N.R. S-pullorum. etc. 309

11 SEP 1946

S12	L,I,H,C. ±	L,I,C ±	L,H,C ±	H,I,C,. −	L,I,H,M. −	Leucine, cystine ...
S13	L,C ± ++	C −	L ±±	L,M ±		leucine, cystine
S14	L,A,M − − +	L,M,C ++ −	L,A,C − ++	L.AMC ++	~~AM.C~~ −	Leucine, methionine, cystine
S15	L − −	O +	LC −	C +	LM.	leucine, ≠S
S16	L − + −	C − ±	LC ++			leucine ≠S
S17	L −	C −	LC −			
S1 (R)	M − − −	T_1 − +	MT_1 ++			methionine, trypt
~~S4~~	~~M~~	~~O~~	~~C~~			
Y50.	O	N,C				

Salmonella - Inv.

S10

1 SEP 1946

1 ml inoc. S1, S17 (S15?) in YB c̄ single controls. Sh. 30°

1230A 11

Plate N13. into T(0) dupl.

no colonies on any plates.

Salmonella: N.R. 3 11

12 SEP 1946

	ENV	EN	NV	EV	O	thioglyc. +a
S18	+	+	+	+	—	+ R. +
19	+	+	+	+	—	✓ + +
? 20	+	+	+	+	—	+ +
21	+	+	— +	+	+	+ ±
22	+	+	± +	+	+	+ +
23	+	+	+	+	—	+ +
24	+	+	+	+	—	+ ++
25	+	+	± +	+	—	+ ++ ±
26	+	+	+	+	+	+ ++ ±
27	+	+	± +	+	—	+ +
28	+	+	+	+	—	+ +
29	+	+	± +	+	+	trough ±
30	+	+	+	+	—	+ +
31	+	+	+	+	—	+ +
32	+	+	+	+	+	— ± + —
33	+	+	+	+	—	+ ++ ±
34	+	+	— ±	— +	—	✓ +
35	+	+	— +	±	—	— +
36	+	+	±	+	—	—
37	+	+	± +	+	—	✓ ±
38	+	+	± +	+	—	— +

(There must be a substitution to acct. for these results; probably cystine + methionine. Check with thioglycollate also)

(also: arginine - prol; glut
alanine - [illegible]
φ-alanine - tyrosine
tryptophane - serine

parathiotroph.
parathiotroph
"
parathiotroph

Ev. no good mutants here!

S19, S32, S34 } parathiotrophs; others are prototrophs

These tests were not too careful.

S35-8 may be mutants.

In retest, only S36, S37 held up as mutant types

Starks for linkage Study.

3/2

18 SEP 1946

1P18 Moc NSB to prepare inocula.

A19 - plate c̄ T1, T3 to obtain resistant mutants.

A22: Titer of T3 is very low + continuous lysis is not obtained!

Y41, TL, BL, BT, are sens.
Y24, BB, LB,*

Y10/1 completely lysed! (~~[illegible]~~ rechuk).

Y10/3 OR for resistance.

TL/1 and TL/3 plates have numerous dense white colonies.
do. LB,

Y24/1 on T3 showed no lysis. (cross-resistance ~~[illegible]~~ — contam!)
3 streakings before used.

BM/3 OR. R.

BB,/1 — mucoid.

Y10/3 OR R.

BT/1 Mucoid.

LB,/1 v.s. colonies v. dry - probably cont.

LB,/3 } look like coli, but green!
TT/3 }

Pick to YB tubes. Test for resistance to 1; 3.

Linkage: BMR x PT; BM x PTR.

18 SEP 1946

1P18. Incr YB to prepare inocula. 679-183 from lyophil.

3P24. ~~Inoculate~~ YB. Plate P26

1. PT x Y24/1 5 tubes. -

2. PT x Y40 5 tubes

3. Y41 x 58-161 5 tubes.

R to T1.

