Culture filtrates.

50 ml cultures, shaken at 30° K-12 inoc 1030A21.
6 - d.

2 ~~3~~. glut 5 mg, pntlactone 1 mg, β-alanine 1 mg
3 ~~3~~. prol 5 mg pnt .5 mg

1. T(0).
4. See 202-9.

To desk top N22
sample a 10P22.

1. Ta 5 ml + F(0) 5 ml. N. crassa 5531.
2. 2a 5 ml + F(0) 5 ml N. crassa 5531.
3. pnt series 1r, 5r, 10r " "
4. 3a .1 ml + 10 ml F(0). " "
5. " .5 ml
6. 4a 5 ml + 5 ml F(0). " "

Inoc N23.

11. 1a 5 ml + T(B) 58-3214. ±
12 2a " " ±
13 Proline 25r + 1a ~~[illegible]~~ .25 cc + glut 25r. ±
14 ~~3a 25cc + T(B).~~
3a 0.25cc + T(B) 10 ml. ±

hydrolyze 1-3 (wash 3 cells) in 6 N HCl. 14 hours.

	total o.d.u.
1.	10.400
2.	12.000
3.	4.240

Dry: analyse 207-1.

Streak out 207-1 on a coli + plate P22. Isolate 10 colonies to H_2O, and test; transfer to ∞ slants. 3P23.

∴ ~~thr~~ threonine not required.

∞	T-B		T-T		T-BT.	
1	+	++	−	−	+	++
2	+	"	−	−	−	"
3	+	"	−	−	−	"
4	+	"	−	"	+	"
5	+	"	−	"	+	"
6	+	"	−	"	−	"
7	+	"	−	"	+	"
8	+	"	−	"	−	"
9	+	"	−	"	−	"
10	+	"	−	"	+	"

Test 207-2-4. 11P23.

	O	B.P.M.T.	BM	BT	MP	MT.	BP	TP
*								24h 32h.
2		− ++	− −	− −	− −	− −	− −	− − ++ ✓ ++ May be a multiple mutant.
3		± ++	− −	− −	− −	− −	− −	− ++
4		+ ++	− −	− −	− −	− −	− −	− + ++
5		− ++	− −	− −	− −	− −	− −	− − ++
8		± ++	± ++	± ++	± ++	± ++	± ++	± ++

Finally: +.

2 -	BPMT, PT.		679-183. ✓ 212B.
3	BT, PT, MT, BPMT.		679-183+
4	BPMT, BT, PT.	MT(-)!	679-183+ 679-58x? 679-183+
5	BPMT, PT		679-183
8			

Note: all these strains were isolated from BT plates!

Retest:

	BPMT	BPM	PMT	MTB	BPT	MT.
207-1						
207-1A.						
207-2						
207-4						
BM						
BP						
PT						

See 212.

Sex in bacteria. Prospectus. 210N.

5/25/46. P25.

From mixed cultures, strains have been obtained which behave peculiarly in their nutritional requirements, behaving for a time like recombination types. For a demonstration of sex, a stable recombination type is essential. An analysis must be made of cultures 207-1 and 207-2.

207-1 behaves like a culture of BT with a small % of B cells. Therefore plate heavily + lightly into T(B) + layer ẽ "T" after B is detected.

207-2 behaves like a culture of BMPT ẽ some PT cells still present. Plate into 1) PT 2) BM. Afterwards layer.

207-4 may have BT cells Plate from 210-4-BT into B. After-layer ẽ T.

4-A Mutants

5/26/46

I Cystine Requirement of Y24.

1426. A. 10mcl T(B, ϕ) + B: .01r ϕ 300r

	Cyst.	8P27
1.	10r	89'
2.	30r	84'
3.	100r	83^2 ← ½ opt.
4.	300r	76^3 like 58·309.
5.	1mg.	68

11. Methionine: 1mg. ± not parathiotrophs.

12. Meth. 1mg+Cyst 1mg. 65.

II Otherstrains:

			T(0)	HC	VITS.
1	B	Y13.			
2	B	Y14			
3	B	Y18			
4	B	Y20			
5	B	Y21			
6	B	Y22			
7	ϕB	197-61.			
8	B	197-32			
9	B.	197-33			
10	Bϕ	-5			
11	Bϕ	-7			
12	Bϕ	-21			
13	Bϕ	-23			

Analysis of 207-cultures
Six.

5/26/46.

Scrape growth from slant, suspend in H_2O + dilute as indicated. ✓ (works well)

12M-1A26. ~~Use T(0) c̄ ½ usual phosphate [T₁.]~~

To obtain clean agar, autoclave 3% agar + T(0) 200% separately. then imp. Mutant detection procedure.

A. 207-1. P27. Layer c̄ 11 P27. Addnl col. 11A28.

1:1000 in:

	1.	B	+++			
B^-M^+	2.	B	+++			
	3.	BT	+++			
	4	T	0			
	1:10⁶					
	11	B	51	: T	0	
(N)	12	B	39.	: T	1	#4
	13	BT	43			
	14	T.	0			

Pick to complete M28.

For analysis see 219.

requires B.

207-2.

1:1000 in:

B.

