

Mice inoc. subcut. BDA

	Total No.	Percent at end of 35 days	Range
Untreated —	40 mice	2%	→
Superinfected — 1 day —			

Toto.
Moss
METS.

OCT 27 1947

TOXOPLASMA NEUTRALIZATION TEST IN MICE

Obj: Can toxoplasma neutralizing antibodies be detected with a mouse test
 & if so, should brain or peritoneal exudate be the preferred source of parasites?

TOXOPLASMA: ^{MB.} Fresh 10% suspension ^{in NaCl} of ² pooled brains. (= 8 cc)
 Filter thru gauze & then dilute ^{0.5 cc} of this = ^{2.0 cc} NaCl to
 make 1:50. of the latter make a series of ten-fold dilutions (0.2 cc + 1.8 cc)
 for a total of 5 tubes. 0.1 cc of each of these dilutions transferred
 to each of two small dilution tubes for serum addition.

Peritoneal Exudate: Harvest peritoneal exudate from each of three mice.
 Prepare each as 1:5 (1 pt exudate, 1 pt hyaline, 3 pts NaCl). Pool aliquots
 & then make ten-fold dilutions for 5 tubes as for MB. Transfer
 0.1 cc amts to dilution tubes.

SERA: a) Control - 0.1 cc amts of inactivated NRS to each control line.
 b) Immune: 0.1 cc amounts of Ralph Blaskin serum (22 Oct) to each test line.

Incubation: Mix toxo & sera & allow to stand ^{for 2 hrs. in 37° C H₂O bath.} @ room temp for 30.

Injection: Each dil into 4 Mice ¹²⁻¹⁴ ~~8-10~~ gm mice. 0.03 ml I.cer.

Observe 21 days.

INTRACEREBRAL ROUTE

TOXO SOURCE	SERUM	FINAL DIL. TOXO.						TITER LD ₅₀	NEUT. INDEX
		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶		
MOUSE BRAIN	NRS	—	4/4	4/4	3/4	0/4	0/4	10 ^{-4.3}	—
	BLACK	—	4/4	2/3	1/4	0/4	0/4	10 ^{-3.5}	6
PERIT. EXUDATE	NRS	4/4	4/4	4/4	4/4	0/3	—	10 ^{-4.5}	—
	BLACK	4/4	4/4	1/4	0/4	0/4	—	10 ^{-2.9}	60

Peritoneal
Exudate I.cer
= preferable test.

OCT 27 1947

MOUSE BRAIN TOXOPLASMA VS NRS & BLACK SERUM**NRS**ICER

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	MORT.	
10 ⁻²	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	
10 ⁻³	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	
4.3 10 ⁻⁴	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3/4	LD ₅₀ Tit. 10 ^{-4.3}
10 ⁻⁵	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/4	
10 ⁻⁶	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/4	

BLACK

	1	2	3	4	5	6	7	8	9	10	11	12	13	14		MORT	
10 ⁻²	-	-	-	-	-	-	-	-	-	-	-	-	-	-		4/4	
10 ⁻³	-	-	-	-	-	-	-	-	-	-	-	-	-	-		2/3	
3.5 10 ⁻⁴	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1/4	LD ₅₀ TIT. 10 ^{-3.5}
10 ⁻⁵	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/4	
10 ⁻⁶	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/4	

Disc 18 Nov 47

2 hrs. at 37°C.

OCT 27 1947

PERITONEAL EXUDATE TOXOPLASMA VS NORMAL RABBIT

4 BLACK SERA

INTRACEREBRAL

NRS

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	MORT.		
10 ⁻¹	-	-	-	-	D																						4/4	33,000	
10 ⁻²	-	-	-	-	D																						4/4	3,300	
10 ⁻³	-	-	-	-	D																						4/4	330	LD ₅₀ T.T. 10 ^{-4.5}
10 ⁻⁴	-	-	-	-	D																						4/4	33	
10 ⁻⁵	-	-	-	-	D																						0/3	3.3	

1cc of undiluted peritoneal exudate has 1,056,000

BLACK

	1	2	3	4	5	6	7	8	9	10	11	12	13	14		MORT.		
10 ⁻¹	-	-	-	-	-	-	-	-	-	-	-	-	-	-		4/4	33,000	
10 ⁻²	-	-	-	-	-	-	-	-	-	-	-	-	-	-		4/4	3,300	
10 ⁻³	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1/4	330	LD ₅₀ T.T. 10 ^{-2.7}
10 ⁻⁴	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/4	33	
10 ⁻⁵	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/4	3.3	

Disc - 18/10/47

Observations on Peritoneal Exudate

Oct. 27, 1947

Used in Toxoplasma Neutralization Test

Time after inoculation - 4 days after i.p. injection of 0.3 cc of fresh 10% m. brain suspension

Appearance of mice - not ill; only 2 of 6 exhibit slight abdominal distention. These 2 were used for puncture

Appearance of exudate - opalescent; 0.5 cc easily obtained. Did not clot spontaneously ~~for~~ after 3 hours at room temperature.

Preparation of exudate for Neutralization Test:

Pool of exudate from 2 mice. 0.5 cc of this added to 0.5 cc of heparin (1:500) [very brown looking] + 1.5 cc saline to make 1:5 dilution of exudate
0.1 cc of the 1:5 was added to each tube for the 1:10
0.2 cc of the 1:5 was added to 1.8 cc saline to make 1:50

Microscopic observations

Coverslip preparation of undiluted exudate - about 2 extracellular toxoplasma per each oil-immersion field. Quite a few cells with intracellular toxoplasma seen.

Examination in Counting Chamber

Undiluted exudate - many white cells - crowded - difficult to count toxoplasma because they appeared in different layers. However the approximate count for 1 square mm was 262 toxo or 2620/cu. mm.

Exudate diluted 1:5 with heparin (1:500) and saline (final dilution of heparin 1:2500). This had been standing about 30 - 60 min.

Very clear uniform distribution - few leukocytes seen in this dilution - many toxoplasma clearly visible under high dry (x450). Almost no cells with intracellular toxo encountered and the count per square mm was 220

Multiplying by 5 for the dilution $\therefore 220 \times 5 \times 10 =$

11,000 toxo per cu. mm. of original exudate.

NOTE: the possibility was considered that ~~most~~ of

the intracellular toxo might have been "liberated" in the 1:5 dilution tube containing the heparin and saline.

On the basis of 11,000 toxo/cu. mm. of undil. exud.

1 cc of undil. exudate	=	11,000,000
0.03 cc "	=	330,000
" 10^{-1} "	=	33,000
" 10^{-2} "	=	3,300
" 10^{-3} "	=	330
" 10^{-4} "	=	33
" 10^{-5} "	=	3.3
" 10^{-6} "	=	0.33

Therefore if one toxoplasma were sufficient to kill ~~se~~ a mouse, one would expect all the mice inoculated with the 10^{-5} dilution to die and only one of each 3 mice inoculated with the 10^{-6} dilution. On this basis the LD₅₀ should be between $10^{-5.5}$ and 10^{-6}

Note on the 10% Mouse Brain Suspension in Saline

Macerated in [REDACTED] - filter through gauze and cotton.

Cover slip preparation under oil-immersion

much tissue debris - only occasional toxo seen in occasional field. Not examined in counting chamber

NOV 24 1947.

SUMMARY OF TOXO. NEUT. EXPT. OF 24 NOV. 47 - MICE I.C.E.R. & I.P.

(EGGS DESTROYED BY HEAT - OMITTED FROM SUMMARY)

Peritoneal Exudate - Source of Toxoplasma

SERUM	INOC. CC.	ROUTE	DIL. OF. TOXO.						PRE-DICTED TITER LD ₅₀	LD ₅₀ TITER	NEUT. INDEX
			10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶			
NRS	0.03	I.C.E.R.	4/4	3/3	4/4	4/4	0/4	0/3	4.9 ^{5.0}	10 ^{-4.5}	—
	0.5	I.P.	4/4	4/4	4/4	4/4	4/4	2/4	5.9 ^{6.0}	10 ^{-6.0}	—
RALPH. BLACK (22 OCT. 47)	0.03	I.C.E.R.	4/4	3/4	1/4	0/4	0/3	0/3	—	10 ^{-2.5}	100
	0.5	I.P.	3/3	4/4	4/4	0/4	0/4	0/3	—	10 ^{-3.5}	320
IM. MONK. (23 DEC. 42)	0.03	I.C.E.R.	4/4	4/4	2/4	0/3	0/4	0/3	—	10 ^{-2.0}	32

NOV 24 1947

Comparative Neutralization of Toxoplasma In Mice & Eggs And The Effect of the Amt of Serum Used.

