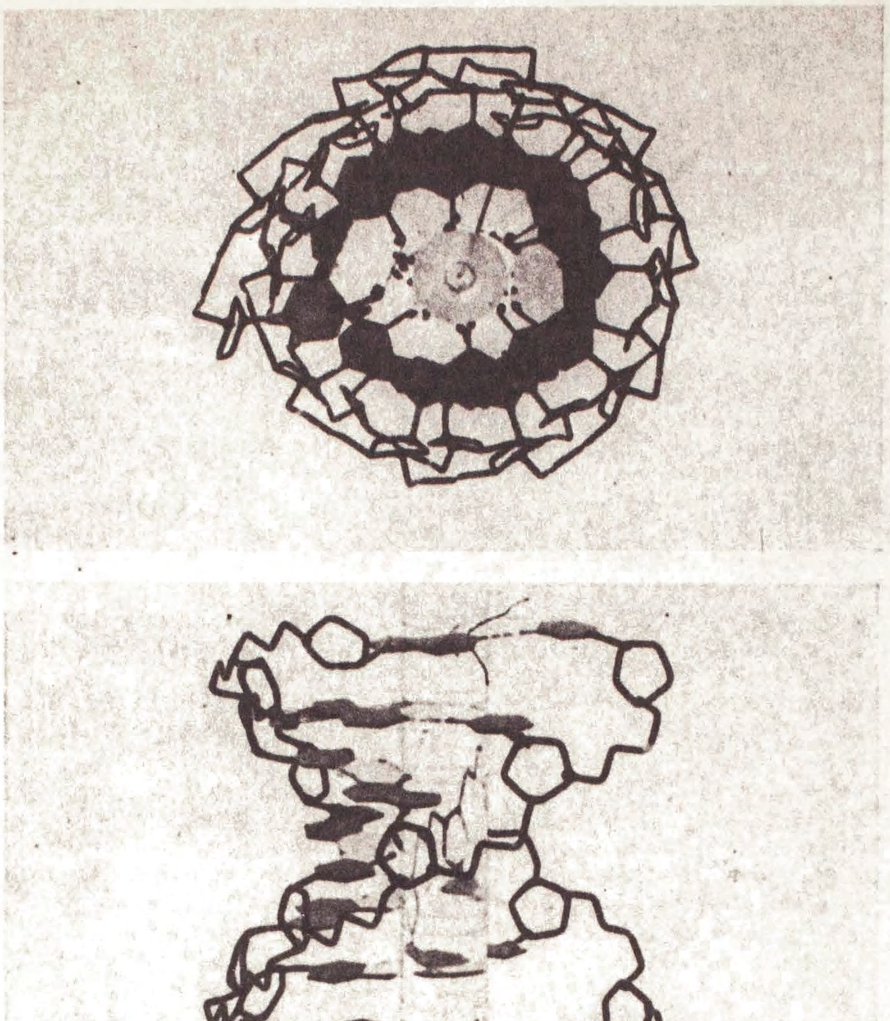
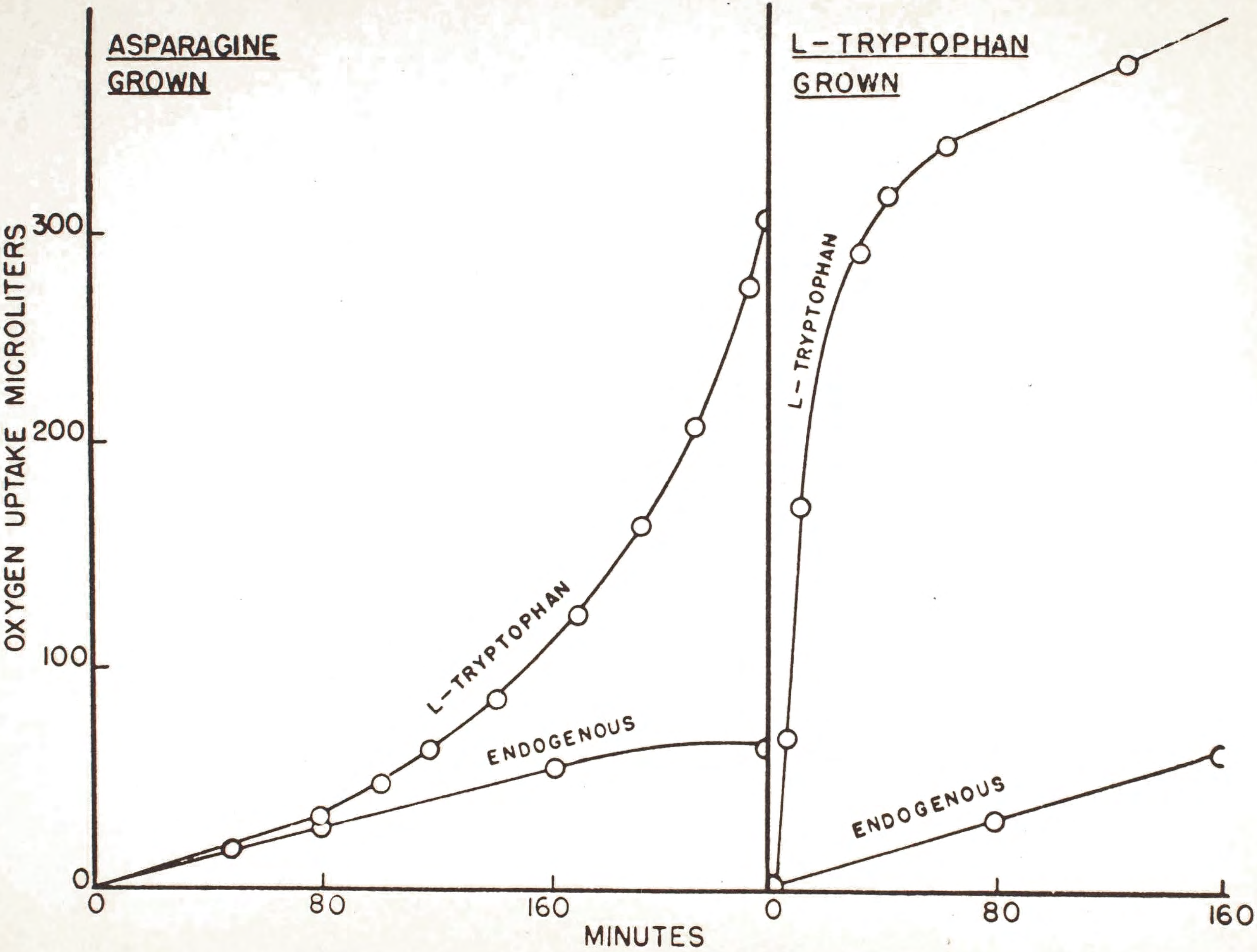
EXTRAS



STRUCTURAL MODEL shows a pair of DNA chains wound as a helix about the fiber axis. The pentose sugars can be plainly seen. From every one on each chain protrudes a base, linked to an opposing one at the same level by a hydrogen bond. These base-to-base links act as horizontal supports, holding the chains together. Upper photograph is a top view.

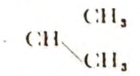
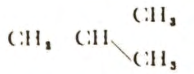
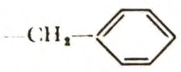
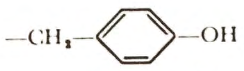
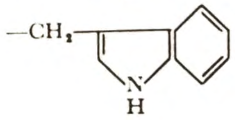
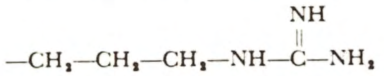
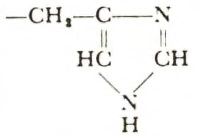
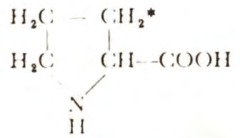
6



ASPARAGINE GROWN
L-TRYPTOPHAN GROWN
OXYGEN UPTAKE MICROLITERS
300
200
100
0
L-TRYPTOPHAN
ENDOGENOUS
L-TRYPTOPHAN
ENDOGENOUS
0
80
160
0
80
160
MINUTES

TABLE I

Common naturally occurring amino acids

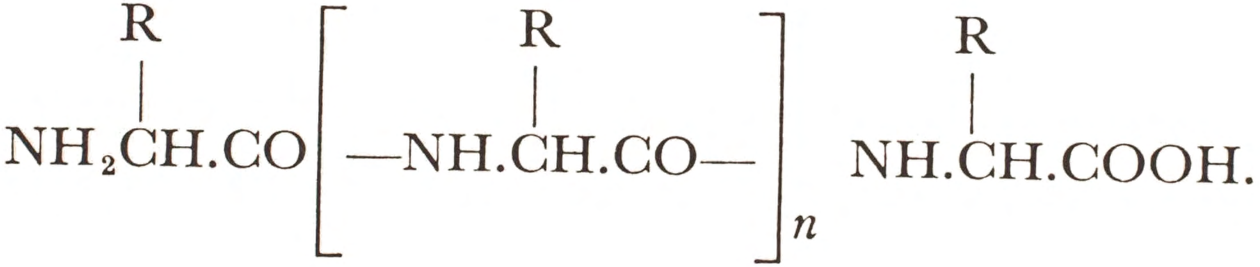
Amino acid	*Structure of R group*	*Abbreviation used for residue* $NH-CH(R)-CO-$	*Number of residues in insulin*
Aliphatic			
Glycine	H	Gly	4
Alanine	CH_3	Ala	3
Valine		Val	5
Leucine		Leu	6
Isoleucine	$-CH(CH_3)-CH_2-CH_3$	Ileu	1
Serine	$-CH_2-OH$	Ser	3
Threonine	$-CH(OH)-CH_3$	Thr	1
Aromatic			
Phenylalanine		Phe	3
Tyrosine		Tyr	4
Tryptophan		Try	0
Acidic			
Aspartic acid	$-CH_2-COOH$	Asp	0
Glutamic acid	$-CH_2-CH_2-COOH$	Glu	4
Acid amides			
Asparagine	$-CH_2-CONH_2$	$AspNH_2$	3
Glutamine	$-CH_2-CH_2-CONH_2$	$GluNH_2$	3
Basic			
Lysine	$-CH_2-CH_2-CH_2-CH_2-NH_2$	Lys	1
Arginine	 $-CH_2-CH_2-CH_2-NH-\overset{\overset{NH}{\parallel}}{C}-NH_2$	Arg	1
Histidine		His	2
S-containing			
Cysteine	$-CH_2-SH$	CySH	0
Cystine	$-CH_2-S-S-CH_2-$	CyS–CyS	3
Methionine	$-CH_2-CH_2-S-CH_3$	Met	0
Cyclized			
Proline		Pro	1

* Complete structure of the amino acid.

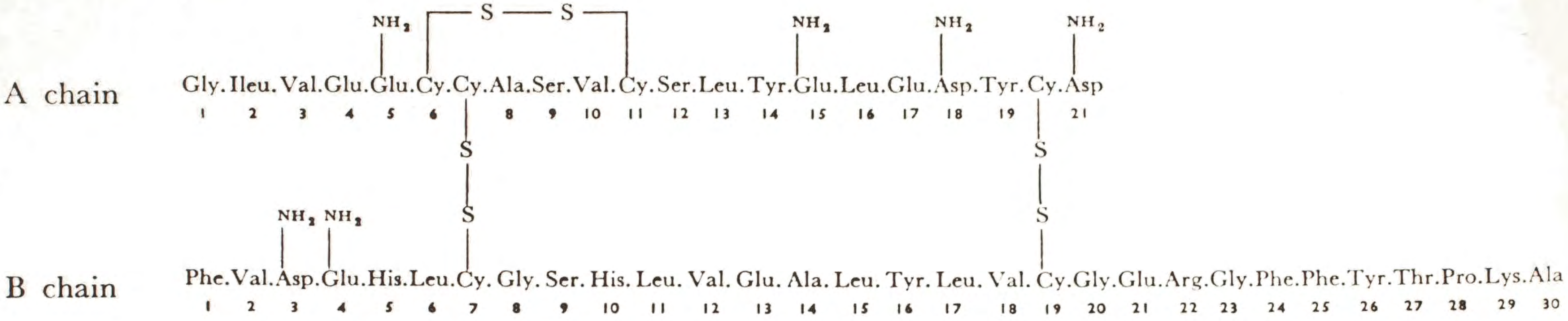
TABLE 1. CO-ORDINATES FOR THE ATOMS OF THE BACKBONE, FOR A SINGLE RESIDUE

atom	ρ (Å)	ϕ	Z (Å)
P	10·0	0·0°	0·0
O_{I}	8·95	− 3·6°	+ 0·8
O_{II}	11·25	+ 0·7°	+ 0·8
O_{III}	9·65	+ 8·9°	− 0·5
O_{IV}	10·35	− 5·3°	− 1·3
$C_{5'}$	9·6	− 22·2°	− 2·8
$C_{4'}$	9·65	− 13·2°	− 3·2
$C_{3'}$	9·2	− 7·3°	− 2·05
$C_{2'}$	8·65	+ 0·4°	− 2·8
$C_{1'}$	8·2	− 3·5°	− 4·15
$O_{1'}$	8·8	− 11·8°	− 4·35
N	6·7	− 4·2°	− 4·15
diad	—	+ 39·0°	− 4·15

$$\mathrm{R.CH(NH_2).COOH.}$$



$$\mathrm{NH_2\overset{\overset{R}{|}}{C}H.CO\left[-NH.\overset{\overset{R}{|}}{C}H.CO-\right]_{\mathit{n}} NH.\overset{\overset{R}{|}}{C}H.COOH.}$$



Structure of cattle insulin.

TABLE II

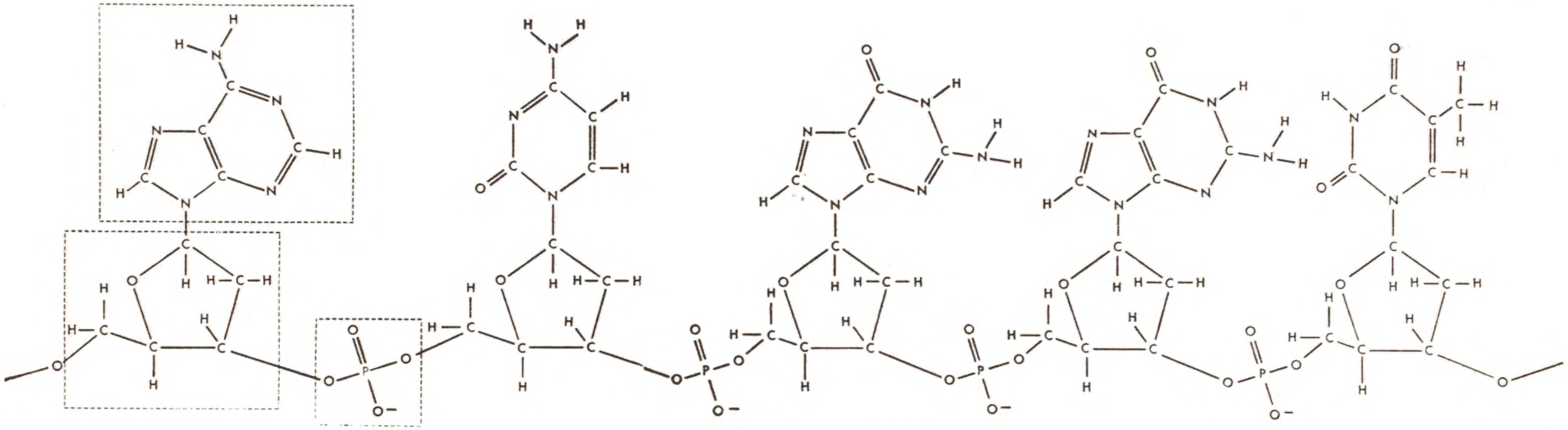
The amino acid sequence in positions A7–10 of insulins from various species

Cattle	$CySO_3H$.Ala.Ser.Val
Pig	$CySO_3H$.Thr.Ser.Ileu
Sheep	$CySO_3H$.Ala.Gly.Val
Horse	$CySO_3H$.Thr.Gly.Ileu
Whale	$CySO_3H$.Thr.Ser.Ileu

SUPPRESSOR GENE

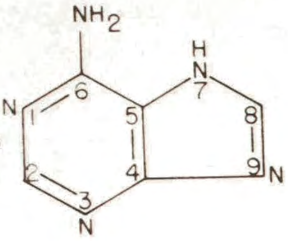
td MUTANT		su_2	su_3	su_{24}	su_6
Type I	td_1	−	−	−	−
Type II	td_2	+	−	−	+
Type III	td_3	−	+	+	−
	td_{24}	−	+	+	−
Type IV	td_6	−	−	−	+

+ = SUPPRESSION

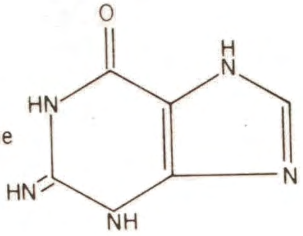


FRAGMENT OF CHAIN of desoxyribonucleic acid shows the three basic units that make up the molecule. Repeated over and over in a long chain, they make it 1,000 times as long as it is thick. The backbone is made up of pentose sugar molecules (marked by the middle colored square), linked by phosphate groups (bottom square). The bases (top square), adenine, cytosine, guanine and thymine protrude off each sugar in irregular order.

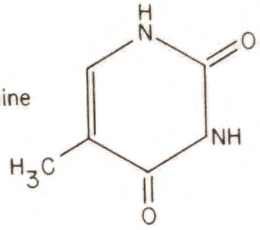
Adenine



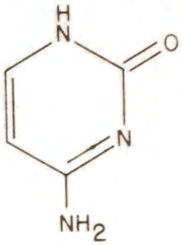
Guanine



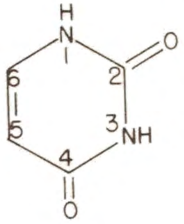
Thymine



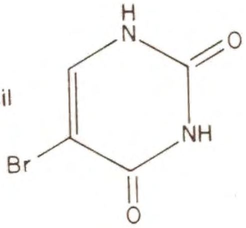
Cytosine



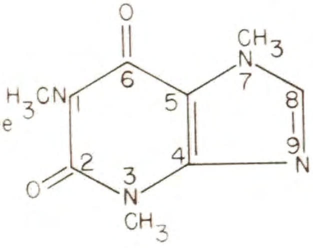
Uracil



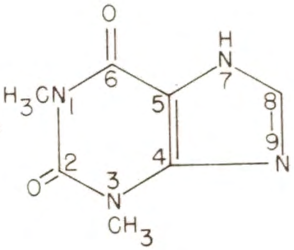
Bromuracil



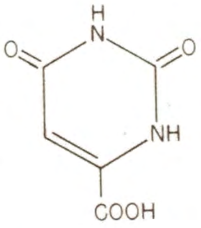
Caffeine



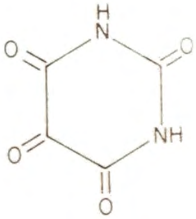
Theophylline



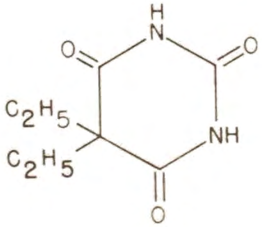
Orotic Acid



Alloxan



Barbital



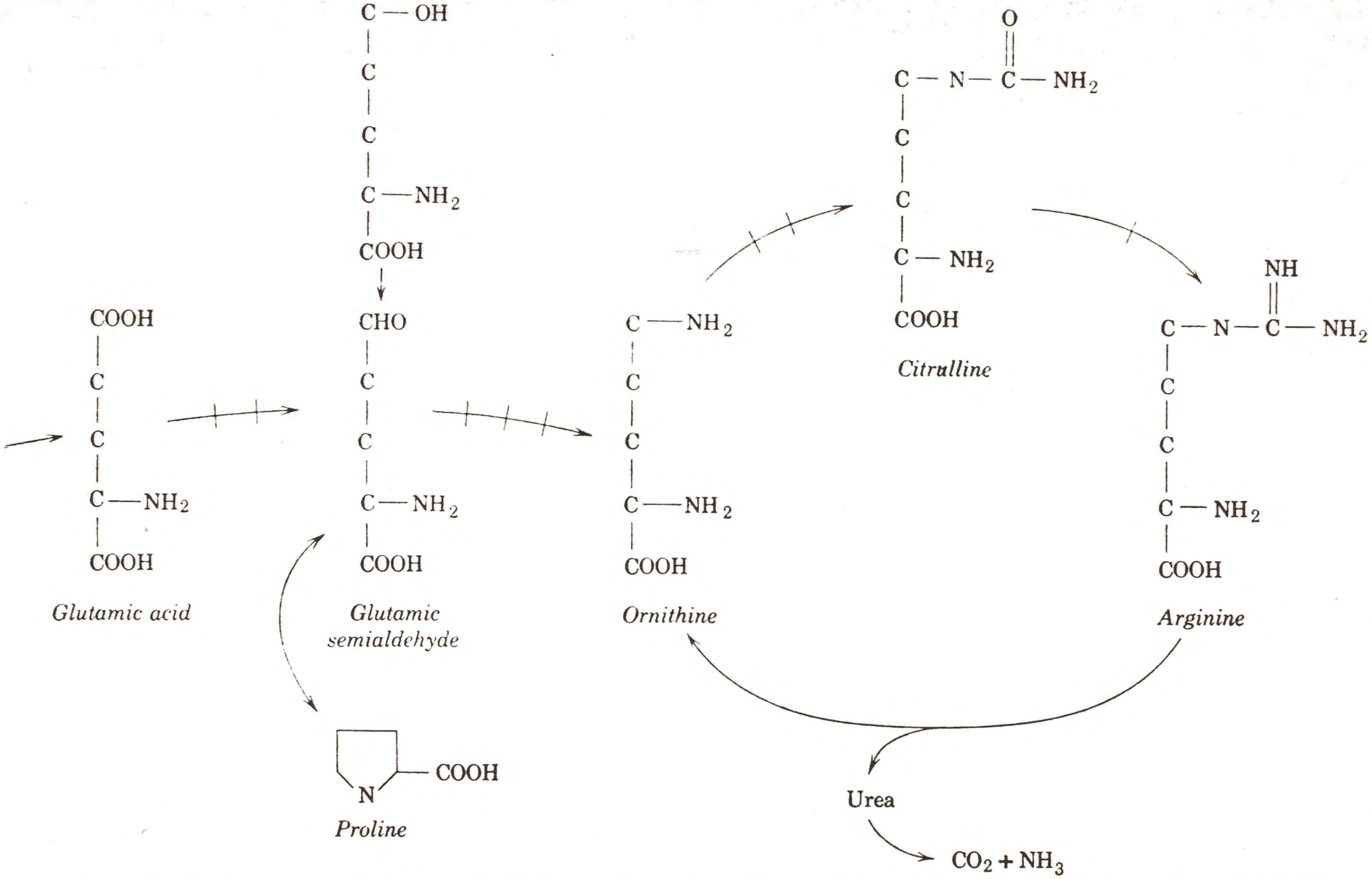


Fig. 56. Arginine and proline biosynthesis. Cross bars indicate apparent positions of known genetic blocks.

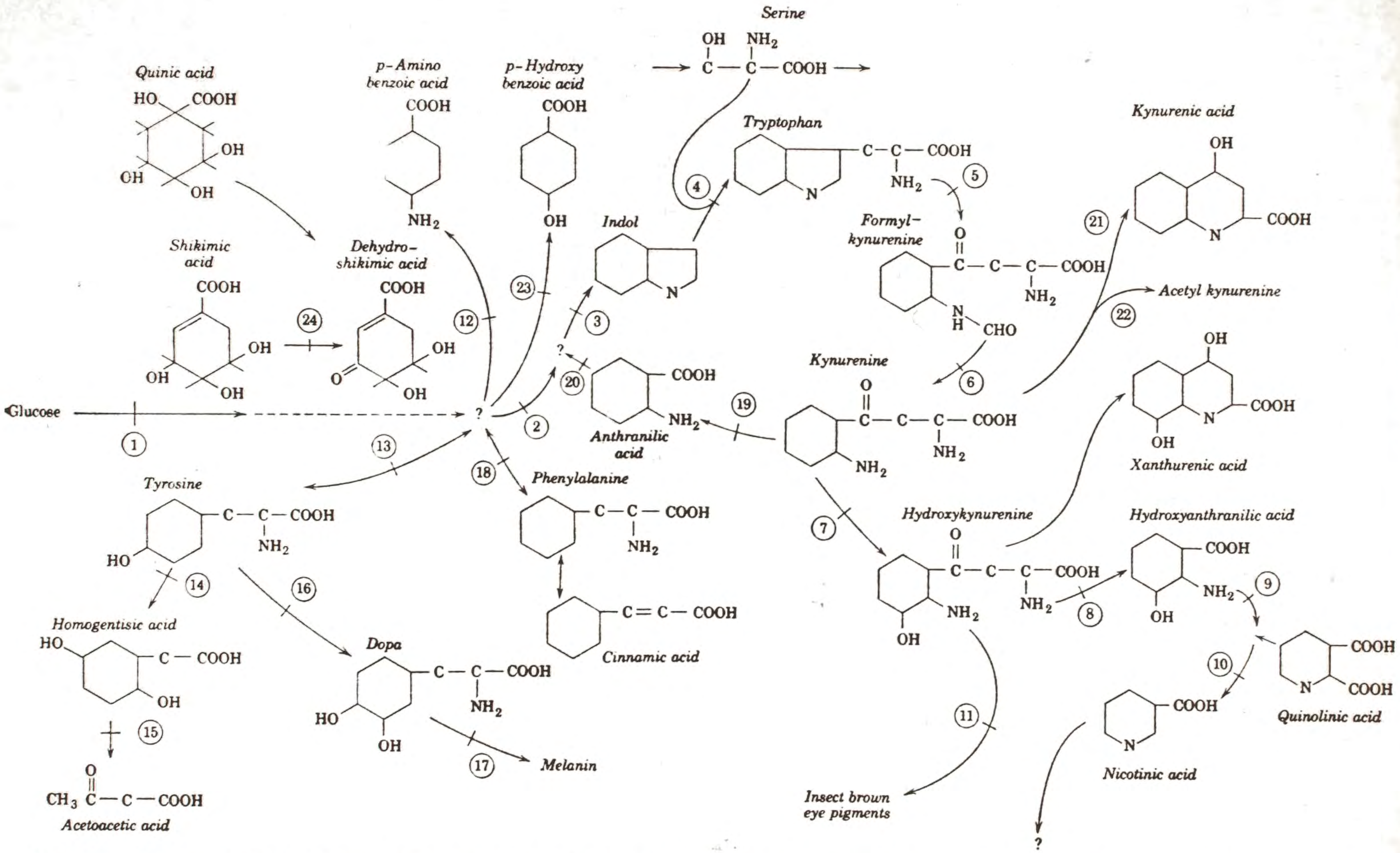


Fig. 53. Biosynthesis of the aromatic amino acids. Known positions of genetic interference in reaction series are indicated by the circled numbers on the cross bars. (See text for full explanation.)