1: 27/30, 20/20, 27/30. } 74/80 = .92 PT x ~~BMR~~ BØC/1

2: 18/20, 26/30, 5/7 } 49/57. .86 PT x BM/1

3. 0/3, 0/1, 4/10, 1/10 } ~~5/28~~ 5/24 .21. PT/1 x BM.

Resume linkage data – phage resistance 314

Ref.

257	BM×PT/1	2/10 R.	0.2
	BM/1×PT	7/10 R	.7

compare 313

259	BΦC × PT/1	2/9 R	.2
278	"	16/60 R	.27
284	"	5/20 R	.25
284	"	5/21 R	.24

313: 30/137 = .22 .78

BΦC/1×PT 27/30, 20/20, 27/30; 24/80 = .92 .92

287-	BΦC × PT/1	2/27 R	.1
	on B.	5/23	.22
293-	BΦC × TLB,/1	10/35 R.	.29
300A	"	10/29 R	.34
300B	BΦC/1 × TLB,	19/26 R	.73
301-1.	BΦC × TLB,/1	20/49 R	.41
	BΦC/1 × TLB,	27/37	.73
SUM: A.	BΦC × TLB,/1	40/113	.35
B.	BΦC/1 × TLB,	46/63	.73
315-	BΦC × TLB,/1	13/73 R	.18
318	BΦC/1 × TLB,	34/40	.85

$\chi^2_1 = 0.8$ for .35 vs. .27 (1-.73)

$\chi^2_1 = 9$ for .35 vs .50

$\chi^2_1 = 5.5$ .73 vs. .50

1.08

R: ~~386~~: Y40 × ~~Y93~~ Y64 × 58-161

353	47/57
358	16/18
364	49/70
359	32/50

SUM:

A.	Y24 × Y10/1	53/186	.28
B.	Y24/1 × Y10.	80/103	.77

313 BM × PT/1

0/3	
0/1	
4/10	
1/10	
5/24	.21

313 BM/1 × PT

18/20	.86
26/30	
5/7	
49/57	

Linkage-/T1. 315

19 SEP 1946.

A. BØC × TLB/1 B', A'. is mixture in old medium

B. BØC/1 × TLB.

Use 1ml moiula for flasks; .1 for 10ml medium in tubes

a). 5 of A; 5 of B in 50ml YB 30° sh. (A_L, B_L)

b) 10 each in 10ml YB in tubes, 30° sh.

Wash + plate N20.

A series: some large col. many small heavily suded microcol.

	Prototrophs.	T-1 R.
A	a. 1	2/8
	2	0/3
	3	0/4
	4	0/13
	5	~~2/7~~

B series: all heavily turbid; maybe contam, or recom. rate may be very high.

A' numerous col. - not coli exc. 1/10 S.
B' like other B. plates

A	b. 1	0/3
	2	0/6
	3	2/7
	4	2/5
	5	1/3
	6	0/5
	7	5/10
	8	1/6
	9	
	10	

Sum total: 13/73 = .18

B.
1
2
3
4
5
6
7
8
9
10.

Y10/1 x Y24/1.

20 SEP 46.

10 ml. YB sh. 30° Also controls

n.g. — Y24/1 cont

start new 8 ocks

Staph. flavoryanea

317

20 SEPT 1946

ENV	NB.	NV	EV	EN
++	+	+	+	−

try on rats – n.g. ~~Reat~~ Repeat!

BØC
TLB, - 1

318

24 Sept 1946

In 10 ml YB, 1P24. do. - spread .1 ml mixture on CC plates.

1. Y24 x Y10/1 (A)
2. Y10 x Y24/1 (B).

(A) + (B).

Wash + plate p. 26.
into 1 (0).

controls: Y24 - no colonies
Y10 - no colonies
Y24/1
Y10/1
in broth

(A) (B). p28 - colonies as in broth tests.
No evidence of marked increase.

B:

T-1 R.
28/33
2/3
1/1
1/1
1/1
1/1

34/40 = .85

A

1/9
4/10

September 29, 1946.