Obj: Using mouse peritoneal exudate, is the egg superior to the mouse (or vice versa) as a vehicle for detecting Toxoplasma-neutralizing antibodies. As a corollary, is the test altered by increasing the amt of antiserum.

Scheme: Serial dilutions of toxoplasma-infected peritoneal exudates made in saline mixed in equal portions of serum, inc for 20 @ 37° in H₂O bath & then inj into mice & eggs.

Toxoplasma: The 12 mice inj IP on 20 Nov. are all sick to-day. Some in generalist signs & all in varying degrees of abdominal enlargement. 10 of these were killed in CCl₄, dipped in lysol & pinned out, belly up. Skin carefully dissected so as to leave peritoneum intact. Lateral then P & A in ether & pierced in capillary & fluid withdrawn. Small amts of turbid, opalescent fluids obtained from each. Total: 1.5 cc.
1.2 cc exudate + 1.2 cc lysatin (1500 same as used 27 Oct.) + 3.6 No 4 = 1.5 tube.
tenfold dilutions then made in saline. These latter then mixed in sera as follows:

Peri. E + BAP = 000

A = 0.15 cc toxo dil + 0.15 cc ^{NRS (inact.)} ~~same as above~~. (for since 12-23-42. taken up in 1 cc No 4.)

B = 1.8 cc toxo dil + 1.8 cc NRS (inact.)

C = 0.15 cc toxo dil + 0.15 cc Purple Black (frozen since — 22 Oct 47)

D = 1.2 cc " " + 1.2 cc " " " " " " " "

E = 0.15 cc toxo dil + 0.15 cc imm mark se. (for since ^{23 Dec. 42} 12-23-42 taken up in 1 cc No 4.)

Dilutions carried to 10⁻⁶.

These then well shaken, placed in H₂O bath for 20 @ 37°. Then mice. Eggs first, then mice.

Eggs = 50 BR - all eggs run hand in sequence & placed in 35° C inc.

Mice = Max 8-10 gms.

A-series = 0.03 ml Icr (mice) & 0.03 ml vs of eggs.

B- " = 0.5 ml (0.25 ml serum) IP (mice) & 0.5 ml vs.

C " = as in A

D " = as in B

E " = as in A.

4 mice or eggs used per dil & as noted in protocols. These deficiencies also result from accidental egg shortage.

Partial Erythrocyte Counts:

a) Undil & dil 1/5 exudate placed in same chamber & counted within hour after mixing.

1) Undil - 1 sq. mm. = $\begin{matrix} 6 & 2 & 4 & 5 \\ 3 & 7 & 3 & 3 \\ 2 & 8 & 3 & 6 \\ 1 & 7 & 8 & 4 \end{matrix} \left\{ \begin{matrix} 72/\text{mm}^2 = 720 \text{ mm}^3 = \\ 720,000 \text{ ml.} \end{matrix} \right.$

As previously noted by ABS - diff. to count - numerous cells but distribution appears valid.

2) Dil 1/5 = 4 squares = 42, 41, 42, 41 thus 1 sq. mm = 41.5
Much easier counting. & few leucos. $\text{mm}^3 = 415 \times 5 = 2075$
Tox. free & distinct. Cell under high dry. $\text{ml} = 2,075,000$
Each square small square counts extremely regular.

Thus 0.03 ml = 62,879 toxo (using 2,075,000 as count)

$$10^{-1} = 6287.9$$

$$10^{-2} = 628.8$$

$$10^{-3} = 62.8$$

$$10^{-4} = 6.3$$

$$10^{-5} = 0.63$$

thus titer should be ca $10^{-4.9}$

$$10^{-4.9} = 1.17 \text{ toxo / dose.}$$

@ 0.25 ml dose = 518,750 toxo.

$$10^{-1} = 51875$$

$$10^{-2} = 5187.5$$

$$10^{-3} = 518.8$$

$$10^{-4} = 51.8$$

$$10^{-5} = 5.2$$

$$10^{-6} = 0.5$$

Thus titer should be ca $10^{-5.9}$

$$10^{-5.9} = 1.0 \text{ toxo / dose.}$$

Leucos: Ca 4 hrs after mixing (2 hrs in H_2O bath & 2 hrs at room temp) all 10^{-1} tubes showed a few heavy drops & leucos to dry down. Wright-stained:

A-1 = TOXO V. PLentiful & NORMAL, LEUCOS, MAINLY, ARRANGED AROUND

B-1 = —

C-1 = VERY RARE TOXO SEEN

D-1 = VERY RARE TOXO SEEN.

E-1 = PLentiful TOXO (NO FEWER THAN "A")

Origin of Dye Test
Swears made by ABS before going home

LOW COUNT

TOXOPLASMA NEUTRALIZATION - Per. Ex. (TOXO. VS NPS)

NOV 24 1947

MICE - 0.03 ml ICER.

MAX 8-10 gms.

Intracerebral - 2 hrs. 37°C.

ICER. NORMAL

FINAL DIL.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	MORT	
10 ⁻¹	-	-	-	D																									4/4	62.88
10 ⁻²	-	-	-	-	CAS	D																							3/3	62.9
10 ⁻³	-	-	-	-	-	-	D																						4/4	LD 50 10 ^{-4.5} 63
10 ⁻⁴	-	-	-	-	-	-	-	CAS																					4/4	6.3
10 ⁻⁵	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		9/4	0.63
10 ⁻⁶	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/3	

EGGS - 0.03 ml VIA YS

FINAL DIL. 1 2 3 4 5 6 7 8 9 10

1cc has 1,056,000

MORT.

FINAL DIL.	1	2	3	4	5	6	7	8	9	10	MORT.	
10 ⁻¹	-	-	-	-	-	-	-	-	-	-		
10 ⁻²	-	-	-	-	-	-	-	-	-	-		
10 ⁻³	-	-	-	-	-	-	-	-	-	-		
10 ⁻⁴	-	-	-	-	-	-	-	-	-	-		
10 ⁻⁵	-	-	-	-	-	-	-	-	-	-		
10 ⁻⁶	-	-	-	-	-	-	-	-	-	-		

NOV 26 1947
Egg incubator
between 40-45°C!
Probably overcooked - poor egg air
circulation.
27 Nov - inc. @ 37°C after
refrigerated + yesterday
Vaccinia egg kept at 27°C this
afternoon.

Sheep

MICE: 0.5 ml IP MAX. 8-10 gms.

TOXO VS NPS (CONT.)

NOV 24 1947

NORMAL INTRAPERITONEAL

FINAL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	MORT.		
10 ⁻¹	-	-	-	-	-	D																							4/4	103,750	
	-	-	-	-	-	D																									
	-	-	-	-	-	D																									
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
10 ⁻²	-	-	-	-	-	D																							4/4	10,375	
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
10 ⁻³	-	-	-	-	-	D																							4/4	LD ₅₀ 16-6.0 1037	
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
10 ⁻⁴	-	-	-	-	-	D																							4/4	104	
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
10 ⁻⁵	-	-	-	-	-	D																							4/4	10	
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
10 ⁻⁶	-	-	-	-	-	D																							2/4	1	
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		
	-	-	-	-	-	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS	CNS		

Sec loc = 2,000,000 LD₅₀

EGGS - 0.5 ml VIA YS.

FINAL	1	2	3	4	5	6	7	8	9	10	MORT.	
10 ⁻¹	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
10 ⁻²	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
10 ⁻³	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
10 ⁻⁴	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
10 ⁻⁵	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
10 ⁻⁶	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									
	-	-	D									

Brain smear = neg for tox.

Body autolyzed.

(TORO VS RALPH BLACK)

MICE: 0.03 ml ICER. MAX. 8-10 gms.

