October 7, 1949.

A) Reversions of parents. Plate ca 10^9/plate ± or without single components.

Purified cultures

Some tests not recorded owing to subsequent contamination.

W1205:

T(0): 11, 11, 13 37 tested all Lac− Mal+

Hist 32, 33 18 tested on T(0) 7 prototrophs!

Th. Crowded ± contaminants.

1195

T(0) 0, 0

Leuc. 7, 4

B) Crosses.

A 1189 × 1195 B 1189 × 1205 C 1195 − 1191 No prototrophs

all Mal+ on EMMal

7 tested: all Mal+

4 Lac (3 Lac+) 17 Lac (8 Lac+)

25 Mal+

Are these Lac+ Mal+ recombinants?

October 14. 1949.

1 ml (10 x) per plate. Grown in Penassay overnight and washed. Spread on T(0); Nut; Threonine.

O 8, 11
H 448, 380.
T 15, 14

Test T colonies for prototrophy
25 : all prototrophic!

Oct. 17, 1949.

9AM. Reinoculate from overnight Permassay cultures .1 ml / 10.
Grow to 8PM+
A W1177^{SR} B W1217^{42R} C W1177 + W1217
Use nutrientagar + 100u/ml SR + 130 γ/ml Azide (= M/500)
1) Inoculate 2 ml culture / 50 ml nutrient agar med. Pour 10 ml/plate
2) " 5 ml " ....

100 colonies picked from C, streaked on EMB Lac. 34 Lac+ : 66 Lac−

A1 Eventually produces numerous minute colonies, especially when unevenly dispersed. 13 countable colonies / 4 plates.
= 13/2×10^9.

A2 " " very minute 1 " " / 5 plates.

14 small Lac− Xyl− etc.

B1 4 plates. No colonies. Clear plates!

B2 5 plates 1 colony. Clear plates. Pick this colony and streak out on the same plate, as well as EMB Lac.

C1 Many minute colonies. 98, 121, 98, 91 (add 1 one picked)
= 102 / plate average
= 500 / 2×10^9

Lac+ 34. 28 were Mal− Xyl− Mtl−
Xyl+ Mtl+ Mal− #2
Xyl+ Mtl− Mal− #4
Xyl+ Mtl+ Mal+ 1

Lac-66.

A) 1 Xyl+ Mtl+ ~~Xyl~~ Mal+
2 Xyl+ Mtl+ Mal−
1 Xyl− Mtl− Mal+

B) 1

1 Xyl+ Mtl− Mal−

	Total:	Lac m	Xyl	Mtl	Mal	T5	Nutr sec infec
1	61	−	−	−	−	(16R) 45S	.....
2	1	−	+	−	−	R	B_1
3	2	−	+	+	−	1S; 1R	M−
4	2	−	+	+	+	1S; 1R	1M; $1B_1$
5	1	−	−	−	+	S	M−
6	28	+	−	−	−	S	
7	2	+	+	+	−	2 S	M; +
8	4	+	+	−	−	S	2+; $1B_1$; 1L
(9)	1	+	+	+	+	S	M−

Linkages. $\frac{Xyl+/Lac-}{Xyl+/Lac+} : \frac{5/61}{6/28}$

$\frac{Xyl+/Mtl+}{Xyl+/Mtl-} : \frac{6/8}{3/92}$

$\frac{Mal+/Xyl+}{Mal+/Xyl-} : \frac{3/9}{1/91}$

Test 10 type 1 for nutr., phage samples of all other types

Complete phage tests
nutritional

6305

10/25-6/1949.

10 lac- .... - tested (all T_5^s)

1 +
2 B_1
* 3 M
4 +
5 TB_1
6 TB_1
7 TB_1
8 n.g.
9 +
10 +

* useful?

Test lac- .... V_1^R for nutrition

1
2 B_1
3 B_1
4 TLB_1
5 TLB_1 2
6 TLB_1 3
7 B_1
8 B_1
9 TLB_1 4
10 T
11 TLB_1 5
12 T
13 TLB_1 7
14 TLB_1 6
15 TB_1 8

4 B_1 -
2 T -
1 TB_1 -

111-3 coli mm crosses.

Oct 17, 1949

A 1195
B 1205
C 1222
D 1222 × 1195 (grown together overnight)
E 1222 × 1205 "

10/21/49:

A	4 T(O)	3 EMS.	2 colonies.
B	3 "	3 "	4 cols.
C	3 "	4 "	0 cols.
D	8 "	10 "	1? Lac+ colony as EMS
			10 colonies
E	5 "	5 "	5 T/O)
			2 EMS Lac

Ferm. tests:

A 2 Lac- Gal+ Mal+ grew poorly on EMS Lac

B 9 Lac- Gal+ Mal+.

