N.R. S-pullorum. str. 309

11 SEP 1946

	L,I,H,C.	L,I,C	L,H,C	H,I,C.	L,I,H,M.	
S12	±	±	±	−	−	Leucine, cystine ...
S13	L,C	C	L	L,M		leucine, ~~cystine~~
	± ++	−	±±	±~~++~~		
S14	L,A,M	L,M,C	L,A,C	L.AMC	~~M.C~~	Leucine, methionine, cystine
	− +	++ −	− ++	++	−	
S15	L	O	LC	C	LM.	
	−	−	+	−	+	leucine, ≠S
S16	L	C	LC			leucine ≠S
	−	+ −	− ±	++		
S17	L	C	LC			
	−	−	−			
S1.	M	T→	MT→			methionine, trypt
(R)	−	− −	− +	++		
~~S~~	~~M~~	~~O~~	~~C~~			

Y50. O N,C

Salmonella - Durv. 510

1 SEP 1946

Inf mice. S1, S17 (S15?) in YB ± single controls. 8h. 30°

1230A 11

Plate N13. into T(0) dupl.

no colonies many plates.

Salmonella: N.R. 3 11

12 SEP 1946

	ENV	EN	NV	EV	O	thioglyc. +2
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 - prolight
ala - t
ϕ-alanine - tyrosine
tryptophane - serine

paratheotroph.

paratheotroph

paratheotroph

Ev. no good mutants here!

S19, S32, S34 } paratheotrophs; others are prototrophs

S35-8 may be mutants.

These tests were not too careful.

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

Strains for linkage Study. 312

18 SEP 1946

1P18 have NSB to prepare inocula.

A19 – plate ± T1, T3 to obtain resistant mutants.

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

Y41, TL, BL, BT, are sens. Y10/1 completely lysed!
Y24, BB, LB, * (~~no~~ results).

Y10/3 OR for resistance.

TL/1 and TL/3 plates have 4 mucous dense white colonies.
do. LB,

Y24/1 on T3 showed no lysis. (cross-resistance ⇒ — contam.)
3 streaks before used.

BM/3 OR. R. Pick to YB tubes. Test for
BB,/1 — mucoid. resistance to 1; 3.
Y10/3 OR R.
BT/1 Mucoid.
LB,/1 v.s. colonies v. dry - probably cont.
LB,/3 } looks like sol, but green!
TT/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 } ~~5/55~~ 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
287	BΦC × PT/1	2/27 R	.1
	on B:	5/23	.22

313: 30/137 = .22 .78

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

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	40/163	.35	χ^2_1 = 0.8 for .35 vs. .27 (1-.73)
	B. BΦC/1 × TLB,	46/63	.73	χ^2_1 = 9 for .35 vs .50; χ^2_1 = 5.5 .73 vs. .50
315-	BΦC × TLB,/1	13/73 R	.18	
318	BΦC/1 × TLB,	34/40	.85	

1.08

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

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

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

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	

19 SEP 1946.

A. BØC x TLB/1 B', A': is mixture in old medium
B. BØC/1 x TLB.
Use 1ml inocula for flasks; .1 for 10 ml medium in tubes

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

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

Wash + plate N20.

A series: same lazy col. many small heavily coated micro col.

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

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

A' numerous col. - not coli exc. 1/10 3.
B' like other B. plates

D.	1	0/3
	2	0/6
A	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 at 4.30° Also controls

n.g. — Y24/1 cont

Statement Forbes

Staph. flavoguttata

20 SEPT 1946

ENV	NB.	NV	EV	EN
++	+	+	+	−

tryptants – e.g. ~~Acet~~ Repeat!

BØC
TLB, - 1

24 Sept. 1946

In 10 ml YB, 1P24. do. - spread .1 ml mixture on 12 plates.

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

(A) + (B).

Wash + plate p. 26. (10).

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

(A) (B). P28 - colonies as in both tests.
No evidence of marked increase.

	T-1 R.
B:	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 ē 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. 6 drops~~ " ~~plate~~

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

6 see A1.

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

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

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

Plate plate to T(0).

∴ sublethal doses of ultra-violet prevent recombination!

Heterozygote Isolation ?

30 SEP 1946

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

P1. 1. Mix cultures, centrifuge and wash. Plate into T10);

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

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

Salmonella typhi-murium

322

Mutants - (u.v.).

30 SEP 1946

P1. Incr S20 and S24 into YB.

