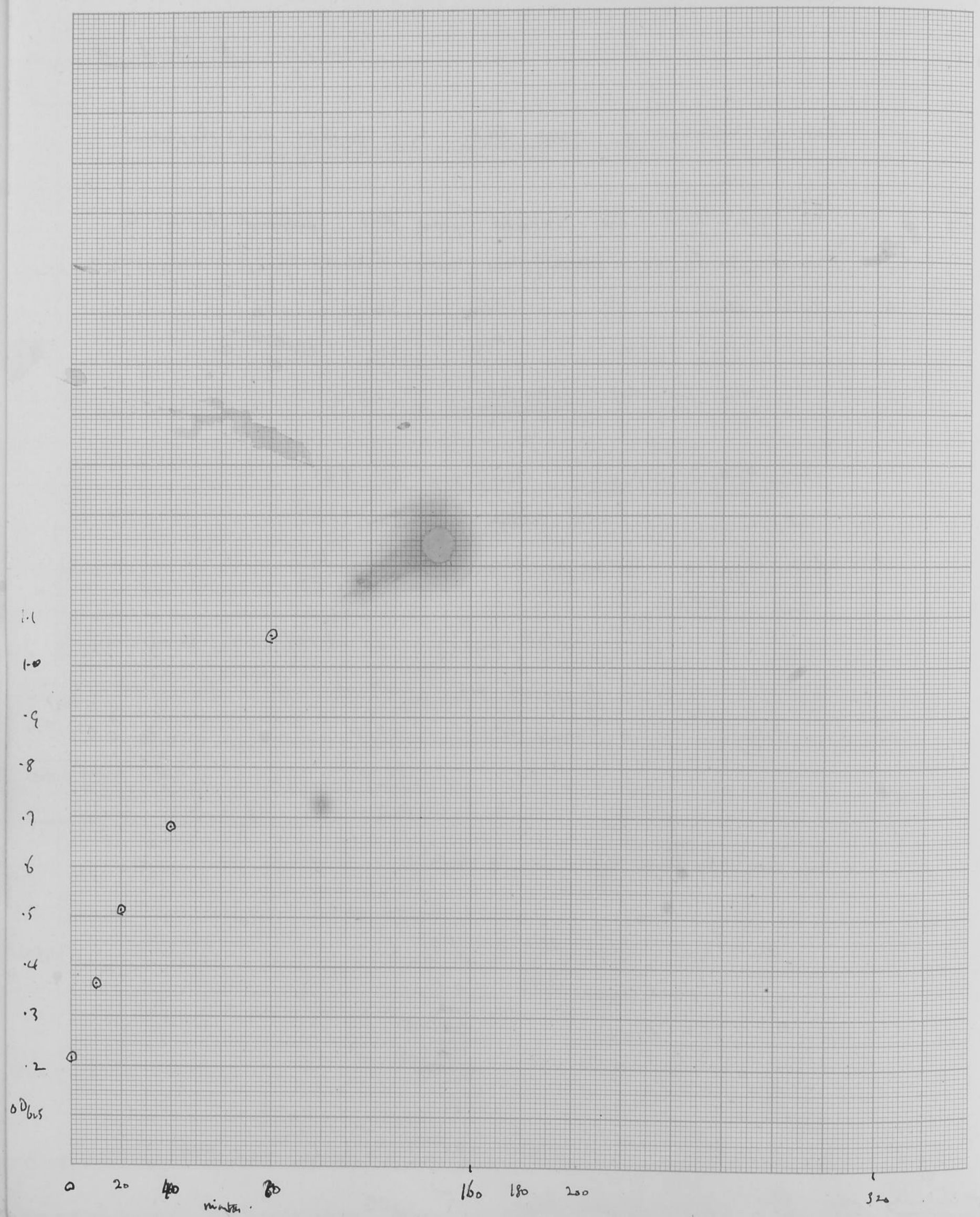
use	yeast RNA	1.0
	diluted enzyme ($\frac{1}{50}$th)	0.1
	M acetate	0.15
	H_2O	0.25
		1.5

Started 1.45 pm.

.05 N acet. all ← cloudy

Then add 1.0 ml of 2.5N NaOH 1.0 (to hydrolyse remaining RNA)

2.5 ml.