C — No growth.

		Gal	Lac	Mal	
D	1	—	—	+	×
	2	—	—	+	×
	3	+	—	+	
	4	+	—	+	×
	5	+	—	+	
	6	—	—	+	×
	7	—	—	+	×
	8	+	+ -	+	×
	9	—	—	+	×
	10	+ —	+ -	+ —	
E	1	+ —	—	+ —	×
	2	+ —	—	+	×
	3	+ —	—	+	× ±
	4	+	—	+	
	5	+	—	+	
	6	+	—	+	
	7	+	—	+	
Parentals	{	+	—	+	
	.	—	+	—	

× grew poorly on EMS Lac

October 24, 1949

Streak on galactose

D

1	5 colonies from each. ~ all Lac- Gal+ Mal+ (P)	Gal±	Lac -	Mal +
2		Gal - P	" -	"
3		Gal + ±	" -	"
4		Gal +	" -	"
5		Gal +	" -	"
6		Gal - P	" -	"
7		Gal - P	" -	-
8	Lac+ Mal- Gal- / Lac - Mal+ Gal+ (P)			
9		Gal - P		
10	Lac+ Mal- Gal- / Lac - Mal+ Gal+ (P)			

E

1	Gal+	Lac -	Mal+	~~Gal +~~
2		"		~~Gal +~~
3	"	"		
4	Gal+	Lac -	Mal+	
5	Gal+	"	Mal+	
6	Gal+	"	Mal+	
7	Gal+	"	Mal+	

No definite recombinants in E. In D, # 1, 2, 6, 7, 9 are Gal- Lac- Mal+ which is a new combination. These are partly mucoid and plaqued!

Renumber as 631: 1-5

Time of prototroph formation.

10/25/49.

58-161 × W677. Spread .1 ml on T(B) plates 11 AM. At stated times, respread with .1 ml saline. Count at 48 hours:

		Time	Numbers of prototrophs / plate	M (m.)
0	11 AM	0	199, 278, 209 Means: 132, 236	211
1	12:10 PM	1:10	170, 202, 207, 213	198
2	2:30	3:30	228, 158	193
3	3:20	4:20	346, 113 (sic!), 380, 307	286
4	4:40	5:40	265 110 240 181	199
5	5:30	6:20	133, 409, 143	262

October 26, 1949.

First prepare inocula overnight. Then mix.1 ml inocula, grow together 36 h.

Wash and plate on T(0).

A. 1117 B. 1135 C. 1197 D. 1222 E. 1222+466 F. 1222 + 477 G 1222+1117
0,0,0 1?0,0,0, 1?,0,0 0,0,1 6,1,3,2,3 1,0 0———

H. 1222+ 1197 I. 1222 + 1135.
000000 0000000
W-1222 is Val- ArgGlut- Mal-Gal-Lac+ Other stocks are Lac-.

Yields (per plate, 3 days) See above

Note prototrophs on E (W-466[K-12?] x W-1222. Streak out on EMB Lac to purify and test:

	Lac	Gal	Mal	Sucr	T5		
W-466	-	+	+	-	S		
W-1222	+	-	-	+s	R		
1	+	-	-	+	R	P	
2	+	-	+	+	S		x
3	+	-	+	+	S		x
4	+	-	-	+	R	P	
5	+	-	-	+	S		x
6	+	-	-	+	R	P	
7	+	-	-	+	R	P	
8	+	-	-	++	R	P?	
9	+	-	-	+	R	P	
10	+	-	-	+	S		x
11	+	-	-	+	R	P	
12	+	-	-	+	R	P	
13	+	+	+	++	R		x
14	+	-	-	+	R	P	

Numerous recombinations! (at least 5)

Try 1113, etc. as x 677, etc.

October 25 "B" 1949.

W 1217 x W-677 on Lac EMS. Good yield. Pick 100 Lac+ and streak out on Lac EMB for Lac~~+~~ v. 14 Lacv found. [All were lambda+ /518]

Reisolate to EMS Lac. 635-1 and 635-8 streaked out, and Lac- and + segregants isolated. Test on NA + Az. : (NA controls all +)

635 -1		-8	
5 Lac-	1r 4s	2r	3s
5 Lac+	5 r 0s	4r	1s
4 Lacv	all r (segregants!)	all r.	

Both these cultures, therefore, are segrgating for Az r/s.