C

FINAL 1 2 3 4 5 6 7 8 9 10

FINAL	1	2	3	4	5	6	7	8	9	10	MORT
10^{-1}	NOT DONE										
10^{-2}	-	2	D								
	?	?	D								
	-	-	D								
	-	-	D								
10^{-3}	-	-	D								
	?	D									
	-	-	D								
	-	-	D								
10^{-4}	-	-	D								
	-	-	D								
	-	-	D								
	-	-	D								
10^{-5}	-	-	?	?	D						
	-	-	?	?	?						
	-	-	?	?	?						
	-	-	D								
	-	-	D								
10^{-6}	-	-	?	?							
	-	-	?	?							
	-	-	?	?							
	-	-	?	?							
	-	-	?	?							

MAX. 8-10 gms.

TOXO. vs RALPH BLACK (CONT.)

NOV 24 1947

[illegible]

E665: 0.5 ml via YS

[illegible]

TOXOPLASMA NEUTRALIZATION INTRACEREB (TOXO. VS IMM. MONK. SER.)

MICE: 0.03 ml ICER

MAX - 8-10 gms.

NOV 24 1947

FINAL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	MORT.	
10 ⁻¹	-	-	-	-	-	D																			4/4
	-	-	-	-	-	D																			
	-	-	-	-	-	D																			
	-	-	-	-	-	D																			
10 ⁻²	-	-	-	-	-	CNS D																			4/4
	-	-	-	-	-	CNS D																			
	-	-	-	-	-	CNS PR D																			
	-	-	-	-	-	CNS CNS D																			
10 ⁻³	-	-	-	-	-	CNS ? ? CNS D																			2/4
	-	-	-	-	-	? PR D																			LD ₅₀
	-	-	-	-	-	-																			10 ^{-3.0}
	-	-	-	-	-	-																			
10 ⁻⁴	-	-	-	-	-	? ? (D) -																			0/3
	-	-	-	-	-	-																			
	-	-	-	-	-	-																			
	-	-	-	-	-	-																			
10 ⁻⁵	-	-	-	-	-	-																			0/3
	-	-	-	-	-	-																			
	-	-	-	-	-	-																			
	-	-	-	-	-	-																			
10 ⁻⁶	-	-	-	-	-	? (D)																			0/3
	-	-	-	-	-	-																			
	-	-	-	-	-	-																			

EGGS: 0.03 ml via y/s

FINAL 1 2 3 4 5 6 7 8 9 10

MORT.

10 ⁻¹	NOT DONE																								
10 ⁻²	-	-	? D																						
	-	-	? SAC - no toxo brain smear.																						
	-	-	? ? D																						
	-	-	D																						
10 ⁻³	-	-	? D																						LD ₅₀
	-	-	? D																						
	-	-	? D																						
	-	-	? D																						
10 ⁻⁴	-	-	? D																						
	-	-	? D																						
	-	-	- ?																						
	-	-	? D																						
10 ⁻⁵	-	-	? ? D																						
	-	-	? ? D																						
	-	-	? D																						
	-	-	-																						
10 ⁻⁶	-	-	? ? D																						
	-	-	? -																						
	-	-	-																						
	-	-	? D																						

No toxo on smear.
No gross lesions in any eggs opened.

Comparative Toxoplasma Neutralization Titers Obtained In
Eggs & Milk (Icer).

19 DEC. 47

Obj: To determine whether the mouse or the egg ~~would be~~ is superior to the other as a toxoplasma neutralization medium.

Toxoplasma: Peritoneal exudate. Pooled from 3 mice - 5th day after IP inoc. All mice very sick - second day of signs. Mice killed in CCl_4 , PE obtained after removing skin from belly & cleaning abdominal wall with ether.

Dilutions: PE dil 1:5 in NaCl & then ten-fold dilutions were made so that the final NaCl-sperm mixture would be 10^{-7} . 0.2 cc amounts of each dil were then mixed & 0.2 cc of either Ralph Blaskin's serum (frozen since 3 Dec 47) or undil NPS as indicated in the protocol.

Incubation: Serum-toxo mixtures incubated 2 hrs. in 37° @ H_2O bath.

Eggs: B-R - hot wash. - 8 days old. Inoc & 0.05 ml units into YS - returned to the 100° egg incubator. 3 eggs/dil. Egg mix first. Syr. & needle changed @ 3rd dil.

Milk: 4 Ch Alb 3-4 wk old/dil. 0.03 ml Icer.

Counts: The 1/50 tube was dil to 1/100 & 1/200 & counted in the Neubauer chamber as follows:

1:100

1 0 0 0	2 1 0 0
0 0 0 2	1 3 3 3
1 2 0 0 ⁽¹⁰⁾	0 1 2 0 ⁽²⁴⁾
0 2 1 1	4 2 2 0

Ave. = 16.7

0 2 0 2	0 3 0 0
2 0 0 1 ⁽¹⁴⁾	3 3 0 0 ⁽¹⁷⁾
1 2 2 0	3 1 1 1
0 3 0 0	2 1 0 1

1:100 = 167 / cu. mm.

1:200

0 0 0 0	1 2 2 0
1 0 0 0	0 2 1 3
0 1 0 0 ⁽²⁾	0 0 1 1 ⁽¹⁵⁾
0 2 2 1	2 0 0 0

Ave. = 7.7

2 0 0 0	0 0 0 0
0 0 1 0	0 0 0 0 ⁽¹⁾
0 1 1 1 ⁽⁸⁾	0 0 1 0
0 0 1 1	0 0 0 0

1:200 = 77 / cu. mm.

or 154 / cu. mm. for total

Average = 16,000,000 per cc of undil exudate

19 DEC 47

COMPARATIVE EGG-MOUSE NEUTRALIZATION TESTSSUMMARY

Mouse Inoculations - Intracerebral

SERUM	HOST	INOC.	TOXO DIL.							LD ₅₀ TITER	NEUT. INDEX
			10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷		
C NRS	MOUSE	0.03	X	X	4/4	4/4	0/4	0/4	0/4	10 ^{-4.5}	—
D BLACK	"	0.03	4/4	3/4	1/4	0/3	0/3	0/4	X	10 ^{-2.5}	100
A NRS	EGG	0.05	3/3	3/3	1/1	1/3	0/3	0/2	0/2	10 ^{-3.9}	—
B BLACK	EGG	0.05	2/3	0/2	0/3	0/3	0/3	0/3	X	10 ^{-1.2}	316

Peritoneal Exudate - Source of Toxoplasma 19 Dec 47
COMPARATIVE EGG-MOUSE TOXOPLASMA NEUTRALIZATION 2 hrs @ 37°C

4 mice/dose. 0.03 ml i.c. - PE/NAC. - Mice - 0.03cc i.c. - Normal

NRS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	MORT.	No. Toxoplasma per dose
10 ⁻³	-	-	-	-	-	-	D																						4/4	484
	-	-	-	-	-	-	D																							
	-	-	-	-	-	-	CNS D																							
	-	-	-	-	-	-	CNS D																							
10 ⁻⁴	-	-	-	-	-	-	CNS CNS	D																					4/4	48
	-	-	-	-	-	-	PP PP	D																						
	-	-	-	-	-	-	- CNS	D																						
	-	-	-	-	-	-	- CNS	D																						
10 ⁻⁵	-	-	-	-	-	-	-	-	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/4	5
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
10 ⁻⁶	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/4	0.5
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
10 ⁻⁷	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/4	0.05
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

Toxo Immune Mice - 0.03cc i.c.

LD50 10-4.5
 1 cc = 1 million LD50

BLACK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	MORT.	
10 ⁻¹	-	-	?	?	-	-	D																						4/4	48,400
	-	-	-	-	-	-	D																							
	-	-	-	-	-	-	D																							
	-	-	-	-	-	-	D																							
10 ⁻²	-	-	?	?	-	-	CNS D																						3/4	4,840
	-	-	?	?	-	-	CNS PR	D																						
	-	-	-	-	-	-	? CNS	D																						
	-	-	-	-	-	-	? ?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
10 ⁻³	-	-	?	-	-	-	CNS D																						1/4	LD50 10-2.5 484
	-	-	?	-	-	-	CNS	D																						
	-	-	-	-	-	-	CNS	CNS ? ?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	-	-	-	-	-	-	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
10 ⁻⁴	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/3	48
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
10 ⁻⁵	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/3	5.4
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
10 ⁻⁶	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0/4	0.5
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

CT HILLED

D Deep loaded's bacteria (CHEWED)

D hand.