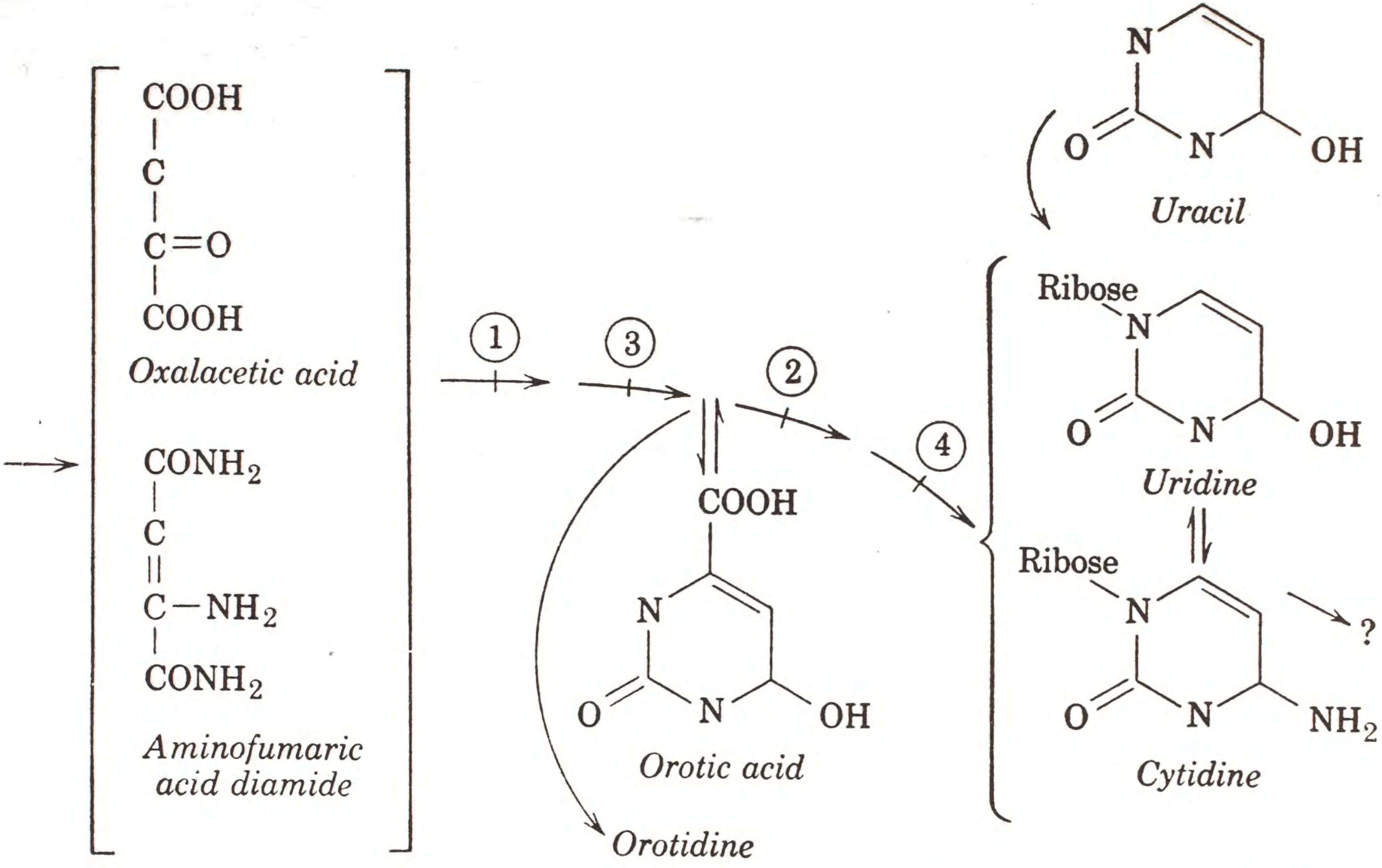


Fig. 60. Biosynthesis of some of the pyrimidines of nucleic acids. Studies of mutants have not established whether orotic acid and orotidine are true intermediates, but enzyme work has shown that orotic acid goes to orotidine phosphate and thence to the phosphates of uridine and cytidine instead of to the free nucleosides as indicated here.

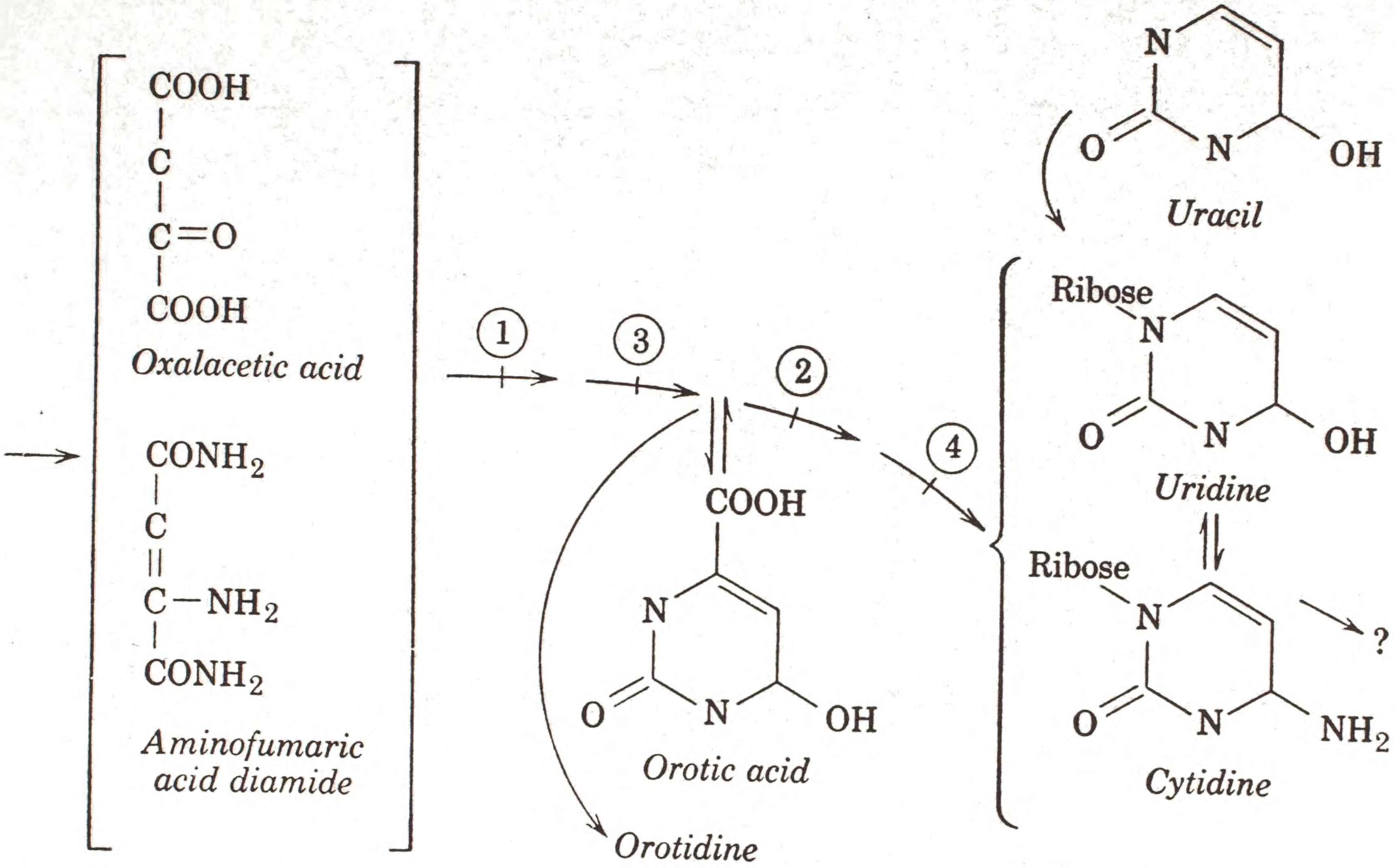


Fig. 60. Biosynthesis of some of the pyrimidines of nucleic acids. Studies of mutants have not established whether orotic acid and orotidine are true intermediates, but enzyme work has shown that orotic acid goes to orotidine phosphate and thence to the phosphates of uridine and cytidine instead of to the free nucleosides as indicated here.

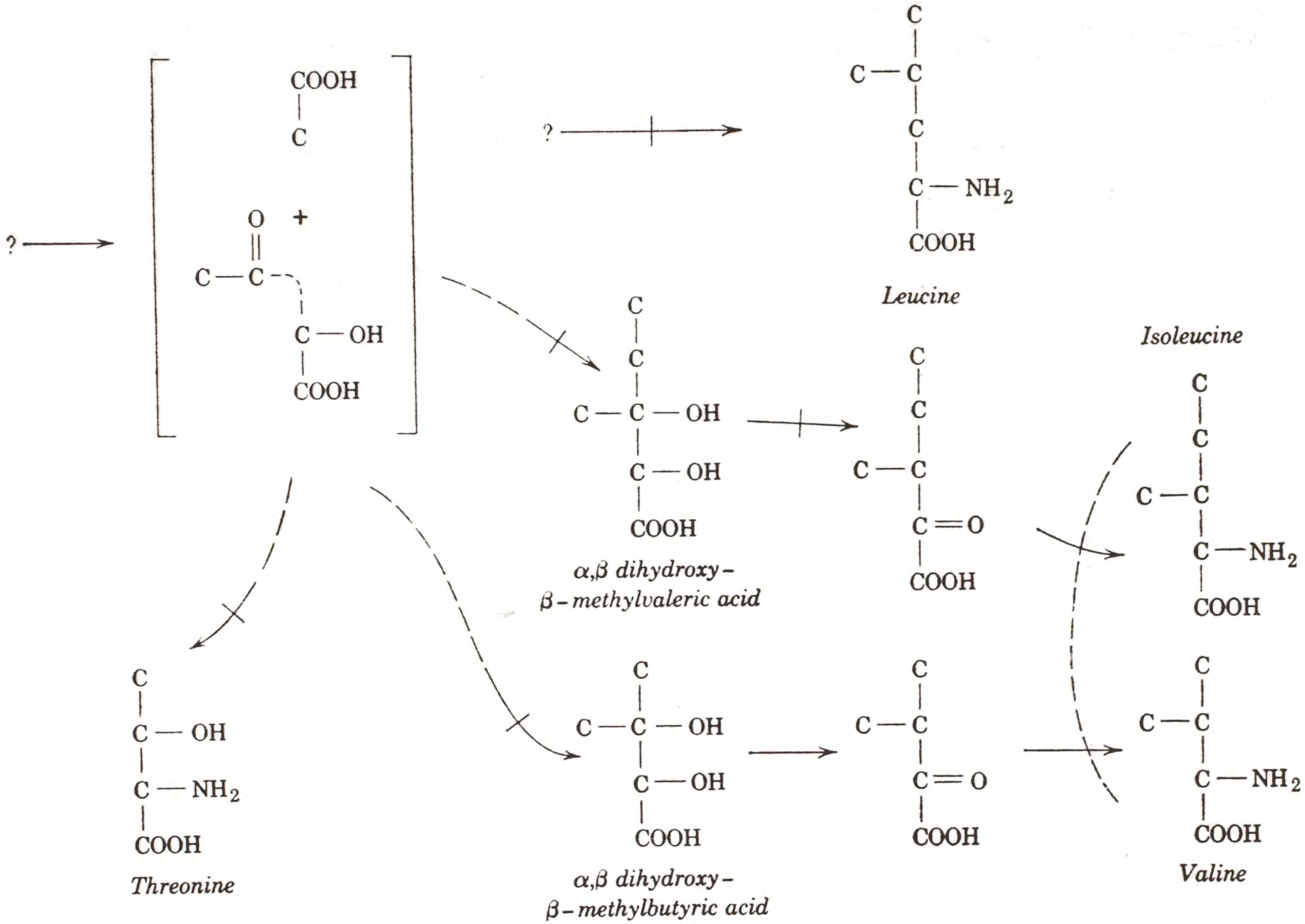


Fig. 58. The biosynthesis of leucine, isoleucine, and valine. It has been shown that a single mutation can block similar reactions in two series as indicated by the cross bar just following the keto acids that correspond to isoleucine and valine.

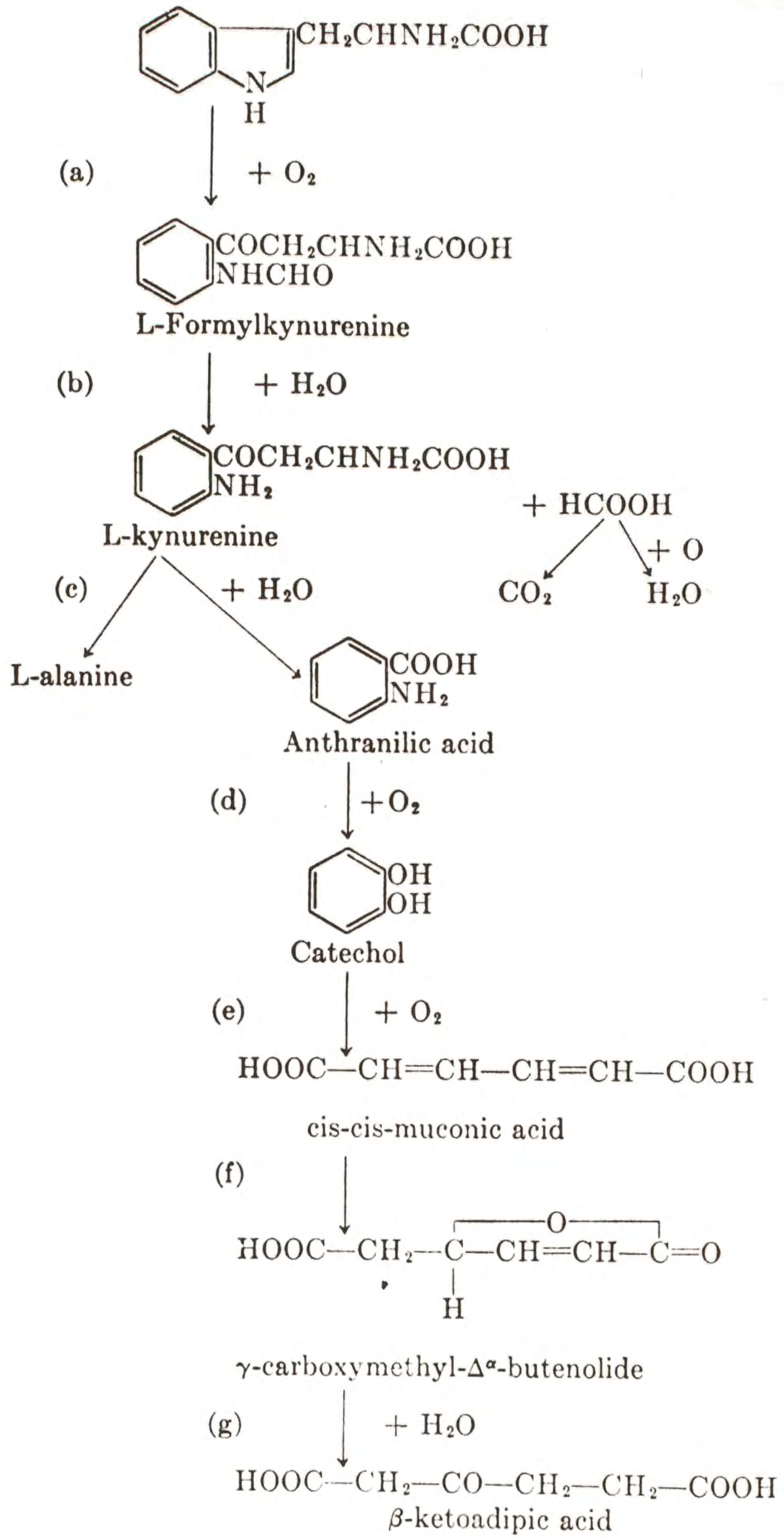


Fig. 2. The biochemical pathway for the oxidation of L-tryptophan by *Pseudomonas fluorescens*. The following inducible enzymes are involved: (a) tryptophan peroxidase + oxidase; (b) formylase; (c) kynureninase; (d) anthranilic acid oxidase (probably a multienzyme system); (e) pyrocatechase; (f) lactonizing enzyme; (g) delactonizing enzyme. The further breakdown of β-ketoadipic is catalyzed by an inducible enzyme, the decomposition of alanine is a constitutive reaction.

33

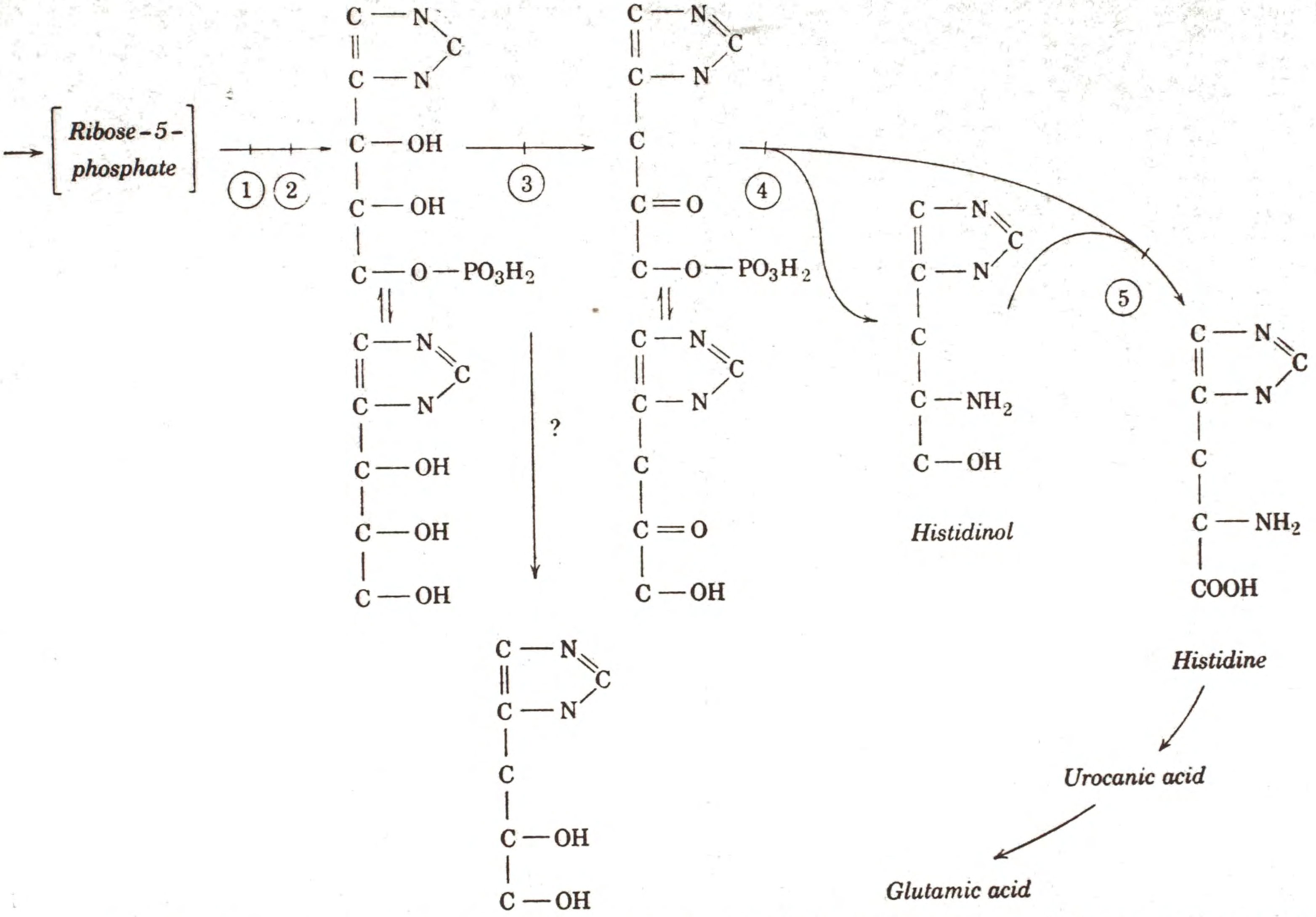


Fig. 55. The biosynthesis and degradation of histidine. Circled numbers indicate different mutant types known in *Neurospora* or *E. coli*.

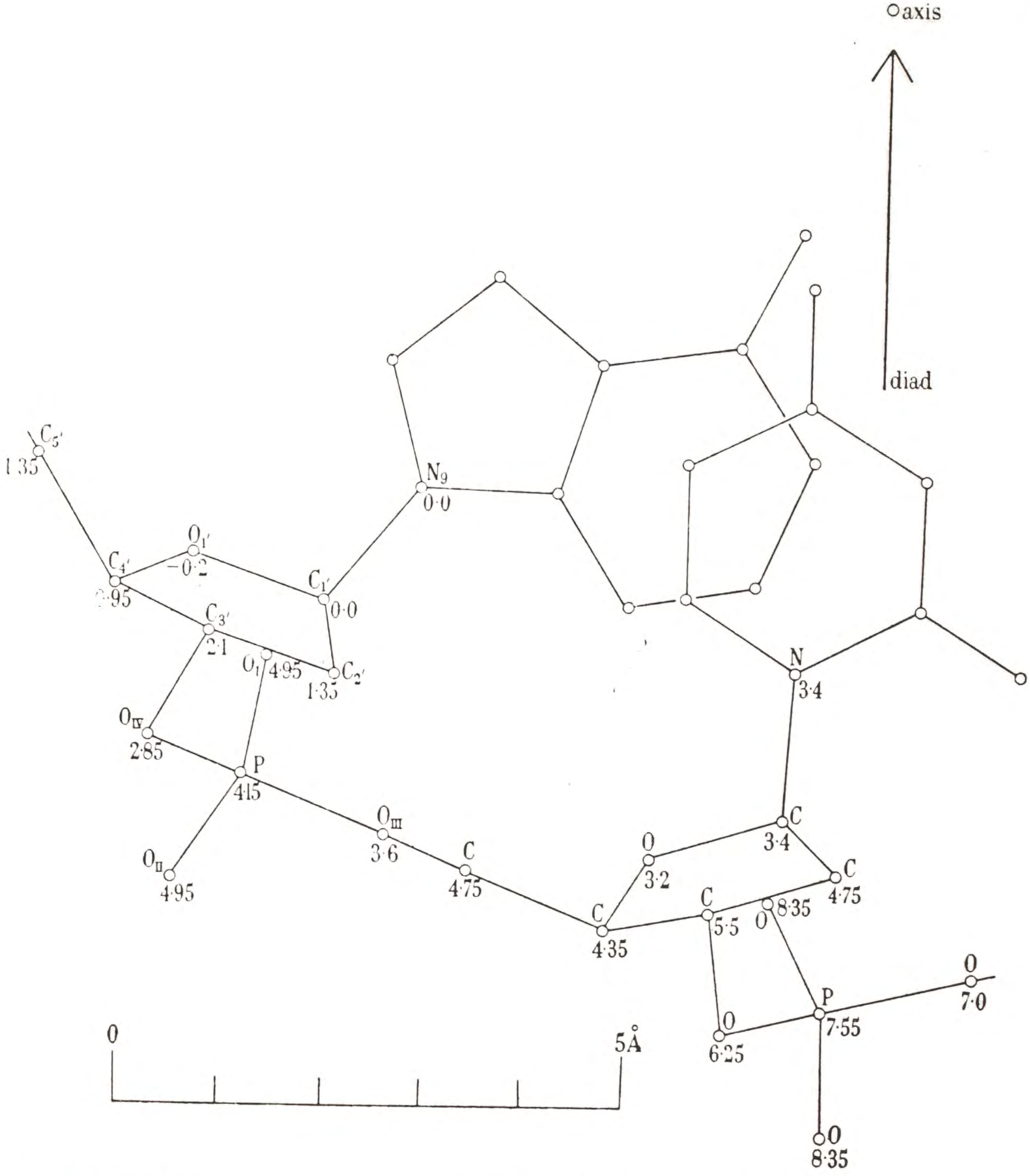


FIGURE 7. A projection of two successive residues of *one* chain of the structure. The direction of projection is parallel to the fibre axis. The figures show the height of each atom (in ångströms) above the level of the lower base.