Note totals:

Lac- 3:7 Lac+ 8:1 indicating linkage of Az to Lac, with frequent crossovers.

Re~~s~~test diploids on synthetic medium.

Oct. 25 1949 "C"

58-161 x 677. EMS Mal. Look for Mal+/- sectored prototrophs.

7 found. Streak out on EMS Mal to seprate components.

from severak hundred. Retest.

	Mal		Lac		Xyl		Mtl		TS	
1	+	−	+	+	−	−	−	−	S	S
2	+	−	−	−	+	−	+	−	S	S
3	+	−	+	+	−	−	−	−	S	S
4	+	+	−	−	+	+	+	+	R	R
5	+	−	−	−	−	−	−	−	S	S
6	+	−	+	−	+	−	+	−	S	S
7	+	−	+	−	+	−	+	−	S	S

No. 4 is an error. No. 6-7 not especially concluded.

In others, possibility of Mal- revertants must be considered.

Nov. 7, 1949.

A. 1235 x 1239

B. 1243 x 1239

C. 1238 x 1236

D. 1238 x 1242

EMS Lac B_1 Crosses by NZ

A) heavy - background 20 small +/pl

B) about 200 large +/pl -indisting from bckg

C) Heavy background 200+ several -, many intermediates

D) Heavy bckg no + many large+

100 from each to EMB Lac to look for Lac v

Sectoring on EMS Lac also observed.

A [1] Lac v Mal -

B [1] Lac v Mal+

Streak out single colonies of Lac v from B for auth. tests: (T, L)

			Lac-	Lac+
1	A1	+ only	1 (T) L	T
2	A1	+, -	2 (T) L	T
3	A1	+, -	3 (T) PROTOTROPH	T
4			4 (T) L	T
			5 (T) L	T
			6 (T) L	T v
			7 L	T
			8 L	T
			9 L	T
			10 L	T

No3 crossovers.

Restreak #3 Lac-

Symmetry test Recrosses. 638

November 10, 1949.

Repeat 637

A 1235 x 1239 dilute, use .1 ml ≐ suspension/plate

B 1243 x 1239 EMS Lac Add B, to susp.

C 1235 } Turbid!

D 1243 } Controls Turbid!

E 1239 } clear.

Most plates with background much too heavy! Pick 120 Lac+ from B and look for Lac^v. 2 ?? Restreak on EMB Lac; Mal.

None Mal^v.

Drug resistance mutants for colicins.

November 13, 1949

Grow K-12, W1113 ~~and W1115~~ in Penassay aer. overn.

W1113/Sr and K12/A2 found. No K-12/Sr noted within run. Ca ~~10~~ 2×10^9 ca. tested.

Keep as W1247-1254.

Mg effects on recombination

November 13, 1949.

5 P13 inoculate 1:5.

8+ P13 Wash W1217 and W1177. Minimal and plates as:

1) T(0)

2 Davis minimal

3 " " + .1ml $MgSO_4$ Molar. Spread

4. " " Drop in center of plate for diffusion effect.

K12 x W1113
with drug resistance

Nov. 23, 1949

From overn. cultures, take 1ml inocula 1244 and 1247 into Permassay 10AM.

A) 7^30 PM. Plate 2ml into 4 plates Nutr. Agar (+A2+Streptomycin) to select possible recombinants

B 12^30 PM 11/24. 5ml/50ml agar in 4 plates

C. do. 2ml " "

K-12 Mutant Rem (Sam Tove)

11/25/49.

Pilot run to search for liver extract requiring mutants.
K-12 subjected to penicillin selection in Y2 broth, then plated on same + liver extract. Colonies tested on T(0); EMBlac; Y2+L.E.
No L.Ex differentials found, but 6/103 tested failed to grow on T(0) on first test. Retest from EMBlac.

[1 Lac- also noted. Streak out and test purified culture on glucose on EMBlac plating. Glut+]

4/6 grew on T(0) this test. → A5; HC; Y.Li.

1 gave a few colonies on T(0) Restreak.

1 did not grow on T(0). Rundown.

K-12 × W1113. 644

Nov 20ff. 1944

		Yields	colo.	
A. W466 × 1229	3		0	
B. 478 × 1118	6		10-20/	Pick at random
C. × 1153	6		2	
D × 1156	7		24+	Mostly Suc−
E × 1159	6		#10/	Pick 4
F × 1160	6		4	

In streakings, a "lytic?" effect of Suc+ on Suc− was noted. On verification, W1156 was found to lyse W478, indicating a lysogenic phage! active on K-12, even though λ+.