1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
OD625
0
20
40
60
160
180
200
320
minutes

2.5

To assay use ·3 + ·4 = ·7 ml (60%) PCA

and 1·2 ml H_2O

Amidol regen 0·4

8·3% Amm. molybdate 0·2

5·0 ml measure OD_{625}

(·3 × 9N = 2·7
1·0 × 2·5N = 2·5)

A_{23} MP (pub 10 mg/ml) ·1

M acetone ·15

H_2O 1·15

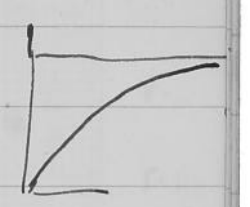
diluted enzyme ($\frac{1}{50}$ lb) ·1 ·3

1·5 ml.

Then treated as above. ·3

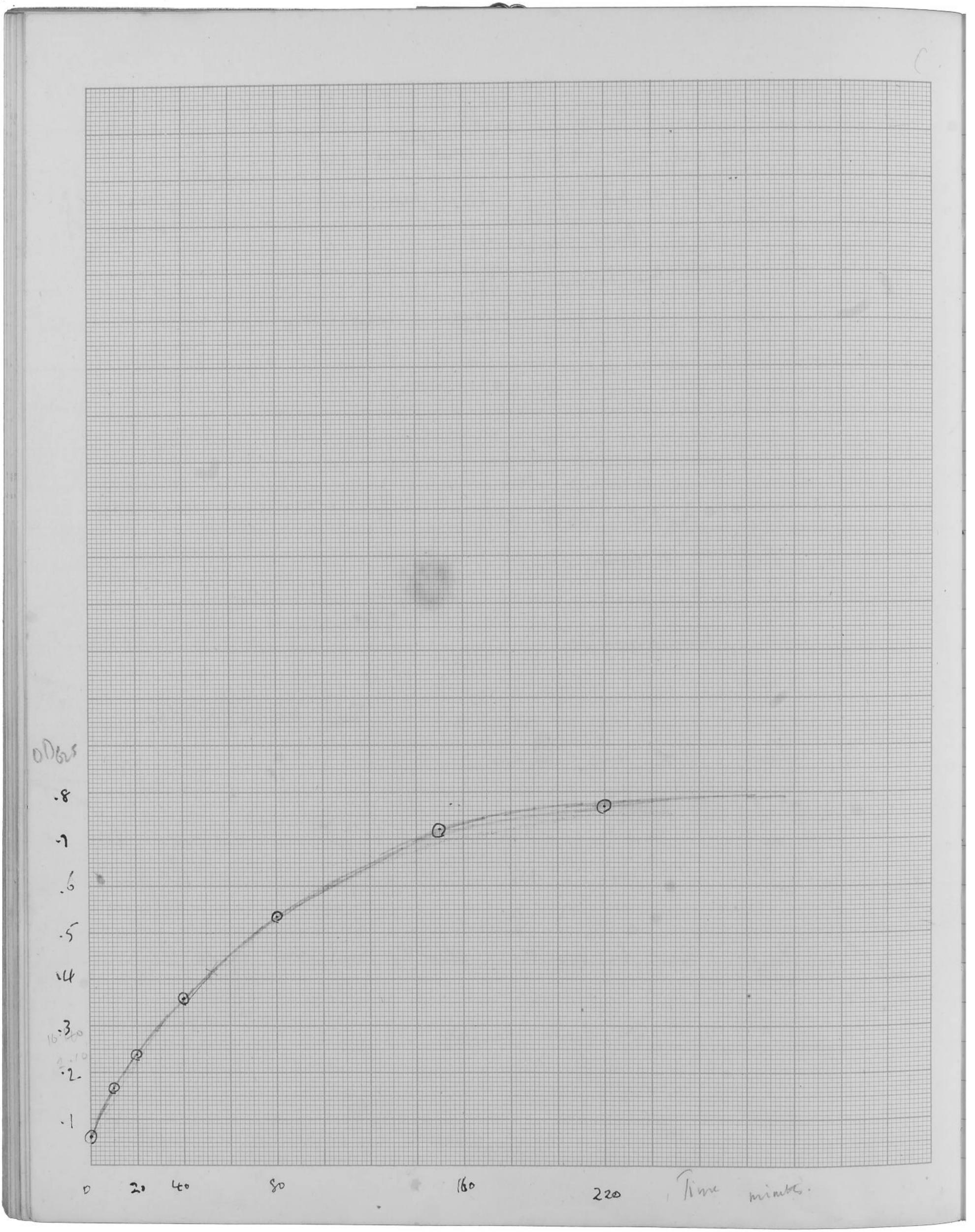
Times.	4 min.	25 mins.
m.	6·4	6·5
ou	6·8	6·30
OD_{260}	1·14	1·55