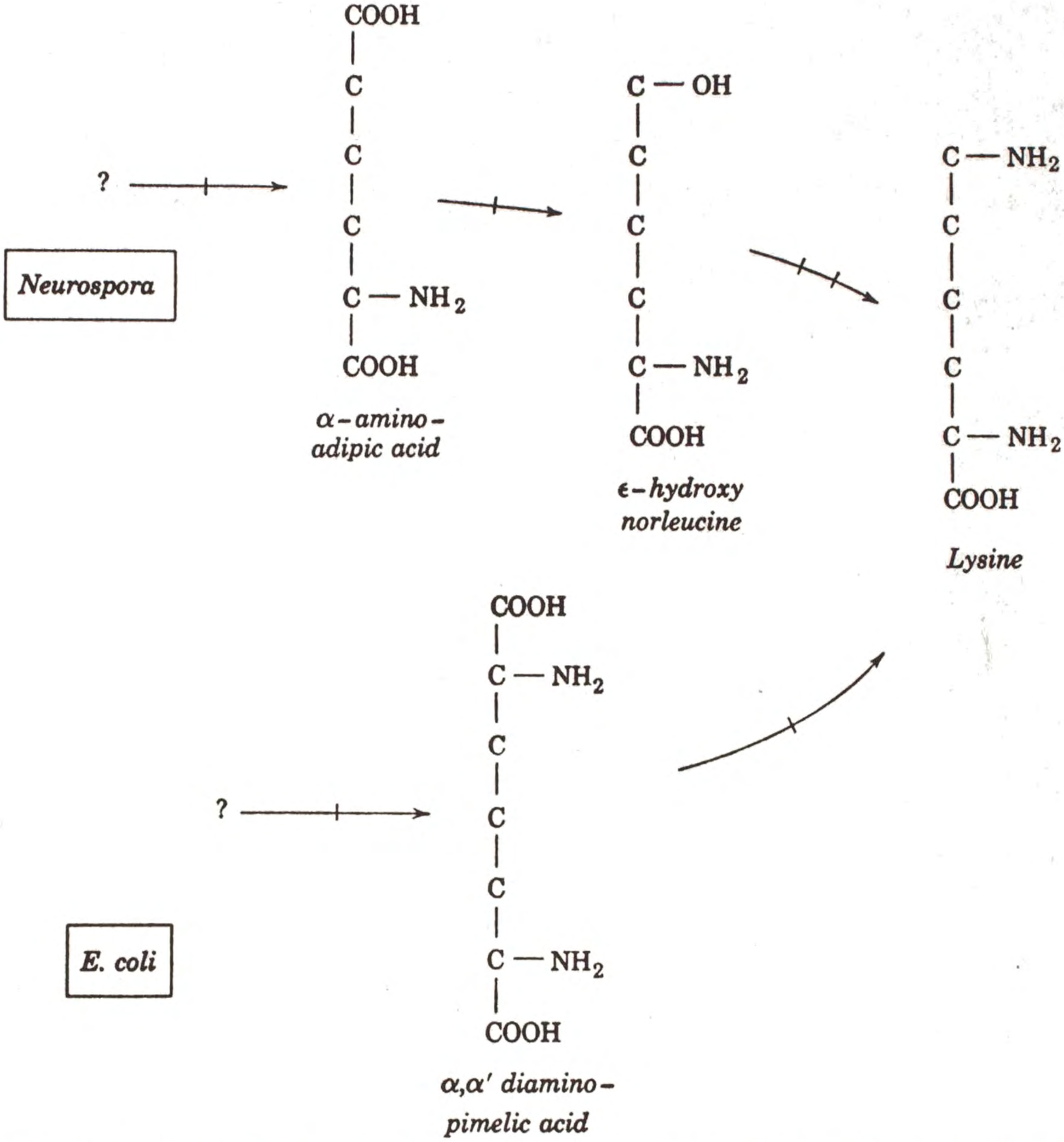


Fig. 57. The biosynthesis of lysine in *Neurospora* and *E. coli*. Two distinct pathways for biosynthesis are indicated.

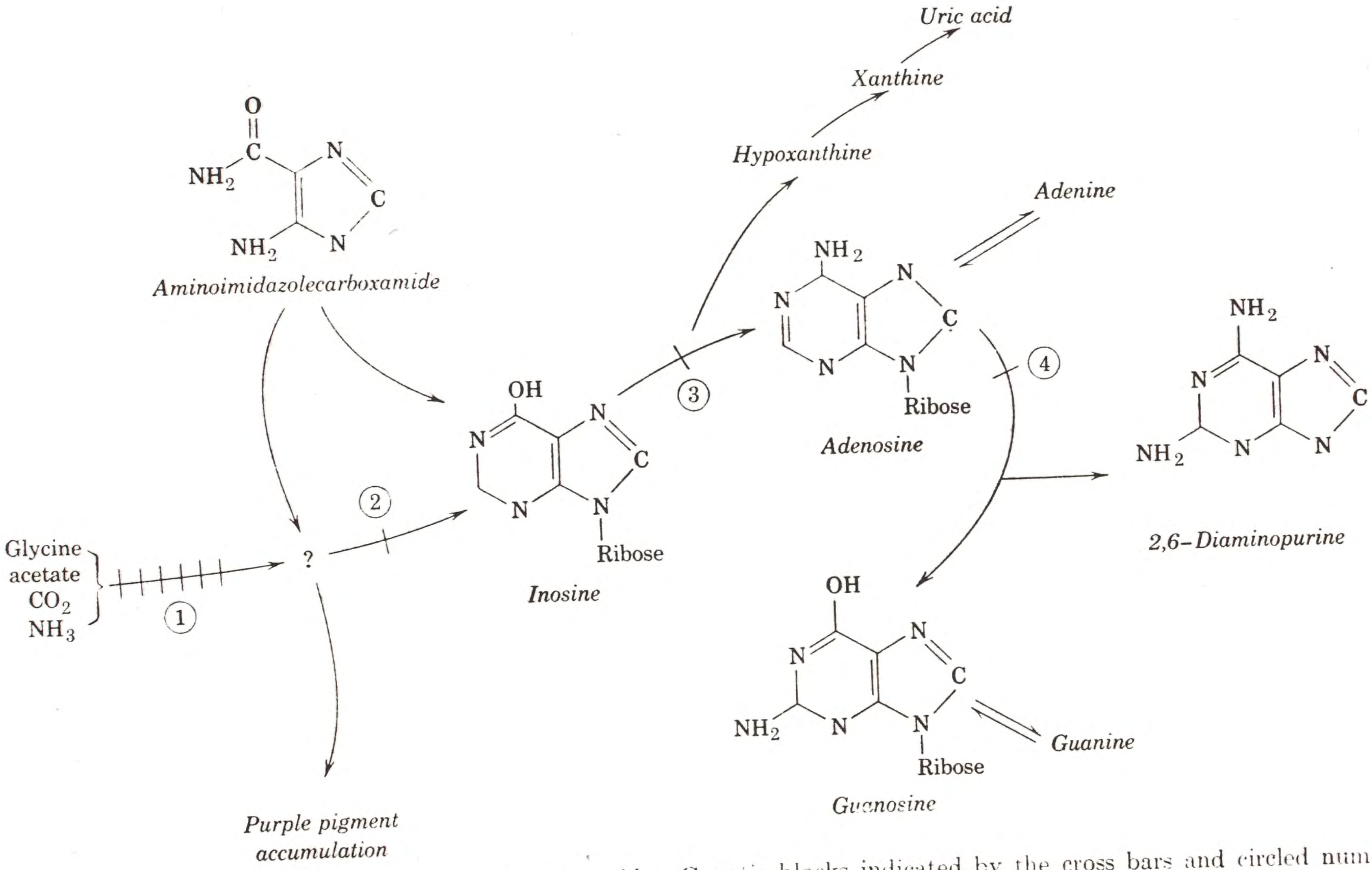


Fig. 59. Biosynthesis of the purines of nucleic acids. Genetic blocks indicated by the cross bars and circled numbers are discussed in detail in the text.

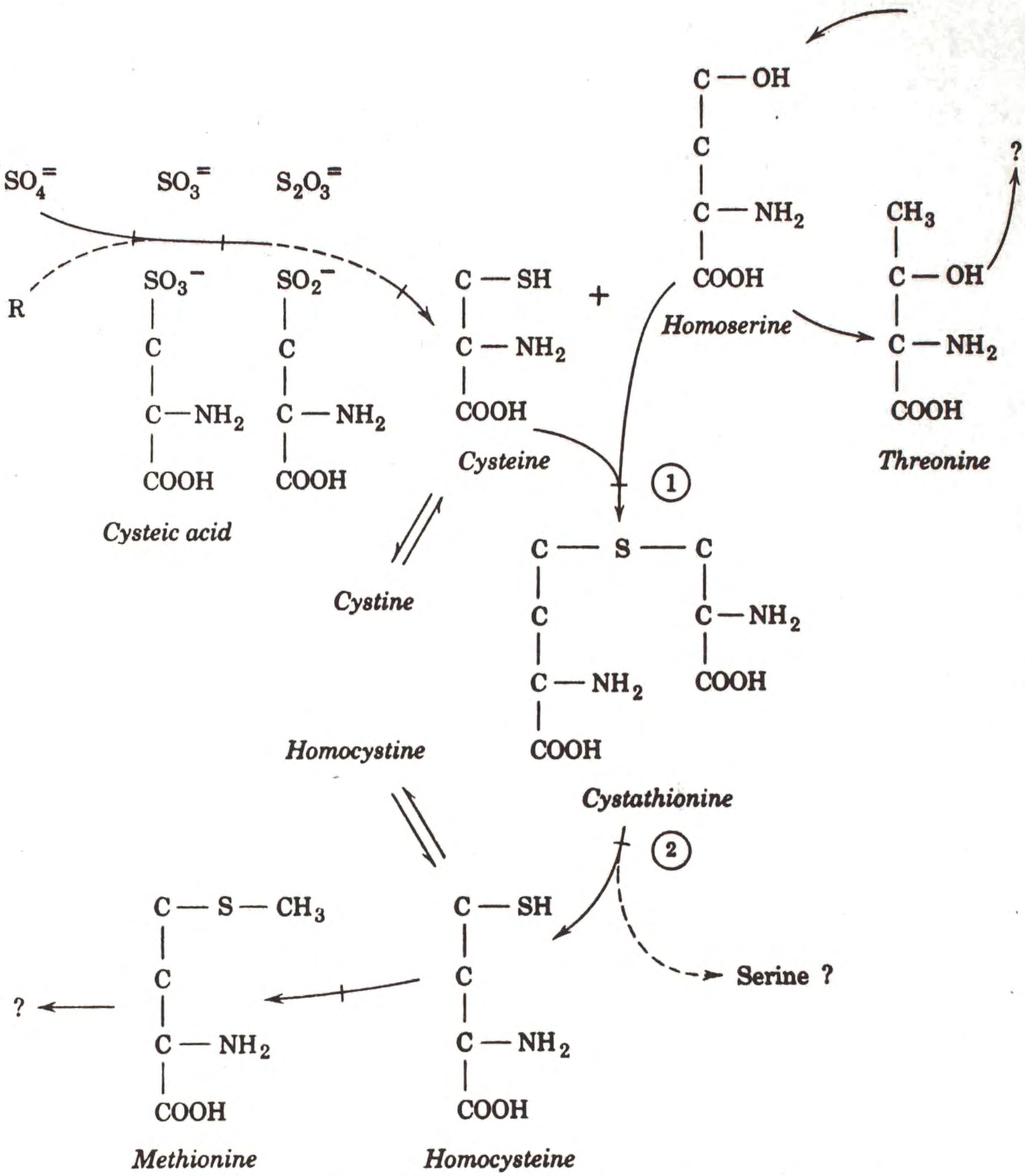
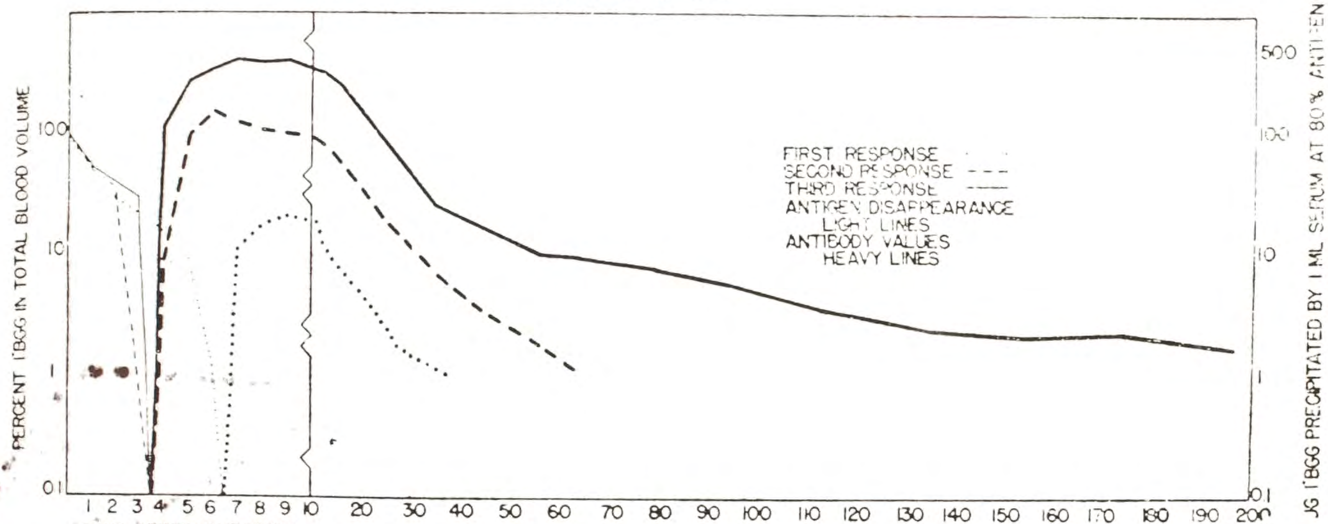


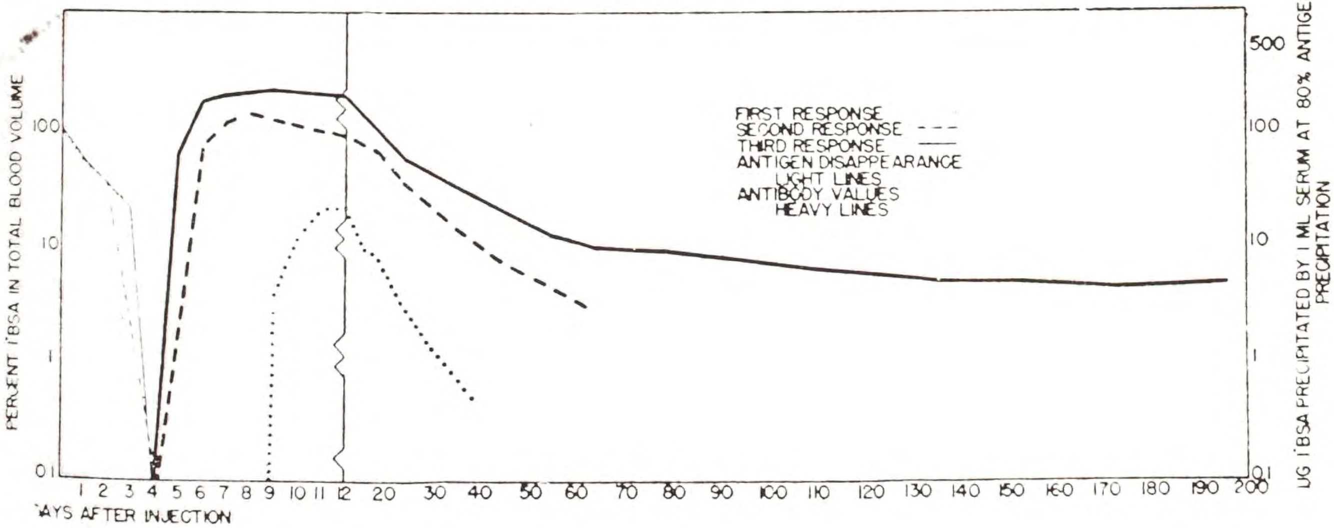
Fig. 54. Biosynthesis of the sulfur amino acids and threonine. The cross bars indicate positions of known genetic blocks.