Test purified (streaked on EMB Suc.) isolates on Suc, Lac, T5, T6:

B: 12 - all Suc+ Lac+ $T6^R$ $T5^R$

C 2 " " "

E 4 " " "

F 4 " " "

D 24: 23 Suc− Lac+ T_6^S $T_5^{S (= P)}$ No unselected recombinations

1 Suc+ Lac+ T_6^R T_5^R observed.

For these crosses, mutually resistant or mutually desirable parents should be used! But try W1156 × W677.

Dominance at the S2 locus.

12/3/49.

W1246 x W1178. 10 plates. Only 5 ~~colonies~~ prototrophs

#lac+3 is lac v. Test segregants on SR.

all Mal −

#1 is $V1^R$; others are V_1^S

#3 appears partially streptomycin sensitive, showing slow growth

In cross B, lac- 2, 3 are S^R; others are S^S

Streak out A3 from EMS Mal on EMB lac and EMB lac+SR 100.

UV effects on diploid

12/3/43 ff.

4 Mal-colo. from H213 10^{-6}/3s. UV streaked on Mtl.

1,2 Mtl+ 3,4 Mtl−

10^{-1}/30s. UV.

1 Mtl+
2,3 Mtl−
4. Mtl ± or ±.

⟶ Residuals possible Mtl± colonies. = 646 − 1

None. Just Mtl+; Mtl−.

Irradiate H213.

12/6/49.

Spread 10^{-6} and 10^{-7} ml H213 (from EMS stock) suspensions on EMB Mal. Expose some to UV, 3 seconds.

	UV.	+	−	V	?	
10^{-7}	0	1	2	10		1
		0	4	11		2
		1	9	9		3
		0	6	9		4
TOTAL		2	21	39	/62	
	%					
10^{-6}	3	2	10	2		11
		5	5	3		12
		3	6	9		13
		5	4	4		14
		1	7	2		15
10^{-7}	3	1				
		1	1	1		
		0	0	2		
TOTAL		18	33	23		
			41	156		
10^{-6}	0	8	[illegible]	[illegible]		21
		3	[illegible] 23	70		22
						23
		6	35	93		
		[illegible]	25	76		24
		2	23	96		25.
		22	147	491		

Re examine 7-0.

1	1	3	10
2	0	2	13
3	1	9	9
4	0	5	11
	2	19	43

χ^2:

2	21	39	62
18	33	23	74
20	54	62	136

6/66.

10^{-1} 8 sec.

	+	−	v
31	6	16	26
32	2	16	22
33	2	22	16
34	0	14	10
35	5	15	10
36	7	11	15
37	1	6	13
38	4	12	19
39	5	13	39
	32	125	170

Some colonies ● counted as +.

Unirradiated:

	+	−	v	Tot.
A	24	166	534	724
%	3.3	22.9	73.8	
B Irradiated 3 secs. ca. 10^{-1} surv.	18	33	23	74
	24	44	31	
C Irradiated 8 secs. ca 2×10^{-7}	32	125	170	327
	10	38	52	

W1205 v W814

~~##~~ Words from EMStac

130 +

20 −

No Lacy.

12/5/49

W1156 x W1257 "B".

Studies on EMB Lac.

c W1257 x W1229 (11/15/49)

NEW YORK STATE COLLEGE OF AGRICULTURE
CORNELL UNIVERSITY

Wednesday
Dec 14

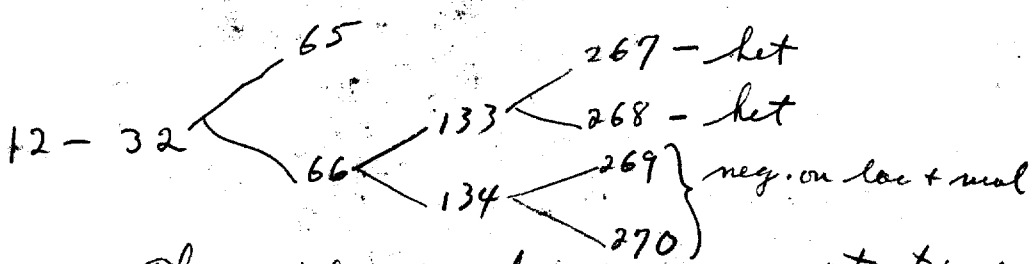
Dear Josh.

I have 4 series, 10, 11, 12 & 13 with H206-1., a strain I have maintained from the original cultures you sent.