IIP2. Incubate each culture 1, 2, 5 mins. + incr 1 ml samples into 10 ml YB. Detection plates SP3. 25°.

1 Y20-1m — 2000 cells sampled. 7 small colonies.
2 2m
A 3 5m Use.
Picks 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 & cent.
	2	+	+	→				green pink cent.
	3	+	+	→				
	4	+++		→				ok. in color yellow
	5	+		→				
	6	+++	−	+++	+++	−	+++	← ok. S70.
	7	+++		→				ok. in color.
B	8	+		→				green cent.
B	9.	+		→				yellow

some app. do not grow well even on M

S70:

paratyphoid

Salmonella - N.R.

30 SEP 1946

Pl:

	T(o)	ENV	EN	EV	NV	
SR4 abeq.	− −	+++ ++	+ ++	+ ++	± ++	? (E stim) grows well as T(o). [Test for adapt]
36 gall.	− −	++	± +	++	± +±	V, E. Thiamin
37 dub.	− ++	+ ++	± ++	+ ++	± ++	( ) Thiamin.
42 t. m.	− ++	+ ++	± ++	± ++	− − ✓	(E) Tryp.
45 ent.	− −	±R ++	±R ++	±R ++	− − ✓	E. Arginine ✓
50 para A	− −	− ++	− − ✓	− (±) ✓	− − ✓	E, N, V. BIOTIN, TRY.
51 B	− − ✓	+ ++ ✓	+ +± ✓	+ ++ ✓	+ ++ ✓	(v?)
52 B	− −	− −	− −	− −	− −	? - clear?
55 chols.	− − ✓	± ++ ✓	− ++ ✓	− + ✓	− − ✓	E (N) TRYPTOPHANE; (~~methionine~~); ~~arginine~~ ~~SERINE~~.
56 ch. suis.	− ++ ✓	± ++ ✓	± ++ ✓	± ++ ✓	− ++ ✓	( )

The clear cut 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

S52 - did not grow

42 no. gr. Trypt.
✓ 45 Arginine. (citr ✓±) (orn)
50 Biotin; TRYPT; grows S N.E.A. ([redacted]; meth) BTCM n.g.
no!! 55 TRYPTOPHANE; SERINE T+S n.g.
36 ENV+++ V++ E++ N- 0- ? V or E +- B₁
37 ENV+++ V++ E++ N- 0- Try Valine V or E +- B₁.
56 ENV+++ V-++ E+++ NE++ 0- ~~Try~~. E or NE
[redacted]
|| 52 ENV+4 EV+ EN- NV- [redacted]. 0- they all.
|| 51. ENV+++ V+ E+ NE- 0-

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 unfiltered.

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	—					E or N? methionine. Campaal 56 etc. do.
61	Taube I	—					
62	Taube II	—					
63	P-1	—					
64	P-2		+++	++	+++	+++	
65	P-11	—					
66	1181-1	—					
67	1181-2	—					
68	M66	—					
69	Pigeon 1366.	—					
70	322/6 S 20.		E; N. + methionine. (SH.).				

Barth Israel 10/21/46

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

Kauffmann

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

Linkage.

20 OCT 1946

1. P2 Y24 x Y10/1.

[2. Y24 x 183]

[3. 183]

4. Y24 x Y41

5. Y41.

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

Plate .5 ml eg. Y41 into O, T, P. 20 colonies on [illegible]! crowded on [illegible].

Exp 1. 1:

12 OCT 1946

Inoc 1 drop of 12 hr. cultures into 10 ml 1/3. 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 + para A !!!!]. Needs rechecking.
Later found that S1 sweets.

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 550. 10/16/46. Plate into T(o), T(trypt).

very many minute colonies in presence of tryptophane. Should be repeated ē
(ca 1/2) a more dilutid inoculum. v. much smaller (a few cells / colony) ∴ trypt.

Evolution

20 Oct 1946

Mix 2 grown cultures. N2. (YB) 30° 2h.

Y24 x Y10 f1 Plate 3P3

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

2 colonies. / 1 mfeq.

Trilateral conbinations 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.	
BL	—	B —	L —	
BT	—	B +	T. +.	

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.

545, 550, 555.

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

	NV-TR. ✓	NV-TRM.	V-TR ✓	V-TRM. ✓	N-B-E ✓	BIOTIN+E. ✓	E ✓	NE+E ✓	N.E.V. ✓
550. ✓	—	±	—	±	±	—	—	++	+++

	SERINE-E.	NE-TR.	Ser-Tr.	NE-E ✓	E	NE ✓		
555 ✓	+++	+++	—	+	+++	+	++	+++	try Trypt+meth.