P29. BM/3 x PT/1 in YB Broth 10ml.

P1. Plate into T10). only a few colonies.

Effects of dilution and of u-v killing on recombination

30 SEP 1946

N30.

A. To 10ml YB, add 1 drop 24hr. Y24/1 and

10^{-2} 1. 1 drop 679-183 (PT) * compare ∑ final PT population. 1 drop = .1 ml.

10^{-3} 2. 10^{-2} drops. "

Dilution. 10^{-2} 3 10^{-4} drops "

15. 4. 10^{-8} drops. " (plate .1 ml.). original = ca 10^9/ml OK.

~~5. 10^{-8} drops~~ " ~~plate~~

B. To 10ml YB, add 1 drop. 24hr. Y24/1 and 1ml of the following.

6 see A1.

0 7. 1ml u-v irradiated 5 mins. plate .1 ml. 10^4

0 8. 1ml u-v irr. 10 mins. 20

0. 9. 1ml u-v. irr. 15 mins. 20

Plate plus to T(0).

∴ sublethal doses of ultra-violet prevent recombination!

Heterozygote Isolation ?

30 SEP 1946

11P30. Inoc 10ml YD Y10/1 ; Y24.

P1. 1. Mix cultures, centrifuge and wash. Plate into T/0);

A. Centrifuge together 3-4 hours to pack cells together. 0, 1, colony.

B. Wash separately + plate together. 4, 2, 0 colonies. too dry to pick.
This, therefore, is the better procedure.

Salmonella typhi-murium

Mutants - (u.v.).

30 SEP 1946

P1. Inoc S20 and S21 into YB.

11P2. Irradiate each culture 1, 2, 5 mins. + inoc 1 ml samples into 10 ml YB. Detection plates 5P3. 25°.

	1	Y20 - 1m		2000 cells sampled. 7 small colonies.
	2	2m		
A	3	5m	Use.	
				Pick A 8.
	4	Y21 1		
	5	2		
B	6	5	Use.	1200 sampled 2 small colonies.

		∞ YB.	O.	E	N	V	ENV	
A	1	+	+	→	→	→	→	green } cont.
"	2	+	+	→	→	→	→	green
"	3	+	+	→	→	→	→	pink. cont.
"	4	+++	→	→	→	→	→	OK. in color
"	5	+	→	→	→	→	→	yellow
"	6	+++	−	+++	+++	−	+++	OK. S70.
	7	+++	→	→	→	→	→	OK. in color.
B	8	+	→	→	→	→	→	green cont.
B	9.	+	→	→	→	→	→	yellow

some app. do not grow well even on ∞

S70:

parathiotrophic

Salmonella - N.R.

30 SEP 1946 P1:

	T(o)	ENV	EN	EV	NV	
S24 ab.eq.	-	- ++ +	++ +	++ +	++ ±	++
36 gall.	-	- ++	± +	++	±	+±
37 dub.	-	++ +	++ ±	++ +	++ ±	++
42 t.n.	-	++ +	++ ±	++ ±	++ -	- ✓
45 ent.	-	- ±R	++ ±R	++ ±R	++ -	- ✓
50 para A	-	- -	++ -	- ✓ -	(±) ✓ -	- ✓
51 B	-	- ✓ +	++ ✓ +	++ ✓ +	++ ✓ +	++ ✓
52 B	-	- -	- -	- -	- -	- -
55 chols.	-	- ✓ ±	++ ✓ -	++ ✓ -	+ ✓ -	- ✓
56 ch.suis.	-	++ ✓ ±	++ ✓ ±	++ ✓ ±	++ ✓ -	++ ✓

? (Estim) grows well as T(o). [Test for adapt]
V, E. Thiamin
( ) Thiamin.
(E) Tryp.
E. Arginine ✓
E, N, V. BIOTIN, TRY.
(N)
? - chip?
E (N) TRYPTOPHANE; (~~methionine~~), ~~arginine~~ ~~SERINE~~.
( )