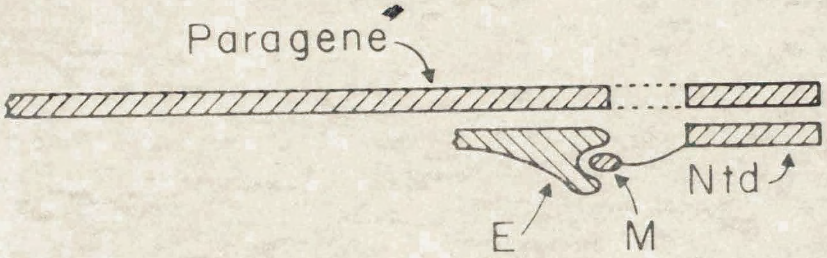
37

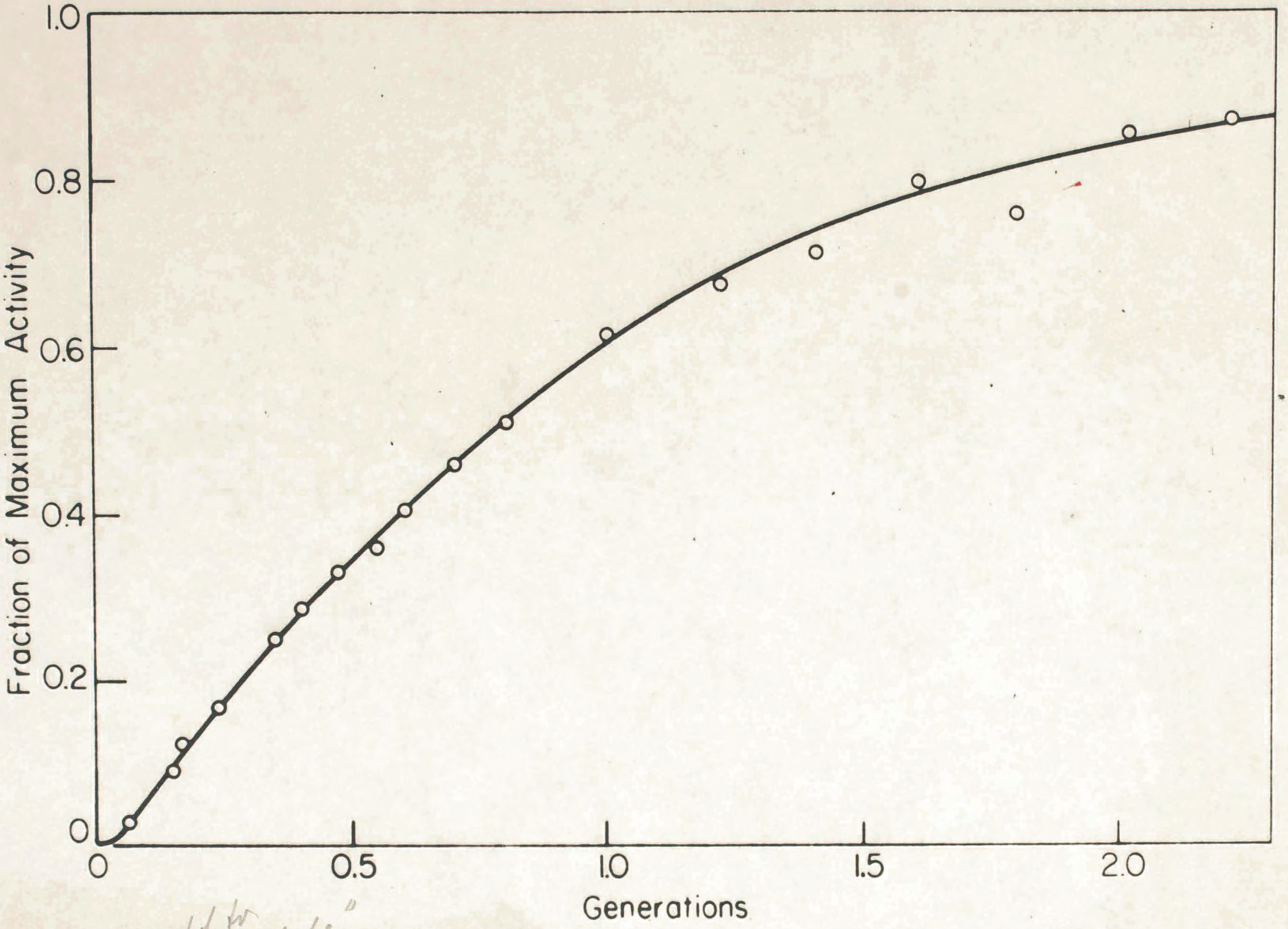
RESPONSES TO FIRST, SECOND AND THIRD INJECTIONS OF I'BGG
RABBIT # 977



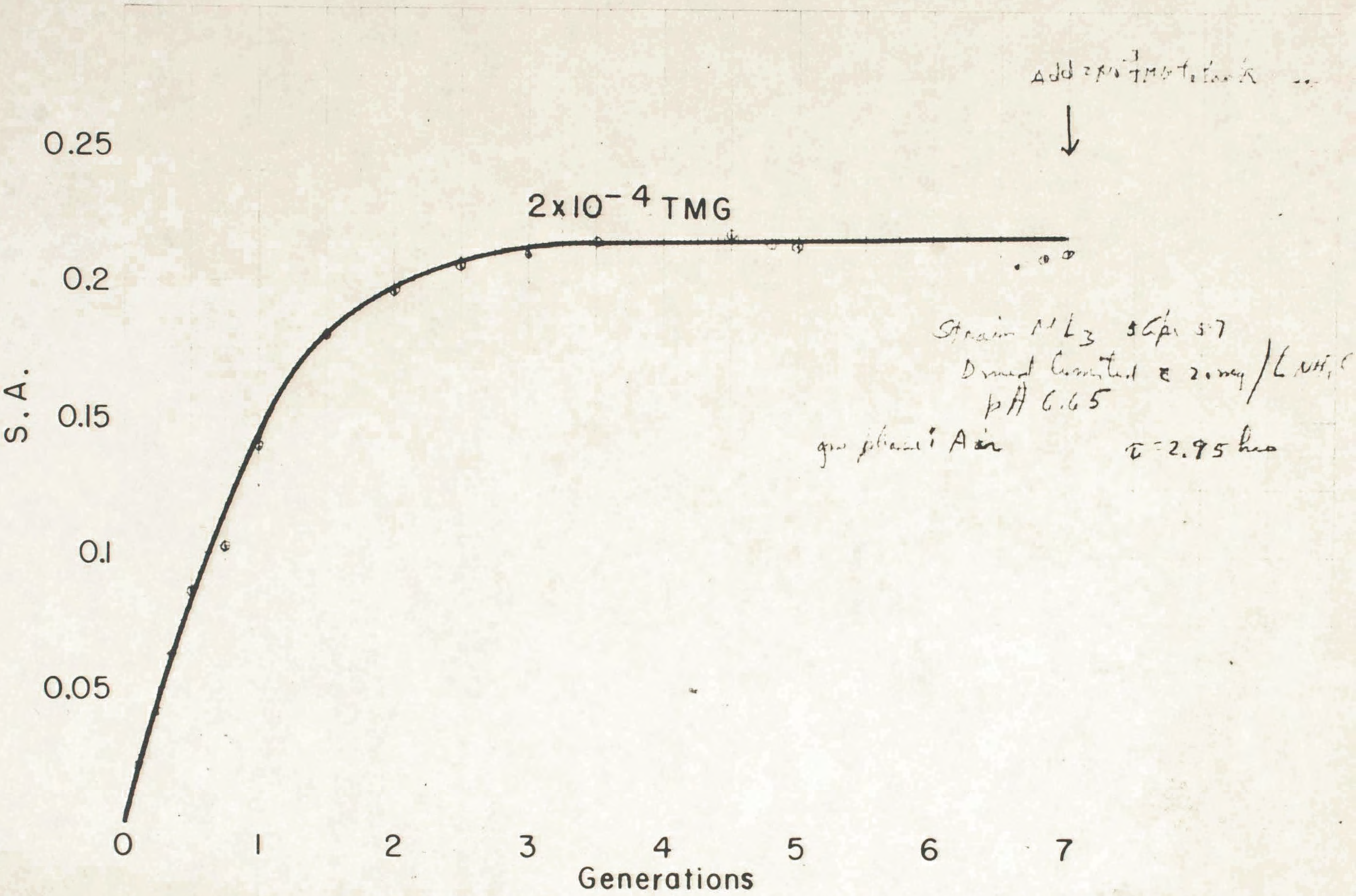
RESPONSES TO FIRST, SECOND AND THIRD INJECTIONS OF I'BSA
RABBIT # 984




Paragene
Ntd
E
M

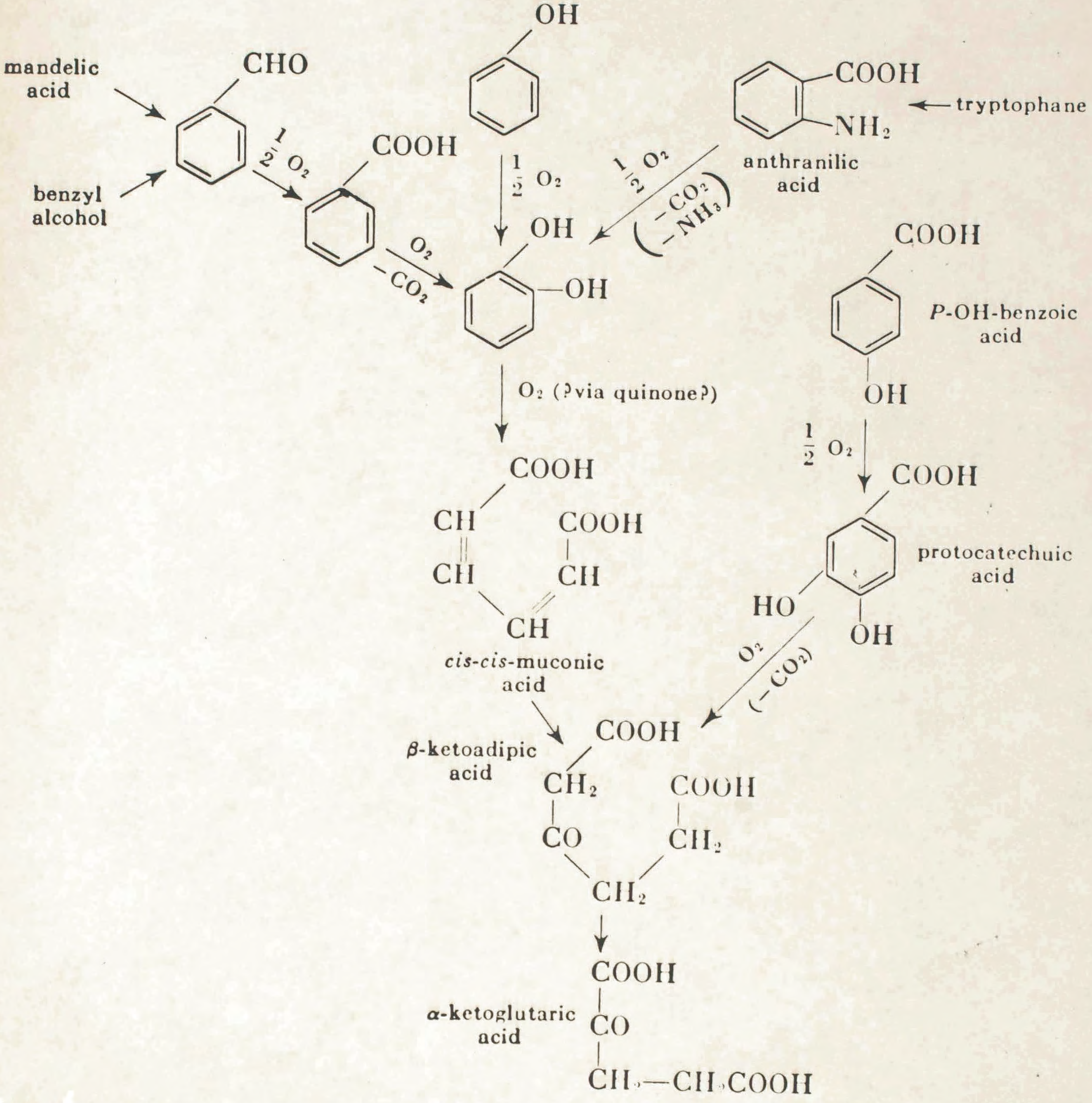


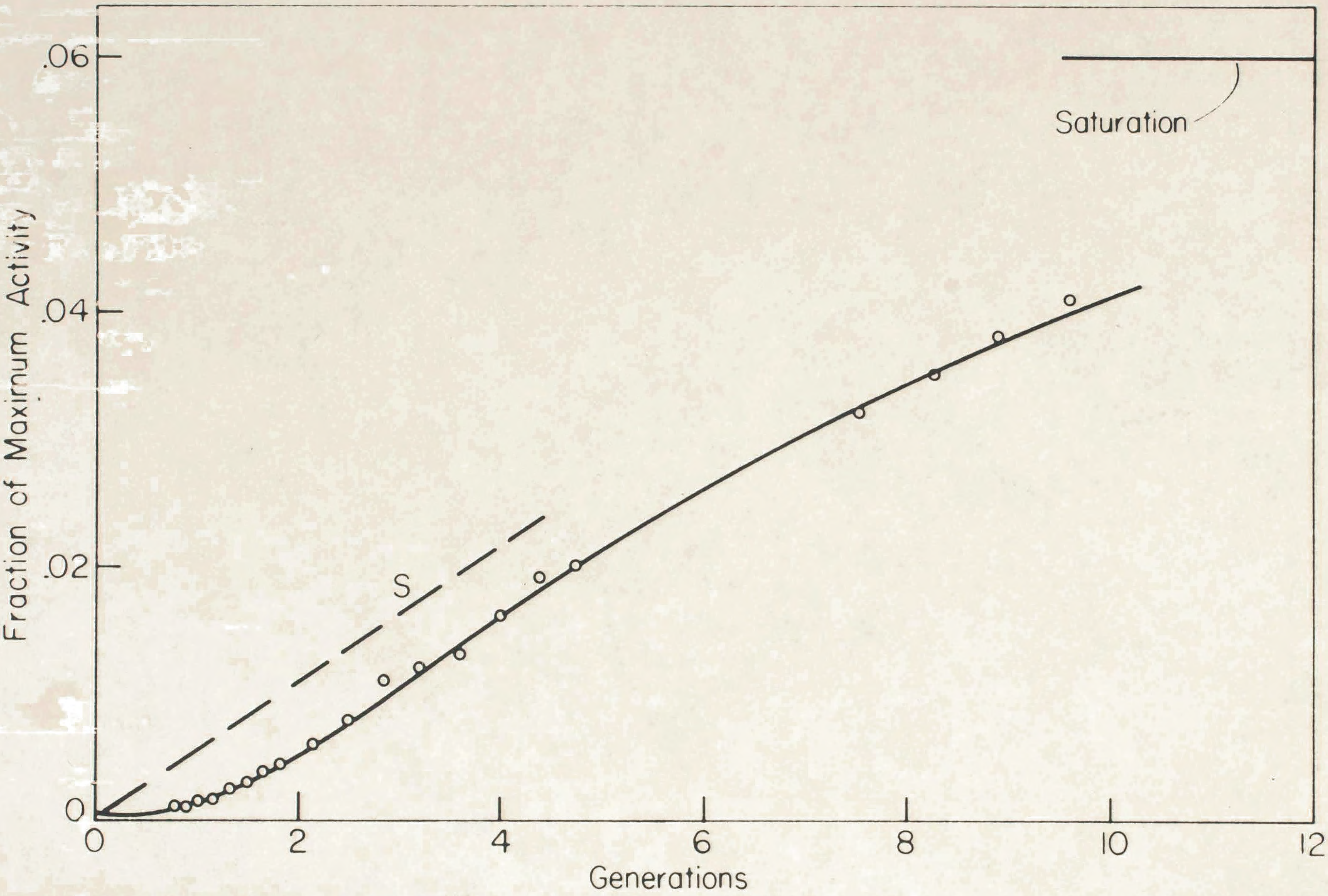
asked for
"complete"

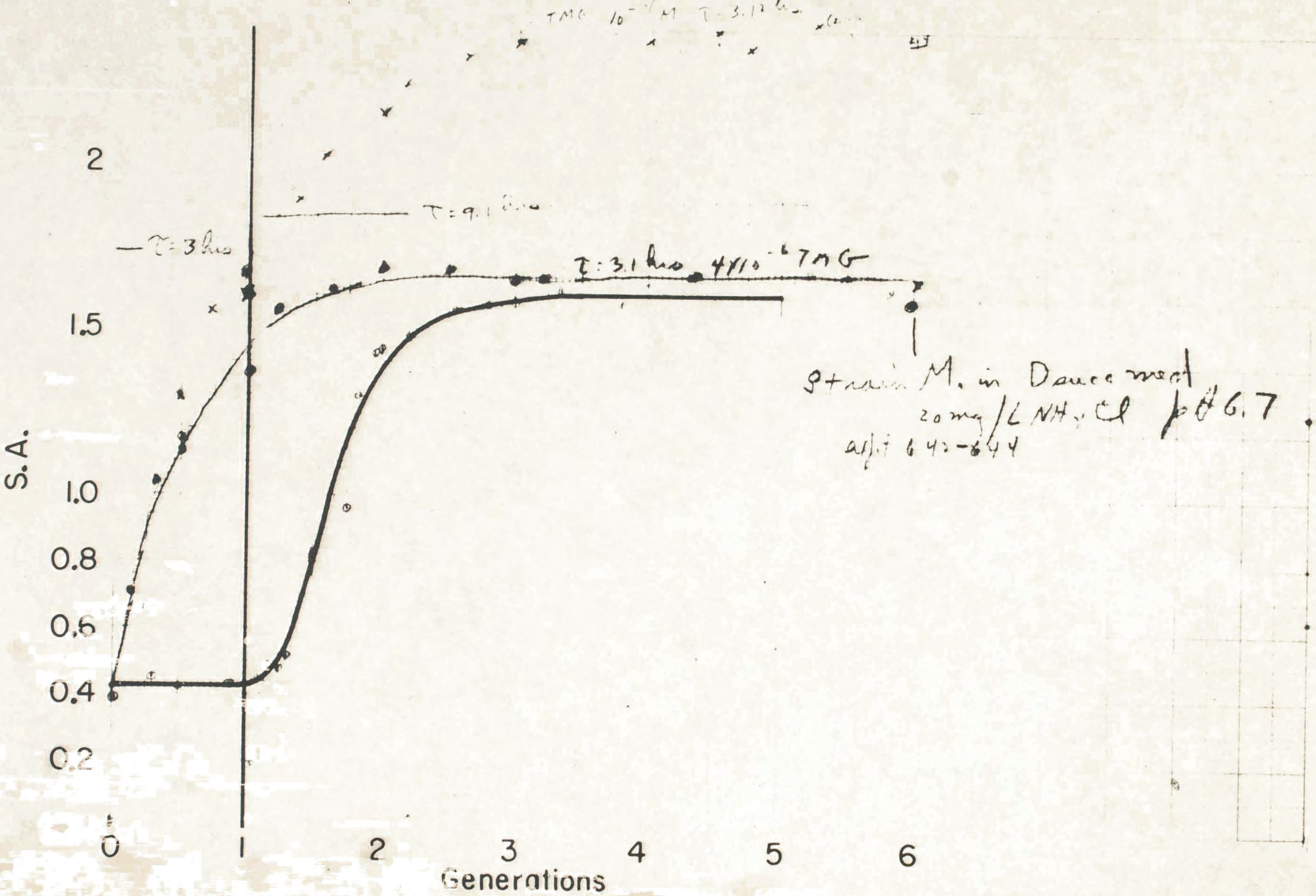


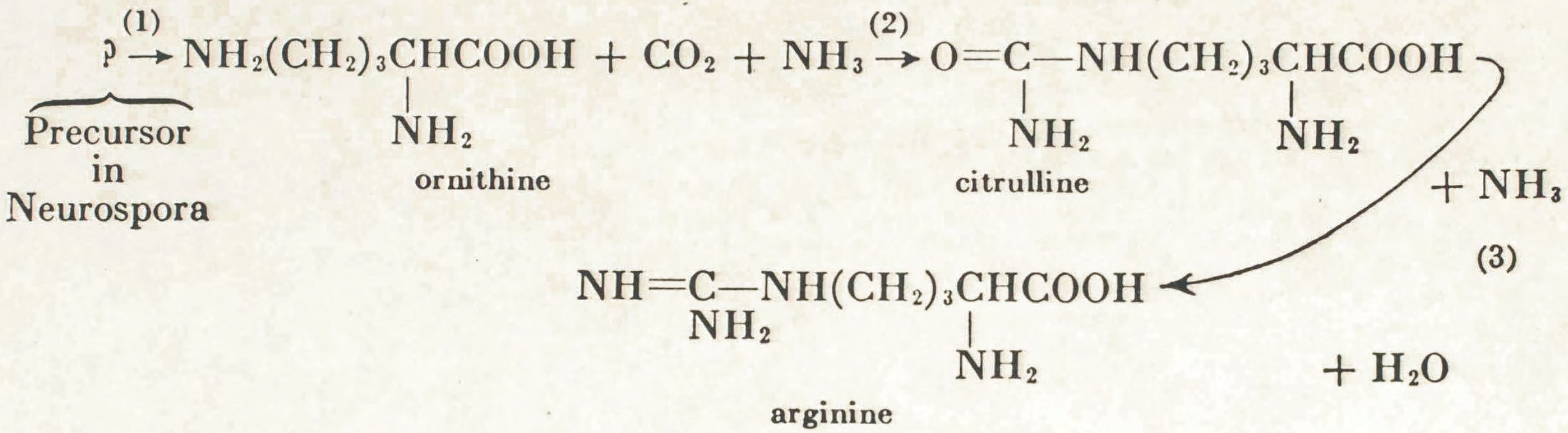
Add 2×10^{-3} TMG to tank ...

Strain ML$_3$ 5Cpi 5-7
Dmed Limited c̄ 2.0 mg/L NH_4Cl
pH 6.65
gas phase: Air
τ = 2.95 hrs

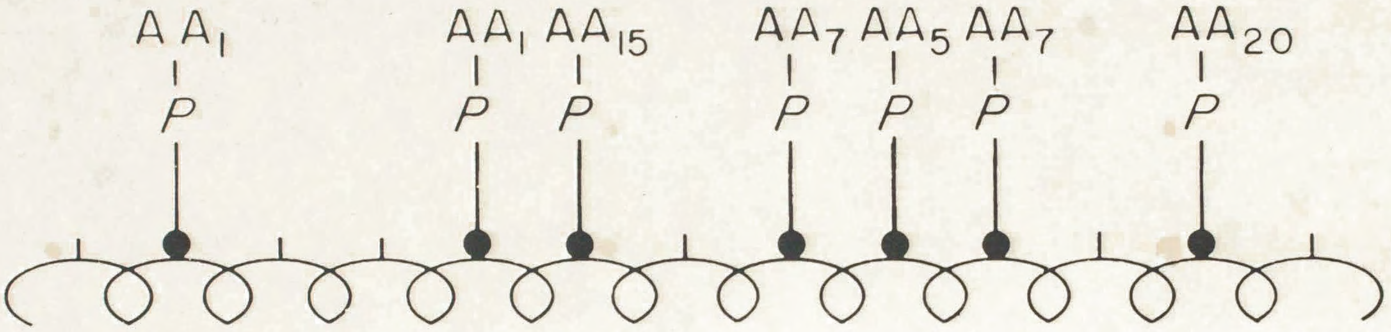

mandelic acid
benzyl alcohol
CHO
1/2 O₂
COOH
OH
1/2 O₂
COOH
NH₂
← tryptophane
anthranilic acid
1/2 O₂ (−CO₂ −NH₃)
O₂ −CO₂
OH
—OH
COOH
P-OH-benzoic acid
OH
O₂ (?via quinone?)
1/2 O₂
COOH
CH COOH
CH CH
CH
cis-cis-muconic acid
COOH
protocatechuic acid
HO
OH
O₂ (−CO₂)
β-ketoadipic acid
COOH
CH₂ COOH
CO CH₂
CH₂
COOH
α-ketoglutaric acid
CO
CH₂—CH₂COOH


Fraction of Maximum Activity
.06
.04
.02
0
0
2
4
6
8
10
12
Generations
Saturation
S

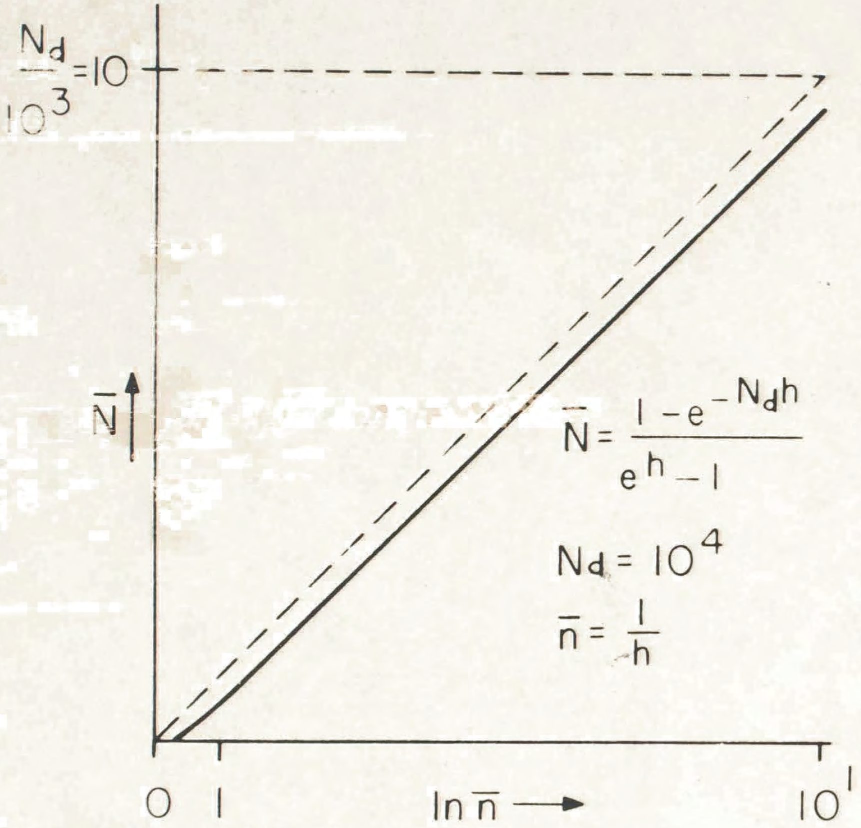

TMG 10⁻⁴M T=3.12 hrs
T=9.1 hrs
—T=3 hrs
T:3.1 hrs 4x10⁻⁶ TMG
Strain M. in Dance med
20 mg/L NH4Cl pH 6.7
642-644
S.A.
2
1.5
1.0
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
Generations

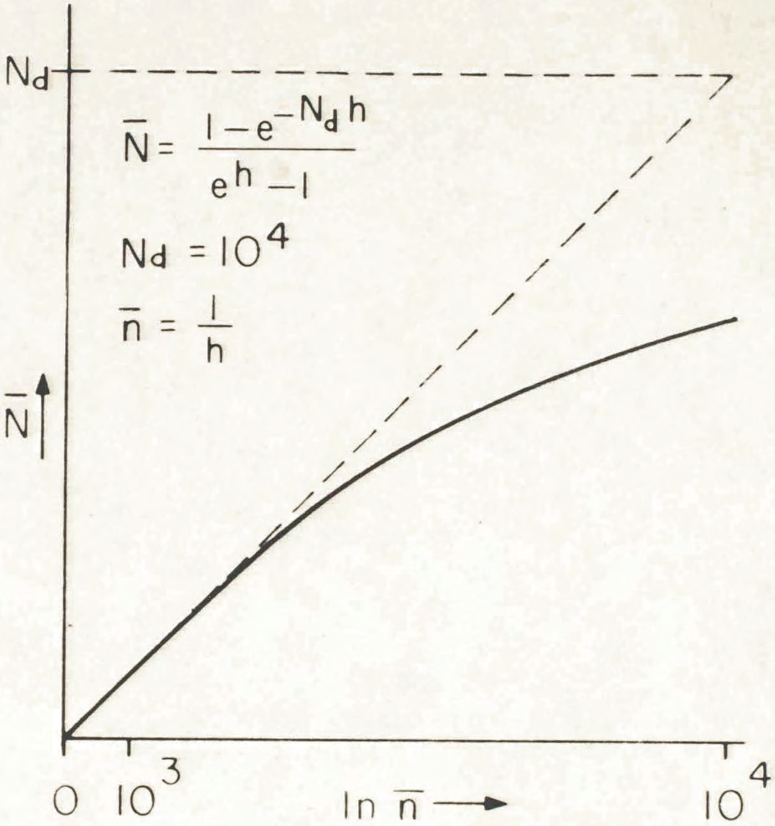


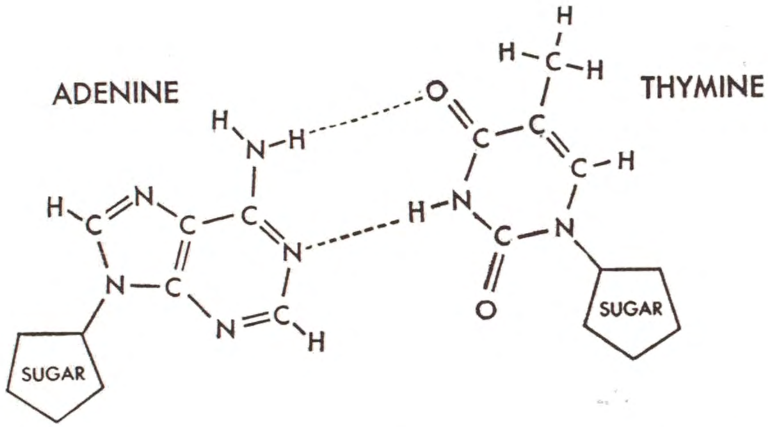
$$\underbrace{?}_{\text{Precursor in Neurospora}} \xrightarrow{(1)} \underset{\substack{|\\ NH_2}}{NH_2(CH_2)_3CHCOOH} + CO_2 + NH_3 \xrightarrow{(2)} \underset{\substack{|\\ NH_2}}{O{=}C}{-}\underset{\substack{|\\ NH_2}}{NH(CH_2)_3CHCOOH} \xrightarrow[+\,NH_3]{(3)} \underset{NH_2}{NH{=}C}{-}\underset{\substack{|\\ NH_2}}{NH(CH_2)_3CHCOOH} + H_2O$$



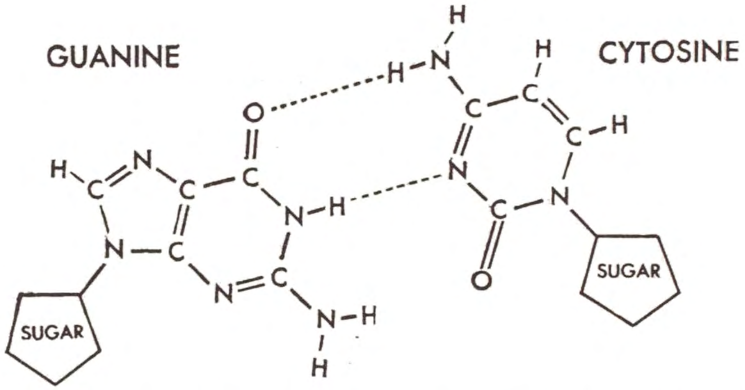
Paragene


$\frac{N_d}{10^3} = 10$
$\bar{N}$
$\bar{N} = \frac{1 - e^{-N_d h}}{e^h - 1}$
$N_d = 10^4$
$\bar{n} = \frac{1}{h}$
0
1
$\ln \bar{n}$ →
10^1