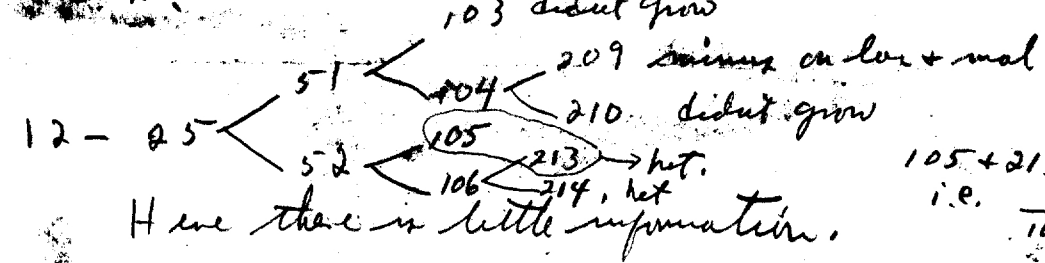
I have been time to give the complete story but results so far are somewhat disappointing. I am sending a few cultures and the portions of the pedigrees pertinent.

Series 10 + 11, with about 100 cultures in each gave no segregants. I thought I had a couple of + segregants (have used + 113 lactose mainly, maltose to a certain extent) but in every case could isolate a het colony.

In series 12, the only true segregants so far occurred. These are; 12-269, 12-270 and 12-209.



This indicates the same type of situation as before, i.e. two complementary types.



105 + 213 could not be separated
i.e. 105 213 214

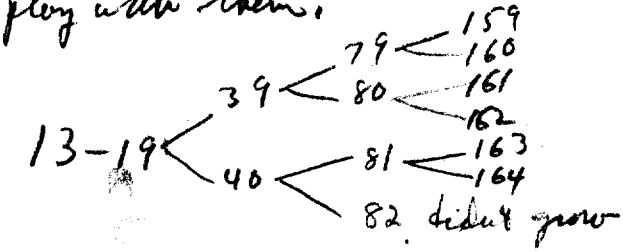
Here there is little information.

12-27 was a filamentous seed & could not further separate. It was largely minus lac, plus mal but there were a few hets also.

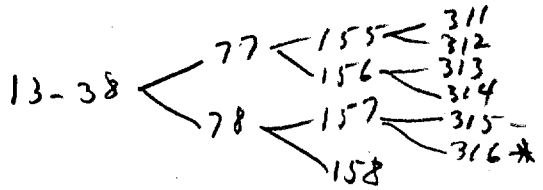
In addition 12-198 and 12-196 looked to be all positive on lactose but again I could find some small het colonies. These were in every case smaller colonies with a positive center + were not sharply margins. But on streaking they proved to be 13-316 and 13-163 behaved similarly, i.e. < 1% hets, and negatives, not positives

NEW YORK STATE COLLEGE OF AGRICULTURE
CORNELL UNIVERSITY

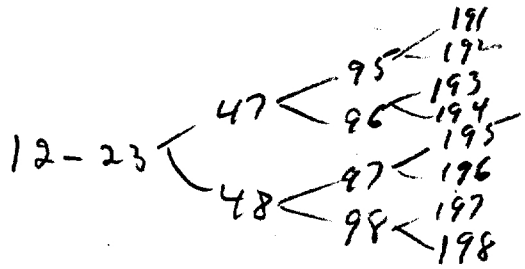
on lactose. It strikes me as curious that so many cells could be hets, segregate to positive very early and end up with a microcolony composed a few het cells and almost no negatives. I wonder if all the positive cells are alike in other segregating characteristics. On replating 12-196 showed a few negatives but 12-198 showed no negatives among some 3 or 4 colonies. Similarly 13-316 and 13-163 showed a few negatives on replating. Probably in all these cases, a segregation occurred in the first division of the cell but the very unequal + to − ratio probably indicates also that complementary types are not formed at the time of segregation. I'm sending some of these in case you want to play with them.



159, 160, 161, 162, 164 were typical hets cultures
163 as above indicates.



158, 311, 312, 314 were typical hets
313 mostly + 3 few means + negatives.
316 mostly +, <1% hets + negatives.



all typical hets but 196 + 198

I have observed some unusual forms of cells in these cultures. Haven't yet worked your latest H306, will do so next week. Sorry to be so shabby but must leave for Washington

Sincerely
Max

NEW YORK STATE COLLEGE OF AGRICULTURE
CORNELL UNIVERSITY

4 Jan 1950

Dear Jork,

Sorry to be so hurried in getting this off but at the moment life is a rat race. So I'll try to give the pertinent information & get this off.

1- Source for the 1231-pedigree was your new culture of H206. This culture behaves differently in the moist chamber. Fewer filaments, fewer cells not growing, no Y forms & weakened.