21	PT	+++			
22	PT	+++			
23	BMBT	+++			
24	BM	—			
1:1000000					
31.	PT	9			
32	PT	8			
33	PT	15	: ∞	1	#1
34	PT	14	: ∞	1	#2
35	BPT	12			
36	MPT	12			
37	BMP	0			
38	BMT	0			
39	BMPT.	14			

C. 207-4

1:1000					
41.	T	++			
42.	B	0			
1:10⁶					
51	B	0			
52	T.	3	'B	1	#3

FPA.

dl-fluorophenylalanine

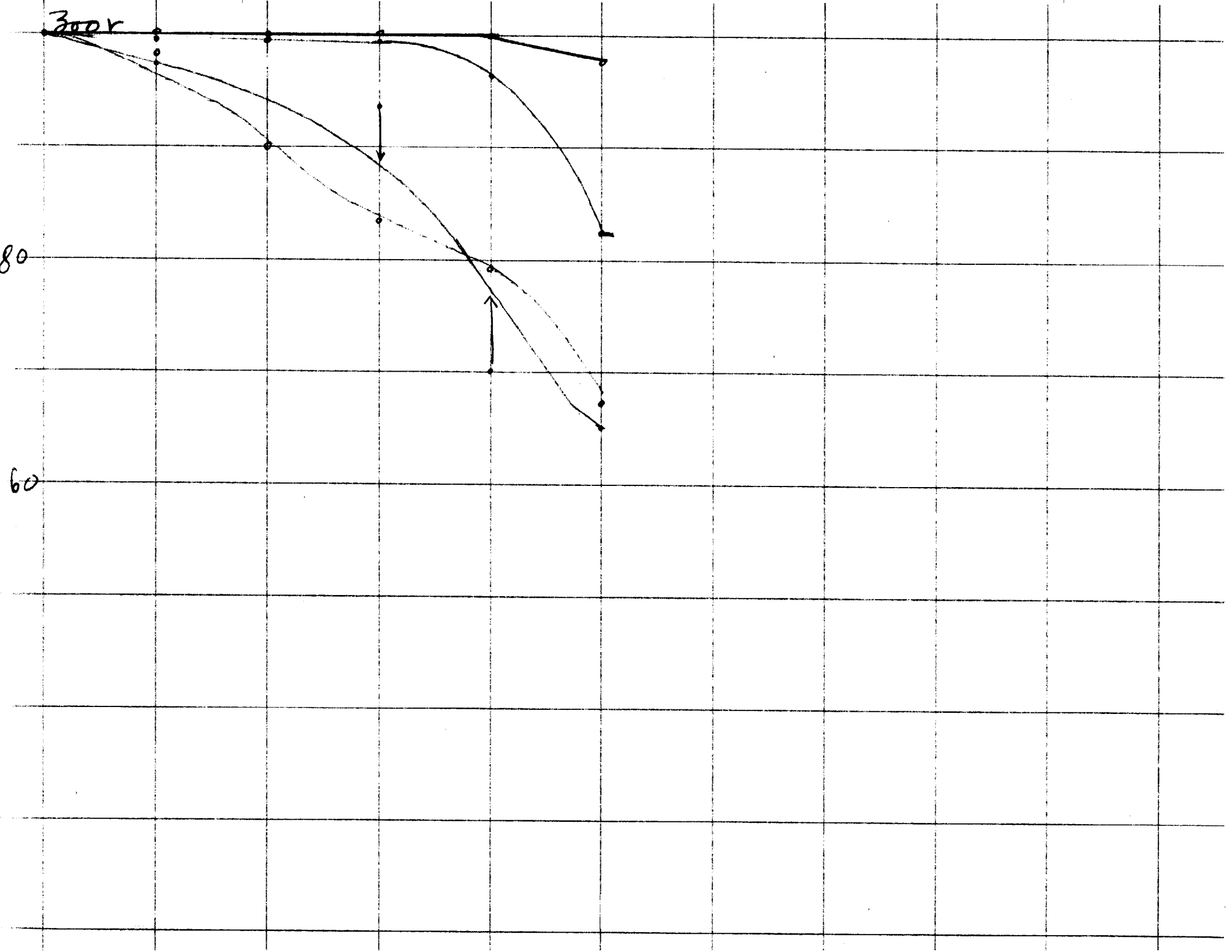
5/24/46.

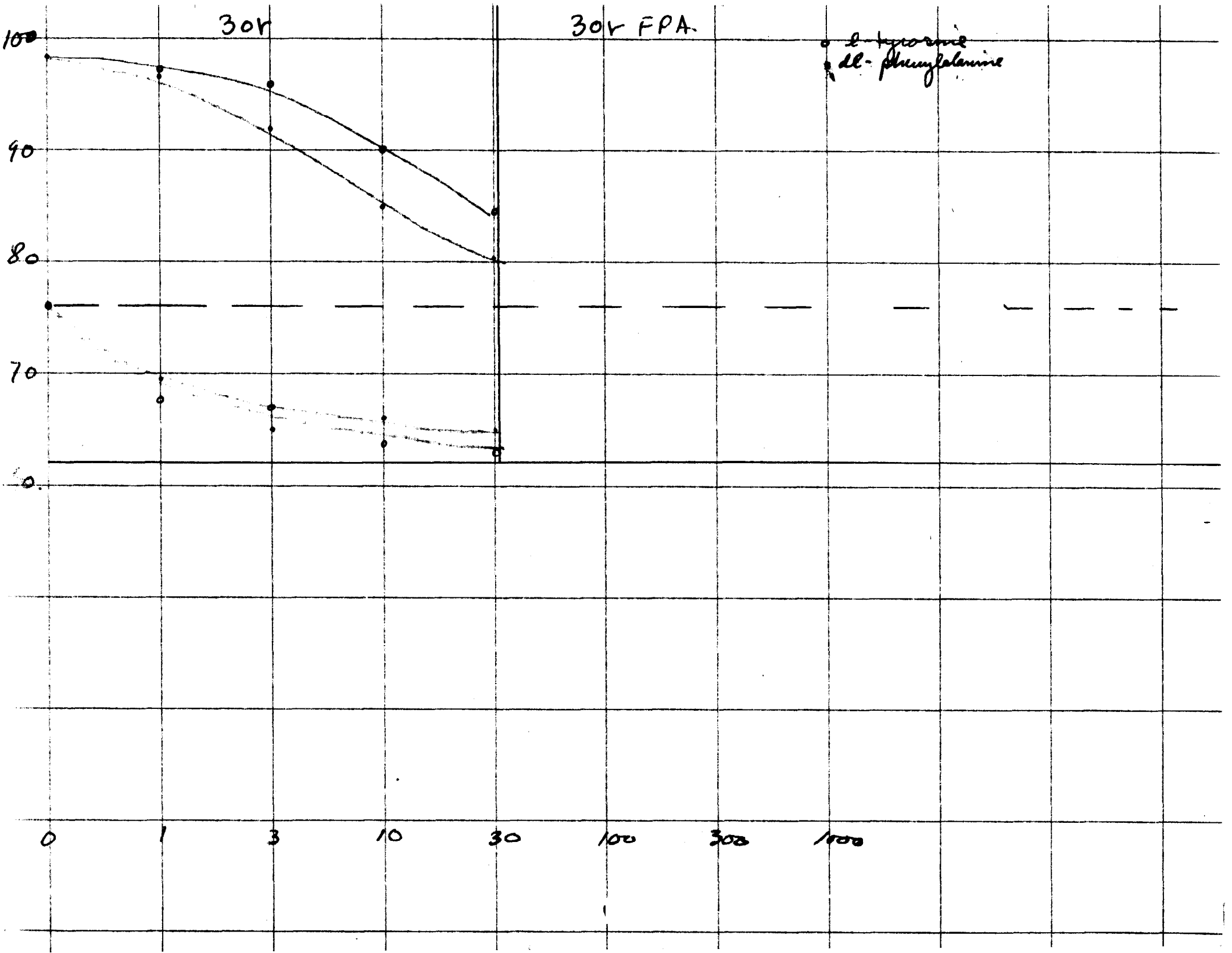
10ml T(B). 30°. in colorimeter tubes. broc. 1A26.