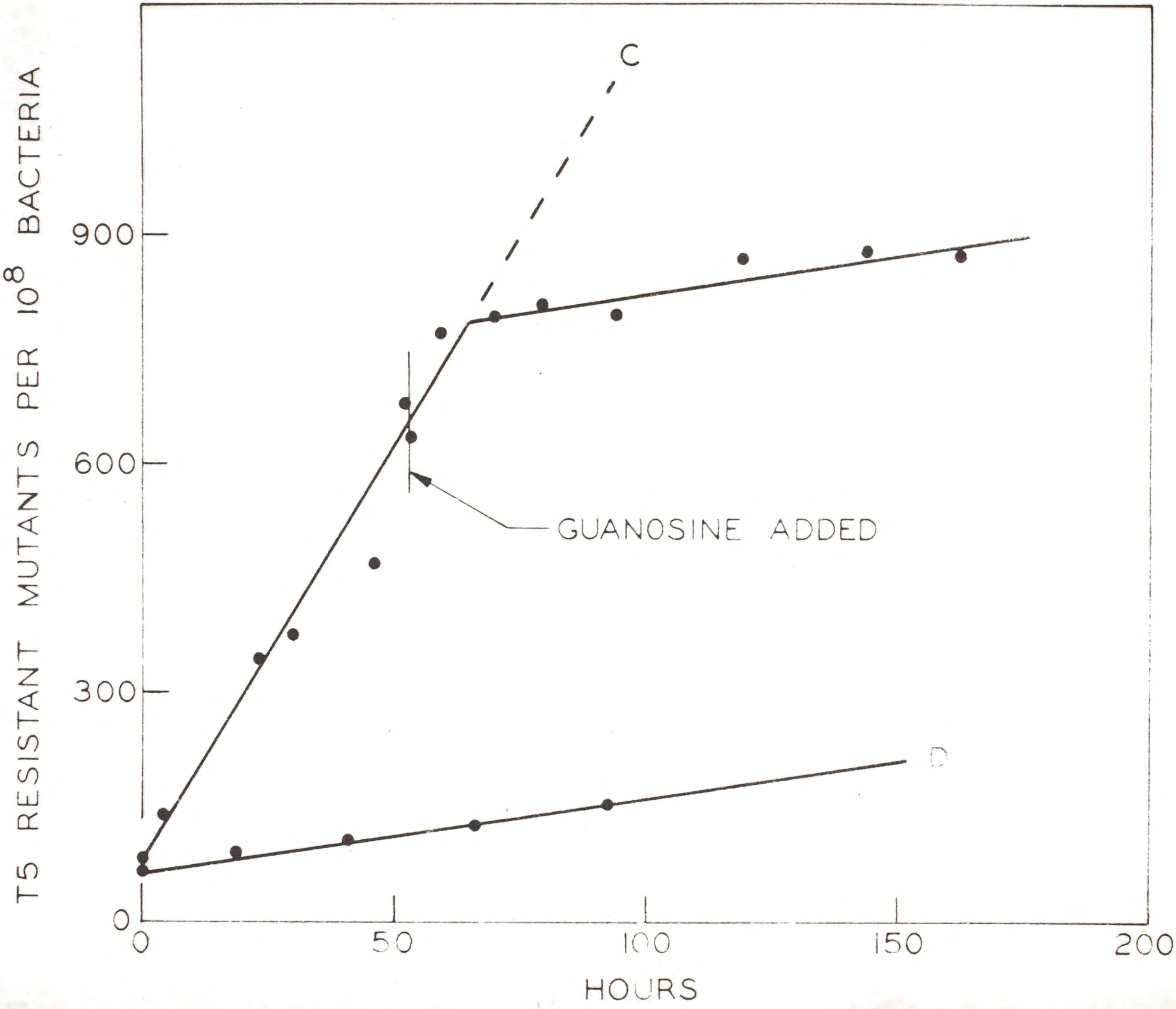

N_d
$\bar{N} = \frac{1 - e^{-N_d h}}{e^h - 1}$
$N_d = 10^4$
$\bar{n} = \frac{1}{h}$
$\bar{N}$
0
10^3
$\ln \bar{n}$ →
10^4

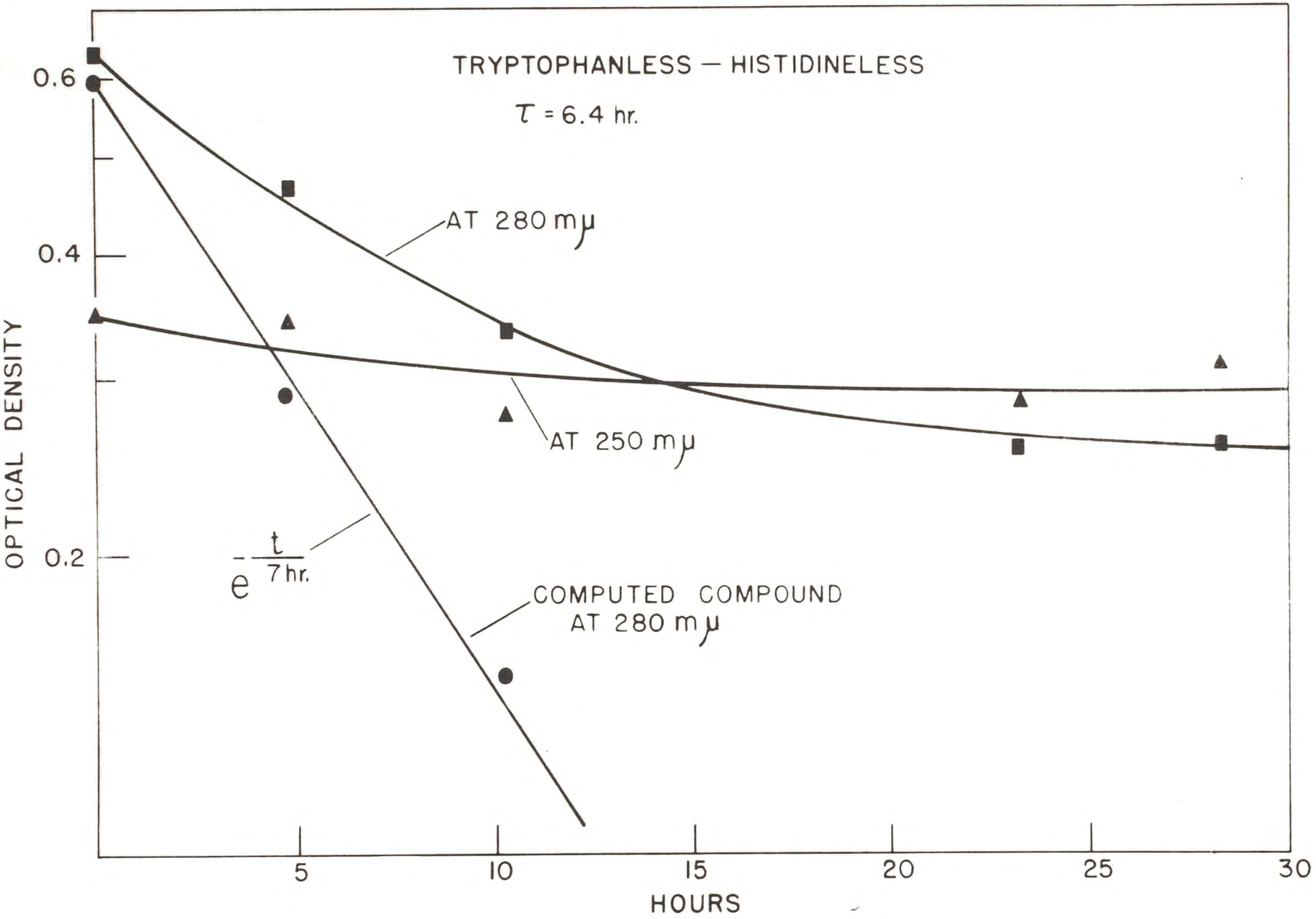


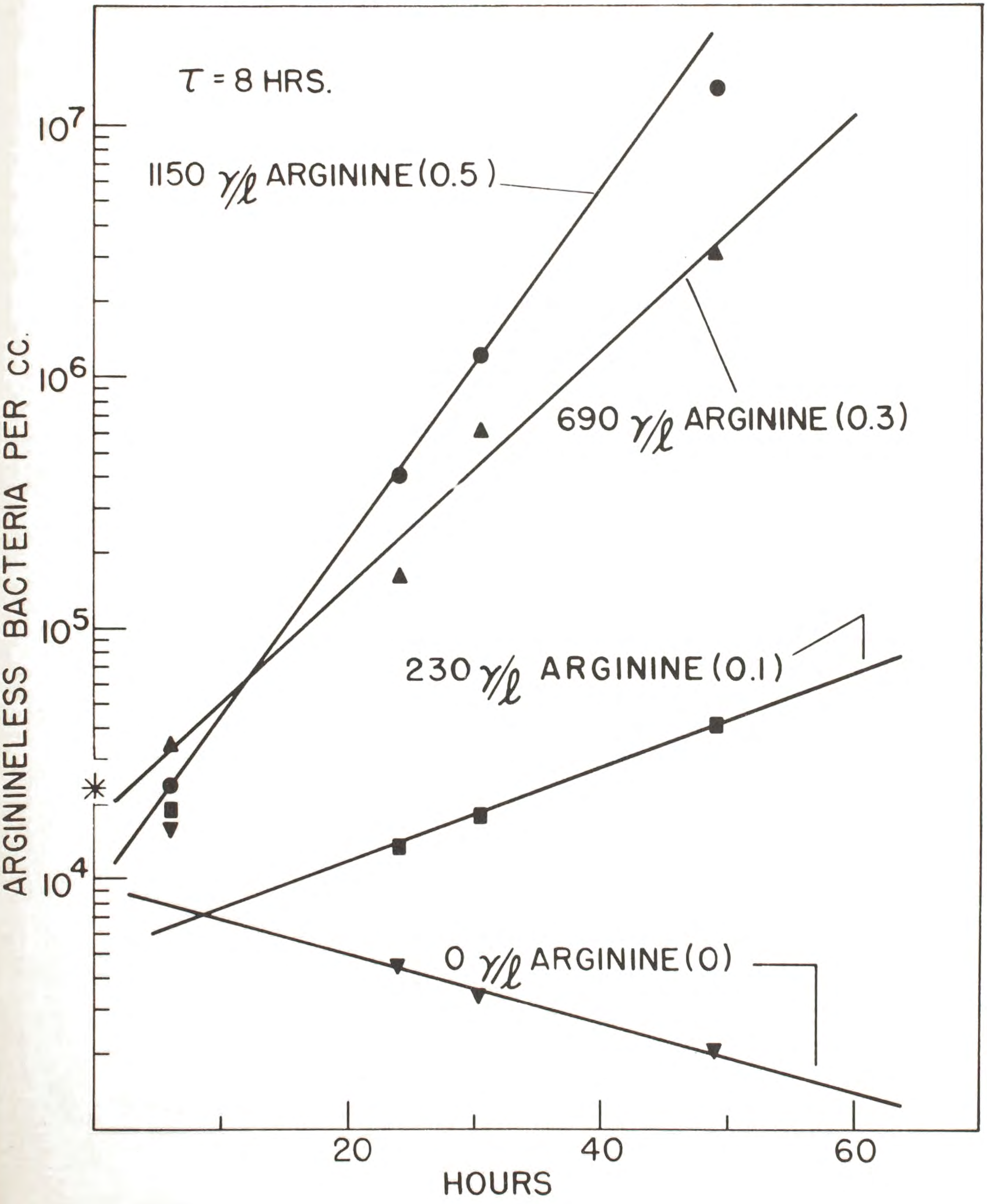
ONE LINKAGE of base to base across the pair of DNA chains is between adenine and thymine. For the structure proposed, the link of a large base with a small one is required to fit chains together.

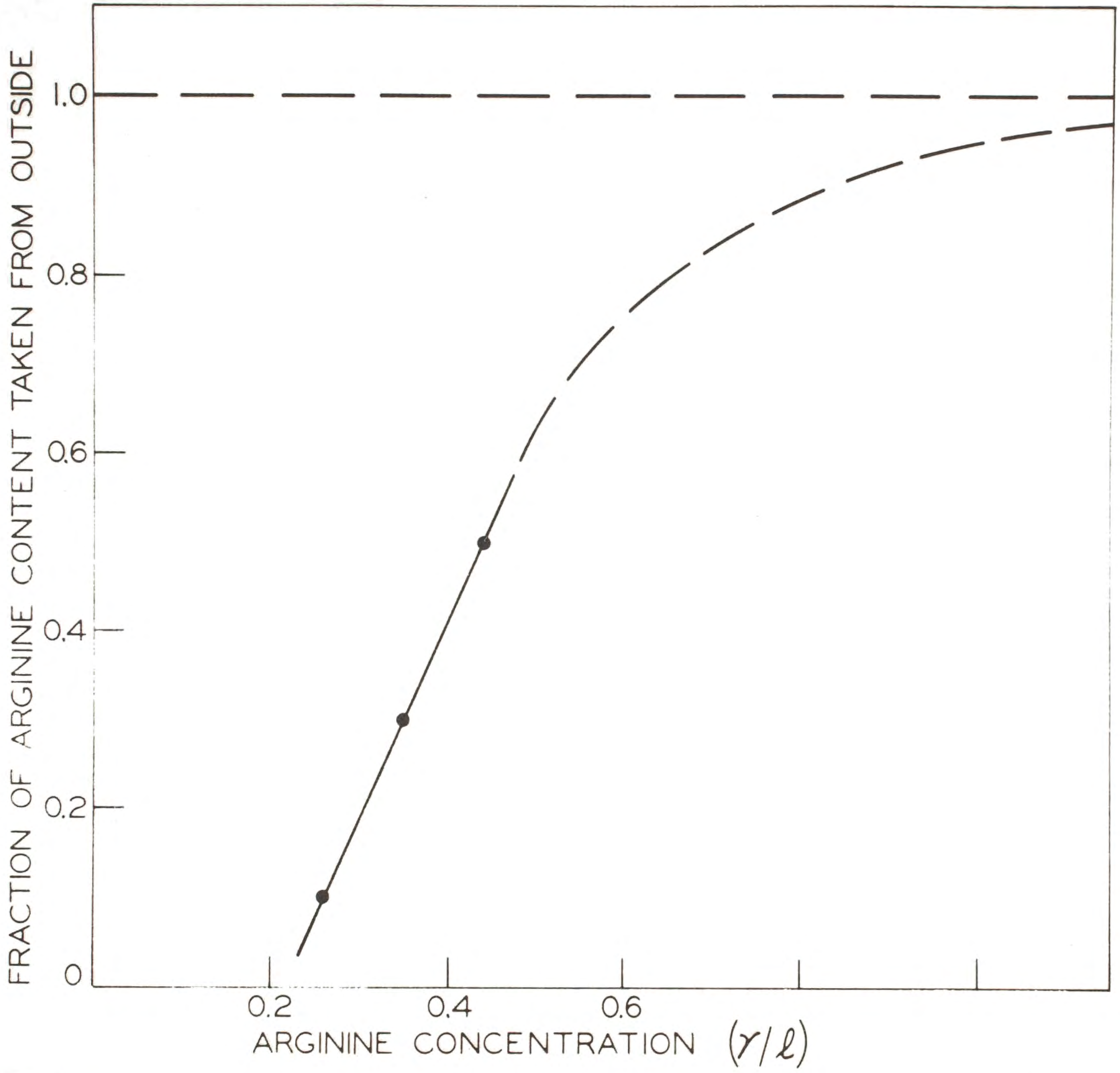


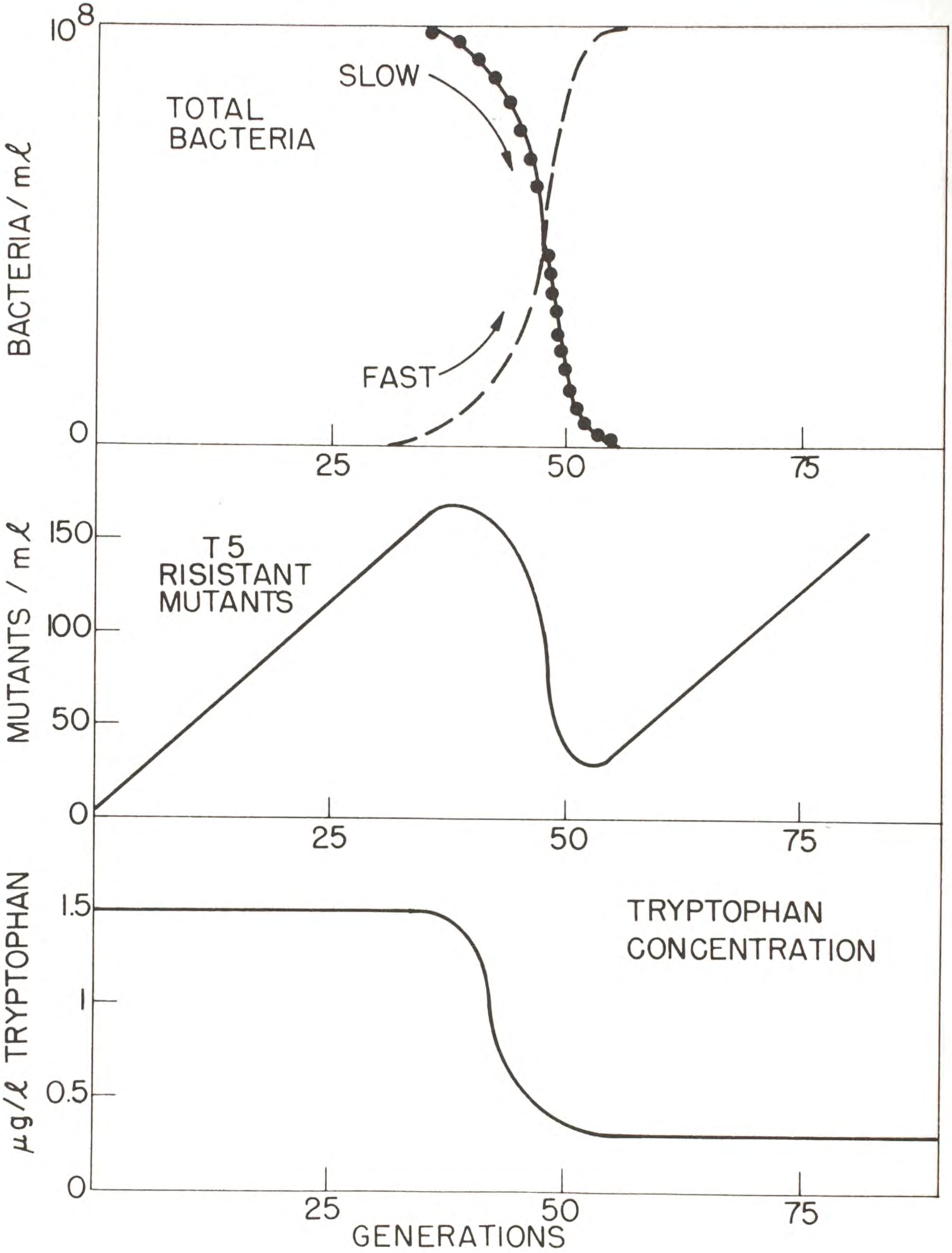
ANOTHER LINKAGE is comprised of guanine with cytosine. Assuming the existence of hydrogen bonds between the bases, these two pairings, and only these, will explain the actual configuration.

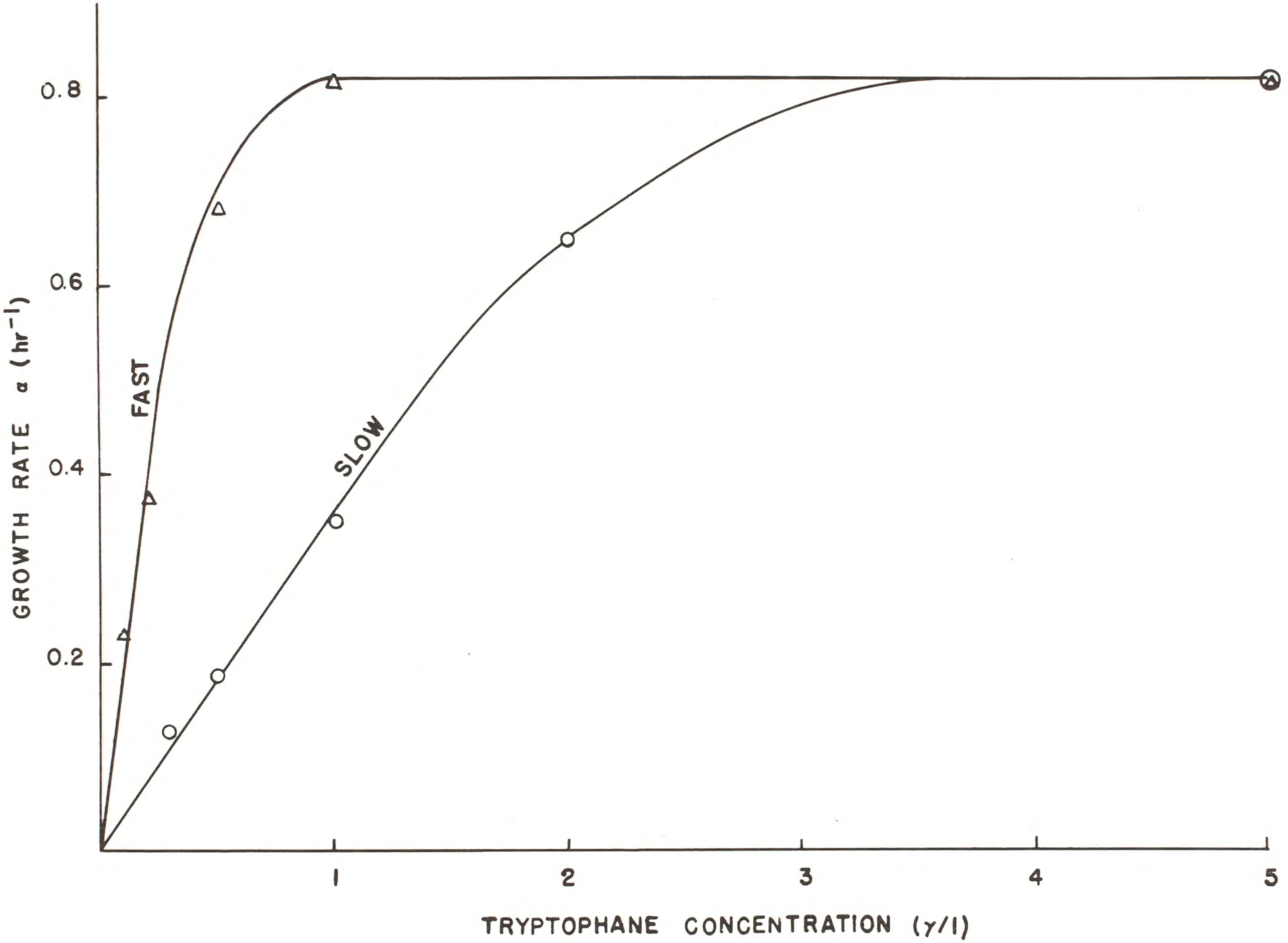

T5 RESISTANT MUTANTS PER 10^8 BACTERIA
900
600
300
0
C
GUANOSINE ADDED
D
0
50
100
150
200
HOURS


TRYPTOPHANLESS — HISTIDINELESS
τ = 6.4 hr.
AT 280 mμ
AT 250 mμ
$e^{-\frac{t}{7\,hr.}}$
COMPUTED COMPOUND
AT 280 mμ
OPTICAL DENSITY
0.6
0.4
0.2
HOURS
5
10
15
20
25
30

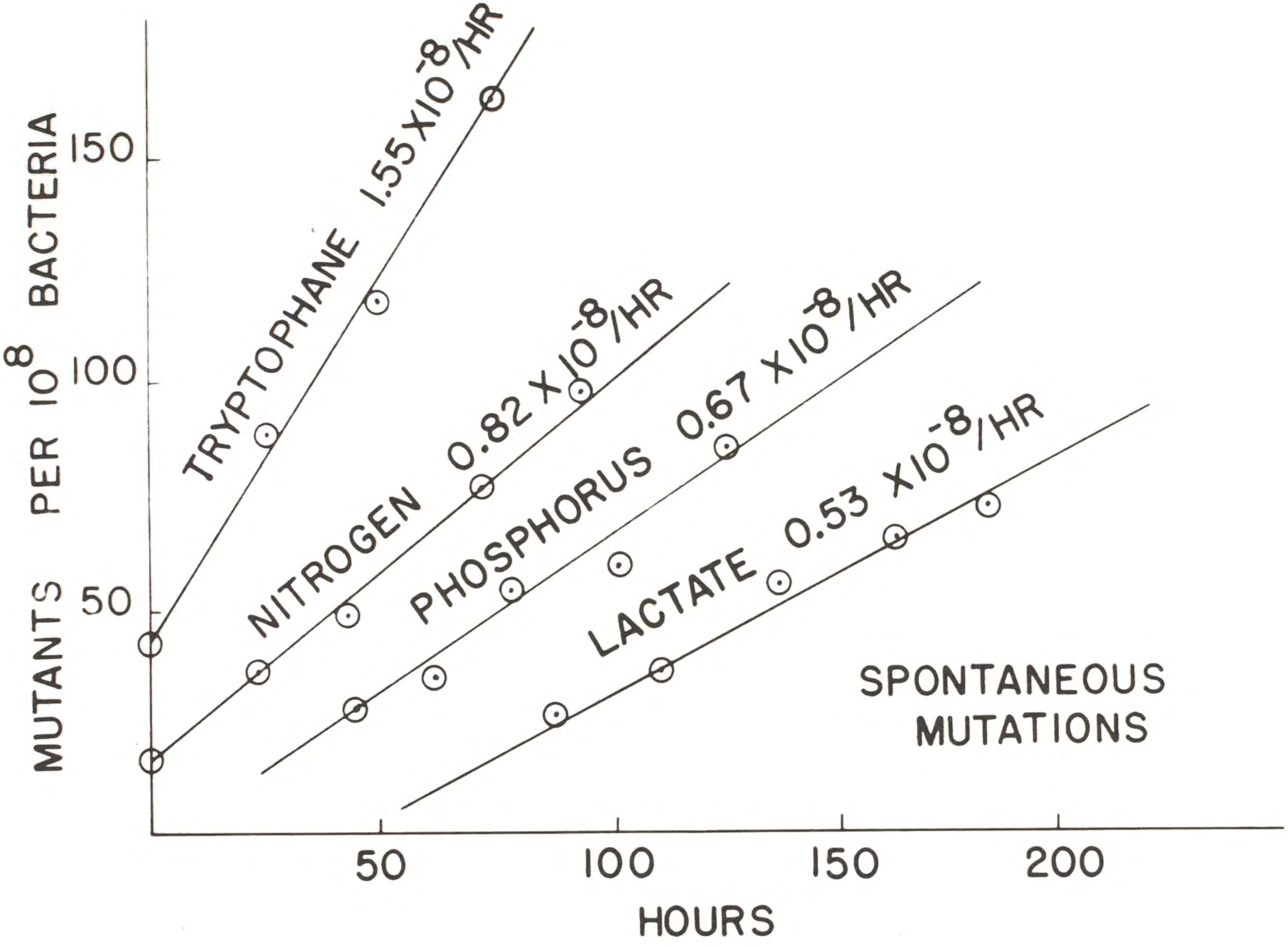

τ = 8 HRS.
1150 γ/ℓ ARGININE (0.5)
690 γ/ℓ ARGININE (0.3)
230 γ/ℓ ARGININE (0.1)
0 γ/ℓ ARGININE (0)
10⁷
10⁶
10⁵
10⁴
*
ARGININELESS BACTERIA PER CC.
20
40
60
HOURS