The cleanup ones are:

S42 - typhimurium - (E)
✓ S45 - enteritidis (E)
✓ S50 - para A (ENV)
✓ S55 cholerae suis (E)(N)

others are:

S24 - prototrophic

gallinarum V, E
dublin, } stim? no sp rq.
S56 chol suis }
S50 para B

552 - did not grow

42 no.gr. Trypt.
✓ 45 Arginine. (citr ✓ +) (orn)
50 Biotin; TRYPT; grows S N.E.A. ([redacted]; meth) BTCM n.q.
no!! 55 TRYPTOPHANE; SERINE T+S n.q.
36 ENV +++ V-++ E+++ N- O- ? V or E +- B1
37 ENV +++ V++ E++ N- O- Try V alone V or E +- B1.
56 ENV +++ V-++ E+++ NE+++ O-- ~~T~~ E or NE
[redacted]
52 ENV +4 EV+ EN- NV- [redacted]. O- Hey all.
51. ENV +++ V+ E+ NE- O-

Penicillin

1 OCT 1946

add ind. amts. of Penicillin (Oxford units) to 10 ml YB broth.
add 2 drops of Y24 + Y10/1. 10 P 1. Sh. 30°.

		Penicillin (per 10 ml).	10 A 2.
3	1.	0	
1	2.	100.	some very motile motility diminished/a few filaments.
0	3.	200	v. "stringy" turbidity v. filamentous
0	4.	500.	partial inhibition. v. filamentous; many "zygospores"

Wash + plate.

10 A 2 Also anon. 1 ml each into 10 ml YB.

1	1'
7	2'
2	3'
8	4'.

penicillin is suppressed.

Edwards 10/9/46.

	S		O	ENV	EN	EV	NV	
57	typhi Smith		—	++	—	—	—	ENV
58	abortus ovis		—	+++	++	+++	—	E
59	sendai		—	+++	+++	+++	+ ++	Try E. (necessary 9 EAA)
60	sendai 571		—					
61	Taube I		—					E or N? methionine. Campaic 56 etc. do.
62	Taube II		—					
63	P-1		—					
64	P-2			+++	++	+++	+++	
65	P-11		—					
66	1181-1		—					
67	1181-2		—					
68	H66		—					
69	Pigeon 1366.		—					
70	322/6 S 20.			E; N. + methionine. (SH.).				

Bath Israel 10/21/46

71	typhi 2943 Smith	
72	sendai 3007	
73	para A 3280	
74	para A 3089	
75	typhi 3166	

Kaufmann

76	S. para A	
77	" Durazzo	
78	typhi 2	
79	" Wolfram	
80	S. sendai	
81	" negram	−
82	"	+

Linkage. 325

2 OCT 1946

1. P2 Y24 x Y10/1.

[2. Y24 x 183]

[3. 183]

4. Y24 x Y41

5. Y41.

Plate .5 ml eq. (1) .2 ml eq. 4,5 into T(0) and suppl. to detect linkage of R.

Plate .5 ml eq. Y41 into O, T, P. 20 colonies on T(0)! crowded on proline.

Exp. n. 1.

Salmonella crosses. 325

12 OCT 1946

Mor 1 drop of 12 hr. cultures into 10 ml HB. Plate 10/14/46.

S1 x 13
1 x 45
1 x 50
1 x 55
coli) 1 x TL.

1 colony found. [para A x para A !!!!]. Needs rechecking.
Later found that S1 reverts.

13 x 45
13 x 50
13 x 55
coli) 13 x BT

45 x 50
45 x 55
coli) 45 x TL

50 x 55
50 x TL
coli) 55 x TL

1
13
45
55
TL
BT

repeat. S1 x S50. 10/16/46. Plate into T(0), T(trypt).

very many minute colonies in presence of tryptophane. Should be repeated c̄
(ca ½) a more diluted inoculum. v. much smaller (a few cells/colony) ≤ trypt.