3 Egg / dil. 0.05 ml into ys. 100°F inc. 8 d EMB.

Eggs - 0.05 cc

NOT SAVED		2995 - 0.05 cc														No. Toxic per doz	
NPS	1	2	3	4	5	6	7	8	9	10	11	12	14	PORT.			
10 ⁻¹	→	-	-	-	-	-	D	-	-	-	-	-	-	3/3	80,000		
	-	-	-	-	-	-	D	-	-	-	-	-	-				
	-	-	-	-	-	-	D	-	-	-	-	-	-				
10 ⁻²	-	-	-	-	-	-	D	-	-	-	-	-	-	3/3	8000		
	-	-	-	-	-	-	D	-	-	-	-	-	-				
	-	-	-	-	-	-	D	-	-	-	-	-	-				
10 ⁻³	-	-	-	-	-	-	D	-	-	-	-	-	-	1/1	800		
	-	-	-	-	-	-	D	-	-	-	-	-	-				
	-	-	-	-	-	-	D	-	-	-	-	-	-				
10 ⁻⁴	-	-	-	-	-	-	D	-	-	-	-	-	-	1/3	80		
	-	-	-	-	-	-	D	-	-	-	-	-	-		LD ₅₀ 10 ^{-3.9}		
	-	-	-	-	-	-	D	-	-	-	-	-	-				
10 ⁻⁵	-	-	-	-	-	-	D	-	-	-	-	-	-	0/3	8		
	-	-	-	-	-	-	D	-	-	-	-	-	-				
	-	-	-	-	-	-	D	-	-	-	-	-	-				
10 ⁻⁶	-	-	-	-	-	-	D	-	-	-	-	-	-	0/2	0.8		
	-	-	-	-	-	-	D	-	-	-	-	-	-				
	-	-	-	-	-	-	D	-	-	-	-	-	-				
10 ⁻⁷	-	-	-	-	-	-	D	-	-	-	-	-	-	0/2	0.08		
	-	-	-	-	-	-	D	-	-	-	-	-	-				
	-	-	-	-	-	-	D	-	-	-	-	-	-				

mouth - many sm lesion on C-A. many tofo in smear.

mouth - number of sm. lesions on amnion - tofo in sm of amnion.

" " " " " " CA - " " "

lesions in amnion + CA - many tofo.

tofo lesion on CA

" " " "

No lesions on mem. Brain sm = neg for tofo.

FEW TOFO LESIONS ON C-A.

lesions - ting on CA - many tofo in smear.

H

H

H

H

H

H

H

No lesions on mem. Brain sm = neg. for tofo.

H

opened by carding sm - alive & well. No lesions.

opened - v. recent dead, C-A = few ? foci - lesion of brain - no tofo seen. Falc & hemorhagic.

■ = toxoplasma - lesions 1/2 smears - demonstrated.

H: hatched

BLACK	1	2	3	4	5	6	7	8	9	10	11	12	14	PORT.
	-	-	-	-	-	-	-	D	-	-	-	-	GA - 70% Lesions	
10 ⁻¹	-	-	-	-	-	-	-	-	D	-	-	-	" - " "	2/3
	-	-	-	-	-	-	-	-	-	-	-	-	H	
	-	-	-	-	-	-	-	-	-	-	-	-	H	
10 ⁻²	-	-	-	-	?	⑤	-	-	-	-	-	-	No lesions on mem. Emb. <u>not dead</u> . -ok. Br. sm. = neg for tox	0/2
	-	-	-	-	-	-	-	-	-	-	-	-	H	
	-	-	-	-	-	-	-	-	-	-	-	-	H	
10 ⁻³	-	-	-	-	-	-	-	-	-	-	-	-	H	0/3
	-	-	-	-	-	-	-	-	-	-	-	-	H	
	-	-	-	-	-	-	-	-	-	-	-	-	H	
10 ⁻⁴	-	-	-	-	-	-	-	-	-	-	-	-	H	0/3
	-	-	-	-	-	-	-	-	-	-	-	-	H	
	-	-	-	-	-	-	-	-	-	-	-	-	H	
10 ⁻⁵	-	-	-	-	-	-	-	-	-	-	-	-	H	0/3
	-	-	-	-	-	-	-	-	-	-	-	-	H	
	-	-	-	-	-	-	-	-	-	-	-	-	H	
10 ⁻⁶	-	-	-	-	-	-	-	-	-	-	-	-	H	0/3
	-	-	-	-	-	-	-	-	-	-	-	-	H	
	-	-	-	-	-	-	-	-	-	-	-	-	H	

JAN 12 1948

TOXOPLASMA NEUTRALIZATIONPERITONEAL EXUDATE - INTRAPERITONEAL0.1 cc TOTAL DOSE

TOXOPLASMA: PER. EX. Pool of two mice (Pen 362 IP) - both very ill & large abdomens. Fluid readily obtained by capillary. Free of blood. ~~PER. EX. MOUSE IP.~~

DILUTIONS: Immediately after with-drawal, this PE dil in NaCl to:

1/5, 1/50, 1/500 etc to 1/5,000,000. Distributed in 0.25 cc amt's to both series of dilution tubes.

SERA: NRS - INAC. POOL. = CONTROL (10⁻³ - 10⁻⁷)

[REDACTED] - frozen since 3 Dec 47. (10⁻¹ - 10⁻⁶)

Test: 0.25 cc of serum added to each tube of the appropriate series, tubes shaken & placed in 37° @ H₂O bath for 2 hrs. At that time, inoc into Ch Alb ca 3-4 wks old. 4 mice/dil. 0.1 ml IP/mouse. !!!

Series A = NRS Control. Series B = R. Black.

COUNT: Shortly after dil, the 1/50 tube dil to 1/100 in NaCl, well mixed & counted in chamber. 4 squares counted = 15, 9, 20, 9 = Ave = 13.25 / sq mm.

= 13,250,000 TOXO/CC EXUDATE

SUMMARY - TOXO. NEUT. PER. EX. IP CH ALB.

TOT. INOC = 0.1 cc / MOUSE.

SERUM	ROUTE	DOSE / MOUSE	TOXO. (FINAL DIL.)							LD ₅₀ TITER	NEUT. INDEX.
			-1	-2	-3	-4	-5	-6	-7		
NRS	IP	0.1 cc	X	X	4/4						—
R. BLACK	IP	0.1 cc	4/4	4/4					X		

CH. ALB. - 0.1cc IP

Normal Rabbit Control

WAS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	MORT	# PAR DOSE
-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	1325
-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3/4	132.5
-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/4	13.25
-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/4	1.325
-7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/4	0.13

Wrong
Dead
Wrong

LD50 TITER

10^{-4.5}

BLACK TOXO IMMUNE HUMAN - RALPH BLACK

BLACK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	MORT	# PAR DOSE
-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	132,500
-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	13,250
-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3/4	1325
-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/4	132.5
-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/4	13.25
-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/4	1.325

Someday

LD50 TITER

10^{-3.5}

TOXO PRESENT IN SMEAR OF LUNGS

TOXO PRESENT IN SMEAR OF LUNGS

INFECTIVITY OF TOXOPLASMA IN PERITONEAL EXUDATE JAN 22 1948

By INTRAPERITONEAL ROUTE IN MICE

PURPOSE

1. Check on No. of toxoplasma per LD₅₀ when 0.1cc is given intraperitoneally
2. Effect of incubation of diluted mixtures for
 - a) - No time
 - b) - 2 hours at 37°C - water bath
 - c) - 4 hours at 37°C - " "
 - d) - 4 hours at 37°C and 20 hours in refrigerator

Source of Toxoplasma - Peritoneal exudate of 2 mice inoc. i.p. 1-17-48

No. of toxoplasma - Preliminary counts of 1:100 dilution in saline

Lower chamber = 37 in 4 sq. mm.