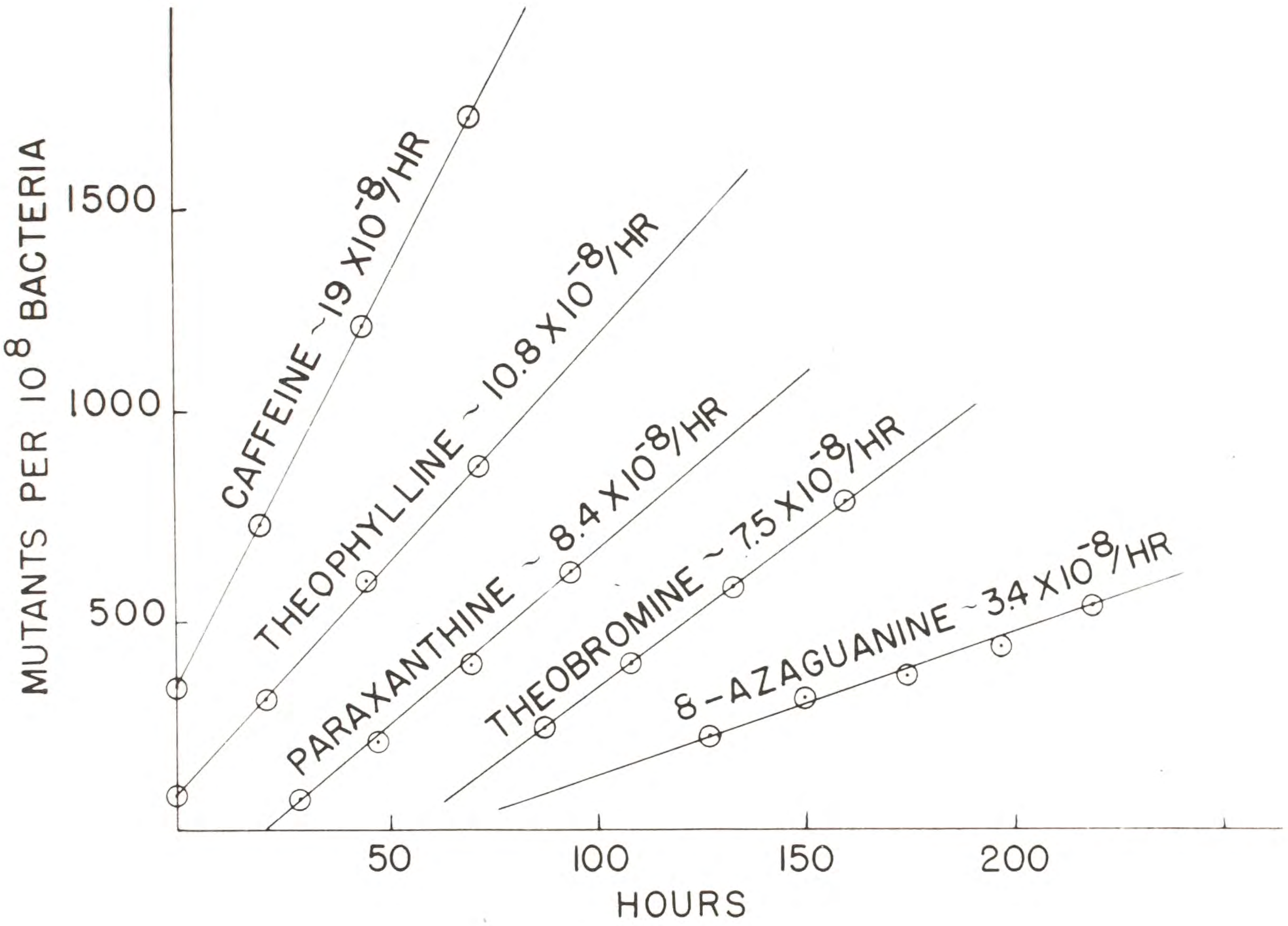

FRACTION OF ARGININE CONTENT TAKEN FROM OUTSIDE
1.0
0.8
0.6
0.4
0.2
0
0.2
0.4
0.6
ARGININE CONCENTRATION (γ/ℓ)

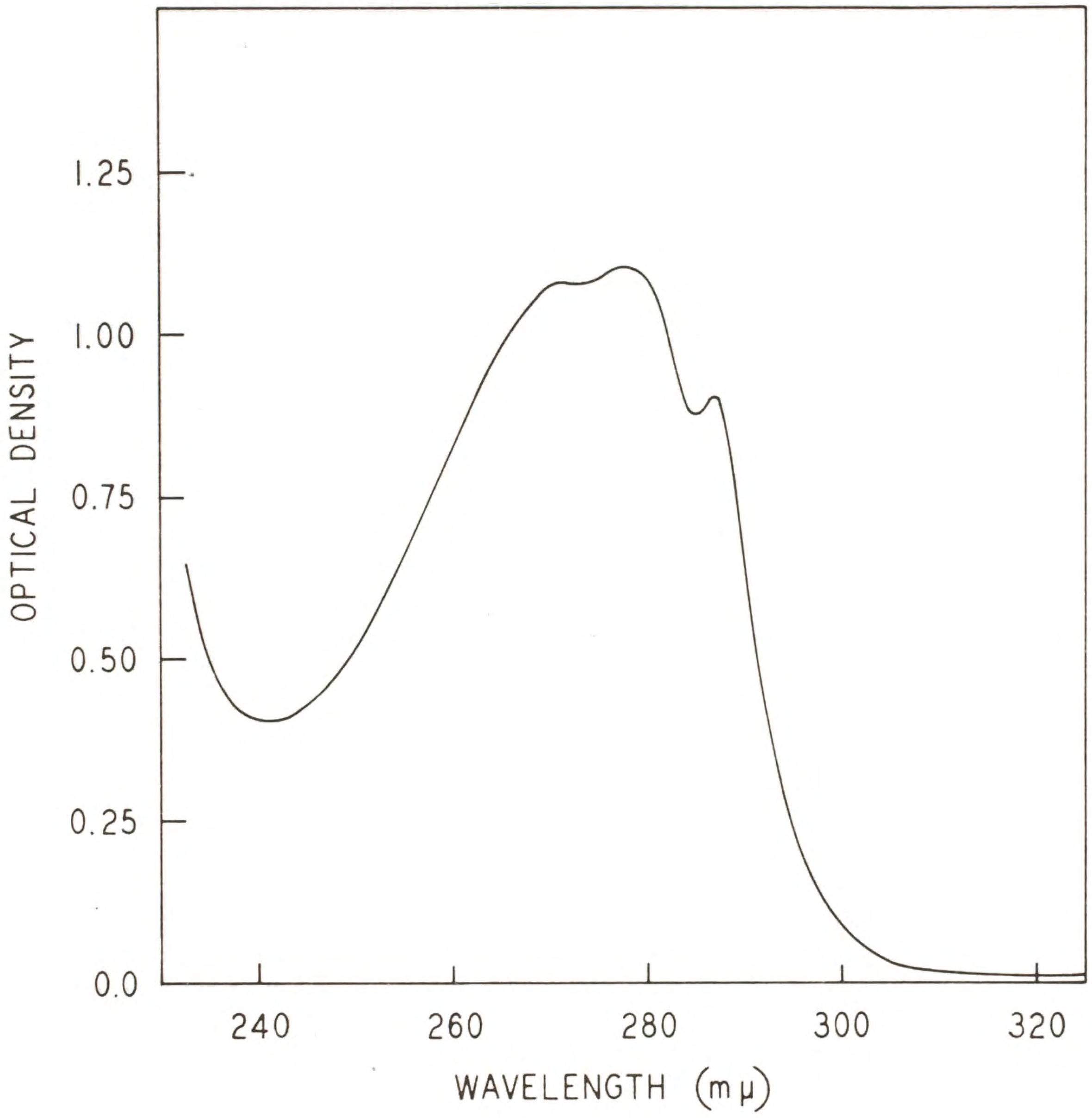

10⁸
SLOW
TOTAL BACTERIA
BACTERIA / mℓ
FAST
0
25
50
75
T 5 RISISTANT MUTANTS
MUTANTS / mℓ
150
100
50
0
25
50
75
μg/ℓ TRYPTOPHAN
1.5
1
0.5
0
TRYPTOPHAN CONCENTRATION
25
50
75
GENERATIONS



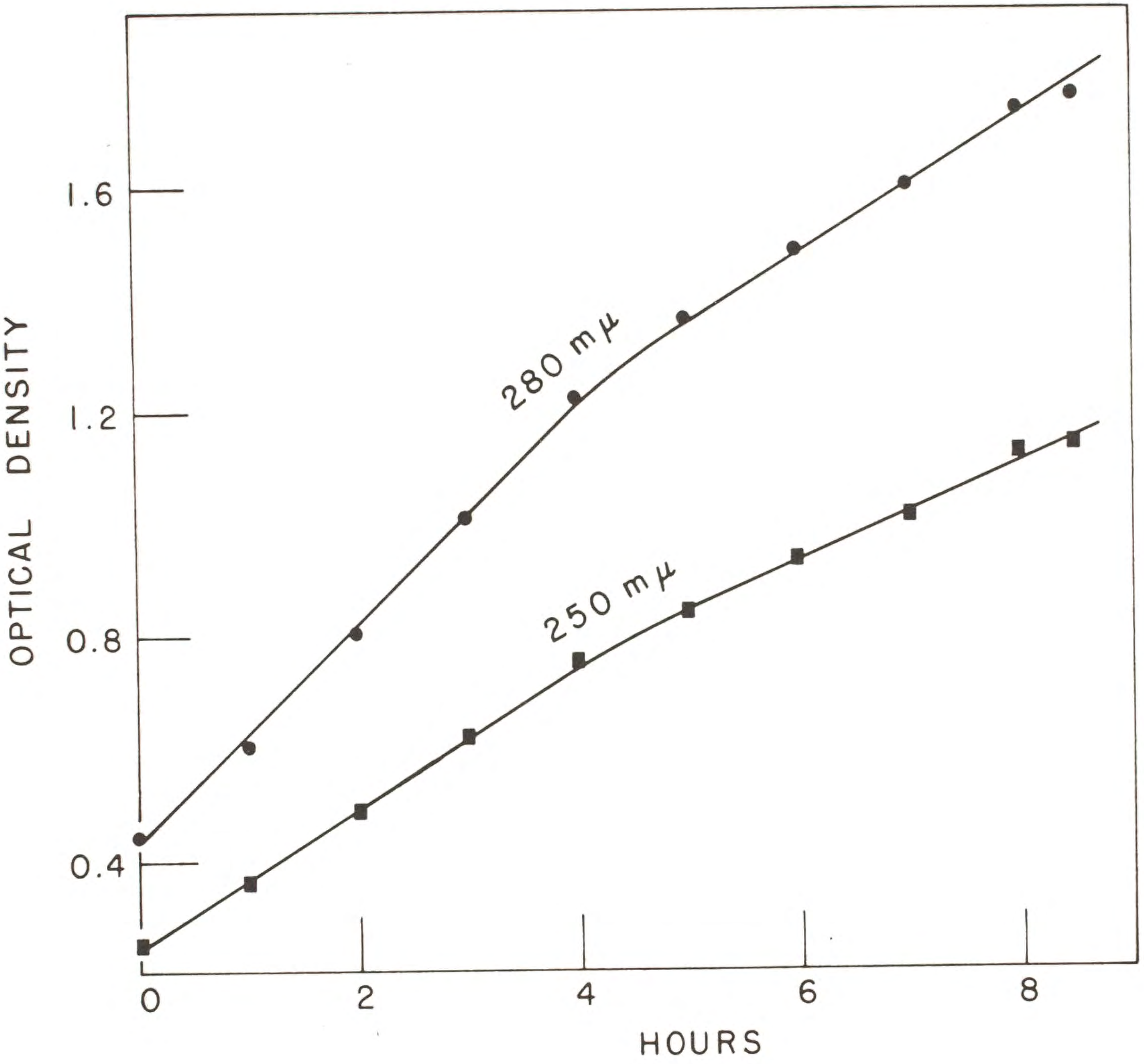
0.8
0.6
0.4
0.2
GROWTH RATE α (hr⁻¹)
FAST
SLOW
1
2
3
4
5
TRYPTOPHANE CONCENTRATION (γ/l)

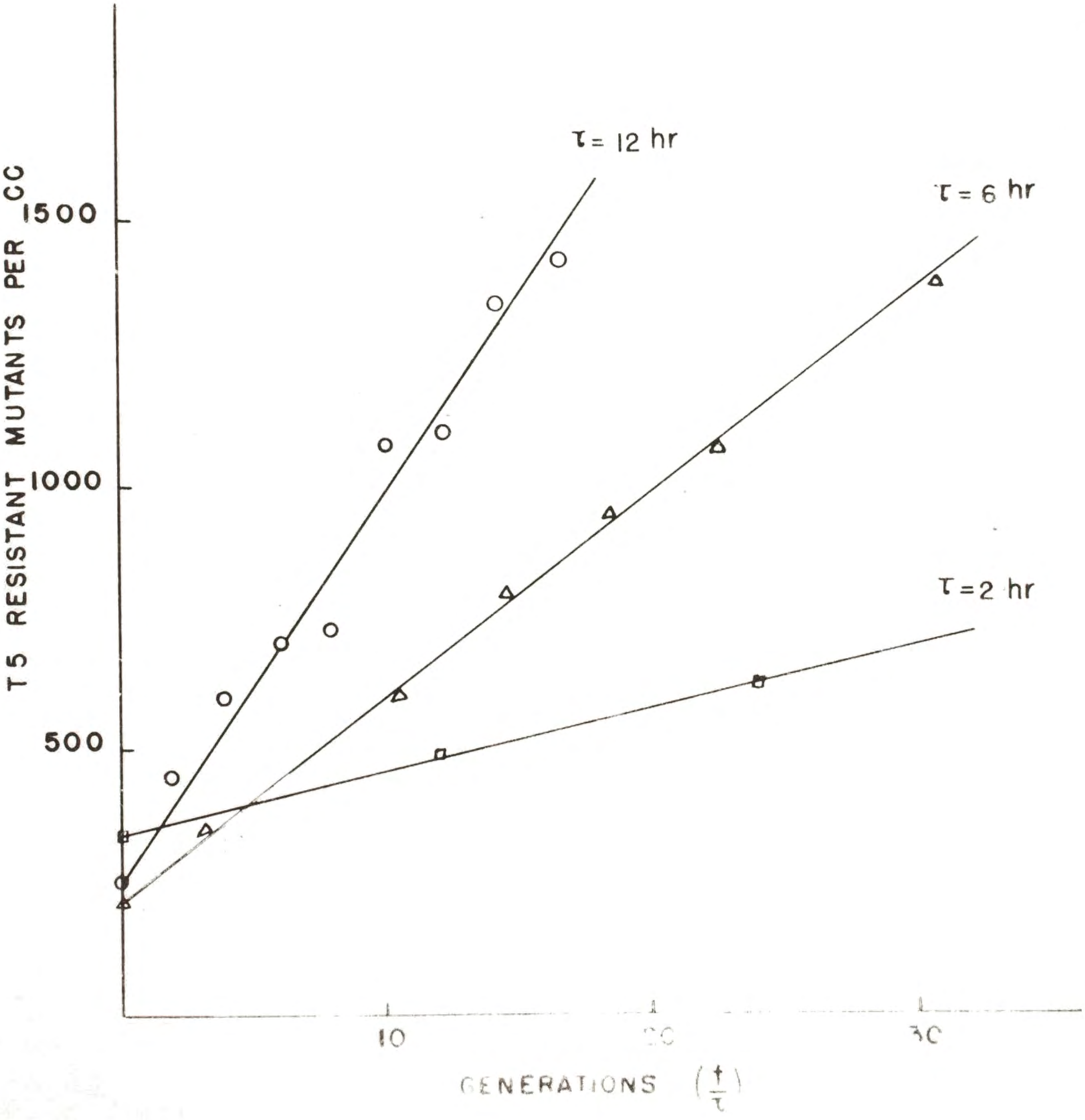

MUTANTS PER 10^8 BACTERIA
150
100
50
TRYPTOPHANE 1.55 X 10^-8/HR
NITROGEN 0.82 X 10^-8/HR
PHOSPHORUS 0.67 X 10^-8/HR
LACTATE 0.53 X 10^-8/HR
SPONTANEOUS MUTATIONS
50
100
150
200
HOURS

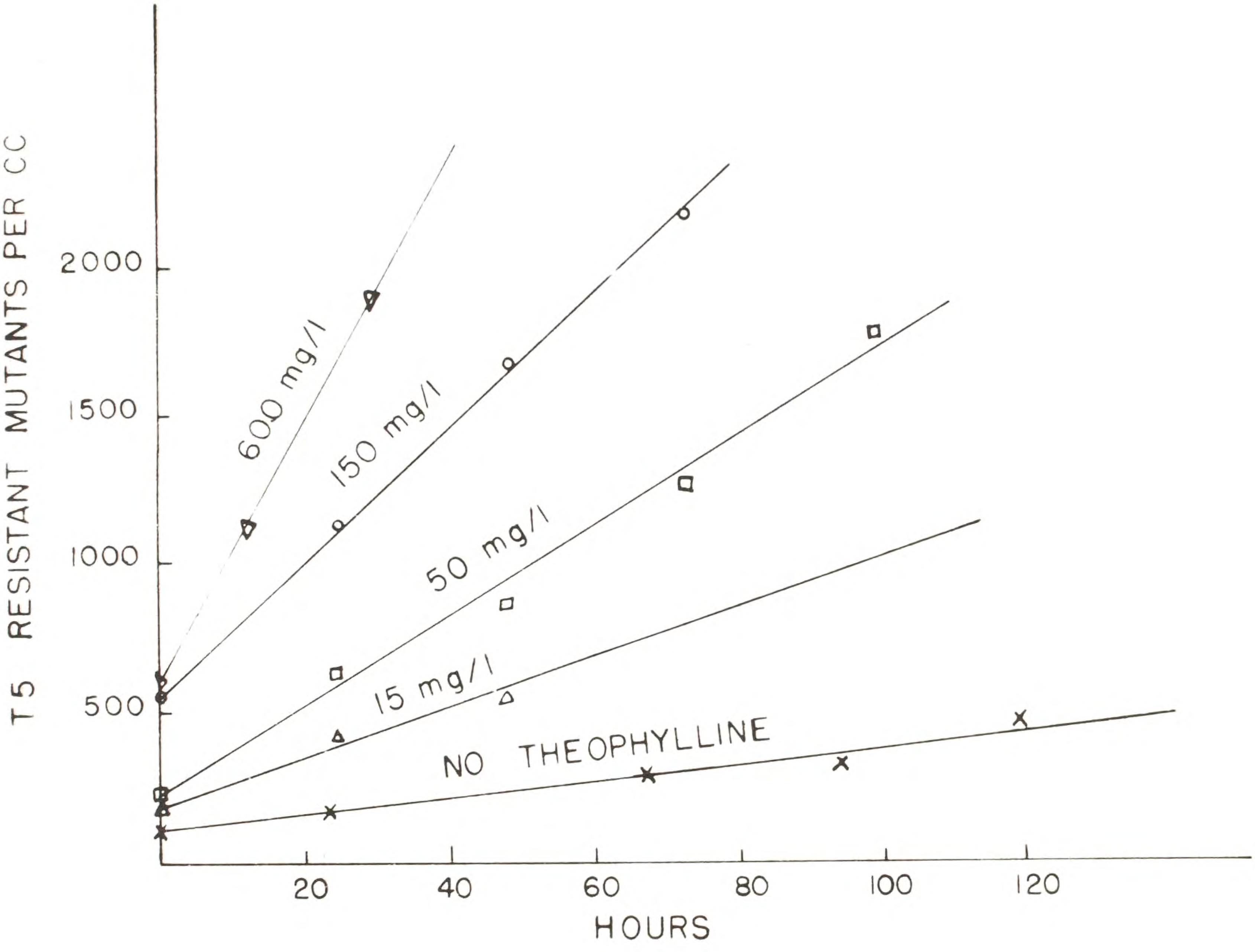

MUTANTS PER 10⁸ BACTERIA
1500
1000
500
50
100
150
200
HOURS
CAFFEINE ~ 19 X10⁻⁸/HR
THEOPHYLLINE ~ 10.8 X10⁻⁸/HR
PARAXANTHINE ~ 8.4 X10⁻⁸/HR
THEOBROMINE ~ 7.5 X10⁻⁸/HR
8-AZAGUANINE ~ 3.4 X10⁻⁸/HR

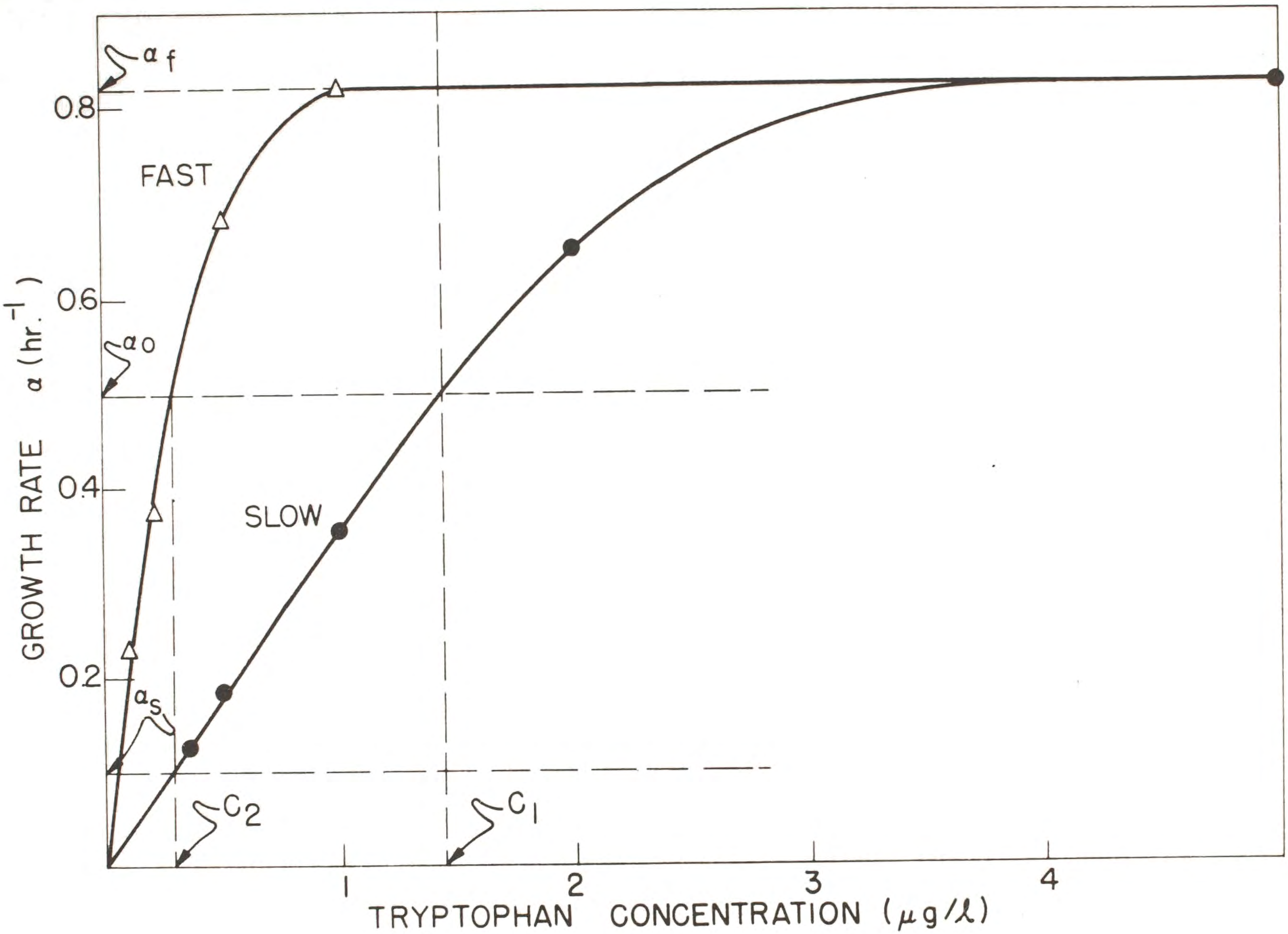


1.25
1.00
0.75
0.50
0.25
0.0
OPTICAL DENSITY
240
260
280
300
320
WAVELENGTH (mμ)

