O.D.

Assume M solution (in nucleotides) will have OD_{260} ~ 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

33 ~~[illegible]~~

2½ N NaOH in 100 gm [illegible].
∴ 5 gm in 50 ml.

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

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

i.e. less than Michaelis constant.

Then probably a factor of 5 due to this?

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

∴ [illegible] point should be at ~ 4 minutes. [illegible] conc. of enzyme [illegible] ∴ 10 [illegible]

∴ 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))	
at <u>37°C</u> Time out.	(1.55)	1.55	2.5	2.25	3.5	4.40 (f 6.50)	6.50 (f .650)	
OD_{625}	(no enzyme) ~~[illegible]~~ .215	.367	.525	.680	1.06	455 / 5.45		\ abandoned

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 .289

thus prob about 3 mg/ml

~~Ac~~ 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 ~~A~~ AMP is thus ≈ 6×10^{-3} M

Michaelis constant appears to be 2×10^{-3} M

This gave ½ digestion in about 2 minutes.

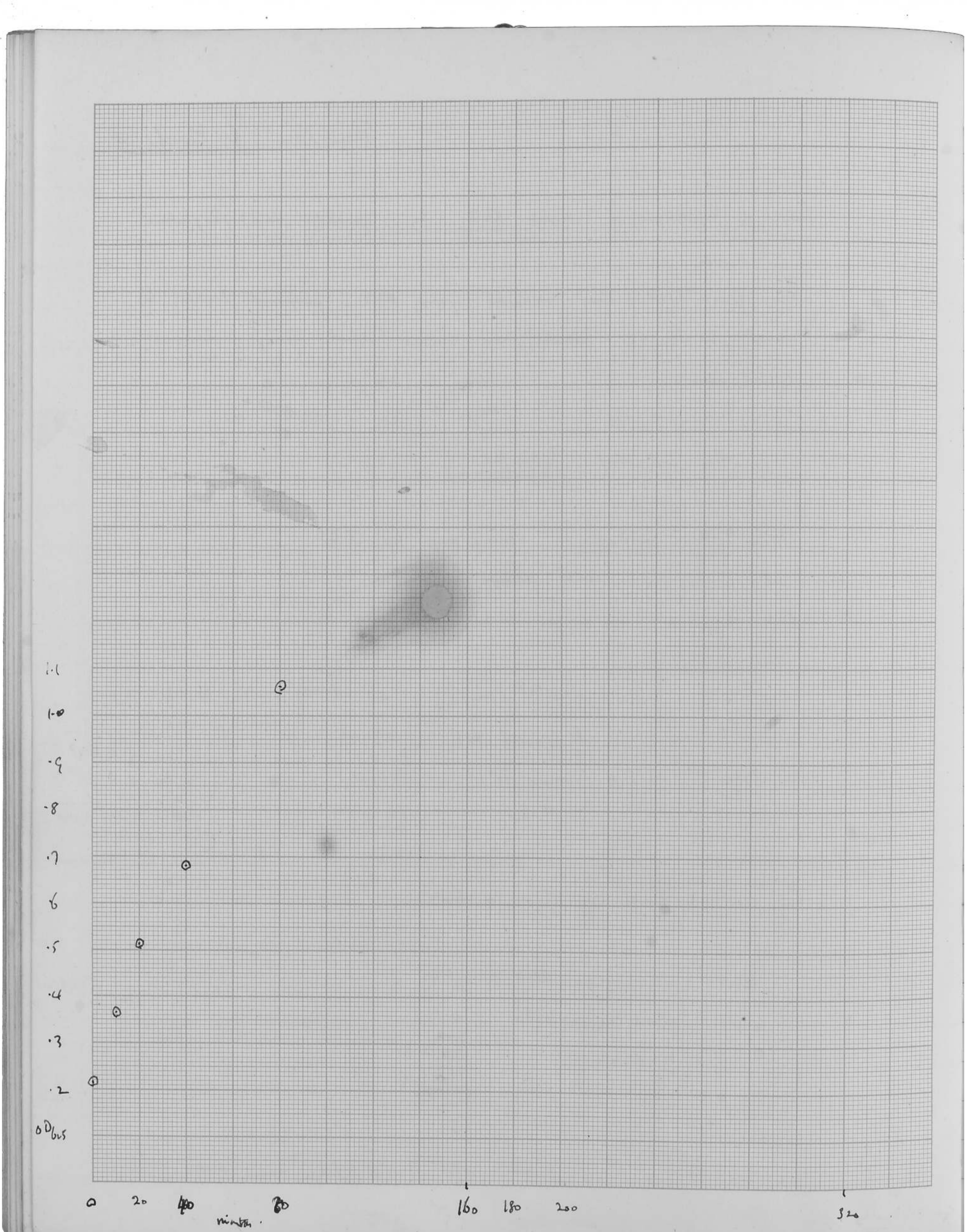
Use	yeast RNA	1.0
	diluted enzyme ($\frac{1}{50}$th)	0.1
	M acetate	~~0.2~~ 0.15
	H_2O	~~0.2~~ 0.25
		1.5