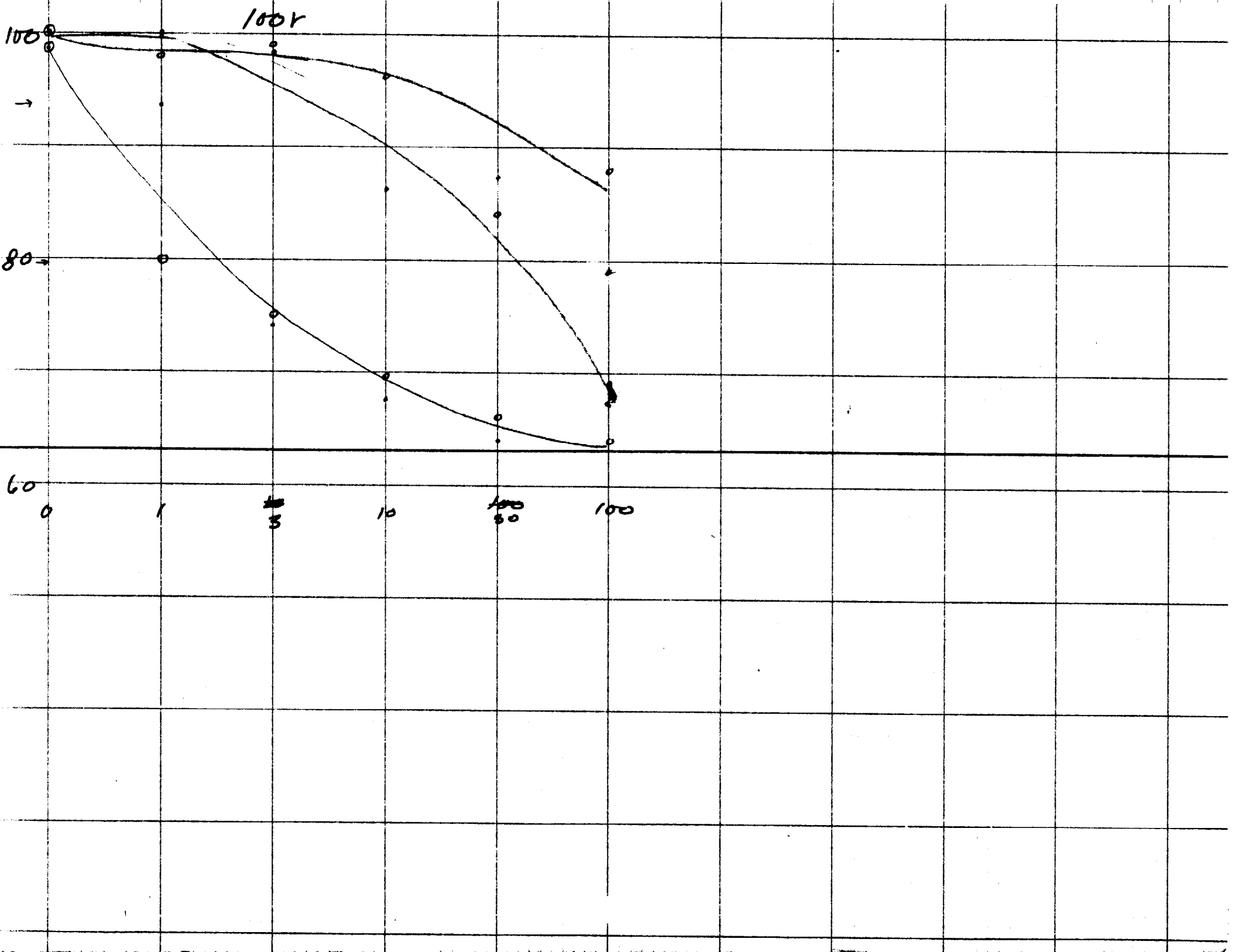
broc. dl ΦAl. dl FPA. l-tyr. 604.