Upper " = 40 in 4 sq. mm.

$$77/8 = 9.6 \text{ per sq. mm.}$$

∴ 96 per cc. mm. of 1:100

∴ 96,000 per cc of 10^{-2} or 9,600 per 0.1cc of 10^{-2}

∴ 0.1cc of 10^{-3} should have 960 toxoplasma

"	"	10^{-4}	"	"	96	"
"	"	10^{-5}	"	"	9.6	"
"	"	10^{-6}	"	"	0.96	"
"	"	10^{-7}	"	"	0.1	"

DILUTIONS - in 50 per cent rabbit serum saline.

0.2cc exud. + 1.8cc 50% NRS = 10^{-1}

" 10^{-1} + " " = 10^{-2}

" 10^{-2} + " " = 10^{-3}

0.4cc 10^{-3} + 3.6cc " = 10^{-4}

etc.

Re-count of 1:100 dilution in 50% NRS = 9,700 toxo per 0.1cc dose of 10^{-2}

Inoculations - The first group inoculated immediately on completion of dilutions - others as indicated. 1cc tuberculin syringe

Mice

- First group - C H albino

Others - Maxfield @ 3 wks old

IMMEDIATE

10^{-4} - 1 - - - - CNS D
 2 - - - - CNS D
 3 - - - - ? D
 4 - - - - CNS CNS } in extremities 1/31 - exudate
 5 - - - - ? CNS } used for tests

10^{-5} - 1 - - - - ? D
 2 - - - - D
 3 - - - - CNS D
 4 - - - - CNS D
 5 - - - - CNS CNS } 1/31 in extremities; exudate used

10^{-6} - 1 - - - - D CHEWED - LUNG SLIDERS NEG. FOR TOXO. PASS. TO 4 ALB ICEA & IP

2 - - - - ? D
 3 - - - - ? D
 4 - - - - ? CNS D } Peritoneal exudate of each examined } same as frozen
 5 - - - - ? CNS D } in hanging drop with methylene blue } showed +
 6 - - - - ? CNS D } Many toxoplasma but not stained } immune pr
 7 - - - - ? CNS D } Aschoff punch 2/1 - many toxo } Lungs full of toxo
 8 - - - - ? CNS ? ? ? ? ?
 9 - - - - ? CNS
 10 - - - -

10^{-7} - 1 - - - - ? D - Many toxo in peritoneum - but not stained w. meth. blue

2 - - - - ? D
 3 - - - - ? D
 4 - - - - ?
 5 - - - -
 6 - - - -
 7 - - - -
 8 - - - -
 9 - - - -
 10 - - - -

2 hrs. at 37°C

10^{-3} - 1 - - - - D
 2 - - - - D
 3 - - - - D
 4 - - - - D

10^{-4} - 1 - - - - ? D
 2 - - - - ? D
 3 - - - - D
 4 - - - - CNS

- SACR. 30 JAN FOR SLIDE-TOXO TESTS - M ASSIVE TOXO PL

10^{-5} - 1 - - - - D
 2 - - - - D
 3 - - - - D
 4 - - - - CNS CNS D

10^{-6} - 1 - D

2 - - - - CNS CNS D
 3 - - - - CNS CNS D
 4 - - - - ? CNS 2/1 - killed for spec. - imm. Toxo

↑
 Mouse found
 dead 2/22
 looked as if
 it had been
 dead for days

(continued)

4 hrs. at
37°C

10⁻⁴ - 1 - - - - ? D
2 - - - - - D
3 - - - - - D
4 - - - - - (MS)

SACR. 30 JAN FOR SLIDE PUNCH TESTS = MAGNIFIC TOXO PE

10^{-6} -

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cus D

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JAN 23 1947

4 hrs. - -37°C
20 hrs. - Refrig.

10^{-4}

1	-	-	-	-	CMS - Zpp?	? ? ? ?	? ? ? ? ? ? ? ? - D
2	-	-	-	-	CMS	- - - - ✓ -	- - - - - - - - - -
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4	-	-	-	-	-	- - - - - - -	- - - - - - - - - -

6 boxes
etc.

96 to 100 per to 100

[illegible]

Passage of Mouse Lung from Jan 22 Tox Titration

JAN 28 1949

One mouse of the 10^{-6} dilution [Immediate Passage] was found dead today. Lung was smeared (head chewed) and stained with Wrights - no toxoplasma, but occasional bacteria. Portion of lung minced, ground in 2 cc. saline, and passaged (0.02 ml. ser. + 0.03 I.P.) to four Masfield mice (8-109.)

Mouse

	<u>Day</u>																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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BAP (#1) - gram positive
cocci-bacilli
and short gram
negative rods.
Ca 20 col.

FEB 3 1948

INTRAPERITONEAL NEUTRALIZATION TEST IN MICE - TOXOPLASMA.

Procedure - obtain peritoneal exudate from several mice - count to 50

Prepare indicated dilutions in 50% ^{heated} rabbit serum saline so that after mixture = equal amount of serum = $10^{-2}, 10^{-3}$, etc.

Mixtures - 0.25cc of serum + 0.25cc indicated dilut.

Code	SERA	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
A -	CONTROL-RABBIT (heated)		redone	4	4	10	10
B -		4	4	4	4		
C -		4	4	4	4		
D -		4	4	4	4		
E -		4	4	4	4		
F -		4	4	4	4		
G -			4	4	4	4	
H -			4	4	4	4	
I -			4	4	4	4	
J -			4	4	4	4	
K -			4	4	4	4	
L -			4	4	4	4	
M -			4	4	4	4	
N -			4	4	4	4	
O -			4	4	4	4	
P -			3	3			
		9.25	3.7	3.95	3.75	3.0	0.175

MIXTURES INCUBATED IN WATER BATH - 2 HRS. AT 37°C

PE $1/100 = 6, 7, 8, 5 = 26/4 \text{ mm}^2 = 6.5 \text{ mil/cc}$

" $1/50 (50\% \text{ R55}) = 10, 18, 15, 16 = 7.35 \text{ mil/cc}$

" $1/15 (50\% \text{ R55}) = 120/\text{mm}^2 = 6.0 \text{ mil/cc}$

Amount of 50% R55	2.7	4.5	4.5	4.5	4.5	1.8
Exud	0.3	0.5	0.5	0.5	0.5	0.2

Toxoplasma Counts on Exudate Used in Neutralization Test

Exudate obtained from 2 mice inoculated i.p. on 1-30-48

Preliminary Count - 1:100 dil. in saline = 6.5 per sq. mm = 65/cu. mm.

1cc of undil. exudate = $65 \times 1000 \times 100 = 6.5$ million

Count of the 1:50 dilution in the ~~1:5~~ 50% rabbit serum saline used in test yielded 7.4 million toxo per cc. of exudate.

Thus

0.1cc	of undil. exud	=	740,000	tox
"	" 10^{-1}	"	=	74,000 "
"	" 10^{-2}	"	=	7,400 "
"	" 10^{-3}	"	=	740 "
"	" 10^{-4}	"	=	74 "
"	" 10^{-5}	"	=	7.4 "
"	" 10^{-6}	"	=	0.74 "
"	" 10^{-7}	"	=	0.07 "

IN VITRO METHYLENE BLUE TEST ON SOME OF SERA USED IN MICE

Procedure - 0.1cc of serum (from same tube) + 0.1cc of perit. exudate 1:5 in saline.
heft at room temperature for 2 hours or more.
0.02cc put on slide + large loop of Loeffler's alkaline methylene blue

Result

ANTIBODY EFFECT

8/50 = 96% stained

10/50 = 100% "

9/50 = 98% "

45/50 = 90% "

2/50 = 4%

3/50 = 6% " (Waldman)

6/50 = 12% "

5/50 = 10% "

9/50 = 18% "

" " (kept at room temp. for 4 days) 32/50 = 64% "

FEB 3

PERITONEAL EXUDATE

1. *Journal of the American Medical Association*, 2000; 283: 2689-2693.