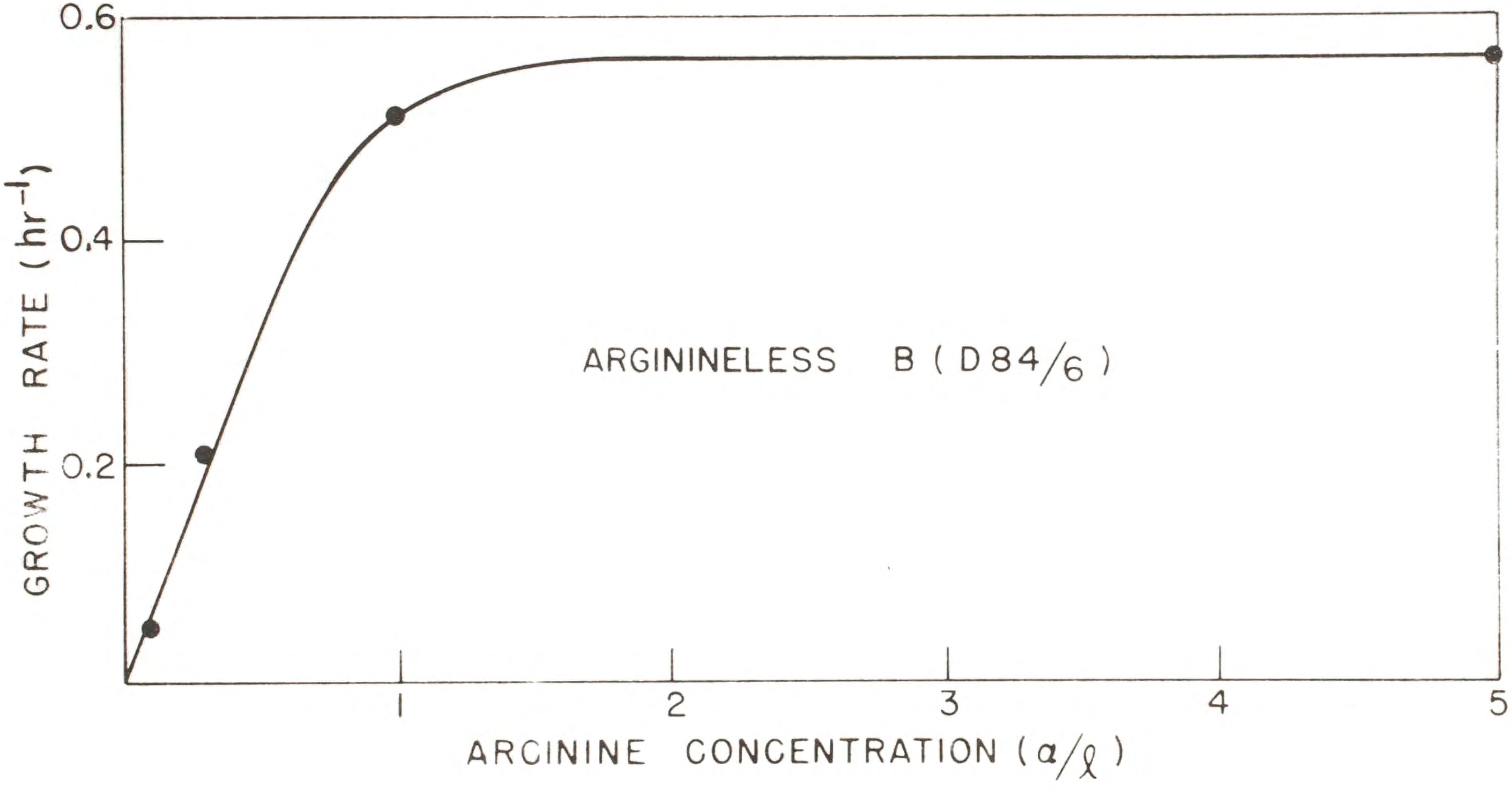

OPTICAL DENSITY
1.6
1.2
0.8
0.4
280 mμ
250 mμ
0
2
4
6
8
HOURS


T5 RESISTANT MUTANTS PER CC
1500
1000
500
10
20
30
GENERATIONS $\left(\frac{t}{\tau}\right)$
τ = 12 hr
τ = 6 hr
τ = 2 hr

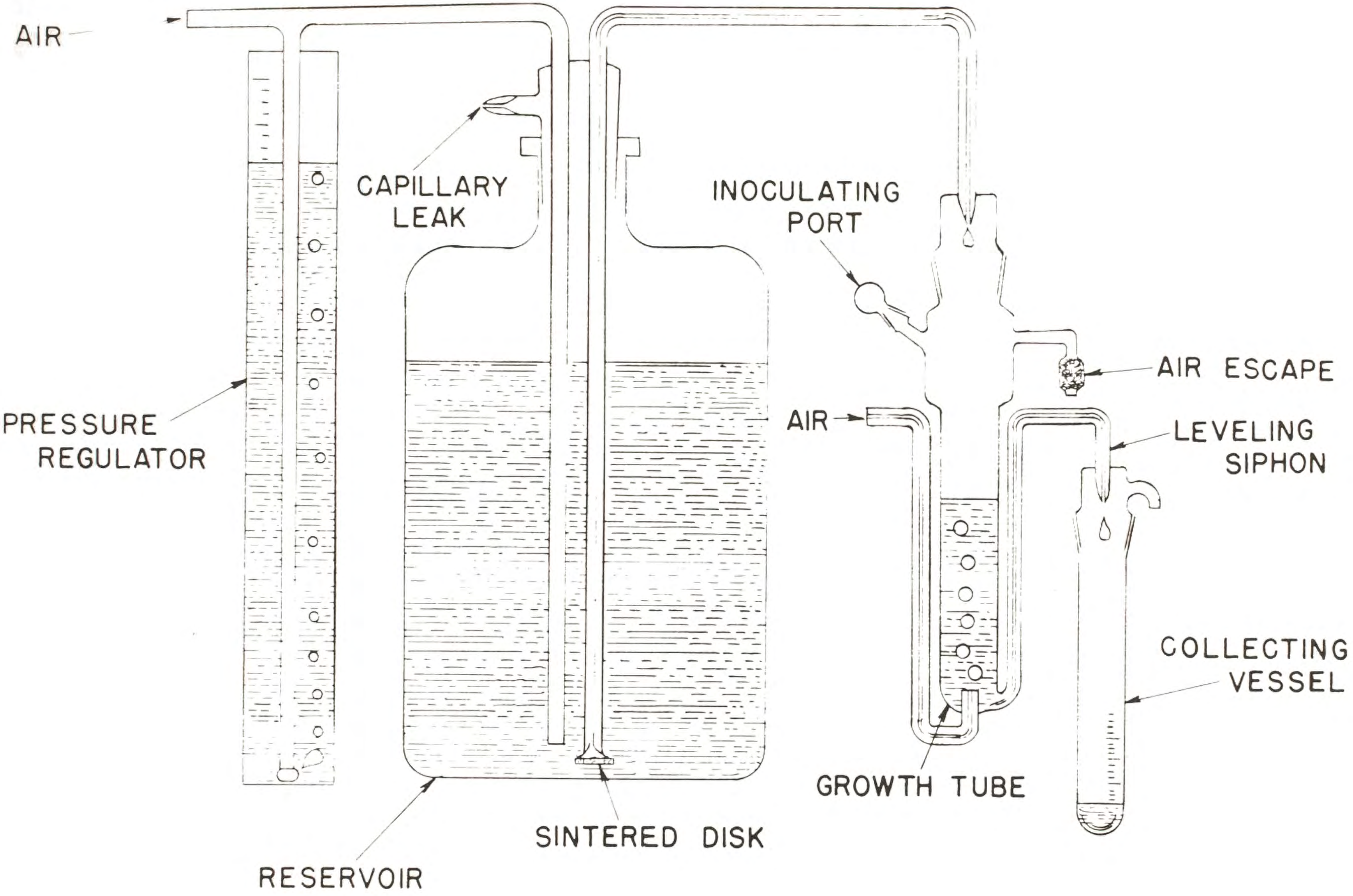

T5 RESISTANT MUTANTS PER CC
2000
1500
1000
500
600 mg/l
150 mg/l
50 mg/l
15 mg/l
NO THEOPHYLLINE
20
40
60
80
100
120
HOURS

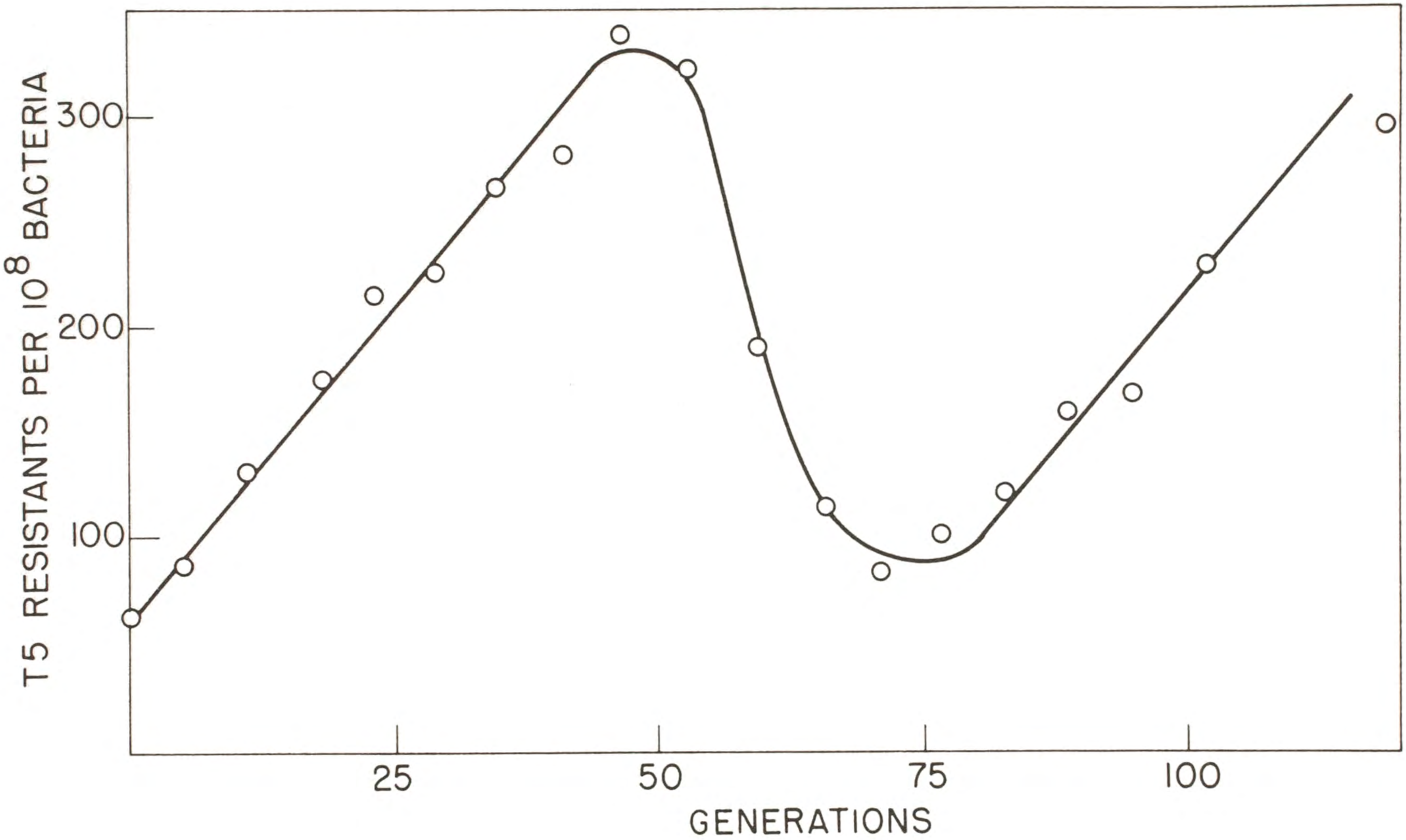


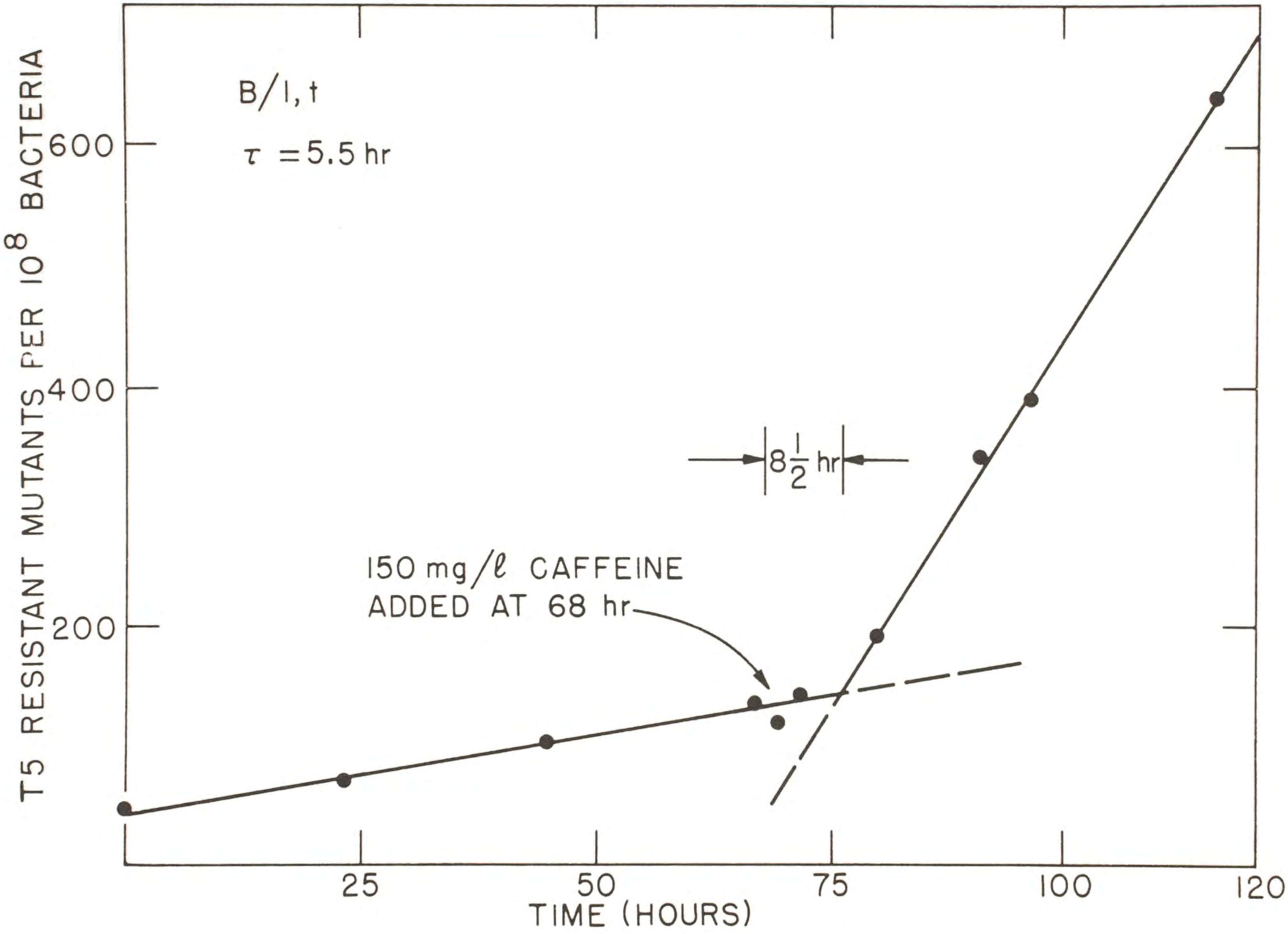
α_f
0.8
FAST
0.6
α_0
0.4
SLOW
0.2
α_s
C_2
C_1
1
2
3
4
GROWTH RATE α (hr.$^{-1}$)
TRYPTOPHAN CONCENTRATION (μg/ℓ)

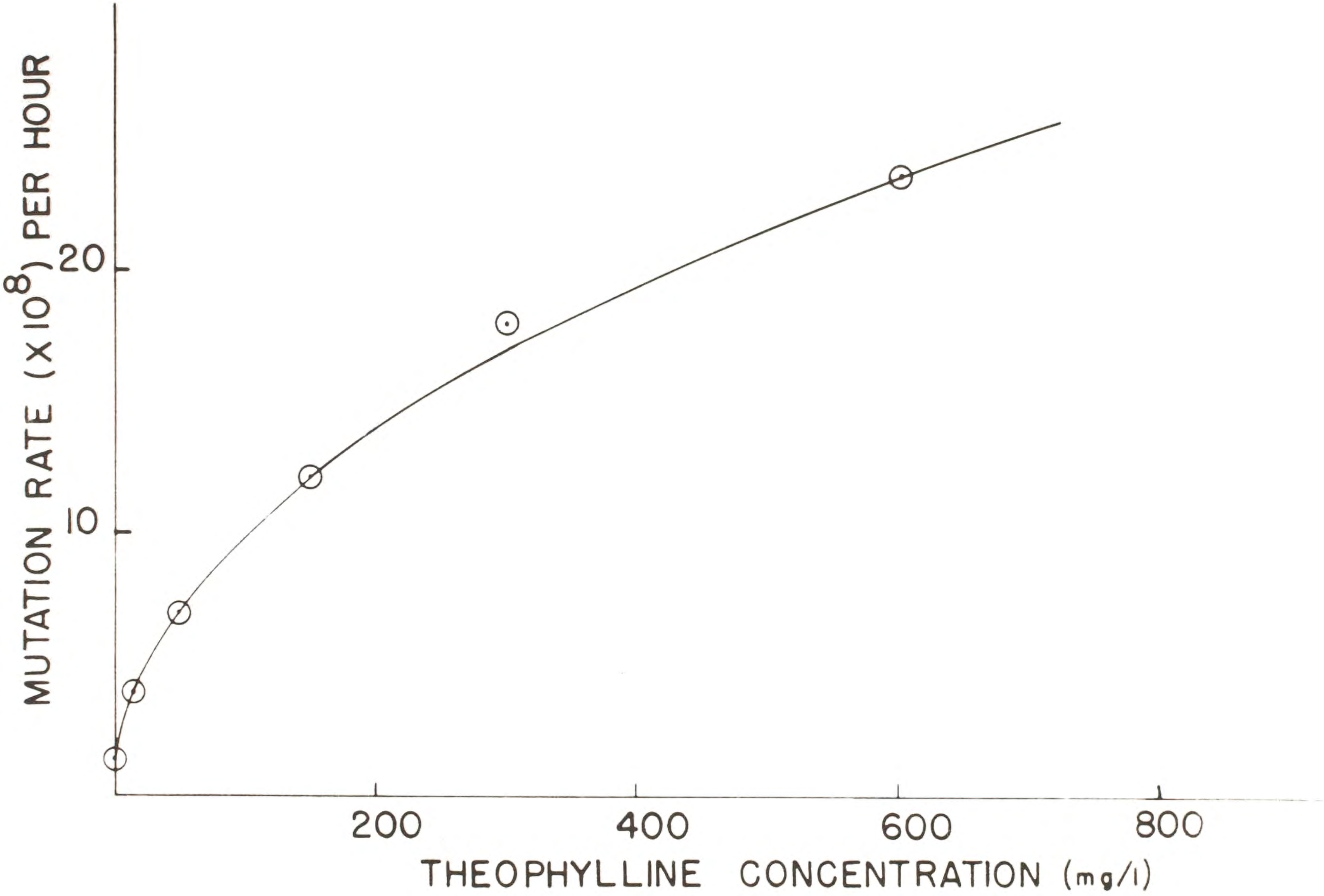

0.6
0.4
0.2
GROWTH RATE (hr⁻¹)
ARGININELESS B (D84/6)
1
2
3
4
5
ARCININE CONCENTRATION (α/ℓ)

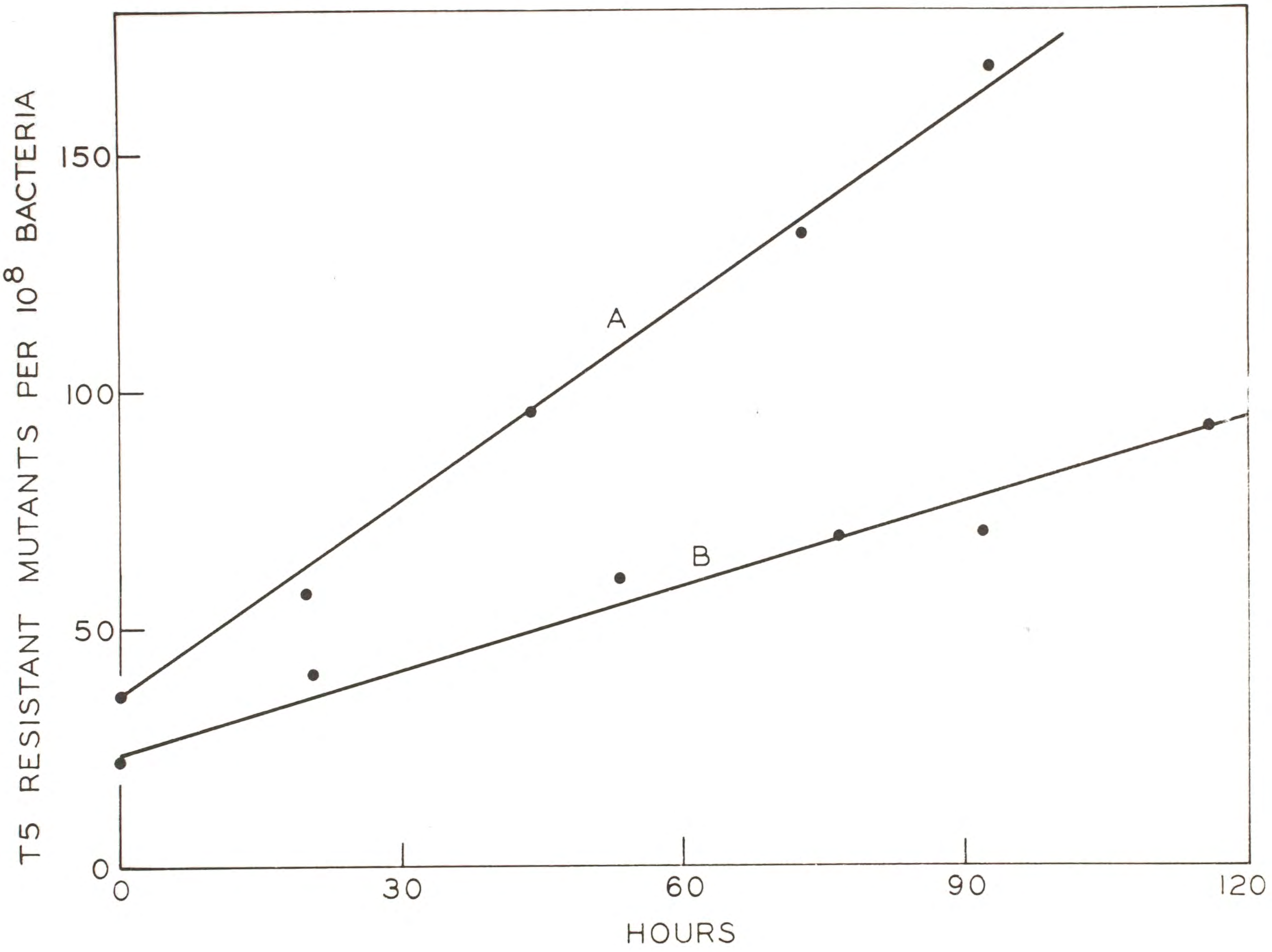
CHEMOSTAT

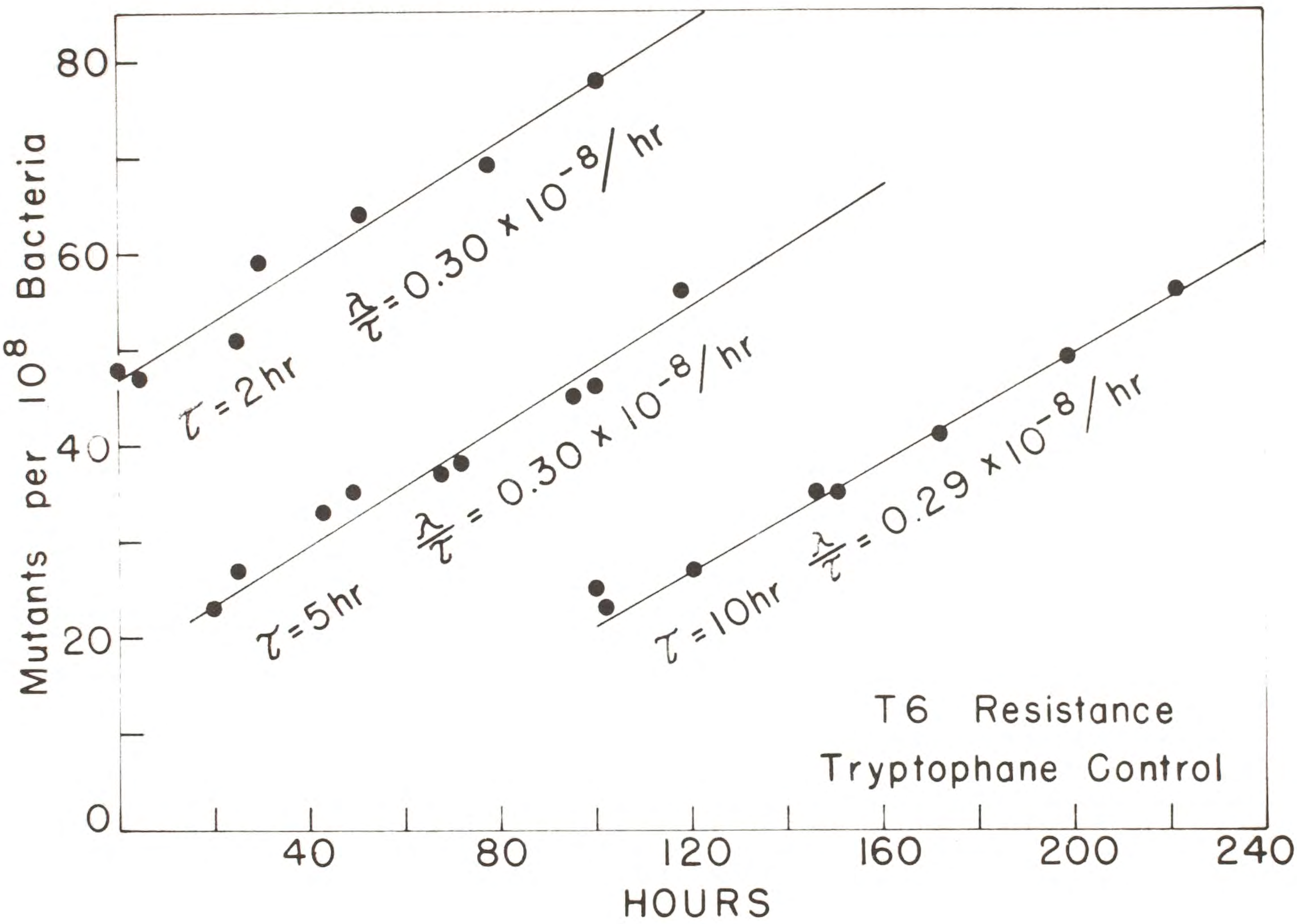

AIR
CAPILLARY LEAK
INOCULATING PORT
AIR ESCAPE
AIR
LEVELING SIPHON
PRESSURE REGULATOR
COLLECTING VESSEL
GROWTH TUBE
SINTERED DISK
RESERVOIR