556. ✓ NE- Tyrosine (??) 0.

536 ✓ V- (B_1) also. 0.

		B_1	B_2	3	4	5	6	7	8	9	10
537 ✓	V+.	B+	+++	B_1 only OK.		Thiamine ✓					

542 E- Tryptophane.

558 E-

559 E- Grows on any combination of 9 EAA.

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

(Heterozygotes) 329.

4 OCT 1946

Drop 24 hr. cultures of Y24 and Y41 into T10 varying grams. (washed separately. Drop 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+679-183. ∓ varying chloral hydrate

		Growth.	Petots:	
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.)

V. f. Rig. – mai. Fruis.
no growth – pat
delayed growth – mi.

21 ... 1946

S 36.	0	B₁	Vits.					
	–	+++	+++	OK. Thiamin.				
556	0	tyr	tyr+glyc	Vits	Tyr+Vits	ØA+Vits		which Vits?
	–	++	++	++	++	++.		
550:	0	ENV	EN	NV	EV	V try	try leuc	try leuc · try meth · NV try leuc
		+++	–	+++	–	–	–	
555.	0	Ser+E	E	Serine Tr.	NE Tryp.	NE−Ser. +tryp.		∴ Tryp. + {NE / Ess.
	– –	+ ++	± ++	– ±	+ +++	± +++		

542. 0 Tryp. n.g. 24h ~~very slow!~~ – – ± +++ **Tryptophane.**

558 0 Tryp.

559 0 Tryp. Single amino ac: E: any a.a. +++ 0 ++. adapted!!

560 0 ~~Tryp.~~ Single amino ac. E: meth +++ others –

567
563
564
etc

570. 0 Tryp. Single amino ac E: meth +++ others –

Sands. Lind. · generous B vits. etc
– pab, folic OK on – folic. ∴ – pab, – nic!
– nic.

23 Oct 1946

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

	O	SH	Cyst	Meth.	Trypt	Nudde Brother
S61	±	–	–	+++		

S70. – ± +++++ +++ ++ +++. true parathiotroph!

S42 – ±++ ✓ ± ++ ✓ – – – anther → ref → trypt. ✓

S62
63
64
65
66
67
68
69 ē Fildes.

59. ± ++ heavy inoc +++ (not adapts!)

S56. Try on all amino acids + vitamins separately. V +++ E +.
Biotin ++✓ no E. alanine ±. lys. ± || 48 hr: Biotin +++
trypt ± tyros. ± alanine ±
tyrosine +++

S55. Trypt + NE (all +; let a time) and ~~Trypt~~ NE (all):
T+N +++
N ++
individually: – – exc. cystine ++ ← OK. Try cyst. alone.
0 – heavy inoc. in 0 +++

later 0: –
any NE. ++
all NE do.

Test growth in proline, for transfer growth 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.

S50 Biotin +: (N)— (EAA) BV + nonegrow.

S56. O Biotin Tyrosine dl-tyrosine Biotin? ?
— ± ± +++
± +++

S55. O Trypt. Cyst. Tr+Cyst. Tr+Prol.
S55. — — — — — muds Trypt, cyst
S55a. — — — ++ — adapts readily.

S57. EN V−: nonegrow! -Leucine found later (tubes allowed to stand)
EV N−:
NV E−:

S58 E− : nonegrow!
O ENV NV EV.

S60

S51 NE+ proline +++. others — NE +++ Serine ±

S52. NV E−

S61. O Meth. NV N-cyst Cyst.
+++ +++ +++ +++ +++ all adapt!

O Meth Tryp.
62 ++ +++ ++
63 + +++ +
64 + +++ +
65 ± +++ —
66 ± +++ —
67 ± +++ —
68 ± +++ —
69. — +++ —

Sugar mutants – E coli.

Yeast

Endo-lactose	K-12	S-[illegible]
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	+++ shows	+++ washes!
c. sucrose	I	
d maltose	+++	–

Try: E coli supp.

Coli (T10)) ½% gluc	n.g.
Fries (N10)) 2% sucr.	requires pab.
* Burkholder's 5% gluc.	only on vits.
Coli T10) 5% gluc	n.g.

* / liter

glucose	20
aspar.	2
KH_2PO_4	1.5
$MgSO_4$	.5
$CaCl_2$	.3
$NH_4)_2SO_4$	2.0

wo KI.7