[illegible]

FEB 3 1948

SERUM	DILUTION OF PERT. EXOD. +	No. TOXOPLASMA	DAYS AFTER INOCULATION																					MORTALITY	LD ₅₀	NEUTRAL INDEX
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FEB 3 1948

SERUM

DILUTION
OF PERIT. EXUD.
+

No. Toxoplasma

DAYS AFTER INOCULATION

MORTALITY

LD₅₀NEUTRAL
INDEX10⁻⁴

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FEB 3 1948

SERUM	DILUTION OF PERIT. EXUD. + NO. TOXOPLASMA	DAYS AFTER INOCULATION																				MORT.	LD ₅₀	NEUTRAL INDEX	
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FEB 3 1948

SERUM	DILUTION OF PERIT. EXUD. + NO. TOXOPLASMA	DAYS AFTER INOCULATION																					MORT.	LD ₅₀	NEUTRAL INDEX
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FEB 3 1948

SERUM	DILUTION OF PERIT. EXUD. + NO. TOXOPLASMA	DAYS AFTER INOCULATION																					MORTALITY	LD ₅₀	NEUTRAL INDEX	
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FEB. 3, 1948

SERUM	RABBIT NEUTRAL. TEST	IN VITRO CYTOPLASM. EFFECT							LD ₅₀	
			10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷		
			4/4		4/4	4/4	6/9	0/10	6.3	
	+	+(82%)	4/4	2/4	1/4	0/4	. . .	. . .	3.2	1300
	+	+(90%)	4/4	2/4	1/4	0/4	. . .	. . .	3.2	1300
	+	+(88%)	4/4	4/4	1/3	0/4	. . .	. . .	3.7	400
	+	+(84%)	4/4	2/4	0/4	0/3	. . .	. . .	3.0	2,000
	+	+	3/4	1/4	0/4	0/4	. . .	. . .	2.5	6,300
	±	+(80%)	4/4	3/4	1/3	0/4	0/3	. . .	3.5	630
	±	+(94%)	. . .	2/4	0/4	0/4	0/4	. . .	3.0?	2,000 +?
	±, +?	± per se + activ.	. . .	3/4	3/4	0/3	0/4		4.2	130
	±	+(96%)	. . .	1/4	0/4	0/4	0/4	. . .	2.7?	4,000 +?
	—	—	. . .	4/4	2/4	1/4	0/4	. . .	4.2	130
	—	—	. . .	4/4	0/4	0/4	0/4	. . .	3.5	500
	—	—	. . .	4/4	1/4	0/4	0/4	. . .	3.7	400
nit.	—	—		3/4	0/4	0/4	0/4	. . .	3.3?	1000 +?
+ sl.?	—	—		4/4	2/4	0/4	0/3	. . .	4.0	200
		— per se + activ.	. . .	2/3	3/3					

Factors Influencing "Neutralization" of Toxoplasma in Mice by "Normal" and "Immune" Human Sera

- Role of Incubation
- Role of Route of inoculation
- Is "neutralizing" factor heat labile in both?

Serum	Route	Incubation	Final Dilution Toxo Suspension						LD ₅₀	N.I.
			10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷		
Heated Rabbit (Control)	I.P.	None	-	-	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{1}{5}$	$\frac{0}{5}$	10 ^{-5.6}	—
	0.1cc	37°C-2h	-	-	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{1}{5}$	$\frac{0}{5}$	10 ^{-5.6}	—
	I.cer.	None	-	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{3}{5}$	$\frac{0}{5}$	-	10 ^{-5.2}	—
"A"	0.03cc	37°C-2h	-	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{2}{5}$	$\frac{0}{5}$	-	10 ^{-4.8}	—
	Fresh negative	I.P.	-	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{3}{4}$	$\frac{1}{4}$	-	10 ^{-5.5}	1
"B"	0.1cc	37°C-2h	-	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{2}{3}$	$\frac{0}{4}$	-	10 ^{-5.2}	2.5
	I.cer.	None	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{3}{3}$	$\frac{2}{4}$	$\frac{1}{4}$	-	10 ^{-5.2}	0
	0.03cc	37°C-2h	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{1}{4}$	$\frac{0}{4}$	$\frac{0}{4}$	-	10 ^{-3.7}	32 12.5
Fresh Toxo - positive human.	I.P.	None	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{2}{4}$	-	-	10 ^{-5.0}	4-7
	0.1cc	37°C-2h	$\frac{4}{4}$	$\frac{2}{4}$	$\frac{0}{4}$	$\frac{0}{4}$	-	-	10 ^{-3.0}	400 398
	I.cer.	None	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{2}{4}$	$\frac{1}{4}$	-	-	10 ^{-4.2}	10
"C"	0.03cc	37°C-2h	$\frac{4}{4}$	$\frac{1}{3}$	$\frac{0}{4}$	$\frac{0}{3}$	-	-	10 ^{-2.8}	350 100
	Heated negative human	I.P.	-	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{2}{4}$	-	10 ^{-6.0}	0
Heated toxo positive	I.P.	37°C-2h.	$\frac{3}{3}$	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{4}{4}$	$\frac{0}{4}$	-	10 ^{-5.5}	1

(OVER)

Conclusions:

1. In Situ incubation of the toxoplasma - serum mixtures is necessary to produce in vivo neutralization.
2. The intra-peritoneal route of inoc. is superior to the intracerebral probably because of the larger inocula.
3. The activity of a ~~normal~~ immune serum is obliterated by heating @ 56° for 30'. (Should be repeated to see whether such sera can be reactivated.)
4. Non-immune human serum yielded no neutralization.

Factors Influencing "Neutralization" of Toxoplasma FEB 25 1948

IN MICE by "Normal" and Immune HUMAN SERA

- Role of Incubation
- Role of Route of Inoculation
- Is "neutralizing" factor heat-labile in both?

Serum	ROUTE	INCUBATION	Final dilution of toxo suspension					LD ₅₀	H. I.
			2	3	4	5	6		
Heated RABBIT (Control) A	I.P. (0.1cc)	None			4	4	5	5	
		37°C-2h			4	4	5	5	
	I.cer. (0.03cc)	None		4	4	5	5		
		37°C-2hrs.		4	4	5	5		
Fresh negative human B	I.P.	None		4	4	4	4		
		37°C-2hrs		4	4	4	4		
	I.cer.	None	4	4	4	4	4		
		37°C-2hrs.	4	4	4	4	4		
Fresh Toxo positive C	I.P.	None	4	4	4	4			
		37°C-2hrs.	4	4	4	4			
	I.cer.	None	4	4	4	4			
		37°C-2hrs	4	4	4	4			
Heated Toxo positive E human	I.P.	37°C-2hrs		4	4	4	4		
		37°C-2hrs	4	4	4	4	4		

32 48 56 58 44 10

For Mice

		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
A	1:4 Heated Rabbit Control -		0.3cc	1cc	1cc	1cc	1cc	
B	1:55 Fresh Neg human -	0.3cc	0.8cc	0.8cc	0.8cc	0.8cc		= 3.8cc
C	2:10 Fresh toxo human -	0.8cc	0.8cc	0.8cc	0.8cc	0.8cc		= 4.0cc
D	Heated neg. human -		0.3cc	0.3cc	0.3cc	0.3cc		= 1.2cc
E	Heated toxo -	0.3cc	0.3cc	0.3cc	0.3cc	0.3cc		= 1.5cc

TOXOPLASMA USED - Peritoneal exudate of two 5-day and two 6-day mice.

Preliminary count of 1:10 in saline
35, 34, 45, 29 = average of 36 toxo per sq. mm.
 $36 \times 10 \times 1000 \times 10 = 3.6$ million/cc exudate

Dilutions

- Although the rabbit serum was said to have been heated before at 56°C for $\frac{1}{2}$ hour, it was reheated just to make certain.
Dilutions were made in 50% heated rabbit serum saline.

Count of Toxo in 1:5 dilution of in 50% h. rabb. ser. saline at five dilutions were made for test yielded 56, 76, 68, 67 = average 67 toxo per sq. mm. or 3.35 million Toxo per cc exud.

$\therefore$ 0.1cc und. has 335,000 - 0.03cc has 10,000
" 10^{-1} " 33,500 - " " 1,000
" 10^{-2} " 3,350 - " " 1,000
" 10^{-3} " 335 - " " 100
" 10^{-4} " 33.5 - " " 10
" 10^{-5} " 3.4 - " " 1
" 10^{-6} " 0.34 - " " 0.1
" 10^{-7} " 0.03

Sera

- Because no more [redacted] serum used in previous tests was available, she was bled again to-day, blood defibrinated and the fresh serum used. Some of it was heated at 56°C for $\frac{1}{2}$ hour.