					8P27.	1P28
1.	R-12	0	0		67	68'
2.	"	"	10 r		80	68'
3	"	"	30 r		97	75^2
4	"	"	100 r		100	89'
5	"	"	300 r		100	100
6	"	"	1 mg.		100	100
11.	58-278	100 r	0		70	70
12	"	"	10 r		66^3	67'
13	"	"	30 r		67	70
14	"	"	100 r		69	67
15	"	"	300 r		70	69^3
16	"	"	1 mg.		99	96^3
21	58-278	200 r	0		72	68^2
22	"	"	10 r		66'	67^2
23	"	"	30 r		67'	68^2
24	"	"	100 r		69	68^2
25	"	"	300 r		69'	70
26	"	"	1 mg.		71^2	71^2
31	58-5030	0	0	0	— 100	100
32	"	"	0	60 r	+++ 77'	77^2
33	"	"	10 r	60 r	77	78'
34		"	100 r	60 r	78^3	79
35		"	1 mg.	60 r	75'	75^3
36.		100 r	100 r	0	— 100	100
37.		100 r	0	0	— 100	100
38.		0	100 r	0	150.	100
					76^5	75^3

compare E 133.


300r
80
60


30r
30r FPA.
l-tyrosine
dl-phenylalanine
100
90
80
70
0
0
1
3
10
30
100
300
1000


100
100r
80
60
0
1
3
10
50
100

FPA.

Inhibition of K-12 and Reversal by phenyl-alanine + tyrosine.

6/2/46.		Inoc K-12		11P2.		25h.	36h.		48h	60h.
		FPA + dl Phe + l-tyr.		4P3		12M3.	1FA4		11P4.	11A5
1	30	0	0	±	98¹	76		63²		
2		1		±	96³	69²		65		
3		3		±	92	65		68		
4	→	10		+	85	66				
5	—	30		+	80¹	65				
6			1	+	97¹	70	67³	67¹		
7			3	+	96	69	67³			
8			10	+	90	70	63³			
9			30	+	84³	[illegible]	63	68		
						63³				
11	100	0		±	100	98³		97¹	71	
12		1		±	100	93³		67²		
13		3		±	99¹	74		67³		
14		10		+	86¹	67³				
15	→	30		+	87¹	64				
16		100		++ slc	69	67¹				
17			1		98	80		70	67	
18			3		99	75		66		
19		→	10		96¹²	69³		73		
20			30	+	84	66		68		
21			100	+	88	64		69¹		
31	300	0			100	100		99³	90³	
32		1			99³	97¹		78¹	69²	←
33		3			100	100		100	97¹	
34		10			99²	93³		72²	71	
35		30			96²	70		67		
36		→ 100		+	82¹	65		70		
37			1		100	98²		98²	74²	
38			3		99³	90		70	70²	
39			10		100	83²		68¹		
40	—		30		100	79¹		68¹		
41			100.	±	97³	67¹		67²		
51	0	1		±	85	61²		65	66	
52		10		+	76	62³		66²		
53		100		+	76	[illegible]65		69²		
61			10	+	77	62³		67²		
62			100	+	85	60		69²		
63			1000	+	74.	66¹		67²		
				(80)	(80.)	(283)		80	80.	

2M 9-43 BIOMSTAT FORM NO. A-2788
SUBJECT

FPA. – Growth Curves. 30°.

SHEET NO.
BY 215a

Inoc. 1130P5

	Inoc.	Medium	1145 A6	210 P6.	450 P6	750 P6	11 P6.	220 A7	944 A7	~~1130P5~~ 1150 A7	350 P7	810 P7	Lag	mgt.
	FROM 11A6.													
Hours + mins			45	190	350	530	~~820~~	920	1365	1490	1730	1990		
1	K-12	0	96	92	84^3	69	58^3	57^1	59				lag 10.34. mgt = 2½4.	
2	K-12	0	97^1	92^3	84^2	69	59^2	58^1	61					
3	K-12 v.small	0	98	98^3	98^2	98^2	98	$96^{2\leftrightarrow 8.5}$	73^3	66	58			144.
4	"	0	99^2	100	~~99~~ 100	99^2	99	95^3	72	64^3	57			
5	K-12	FPA 100r	99	99	99^2	98^2	95^1	90	67^3	~~68~~ 68^1	65^3	67^2		220?
6	K-12	FPA 300r	99	99^2	99^2	98^3	97	96	~~81~~ 81^3	77^2	68^1	67^1		390 ok.
7	K-12	Img. FPA	100	100	99^3	100	100	99^3	98^3	100	97^2	96^3		
8	K-12	ØA 10	100	98^3	93^3	88^2	76	67	69^1	69^2	69^3			175
9	K-12	"	99^2	98	93^3	83^2	74	67^3	~~70~~ 70^3	76^2	70			"
10	K-12 v.sm.	"	100^2	100	100	100	100	100^T	99^T	96	87^2	72^2		140; 205.
11	K-12 ~~K-8~~	FPA 100 Tyn 10	99^3	99^2	95^3	87^1	77^2	68	64^3					220
12	"	"	100	99^1	(96^2)	87^3	75^3	68	64^1					"
13	" v.sm.	" FPA300	100	100	100	100	99	99^T	96	94^3	83	68^3		
14	K-12	ØA 10	97^1	93	82^1	66^3	~~69~~ 69^2	62^1	66^3	65^2	66			~~68~~
15	K-12	FP300 Tyn 10	94^1 but(T)	99^1	99	98^2	97^2	97^1	91^3	89^2	77	67^1		
16	K-12	50%	100	96^2	88^1	74^2	69^1	71	73	73^1	73	73		
			77^1	77^1	~~76~~ 76^2	77								
					77^1		16^2	76^2	76^3	77	76^2	78^2		