2- The number duplicate earlier pedigrees. I started from 1 when I started on H206 + there are 5, 6 & 7. This is not good but not fatal. I remembered the ~~6~~ other but don't know all my numbers here so will leave as is.

3- Some of the cultures gave blank plates. I think they are indicated on the pedigrees. They were inoculated + sent along for you to check.

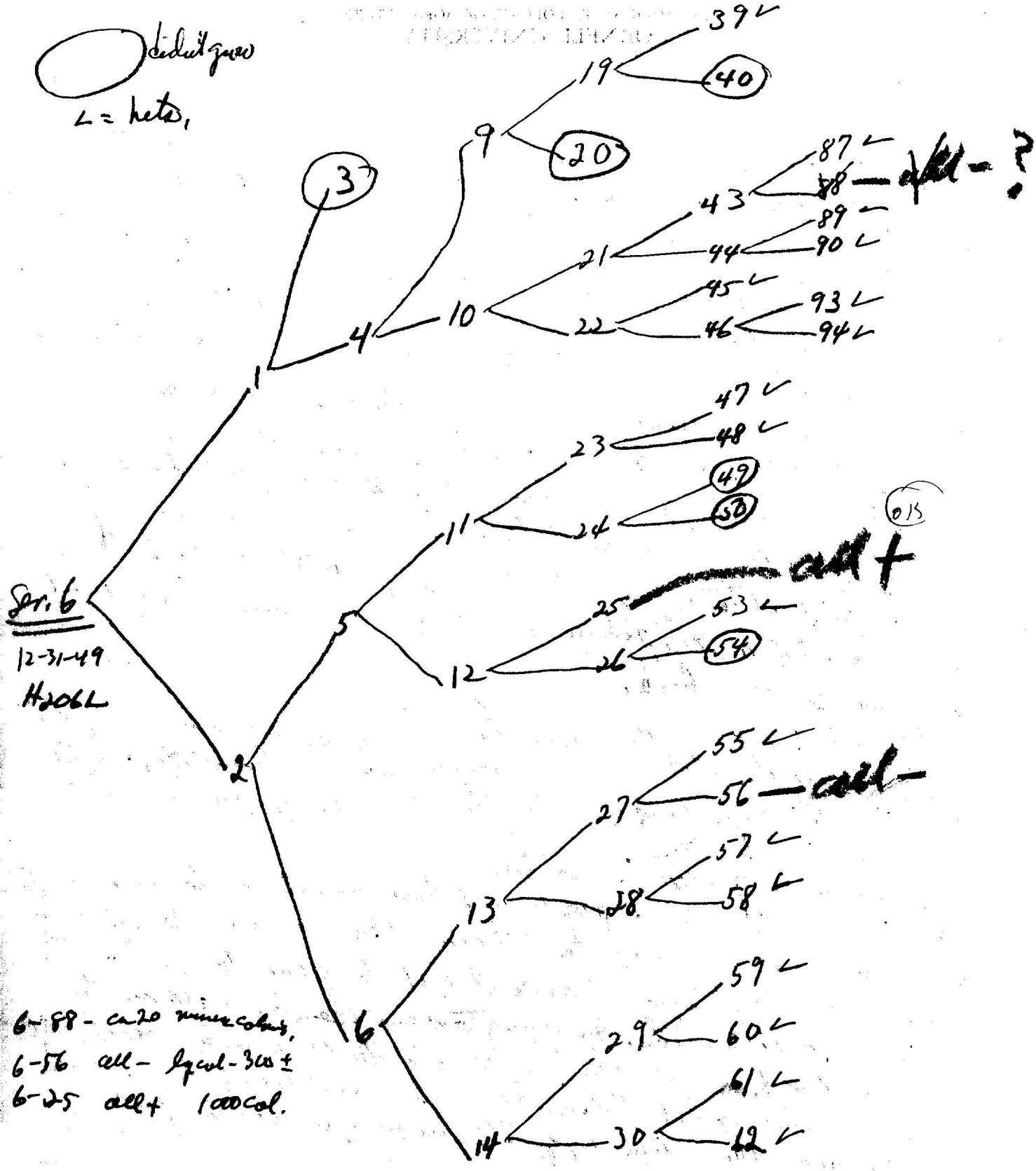
4- I picked 6 + colonies from the plate of 5-223 since they were nearly half the total colonies observed + on the chance they might be all alike and complementary to 5-223. You can do what you wish with them.