PROTOCOLS of TOXOPLASMA NEUTRALIZATION TESTS IN MICE FEB 25 1948

No incubation - inoculated immediately after mixtures were made; ^{at room temp during inoculation}
 37°C - 2 hours - same tubes in water bath

I. P. dose = 0.1cc. ; i.cer. dose = 0.03cc.

SERUM	ROUTE + INCUBATION	DILUTION	Mice No.	DAYS AFTER INOCULATION																					MORT.	LD ₅₀	NEUTRALIZ. INDEX
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	HEATED RABBIT INCUB.	10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
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FEB 25 1948

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[illegible]

FEB 25 1948

SERUM	ROUTE +	DILUTION	M	DAYS AFTER INOCULATION																					MORT	LD ₅₀	NEUTRALIZ INDEX
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D [BACTERIA BUT NO TOXOPLASMA IN LUNG SMEAR]

SERUM	ROUTE + INCUBATION	DILUTION	M No.	DAYS AFTER INCUBATION																					MORT.	LD ₅₀	NEUTRALIZ. INDEX	
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
D HEATED NEGATIVE HUMAN 37°C.	I.P. 2 hrs.	10 ⁻³	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6/4	10 ^{-6.0}		
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MAR 12 1948

To Determine The Residual Viability of Toxoplasma

Exposed To Immune Human Serum & Fresh Rabbit Serum, In Vitro.

Questions: Are toxoplasma rendered non-staining with methylene blue by in vitro treatment with an immune human serum & a non-immune but active-in-the-fresh-state rabbit serum equally viable or non-viable as measured by infectivity for the mouse.

Toxoplasma: Peritoneal exudate pooled from three mice (4th day after IP inoc. on 8 March). Non-bloody, this PE was counted following dilution to $\frac{1}{100}$ in NaCl = 18, 24, 17, 10 = $17.25 / \text{sq. mm.}$ or $17,250,000 / \text{cc.}$ All experiments conducted to day, were made with this exudate. For this experiment, as well as for the one in heated toxoplasma, & the third, to determine the "toxic" effect of NaCl, a $\frac{1}{5}$ pool was made as follows:

PE undil	- 1.5 cc
Hep $\frac{1}{1000}$ in NaCl	- 1.5 cc
NaCl	- 4.5 cc
Tot	= 7.5 cc of PE $\frac{1}{5}$ in Heparin $\frac{1}{5000}$.

Sera: [REDACTED] fresh, frozen since
[REDACTED] fresh, frozen since

Inactivated NRS pool, made on 11 March '48.

Test: 0.3 cc amts of PE $\frac{1}{5}$ (above) + 0.3 cc of each of the above sera, were mixed in Warraman tubes & then placed in the 57°C H_2O bath for ~~tot~~ two hours. At the end of that time, wet films were prepared, using 0.02 cc of the serum-PE mixtures & 3 drops of alc. meth blue, pH 11.0. Differential counts were then made as follows:

(over)

<u>MIXTURE</u>	<u># UNSTAINED</u>	<u># STAINED</u>	<u>% UNSTAINED</u>	
Heated NRS + PE $\frac{1}{5}$ hep NaCl.	4	96	4	-
[REDACTED] + PE $\frac{1}{5}$ hep NaCl.	95	5	95	+
[REDACTED] + PE $\frac{1}{5}$ hep NaCl	94	6	94	+

The tubes were held in the refrigerator until the counts were completed (ca 40 minutes). Then serial ten-fold dilutions were made from each (blue tube = 10^{-1}) in ^{inactivated} 50% RSS (using the same NRS as used above.) & groups of Map 8-10 gm alb injected IP ≈ 0.1 cc as indicated in the protocols that follow. The order of inoculation was: R. Black, Rab # 365, NRS.

MAR 23 1948

TEST WITH TOXO FROM R. BLACK "RESIDUAL" SERIES

PE: Obtained to-day (11th post inc. day) from one mouse in the 10^{-3} group

Test: PE diluted $\frac{1}{5}$ in Schloss serum & incubated for one hour @ 37°C . Jilled.

Mixed, wet film made $\approx$ 3 drops of meth blue & examined:

55/100 unstained = 55% unstained. Toxo of normal appearance & staining.

What does this mean??

MAR 12 1948

SUMMARY OF TEST FOR RESIDUAL VIABILITY
OF TOXO PLASMA EXPOSED TO IMMUNE
HUMAN SERUM (BLACK) AND A FRESH
RABBIT SERUM (#365)

TEST	Final Dilution of Toxoplasma						LD ₅₀	No LD ₅₀ per cc killed	%
	-2	-3	-4	-5	-6	-7			
Control. - heated rabbit serum prot.	—	—	$\frac{5}{5}$	$\frac{5}{5}$	$\frac{4}{10}$	$\frac{0}{10}$	$10^{-5.8}$	630,000	
— fresh	$\frac{5}{5}$	$\frac{3}{5}$	$\frac{2}{10}$	$\frac{0}{10}$	$\frac{0}{10}$	$\frac{0}{10}$	$10^{-3.3}$	2,000	(92.7)
— fresh.	$\frac{5}{5}$	$\frac{5}{5}$	$\frac{5}{10}$	$\frac{1}{10}$	$\frac{0}{10}$	$\frac{0}{10}$	$10^{-4.1}$	14,000	(98.4)

SERUM	DILUTION	NO. M	DAYS AFTER INCUBATION																					TOTAL TOXO PER DOSE	STAINED TOXO PER DOSE	MORT	LD ₅₀
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
CONTROL	10 ⁻⁴	1	-	-	-	-	-	As ^e	As ^e	As ^e	D													17.3	166	5/5	
		2	-	-	-	-	-	? As ^e	As ^e	As ^e	D																
		3	-	-	-	-	-	As ^e	As ^e	As ^e	D																
		4	-	-	-	-	-	? As ^e	As ^e	As ^e	D																
		5	-	-	-	-	-	? As ^e	As ^e	As ^e	D																
HEATED RABBIT SERUM	10 ⁻⁵	1	-	-	-	-	-	As ^e	As ^e	As ^e	D												17.3	16.6	5/5		
		2	-	-	-	-	-	As ^e	As ^e	As ^e	As ^e D	+ toxo present in lung															
		3	-	-	-	-	-	As ^e	As ^e	As ^e	As ^e D																
		4	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e As ^e D																
		5	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e As ^e D																
SERUM POOL	10 ⁻⁶	1	-	-	-	-	-	? As ^e	As ^e	As ^e	D (chewed)	+ toxo present in lung															
		2	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e D	+ toxo present in lung															
		3	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e D	+ toxo present in lung															
		4	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e As ^e As ^e D	+ toxo present in lung															
		5	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e As ^e D												1.7		4/10	5.8	
		6	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e D																
		7	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e D																
		8	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e D												1.7				
		9	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e D																
		10	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e D																
	10 ⁻⁷	1	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e ? As ^e As ^e											0.2					
		2	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e ? ? ?																
		3	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e ? ? ?																
		4	-	-	-	-	-	? As ^e	As ^e	As ^e	As ^e ? ? ?																

MIXTURE 2NRS IN 37°C H₂O BATH, THEN DILUTED IN MAR 12 1948
50% RSS

INOC. = 0.1 cc I.P.

SERUM	DILUTION	No. M E	DAYS AFTER INOCULATION																					TOTAL TOXO PER DOSE	STAINED TOXO PER DOSE	MORT	LD ₅₀
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
	10 ⁻²	1	-	-	-	-	-	-	Ase	Ase	D													17,250	862.5	5/5	
		2	-	-	-	-	-	-	? Ase	D																	
		3	-	-	-	-	-	-	? Ase	D																	
		4	-	-	-	-	-	-	Ase	D																	
		5	-	-	-	-	-	-	?	D																	
	10 ⁻³	1	-	-	-	-	-	-	Ase	Ase sacrificed														17,250	86.3	3/5	
		2	-	-	-	-	-	-	Ase	Ase Ase Be D																	
		3	-	-	-	-	-	-	?	Ase ?																	
		4	-	-	-	-	-	-	-	?																	
		5	-	-	-	-	-	-	-	-																	
	10 ⁻⁴	1	-	-	-	-	-	-	? Ase	D														173	8.6	2/10	3.3
		2	-	-	-	-	-	-	Ase	Ase Ase S D																	
		3	-	-	-	-	-	-	?	Ase ?																	
		4	-	-	-	-	-	-	?	?																	
		5	-	-	-	-	-	-	?	?																	
		6	-	-	-	-	-	-	?	?																	
		7	-	-	-	-	-	-	?	?																	
		8	-	-	-	-	-	-	?	?																	
		9	-	-	-	-	-	-	?	?																	
		10	-	-	-	-	-	-	?	?																	
	10 ⁻⁵	1	-	-	-	-	-	-	Ase	?														17.3	0.9	0/10	
		2	-	-	-	-	-	-	?	?																	
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	10 ⁻⁶	1	-	-	-	-	-	-	Ase	Ase ?														1.7	0.09	0/10	
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		10	-	-	-	-	-	-	?	?																	