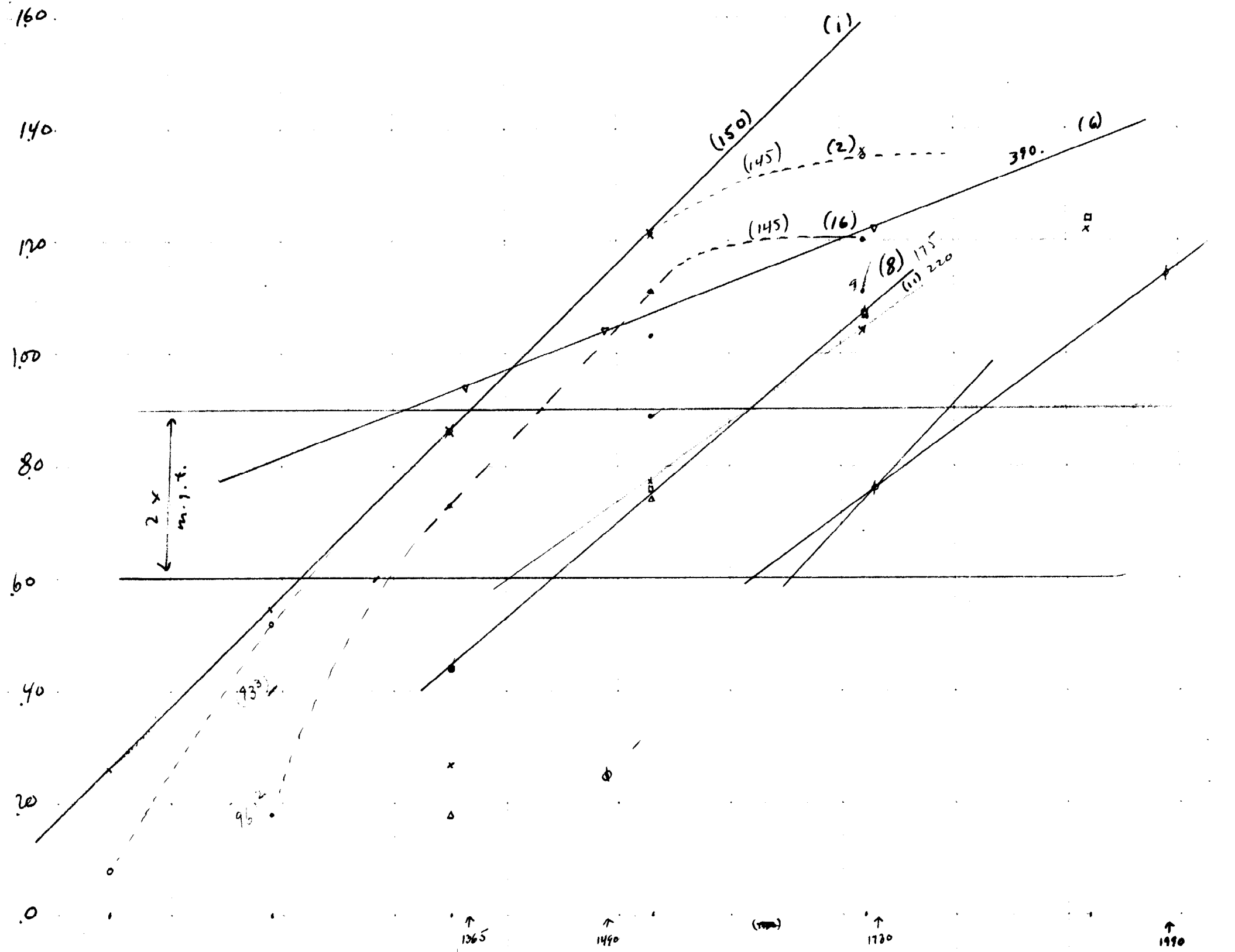
should have a separate blank.