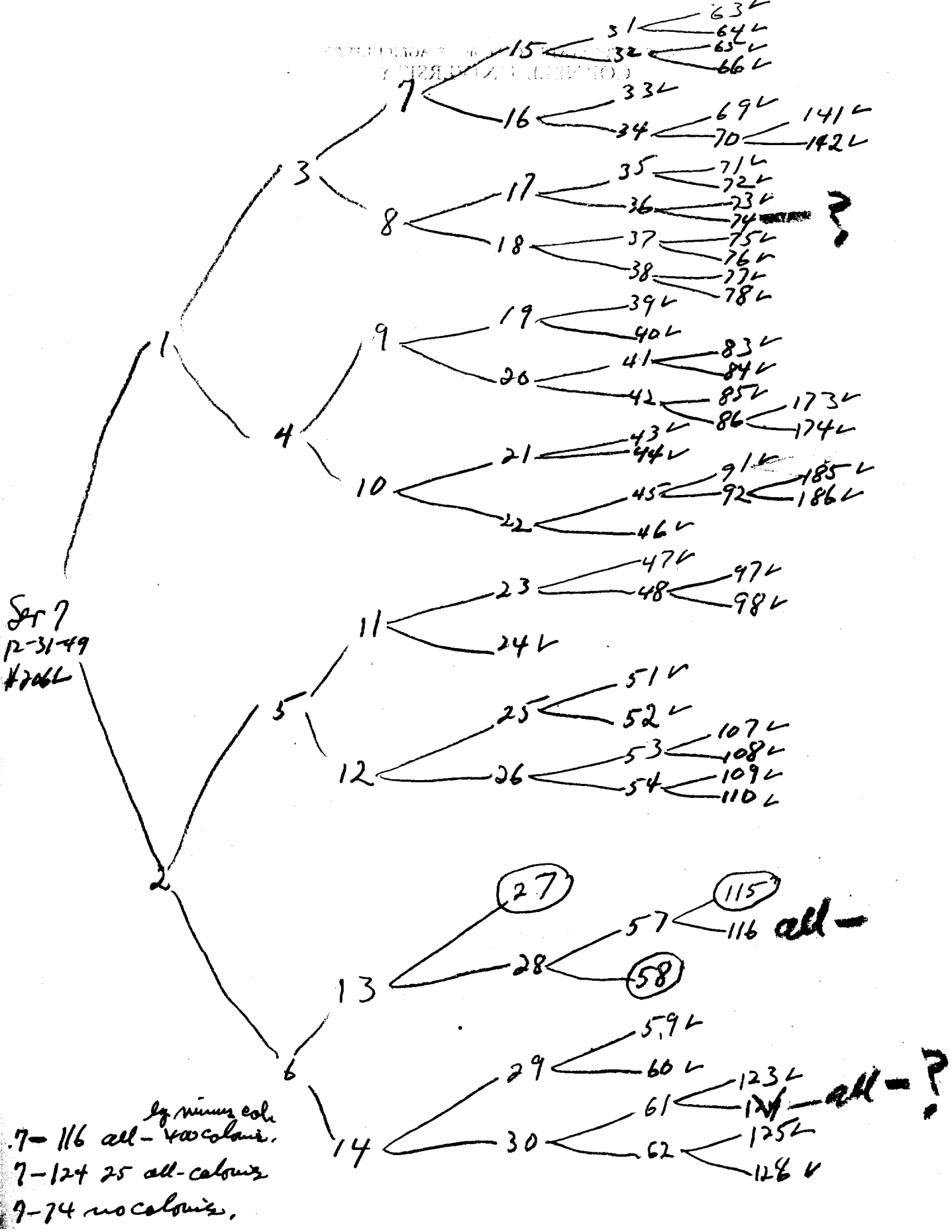
5- Cultures were tested on lactose.

6- I have seen the VIII people and have permission to do some of this down here. I'm going to discuss the weekend to get my equipment. Initially, I think it best to send the cultures to you for classification. I'm going to send them in both in the vials at present. If they are too old to be easily classifiable I think I could do it down here but I don't want to kill the goose laying the golden eggs by moving in here too fast.

So in a couple of weeks I hope to have some more cultures for you. Is it necessary to send them airmail? I inoculate into 4 ml of broth. They can't grow too much to make classification difficult & don't believe. Probably you'll have to send each back occasionally - they are amazingly expensive.

Very truly yours Allan


Sgr. 6
12-31-49
H206L
1
2
3
4
5
6
9
10
11
12
13
14
19
20
21
22
23
24
25
26
27
28
29
30
39
40
43
44
45
46
47
48
49
50
53
54
55
56
57
58
59
60
61
62
87
88
89
90
93
94
015
all +
all -
all -
?
6-88 - ca 20 minor colors.
6-56 all - 1qual - 3cm ±
6-25 all + 1000 col.