Started 1.45 pm.

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

2.5 ml.

.05 NaOH. all cloudy ←


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

To assay use ·3 + ·4 = $\overset{2.5}{·7}$ ml (6.0.) PCA

and 1.2 ml H_2O

Amidol reagent 0.4

8.3% Amm. molybdate 0.2

5.0 ml measure OD_{625}

(·3 × 9N = 2.7)
(1.0 × 2.5N = 2.5)

AMP (soln 10 mg/ml) ·1

M acetate ·15

H_2O 1.15

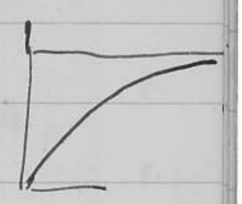
diluted enzyme (1/50th) ·1 ·3

1.5 ml.

Then treat as above. ·3

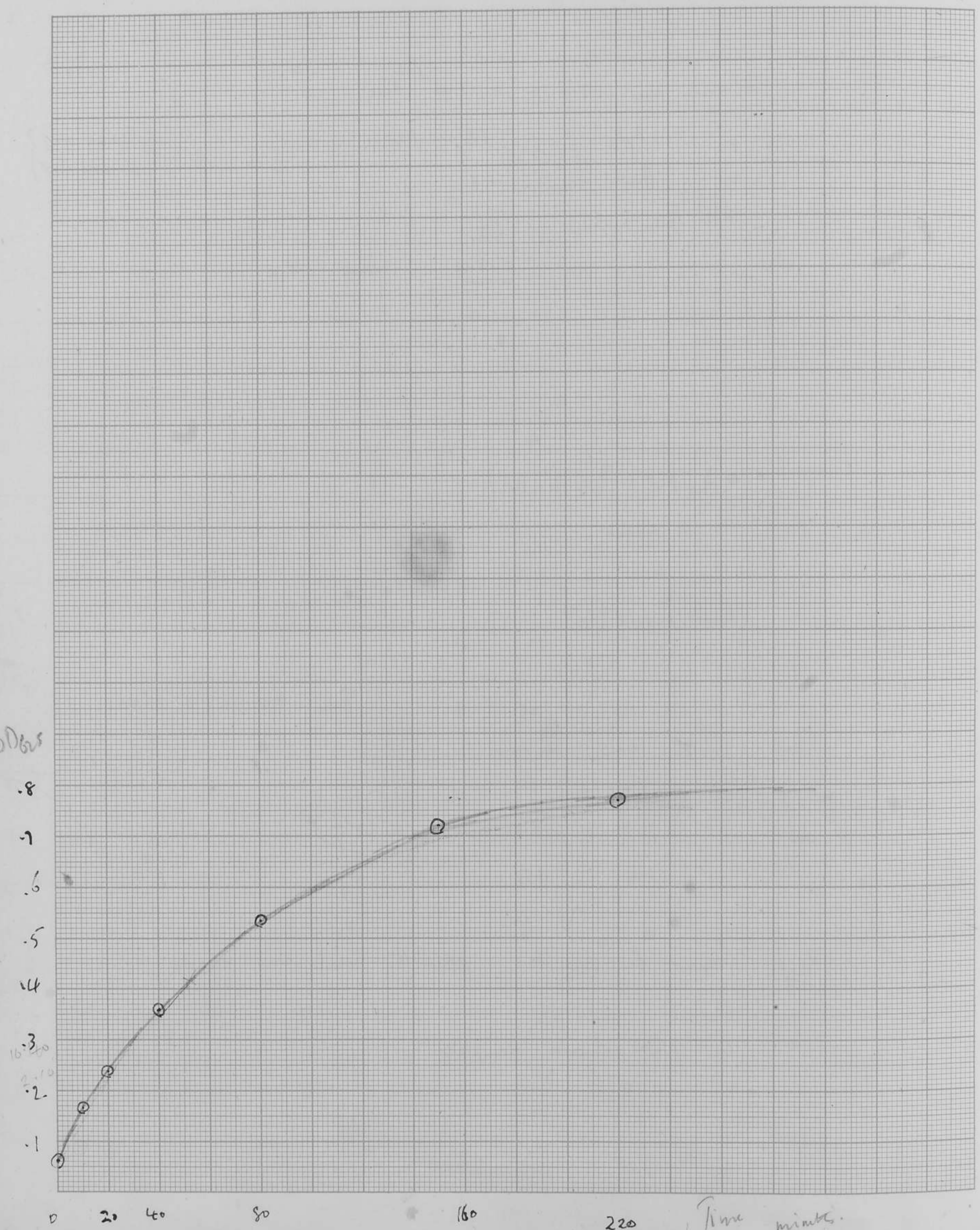
Times.	4 mins.	25 mins.
n.	6.4	6.5
ow	6.8	6.320
OD_{660}	1.44	1.55