$$d = 2 - \log G$$

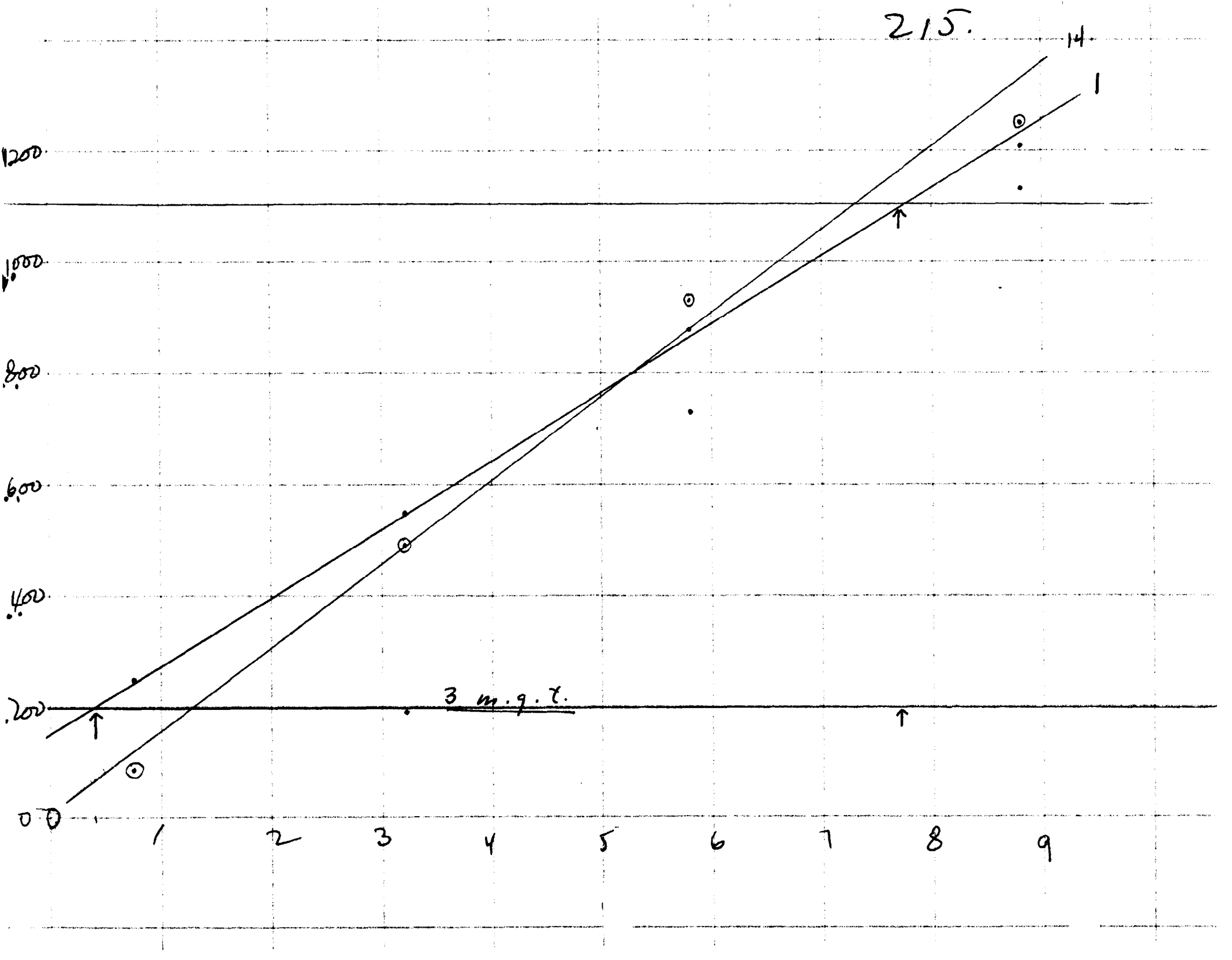
$$t = k \log d.$$

$$t = k \cdot \log(\log G/100)$$

$$= k - \log \log G/100$$



160
140
120
100
80
60
40
20
0
(1)
(150)
(145)
(2)
(6)
390.
(145)
(16)
(8)
175
(11) 220
2 x m.g.t.
93³
96²
1365
1490
1730
1990


215.
14
1
1200
1000
800
600
400
200
0
3 m.g.t.
0
1
2
3
4
5
6
7
8
9

13/2 mutants.

Inoc. 1245A28.

		Arginine	Leucine	Citrulline	21h.	48h.	
Y-38	1	0			99[3]		good response curve.
	2	1			94[1]		1/2 max: ca .03 mg
	3	3			92[1]		
	4	10			83		
	5	30			78[2]		
	6	100			61		
	7	300			57		
	8			1000	100		all up.
Y-39	11		0		100	100	
	12		1		"		
	13		3		"	"	
	14		10		"		
	15		30		"		
	16		100		"		
	17		300		"	"	

(78)

Y-39. 21. L-leucine 100γ

dl-leucine 100γ

dl-isoleucine 100γ

Inoc Y38 in coli of 12M28. Grow on shaker @ 30°.

Mix after growth together →

Controls are questionable —

streptococcus in plate 13 or carryover?

Sex: Analyses of 212 cultures.

219-

	M28 Pick to ∞.		BPTM	−B	−P	−T	−M
x P.T.?		1 ✓	+	+	+	−	+
"	?	2 ✓	−	−	−	−	−
BT ?		3					
BT ?		4 ✓	+	−	+	+	+

Analyse 219-2

Syntrophism in plate cultures.

5/31/46.

1. Inoc 50 ml coli ∝ c α (679-183) and β (58-161) and shake at 30° 11P30 - 1130P31.

T/= Wash and resuspend in 25 ml H_2O.

Turbid Cell count: Turbidity - P4.

1.	α	10^{-7} in ∝ :	24	
2.	β	" " " :	53	

Back-mutation rates: Inoc 1 ml. of suspensions.

3	α	in O.	0		—
4	α	in Threonine exc.	0		±
5	α	in proline " .	2	surface maybe cont but look like coli	
6	β	in O	0		—
7	β	in Biotin	0		—
8	β	in Methionine	0 (T)		+

Syntrophism. Inoc in BT.

9.	β	10^{-7}	Control	1	—	
10	α	10^{-7}	"	0	—	
11	α	10^{-7}	+ 1 ml β ~~[illegible]~~ boiled	0	—	11 very small colonies.
12	β	10^{-7}	+ 1 ml α " .	0	—	—

	α	β				
13	1 ml.	1	0 (T)		++	
14	"	10^{-3}	1	surrounded by local strin. zone	±	
15	"	10^{-5}	1	" " " " "	±	
16	"	10^{-7}	0		±	no colonies.
17	10^{-3}	1	0		—	
18		10^{-3}	0		—	
19		10^{-5}	0		—	—
20		10^{-7}	0		—	
21	10^{-5}	1	0			num. v. sm. col.
22		10^{-3}	0			
23		10^{-5}	0		—	
24		10^{-7}	0		—	
25	10^{-7}	1	0		—	num v. sm. col.
26		10^{-3}	0		—	
27		10^{-5}	0		—	
28		10^{-7}	0	(N)	—	

→ The conclusion that may be drawn from this are limited:
in general diffusion must be limited by the agar to the point
where synthesis is less effective. It is not due to cells sticking
together confirm by plating wild type into concentrated mutant.
delete
Does not adaptation serve the same purpose ??

—

Y38 Ultraviolet killing.

As above but expose by removing transite shutter after 5 min. warm-up period. 5" from tube. Plate initially at $1:10^7$, then take 1 ml samples and determine terminal slopes of eq. 7.

5:15 P31. 1 min. intervals for 10 min.

t=0	10^{-7}		65	650,000,000	ρS
1	1	turbid			
2	1	turbid			
3	1	1 ml	10^5		
4	1		ca 10000		
5	1		ca [redacted] 1200		
6	.1		ca [redacted] 40.		→ 6
7	.1	1 ml	7		
8	.1		0		
9	.1		0		
10	.1		0		
15	1 ml		0		

Because of clumping after [redacted] ing, the counts at lower mortality (?) may be disregarded + the initial count should be considered to be

6/1/46.

~~#30 P31~~. Inoc 50 ml coli ∞ and shake at 30° - Y38.
4. ~~#4~~ P1

1. Dil 1:10⁷ into coli ∞. ca 2000.

shaker [illegible] Irradiate 5 mins as exp. 221. Inoc 5 ml into 50 ml coli ∞ and incubate on shaker. (A). Centrifuge + wash 25 ml + resuspend in saline. Inoculate 0.1 ml into T (arginine) by detection technique, c̄ cover.