37°C



But apart from these two, the blue was cloudy

i.e. Nable digestion incomplete.


OD625
.8
.7
.6
.5
.4
.3
.2
.1
0
20
40
80
160
220
Time minutes.

Friday 7th March 58

Repeat

just as yesterday, except that
Nabk digestion was for 5 hours, at 37°C.
except last two, which were $4\frac{3}{4}$ hours

This time blue was not cloudy.

no enzyme
.1 ml H2O instead

		10	20	40	80	150	220	= Time of incubation at 37°C.
	1	2	3	4	5	6	7	
Time in	—	10.36	10.37	10.38	10.39	10.40	10.41	at 37°C
out (add 1.0 ml 2.5N NaOH)	10.35	10.46	10.57	11.18	11.59	1.10	2.21	at 37°C
						5.55	7.5	
into ice		3.46	3.57	4.18	4.59	6.10	7.21	at 0°C
OD_{625}	.060	.168	.239	.360	.535	.720	.770	

Thus pulls 50 γ of P corresp off from ~ 3 ms RNA
∴ 1 in 6 ends!!

~350 ~~ml~~ ~~mg~~ mg AMP /ml = 1 M

∴ 10 mg /ml ≃ 3×10^{-2} M

Calculation of prostatic phosphates rates

$$\text{Rate} \propto \frac{[E][S]}{K + [S]}$$

where K = Michaelis constant.

Assume Michaelis constant = 2×10^{-3} M

My assay of AMP (eg. 10th Feb.)

½ gone in about 5 minutes, using $\frac{E}{12}$ ·1 ml.

AMP (10 mg/ml) ·1 ml

·2 ml.

all at ~37°C

Thus AMP conc. initially was ~ 15×10^{-3} M

Thus well above Michaelis constant. i.e. near enough the limiting rate.

My assay of ~~[illegible]~~ AMP 6th March

⅔ ml gone in 4 min. very ½ gone in 3 minutes.

AMP conc ≃ 2×10^{-3} M

Thus limiting rate for high conc would be twice this

Thus the new enzyme (diluted $\frac{1}{50}$) is $\frac{5}{\frac{3}{2}}$ ≃ ×3 as strong as $\frac{E}{12}$.

RNA attack

Feb 10th

used .1 ml in $\frac{E}{12}$

plus .1 ml of RNA ② (approx 2 mg/ml)

.2 ml

∴ .04 ml of $\frac{E}{12}$ per 0.2 mg RNA ② is .2 ml

3

Then ~~probably~~ almost certainly in the range of initial slope.

March 6th

used .1 ml enzyme $\frac{1}{50}$th

and 1.0 gm RNA (3 mg/ml)

+ 0.4

to give 1.5 ml total.

∴ above 2 mg RNA/ml ≃ 6×10^{-3} M in nucleotides

∴ again ~~almost certainly~~ probably on the initial slope: but should check with a lower one.

30

Time to half, say, ~ 60 minutes.

Now scale down volume to .2 ml i.e. .014 enzyme ($\frac{1}{50}$th) per .2 ml

~ .4 mg RNA

Then if there had been .2 mg RNA time would still have been ~~30~~ 60 minutes

relative strength of enzymes .014 × 3 ≃ .042 of $\frac{E}{12}$

But before used .04 of $\frac{E}{12}$ i.e. comparable should have done $\frac{1}{2}$ in ~~30~~ 60 minutes

But as I incubated for 1 hour, ~~this was probably ample~~

Then borderline.