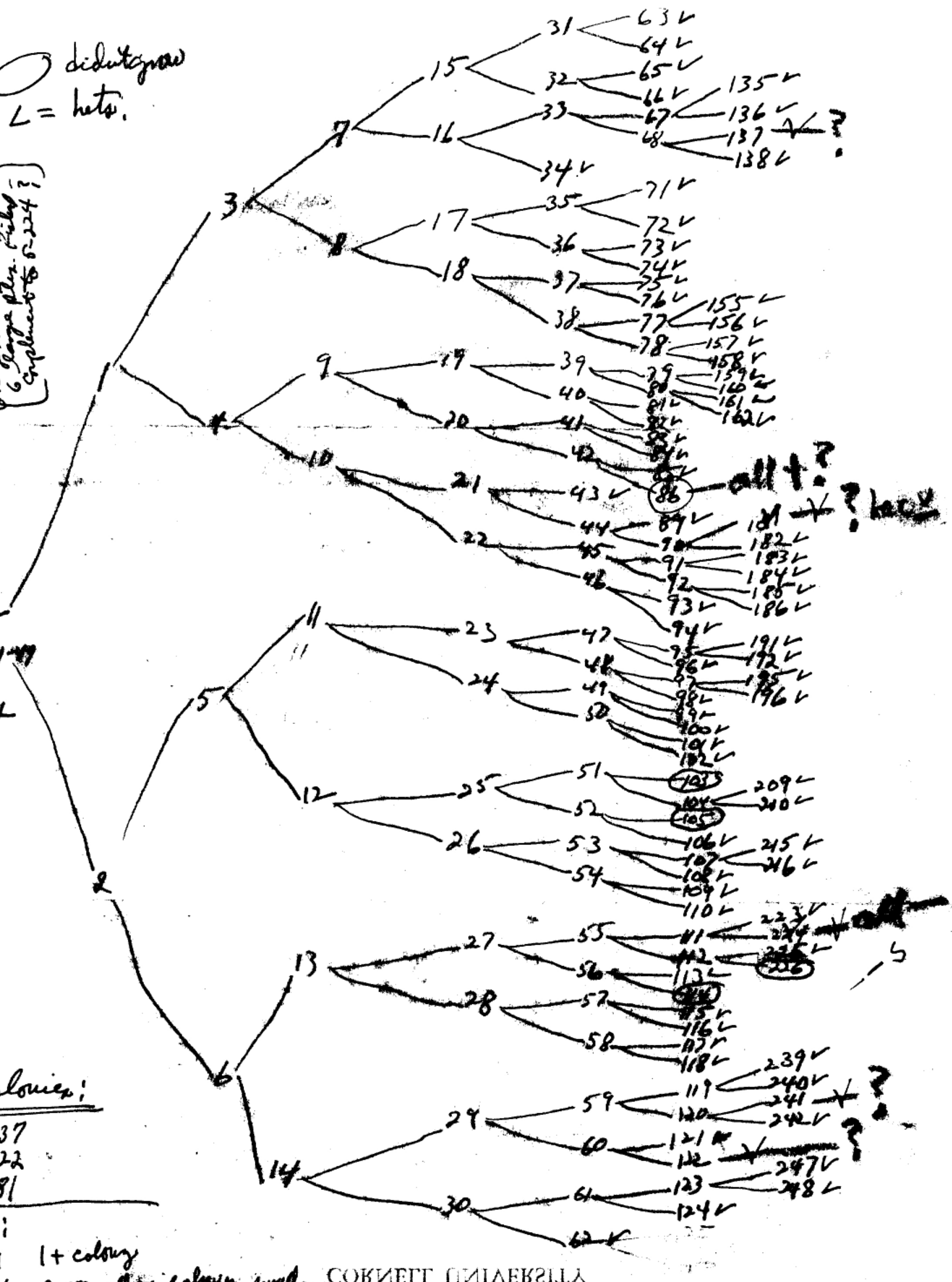


lg minus color
7-116 all-400 colours.
7-124 25 all-colours
7-74 no colours.

◯ didn't grow
∠ = hets.

5-223 { 4 hets
2 small minus
1 lg minus
6 large plus. Fields
complement to 5-224 ? }

Sex 5
12-21-49
H206L



No colonies:

5-137
5-122
5-181

Extras:

5-241 1+ colony
5-224 grew all - colonies, small -
5-86 Hi all + colonies.