Immediately after irradiation: 100 [illegible]
.1 ml = ca 2000 colonies.

1
2
3 $pS_{5\,min.} = 6.$
4
5
6
7
8
9
10.

Dil. A 1:10⁻⁷ and inoc as above.

11
12
13
14
15
16
17
18
19
10.

opt. arginine = 300 r dl – .

Phage T-1.

1/2/46.

Dilute T-1 as received from Demerec (labelled titre 2×10^{10}) 10^{-9} and plate c̄ ca 10^9 cells of: 740P2

1. B/r — Uniform turbidity
2. Y38 (B/r) Arginineless. — Uniform turbidity

3, 4 } Plate ca 10^8 Y38 c̄ 10^{10} T-1 in coli ∞. Turbid! for Y38/T1: Large charcenter c̄ ca 400 secondary colonies; periphery turbid.

5. 10^8 Y38 in coli ∞ ag. When solid, streaks
6. 10^9 B/r in coli ∞ ag. T-1 over surface.

} no response!

~~B3~~ Liquid culture —

4P4 — Inoc coli ∞ c̄ Y38 shake at 37°. ca. 10^8/ml.

1016P — Add 1cc of Phage T-1 "2×10^{10}".

12M - no change

9A5 - no change.

Sex;

Inoc 50ml coli ∞ flasks + shake @ 30°. 11P2 6/2/46.

Shaker broke down A3.

1. 58-161 —
2. 679-183. —
3 58-161 + 679-183. turbid; ca 16 colonies.

11P4 - Centrifuge + wash. Inoc into T(0) ≐ 2cc. culture. per plate + incubate. Also 208-51-2-3. → 0, 0, 0.

As above. No shaking 1130P5. - 1230A7. plate out on T(0).

	P7	
11 58-161	0	
12 679-183	0	
13. } grown together	4	! Fusion or sexuality ??
14. }	5	
15 } grown separately	0	
16. }	0	

P7. Pick 6 colonies from ~~[illegible]~~ to ~~[illegible]~~ coli ∞ ; p8 to slants. 1-6.

See: 227

P10 - numerous additional colonies appear in 13+14
Definite halation around colonies for a diam 2-4mm.

6/5/46.

Inoc. 5 flasks coli ∝ ē Y38. Grow shaken 37° 4P4

1030P 5:

Irradiate individually 5 min. in same quartz tube + determine killing.

	t = 0. 10^{-7}	t = 5. Inoc 1 ml.
1.	ca 100.	ca 19,000
2		
3		
4		
5		

Killing pS at 6 mins. can be taken as: ∝ log : $10^4/10^9$ = (5.)

Y38 - mutants.

6/6/46.

1130 P5 Mor 50 ml coli ∞ c̄ Y38 Shake at 37°.

Irradiate 6 mins. Wash by centr., dilute 1:100 + plate in T(arg.) for mutant detection: ~~[illegible]~~ 2A7

1
2
3
4
5
6
7
8
9
10

Too heavy !! * * ?

ca 10^{4-5}

Mor 1 ml into F(0) and cultivate at 37°.

6/9/46. 9 JUN 1946

1A9 - Plate cultures 1-4 of 224 (1-6) at 10^{-6} and 10^{-7} into T(0).

1a
b

2a
b

3a
b.

1A10 - numerous colonies on plates per expectation. Pick sample colonies from surface each plate ~~and~~ to H_2O + to slant coli ∞ + test on minimal

Plate:		T(0).	T(0).
1.	1	++	
	2	"	
	3	"	
2	11	"	
	12	"	
	13	"	
3	21	"	
	22	"	
	23	"	
4	31	"	
	32	"	
	33	— →	
~~5~~	~~41~~ ~~51~~	see 231.	

A10. from ∞ slant to tube of ∞. Unsh. Use in PM for plating.

Phage T-1.

1 9 JUN 1943

1. Isolated colonies from plate 223-3. to coli ∞. Transfer to slants.
2.
3.
4.

11. Pick agar between colonies from plate 223-3 to the surface of a coli ∞ plate seeded with B/1. No lytics. Probably agar too old.

≡ phage ≡ !!?

12. Incc coli ∞ tube ≡ B/1 heavily transplant. 8 P. 10 P. Incc T-1 (D) by 1130 considerable clearing?

13. " agar plate as above ≡ T-1 (D). → lytic zone.

2A 13A. Y38 + T-1. → lytic zone. Use YB5 agar for coli ∞ in

9. 14. Streak agar plate of B/1 ≡ 12. disturbed lytic zone. this expt.

15. Streak agar plate of Y38 ≡ 12 lytic zone (?); later somewhat turbid

all streaks gave clear areas:

∴ Phage C is effective on coli B/1 + Y38; also Y38 is susc. to T-1.

21. 11A9. Incc 5 ml coli ∞ T-1 "10^10" + 5 ml 10 hour E. coli B/1 from coli ∞ Incubate at 37 in shaker. 1230 P9. - largely cleared.

22. Incc 50 ml ≡ 21-1 ml + B/1 5 ml. 1230 P9. Incubate at R.T. 11P9: - Cleared (faint residual turbidity). Control completely turbid! Use for stock T-1. See 230

23. Plaque count T-1 (D) on B/1. 10^-3 to 10^-9

- results obscured by vigorous fermentation + bubble formation.

No plaques found at 10^{-11}, 10^{-9}, 10^{-7}

Numerous at 10^{-5} (ca 10^3).

Innumerable at 10^{-3}. Should use medium with less glucose for phage enumeration?

Gas bubbles in the agar are cones or lenticulate.

Try threonine. & recovery added Threonine in view of very high requirement.

9 JUN 1946

As 202. K-12 in 50ml T( ) + suppl. Shake at 37°. Inoc 145A9.