Conditions

20 Oct 1946

Mix 2 . groundcultures . N2 . (YB) 30° sh.

Y24 x Y10/1 Plate 3P3

~~Detection plates . T(0) . 10^{-7} . P3~~

2 colonies . / 1 inf eq.

Trilateral combinations test for transformation.

12 OCT 1946

Inoc 1 drop 12 hour cultures into YB Plate 10/14/46.

	O	T	L	B.
TL	—	—	ca 10^4.	
		B	L	
BL	—	—	—	
		B	T.	
BT	—	+	+.	

BT + TL + BL } 1/4 plates (turbid). turbid. ca 10^3 not so numerous →

This expt. is inconclusive, since there were a large number of L⁻ cells present. Quantitatively, it seems to support the recombination hypothesis.

Reisolate TL.

Salmonella - N.R.

S45, S50, S55.

	1	2	3	4	5	6	7	8	9
	O	PROL	GLUT	ORN	CITR	ARG.			
S45. ✓	—	—	—	+++	+++	+++		arginine	

	NV-TR. ✓	NV-TRM. ✓	V-TR ✓	V-TRM. ✓	N-B-E ✓	BIOTIN+E. ✓	E ✓	NE+E ✓	N.E.V. ✓
S50. ✓	—	±	—	±	±	—	—	++	+++

	SERINE-E.	NE-TR.	Ser-TR.	NE-E ✓	E	NE ✓		
S55 ✓	+++	+++	—	+	+++	+	++	+++ ?	try Trypt + meth.

S56. ✓ NE- Tyrosine (??) 0.

S36 ✓ V ■ (B_1) also. 0.

	B_1	B_2	3	4	5	6	7	8	9	10
S37 ✓	V +. B_1 +++	B_1 only OK.					Threonine ✓			

S42 E- Tryptophane.

S58 E-

S59 E- Grows on any combination of 9 EAA.

S61 E- Grows on any combin. of 9 EAA.

(Heterozygotes) 329.

4 OCT 1946

Inoc 24 hr. cultures of Y24 and Y41 into T(0) varying quants. (washed separately. In water together ca. 3 mins. Mix with agar before pouring. A4.

most plates sterile. A few have many non-coli bugs.

15 OCT 1946.

Add Y40+671-183. & varying chloral hydrate

		Growth.	Plate:	
1	1%	±		sl. (?) inhibition, but not feasible for exp. use.
2	.1%	+++	ca 100.	
3	.01%	+++	ca 500	
4	.001%	+++		
5	.0001%	+++	ca 500	
6	.00001%.	+++		
7.	—	+++	ca 10^3	

plate. H18. Compare, however, effect of 5% salt.

But swarming in motility-gelatin-agar is only partially inhibited by .1% chloral hydrate (1-2cm/24h.) Try .2%, .5%.

Saccharomyces sp. (ss.) 329.

Vit. Reg. – mai. Fries.
no growth – pab
delayed growth – nic.

Salmonella NR

329

21 [illegible] 1946

S36. O B. Vits.
— +++ +++ OK. Thiamin.

S56 O tyr tyr+glyc Vits Tyr+Vits ØA+Vits
— ++ ++ ++ ++ ++. which vits?

S50: O ENV EN NV EV V try try lucs try lucc try meth NV try lucm
+++ — +++ — — — — —

S55. O Ser + E E Serin Tr. NE Try. NE-Ser. + tryp.
— — + ++ ± ++ — ± + +++ ± +++ ∴ Tryp. + NE Ess.

S42. O Tryp. n.g. 24/6 ~~very slow!~~
— — ± +++ Tryptophane.

S58 O Tryp.

S59 O Tryp. Single anemio ac: E: arrya.a. +++ O ++. adapted??