T5 RESISTANTS PER 10^8 BACTERIA
300
200
100
25
50
75
100
GENERATIONS


B/1, t
τ = 5.5 hr
$8\frac{1}{2}$ hr
150 mg/ℓ CAFFEINE ADDED AT 68 hr
T5 RESISTANT MUTANTS PER 10^8 BACTERIA
600
400
200
25
50
75
100
120
TIME (HOURS)


MUTATION RATE (X 10^8) PER HOUR
20
10
200
400
600
800
THEOPHYLLINE CONCENTRATION (mg/l)


T5 RESISTANT MUTANTS PER 10^8 BACTERIA
150
100
50
0
0
30
60
90
120
HOURS
A
B

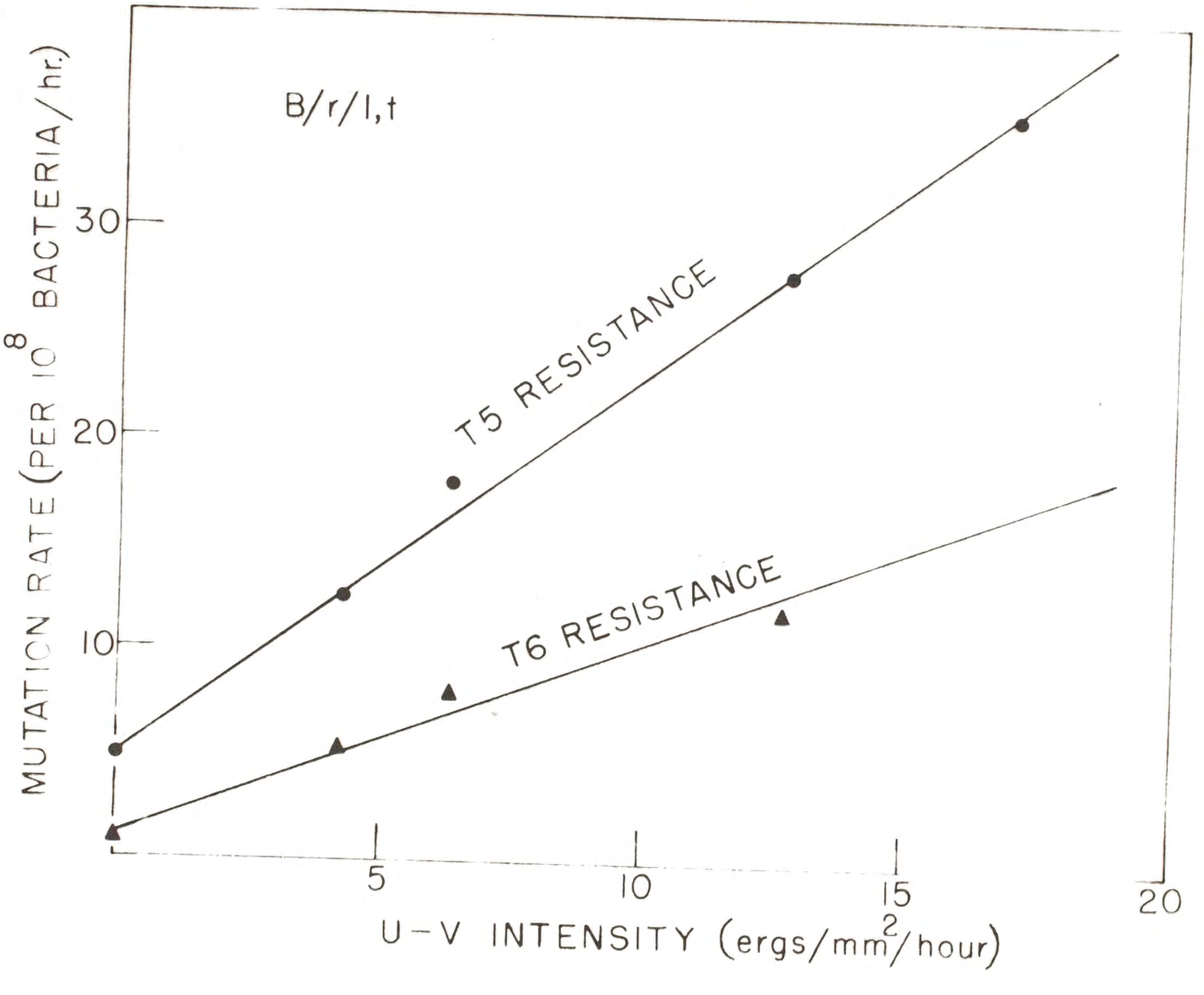


Mutants per 10^8 Bacteria
0
20
40
60
80
40
80
120
160
200
240
HOURS
$\tau = 2hr$
$\frac{\lambda}{\tau} = 0.30 \times 10^{-8}/hr$
$\tau = 5hr$
$\frac{\lambda}{\tau} = 0.30 \times 10^{-8}/hr$
$\tau = 10hr$
$\frac{\lambda}{\tau} = 0.29 \times 10^{-8}/hr$
T6 Resistance
Tryptophane Control

Hypoxanthine
Xanthine
2,6 Diaminopurine
8 Methylxanthine
Imidazole

Uracil
Thymine
6-Methyluracil
5-Bromouracil
5-Aminouracil
3-Methyluracil
Barbituric Acid

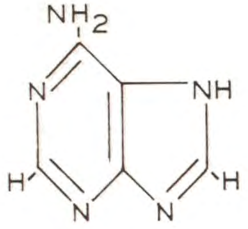
Colchicine
Urethane
Paraldehyde
Allantoin
Acetysalicylic acid
Procaine
Quinine sulfate
Aminopyrine
Atropine


B/r/1,t
T5 RESISTANCE
T6 RESISTANCE
MUTATION RATE (PER 10^8 BACTERIA/hr.)
10
20
30
5
10
15
20
U-V INTENSITY (ergs/mm^2/hour)

	T 5 Resistance	T 6 Resistance
Spontaneous	4.0	1.0
150 mg/l Adenosine	1.2	0.4

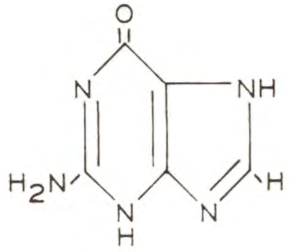
B/r/lt

	Aerobic		Anaerobic	
	T5	T6	T5	T6
Spontaneous	4.0	1.0	0.6	(0.4)
Adenosine	1.2	0.4	0.8	0.6
Theophylline	22	2.1	1.1	1.3
Theophylline + Adenosine	1.4	0.4	--	--
U - V	15	5.6	13.6	7.7
X-Ray	16.4	11.5	7.0	5.4
Spontaneous High Growth Rate (B/lt)	3.8	3.0	3.8	3.0

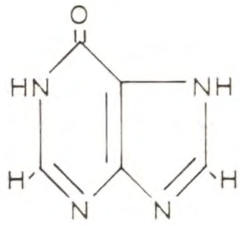


3.0-164

ADENINE

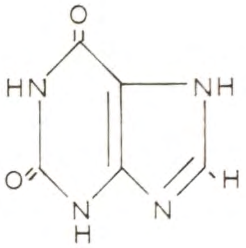


GUANINE

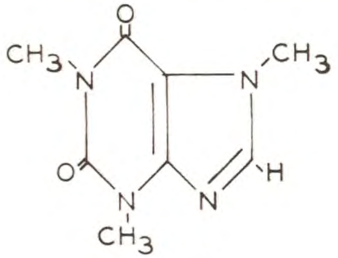


2.0-500

HYPOXANTHINE

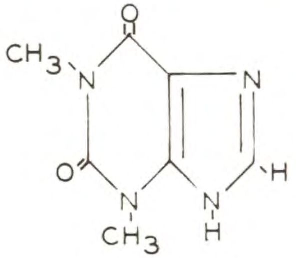


XANTHINE



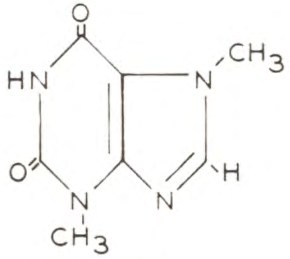
17-150

CAFFEINE



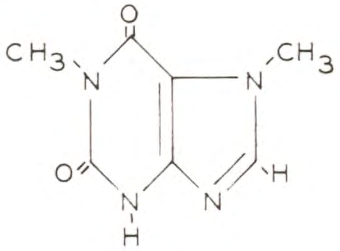
10.8-150

THEOPHYLLINE



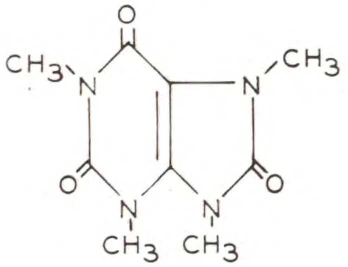
7.5-150

THEOBROMINE



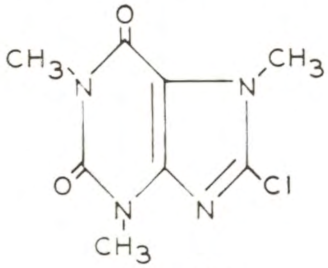
8.4-150

PARAXANTHINE



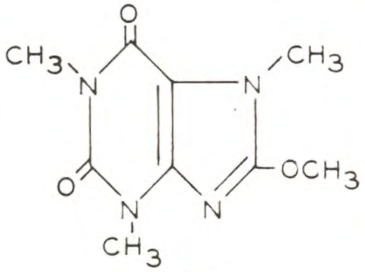
7.6-150

TETRAMETHYL URIC ACID



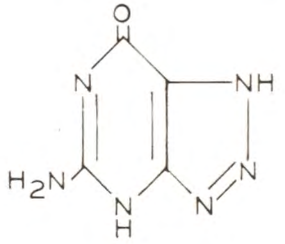
4.3-150

8-CHLOROCAFFEINE



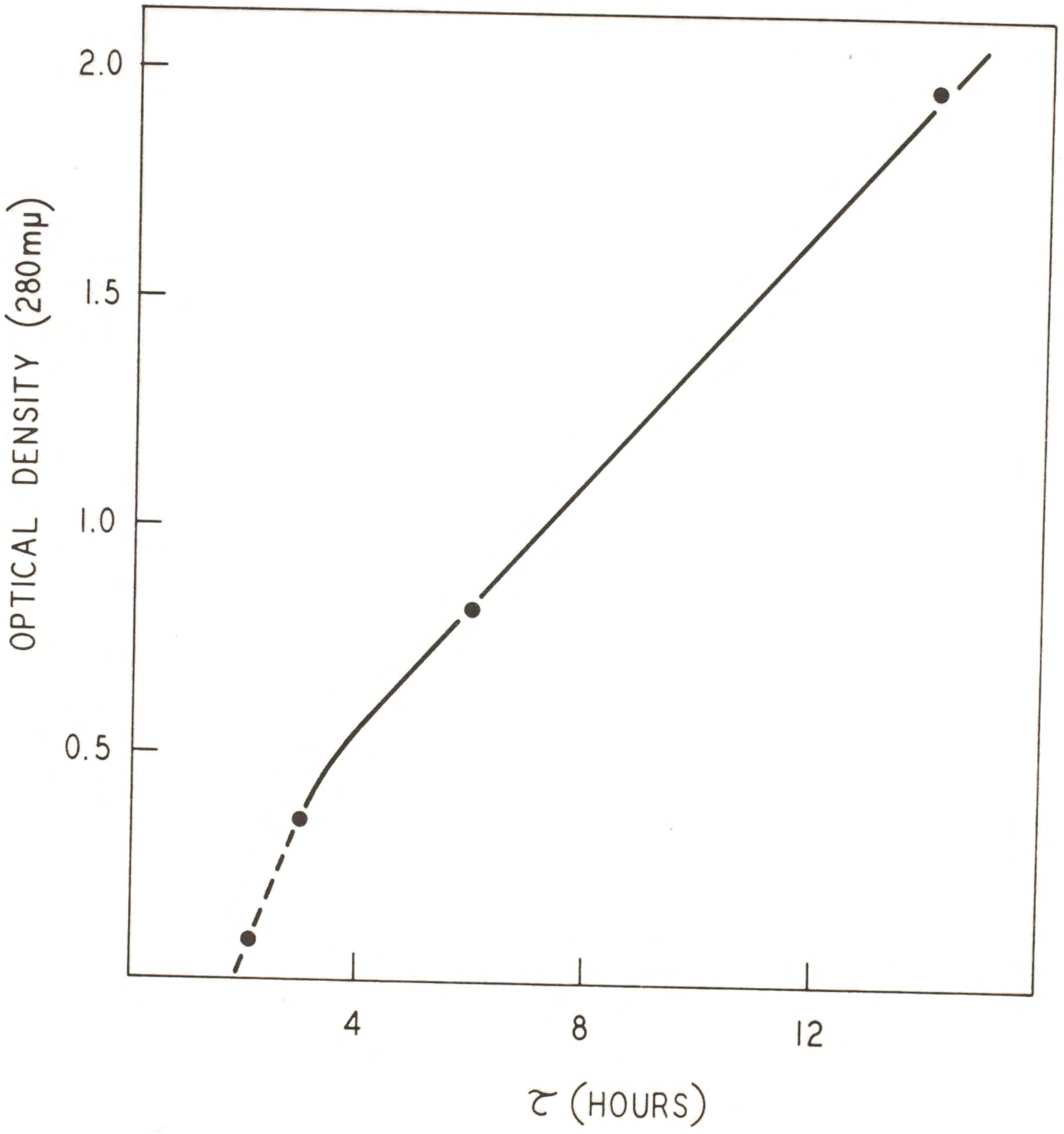
5.2-150

8-METHOXYCAFFEINE

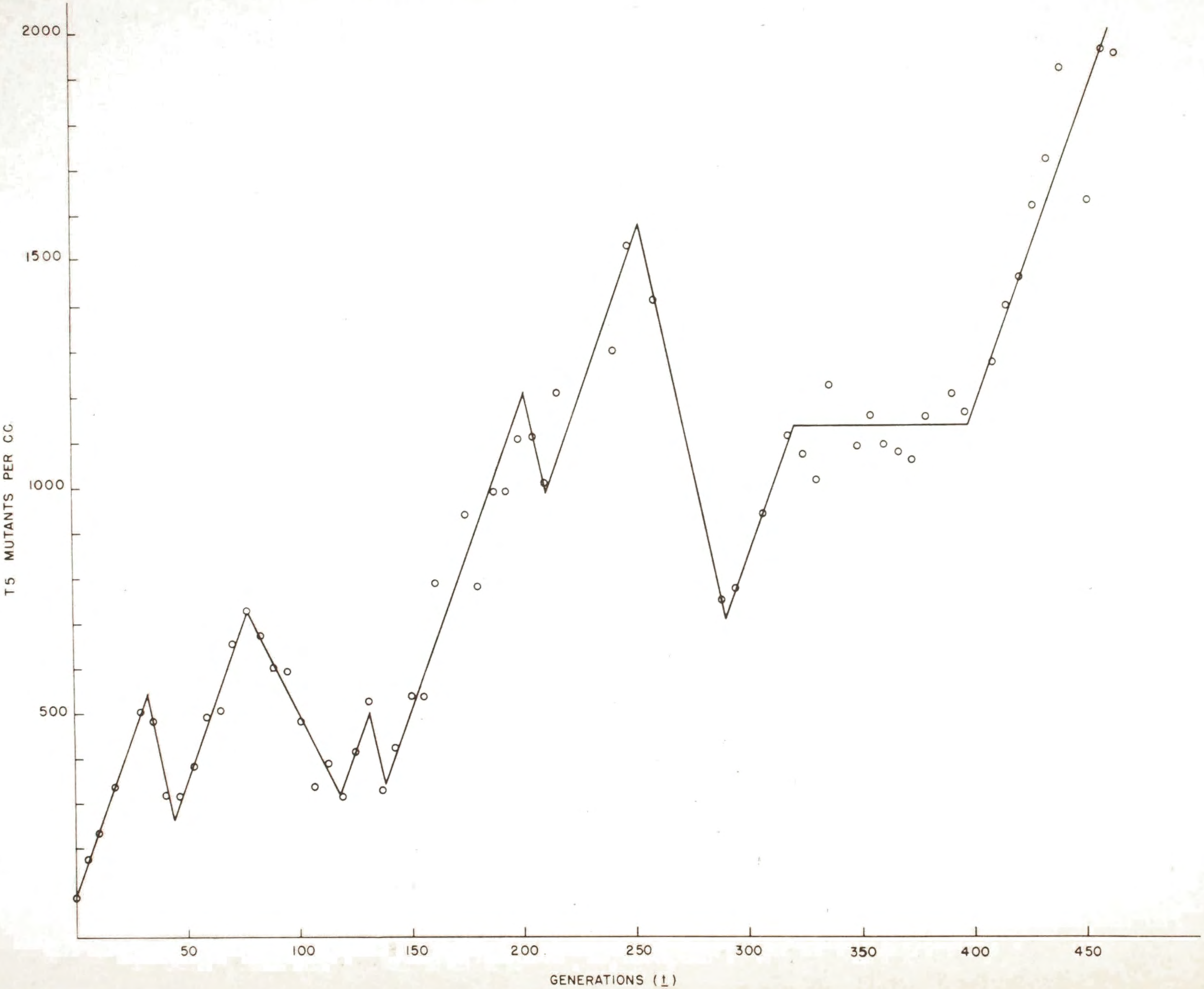


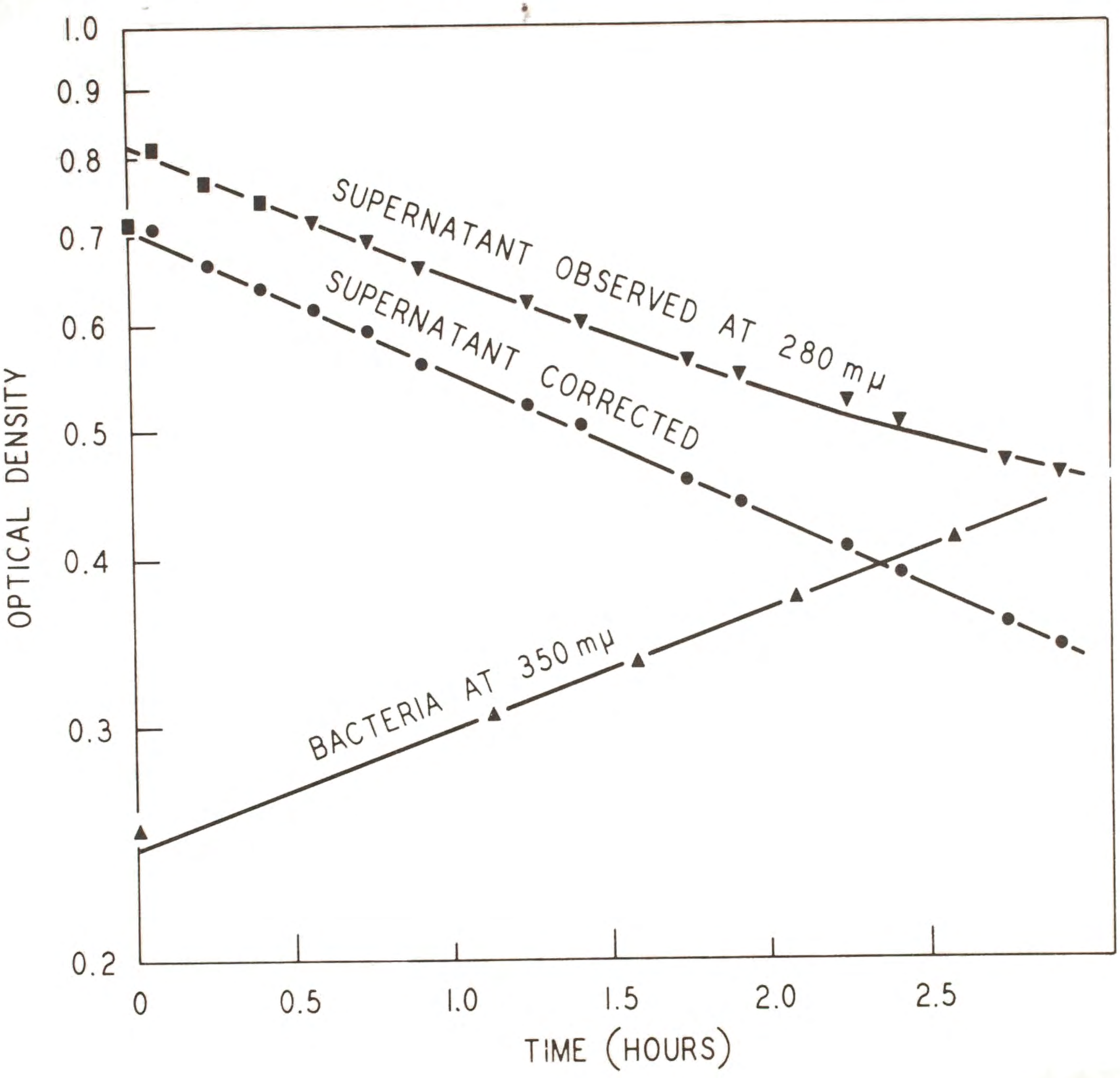
3.4-150

8-AZAGUANINE


2.0
1.5
1.0
0.5
OPTICAL DENSITY (280mμ)
4
8
12
τ (HOURS)

Caffeine	19	8-Methoxycaffeine	5.2
Theophylline	11	8-Chlorocaffeine	4.2
Paraxanthine	8.4	Azaguanine	3.4
Theobromine	7.5	Benzimidazole	3.0
Tetramethyluric acid	7.0	Adenine	2.8


2000
1500
1000
500
T5 MUTANTS PER CC.
50
100
150
200
250
300
350
400
450
GENERATIONS ($\frac{t}{\tau}$)


OPTICAL DENSITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
0.5
1.0
1.5
2.0
2.5
TIME (HOURS)
SUPERNATANT OBSERVED AT 280 mμ
SUPERNATANT CORRECTED
BACTERIA AT 350 mμ