		G (1:10)	d.	mg/25 cc ca.	Hydrolysate cast =		Recovery %
	1. 0	84¹	.744		85¹	.693	93%
prol	2. glut. 5mg	81³	.875		for aq. (74³) = 83²	.783.	89%
indole	3. anthr. 5mg	83²	.783				
put	4. pantolact 1mg β-alanine 1mg.	84	.757				
arg.	5. citrulline 5mg.	83² (73)	.783		(78²)		

See 234.

Harvest 1130P9. Separate 25 ml samples. (a). 1b = 15cc.
Start hydrolysis in 6NHCl 20cc but discontinue due to severe bumping: — for 1, 2, 5 only. Estimate recovery from volume ←

	Volume after hyd.			
1.	~~4.5cc~~ 11cc	[illegible]	to	13.5cc
2.	4.5cc	22.5%		5.6cc
5.	6cc.	30%.		7.5cc

Neutralize. Dilute to % of 25 cc ind. by recovery
1cc of each solution should give mutant responses of magnitude indicated above, in 10 ml medium. See 234. for assays

Glutamic does not increase (proportion) production of proline in wild type
Citrulline " " " " arginine

Note Y38 is a B strain and therefore >100% recovery does not signify anything.

∴ Requirement is α content. (Proline α = c. 3% dry wt.).

Phage T-1

6/10/46.

Sticks - filter 228-22 for T-1 stock. Pool c̄ 228-21
Dispense aliquots variously; some to K.W. for lyophil preservation.
Plague out on β1r at indicated dilutions. 1A1 for titer.

1. 10^6 ca 300
2. 10^8 1
3. 10^{10} 0
4. 10^{12} 0

·/11/46.

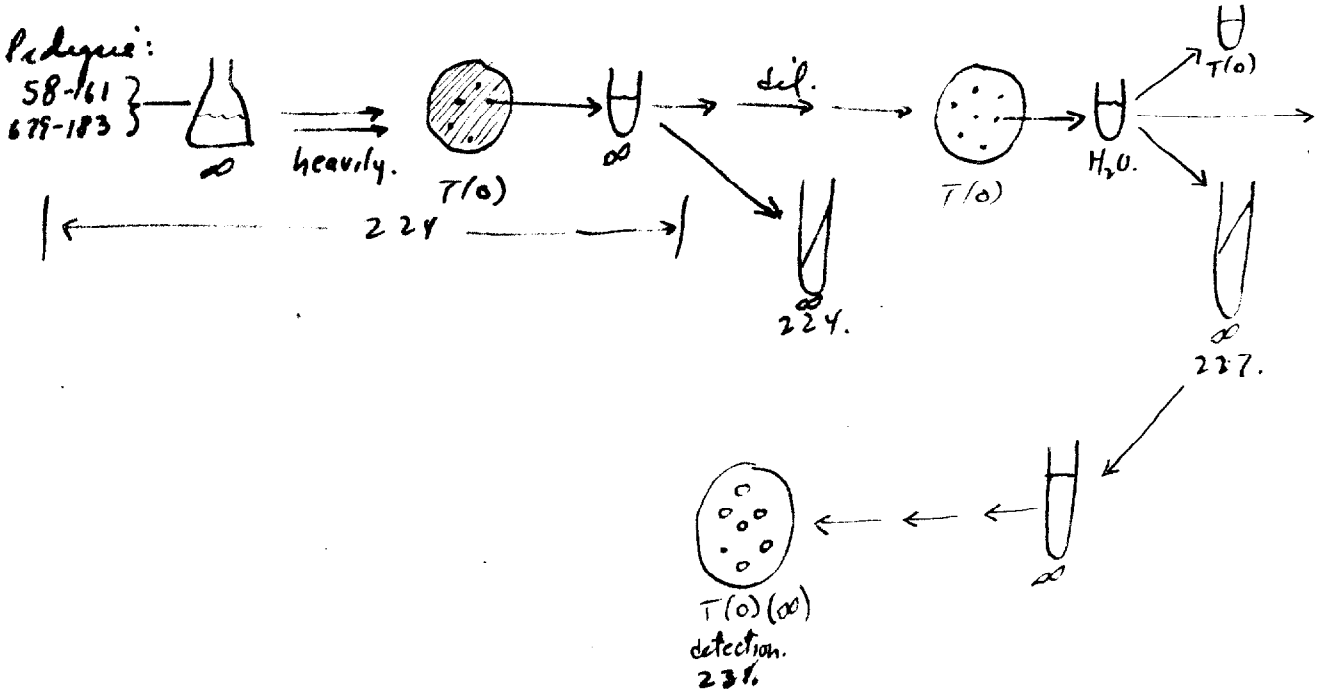
Plate out 227-1, etc. Detection plates, for mutant segregants.
Dil 10^{-6}, 10^{-7}. Unshaken culture in coli ∞ from ∞ slant.
227- 1A11. Layer E coli ∞ 12N12.

1
2 1

3
4 11

5
6 21

7
8 31.



1230A13 - Nothing sign. seen.
11A13 - do. (1 col 0?)

Y38 Mutants.

232

6/12/46.

Inoc coli ∞ 40ml c̄ Y38 + shake at 30° 11P11

Lebit ride

Sex

6/11/46.

Wash and plate heavily into T(0) etc. The cultures:

Mor P5 coli ∞ c̄

1. 58-161

2. 679-183.

3. 58-161 + 679-183.

heavily ≙ 1ml grown culture / plate.

5P11↓ (6 day cultures).

				1230A13	11A13
1.	1	in	O	O	
2.	1	in	O	O	
3	2	in	O	O	"
4	2	in	O	O	
5	3	in	O	O	
6	3	in	O	O	
7	1+2	in	O	O	
8	1+2	in	O.	O	"
9.	1	in	biotin	O	
10	1	in	meth	O	
11	2	in	threonine	O	
12	2	in	proline.	O	
13	3	in	BP	O	"
14	3	"	BT	O	
15	3		MP	O	
16.	3		MT.	O	

OK / throw out.