S61 O ~~Tryp.~~ Single anemio ac. E: meth +++ others —

S07
S63
S64.
etc

S70. O Tryp. Single anemio ac E: meth +++ others —

Sarch. Lind. grew on B vits exc
— pab, folic OK on — folic. ∴ — pab, — nic!
— nic.

Salmonella, N.R. 330

23 Oct 1946

	O	EN	ENV	ENB	ENB.	NV	BN	
S50.	–	–	++++	+++	–	(+)	(+)	∴ Biotin. can disp. ē e.a.a.

	O	SH	Cyst	Meth.	Trypt	Indole	Anther
S61	±	–	–	+++			
S70.	–	±	+ +++	+++	++	+++.	true parathiotroph!
S42	–				±++ ✓	± ++ ✓	– –

anther → ind → trypt. ✓

ē Fildes.

S62
63
64
65
66
67
68
69
59. ± ++

heavy moi +++ (ind. adapts!)

S56. Try on all amino acids + vitamins separately. ✓ +++ E +.
Biotin ++ ✓ noE. alanine ±. lys. ± trypt ± tyros. ± || 48 hr: Biotin +++ alanine ± tyrosine +++

S55. Trypt + NE (all +; let a time) and ~~Trypt~~ NE (all):
T + N ++
N ++
individually: – – exc. cystine ++ ✓ ok. Try cyst. alone.
O – heavy moi. in O ++++

later O: – Test growth in proline, for transfer growth
any NE. ++
all NE do. in minimal, proline; compare original culture.

E coli mutants.

24 OCT 1946

Selective media (lactose)

1. EMB Difco. OK.

2. Endo: lactose 1% pul.
   Na_2SO_3 2.5
   Fuchsin .4 g
   K_2HPO_4 3.5 g
   Nutr. agar
   n.g. as made up.

3. Purple lactose:
   Nutr. agar
   lactose
   BCP .025 g/l. n.g.

Streak K-12, S1 and K-12+S1 on each of these plates, for decision as to most appropr. medium.

Salmonella N.R.

S50 Biotin +: (N~~V~~)– (EAA) BN ~~+~~ + none grew.

S56. ✓ 0 BIOTIN TYROSINE dl-tyrosine Biotin? ?
– ± ± +++
– ± +++

S55. ✓ 0 TRYPT. CYST. TR+CYST. TR+PROL.
– – – – –
S5a. – – – +++ –

nuds Trypt, cyst adapts readily.

S57. EN V–: none grew! –Leucine found later (tubes allowed to stand)
EV N–:
NV E–:

S58 E– : none grew!
0 ENV NV EV.

S60

S51 ✓ NE+ proline +++. others – NE +++ Serine ±

S52. NV E–

S61. ✓ 0 Meth. N~~V~~ N~~V~~-cyst Cyst.
+++ +++ +++ +++ +++ all adapt!

✓ 0 Meth Tryp.

62	++	+++	++	✓
63	+	+++	+.	✓
64	+	+++	+	✓
65	±	+++	–	✓
66	±	+++	–	✓
67	±	+++	–	✓
68	±	+++	–	✓
69.	–	+++	–	✓

Sugar mutants – E coli.

Yeast

Endo-lactose	K-12	S-43
a. Med School	+++	– I
b. made up	+++	–
c Endo-glucose	+++	+++
d. Endo-sucrose.	–	I
e maltose	+++	slow +

EMB.

N.A. + 1% sugar + 2g K_2HPO_4, .4g Eosin Y; .065g MB/l.

a. lactose	–	+++ –
b. glucose	+++ slow	+++ rosheen!
c. sucrose	I	
d maltose	+++	–

Try: E coli suppl.

Coli (T10))
½% glue — n.g.

Fries (N10))
2% sucr. — requires pab.

* Burkholder's
5% gluc. — only on vits.

Coli T10)
5% glue — n.g.

*/liter
glucose 20
aspar. 2
NH_4PO_4 1.5
$MgSO_4$ .5
$CaCl_2$ .3
$NH_4)_2SO_4$ 2.0

work?