37°C



But apart from these two, the slice was cloudy

i.e. Nucleic digestion incomplete.


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

Friday 7th March 58

<u>Repeat</u>

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

This time Slice was <u>not</u> cloudy.

no enzyme
·1 ml H_2O instead
↓

	1	2	3	4	5	6	7	
		10	20	40	80	150 ~~40~~	220 ~~70~~	= Time of incubation at 37°C.
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 / 5·55	2·21 / 7·5	at 37°C
into ice		3·46	3·57	4·18	4·59	6·10	7·21	at 0°C
OD₂₆₀	·060	·168	·239	·360	·535	·720	·770	

Thus put 50 γ of P comes off from ~ 3 mg RNA
∴ 1 in 6 cuts!!

~350 mg AMP/ml = 1 M

∴ 10 mg/ml ≃ 3×10^{-2} M

Calculation of prostatic phosphatase rates

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

where K = Michaelis constant.

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

<u>Dry assay of</u> 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 — I

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

<u>Dry assay of AMP 6th March</u>

$\frac{2}{3}$rd gone in 4 mins. say $\frac{1}{2}$ gone in 3 minutes.

AMP conc ≃ 2×10^{-3} M

Thus limiting rate for high conc would be <u>twice</u> this

Thus the new enzyme (diluted $\frac{1}{50}$) is $\frac{7}{4}\ \frac{5}{\frac{3}{2}}$ ≃ ×3 as strong as $\frac{E}{12}$.

RNA attack

Feb 6th

used ·1 ml of $\frac{E}{12}$ (units)

plus ·1 ml of RNA ② (approx 2 mg/ml)

·2 ml

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

3

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

March 6th

used ·1 ml enzyme $\frac{1}{50}$th

and 1.0 yeast RNA (3 mg/ml)

\+ 0.4

to give 1.5 ml total.

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

∴ again ~~almost certainly~~ probably in 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 $\frac{E}{12}$ ie comparable so should have done $\frac{1}{2}$ in ~~30~~ 60 minutes

But as I incubated for 1 hour, this ~~[illegible]~~ probably ~~[illegible]~~
Then borderline.