0.1cc IP

MAR 12 1948

(OVER)

RESIDUAL VIABILITY (CONT.)

0.1cc IP

MAR 12 1948

RABBIT
#365
FRESH
(CONT.)

SERUM	DILUTION	No. M 1/2	DAYS AFTER INCUBATION																					TOTAL TOXO PER DOSE	STAINED TOXO PER DOSE	MORT	LD ₅₀
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
RABBIT #365 FRESH (CONT.)	10 ⁻⁶	1	-	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	1.7	0.1	0/10	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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MAR 12 1948

The Affinity For Methylene Blue And The Residual Viability
Of Toxoplasma Heated For 15 & 30 Minutes At 50°C.

Toxoplasma: The same PE's with lysin that was made up for the residual viability following exposure to immune sera experiment ^{today} 7.

Procedure: 1.0 cc amounts of these PE's were placed in glass ampoules which were then heat-sealed. One of these was then heated for 15 minutes & the other for 30 minutes in a carefully controlled H₂O bath. The ampoules were totally submerged with weights. Heating was completed at 12⁴⁵ p.m. & the ampoules immediately transferred to the refrigerator. At 3⁰⁰ p.m., the contents of the ampoules were transferred to tubes & wet-film preparations made & counted as follows:

<u>PE's</u>	<u># unstained</u>	<u># stained</u>	<u>% unstained</u>	
Unheated (refrigerated)	6	94	6	
Heated 15'	72	128	36	good staining
Heated 30'	61	139	30.5	" "

At 4³⁰ p.m., the above tubes were serially diluted in the same 50% ^{inoc.} RSS used for the other experiments performed to-day & then, as indicated in the protocols, various groups of Wistar mice were given 0.1 cc IP.

SUMMARY OF EXPT. ON EFFECT
OF HEATING FOR 15 & 30 MIN. @ 50°C
ON VIABILITY OF TOXO PLASMA

MAR 12 1948

TEST	Final Dilutions of Toxoplasma								LD ₅₀
	1/5	-2	-3	-4	-5	-6	-7	-8	
Control - unheated	—	—	—	$\frac{4}{5}$	$\frac{3}{5}$	$\frac{6}{10}$	$\frac{2}{10}$	—	10 ^{-6.1}
Heated 15' at 50°C	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{10}$	$\frac{0}{9}$	—	0
Heated 30' at 50°C	$\frac{0}{5}$	$\frac{0}{4}$	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{8}$	$\frac{0}{10}$	—	0

1
DILUTIONS IN INACTIVATED 50% RSS

INOC: 0.1 cc IP IMMEDIATELY AFTER DILUTION

Dilution	# MICE	Days After Inoculation																					Total Toxo per Dose	Stained Toxo per Dose	Mort.	LD ₅₀
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
10 ⁻⁴	1	-	-	-	-	?	?	Asc	Asc		D												173			
	2	-	-	-	-	?	?	Asc	Asc		D													163	4/5	
	3	-	-	-	-	-	-	Asc	Asc		D															
	4	-	-	-	-	-	-	?	Asc		D															
	5	-	-	-	-	-	-	-	?		?	?	-	-	-	-	-	-	-	-	-	-				
10 ⁻⁵	1	-	-	-	-	?	?	Asc	Asc		D															
	2	-	-	-	-	-	-	Asc	Asc		D															
	3	-	-	-	-	-	-	?	Asc		D													17.3		3/5
	4	-	-	-	-	-	-	-	?		?	-	-	-	-	-	-	-	-	-	-	-		16.3		
	5	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-				
CONTROL HEATED UNHEATED	1	-	-	-	-	?	?	Asc	Asc		D	toxo present in lung														
	2	-	-	-	-	-	-	Asc	Asc		D	toxo present in lung														
	3	-	-	-	-	-	-	?	Asc		D	toxo present in lung														
	4	-	-	-	-	-	-	?	Asc		D	toxo present in lung														
	5	-	-	-	-	-	-	?	?		Asc	Asc	D	"	"	"	"	"	"	"	"	"	1.7			6/10
	6	-	-	-	-	-	-	-	-		?	-	-	-	-	-	-	-	-	-	-	-				
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10 ⁻⁶	1	-	-	-	-	-	-	Asc	Asc		Asc	D	toxo present in lung													
	2	-	-	-	-	-	-	Asc	Asc		Asc	D	toxo present in lung													
	3	-	-	-	-	-	-	?	?		?	?	-	-	-	-	-	-	-	-	-	-				
	4	-	-	-	-	-	-	?	?		-	-	-	-	-	-	-	-	-	-	-	-				
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	9	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-				
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Heated 15 Min (50° C)	1	-	-	-	-	?	?	Asc	Asc		?	-	-	-	-	-	-	-	-	-	-	-	345,000			0/5
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	4	-	-	-	-	-	-	-	Asc		-	-	-	-	-	-	-	-	-	-	-					
	5	-	-	-	-	-	-	-	Asc		-	-	-	-	-	-	-	-	-	-	-					

(over.)

EFFECT OF HEATING @ 50°C.

MAR 12 1948

DILUTIONS IN INAC. 50% RSS

INOC: 0.1cc IP IMMEDIATELY AFTER DILUTION

Dilution	# M.C.E	Days After Inoculation																								Total Toxa per Dose	Stained Toxa per Dose	Mort.	LD ₅₀
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24				
10 ⁻²	1	-	-	-	-	-	-	-	Asc	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17,250		0/5	
	2	-	-	-	-	-	-	-	Asc	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		11,040		
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10 ⁻³	1	-	-	-	-	-	-	-	Asc	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1,725		0/5	
	2	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1,104		
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HEATED

15 MIN.
(50°C.)

(cont)

0

Notoxo ssu

0.2

0.1

EFFECT OF HEATING @ 50°C.

MAR 12 1948

DILUTIONS IN INAC. 50% RSS.

INOC: 0.1cc IP IMMEDIATELY AFTER DILUTION

	Dilution	# MICE	Days After Inoculation																					Total Toxo per Dose	Stained Toxo per Dose	Mort.	LD ₅₀
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
<u>Heated</u> <u>30 Min</u> <u>(50°C)</u>	1/5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	345,000	239,775	0/5	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	10 ⁻²	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1,725	11,300	0/4	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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	10 ⁻³	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1,725	1130	0/5	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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	10 ⁻⁴	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	173	113	0/5	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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	10 ⁻⁵	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17.3	11	0/5	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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	10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.73		0/8	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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		10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				

(over)

EFFECT OF HEATING @ 50°C.

MAR 12 1948

INOC: 0.1cc IP.

	Dilution	# Mice	Days After Inoculation																					Total Toxo per Dose	Stained Toxo per Dose	Mort.	LD ₅₀
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
Heated 30 Min (50°C) (cont)	10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	0.1	0/10	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
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The Residual Viability Of Toxoplasma Exposed To Saline

MAR 12 1948

Obj: What is the relation between the changes in affinity for methylene blue & the residual viability of toxoplasma stored in saline.

Toxoplasma: The same PE '5 made up for the immune serum experiment today.

This was diluted with sterile NaCl to make a 1:10 of the PE.

Procedure: The 1:10 dil. of PE was incubated in the 37°C H₂O bath for one hour & then the tube was placed in the refrigerator for 24 hours.

At this time (11⁰⁰ a.m. MAR 13 1948 13 March 48) a differential count was made with 3 drops of meth. blue. Of 100 parasites counted, 42 were well-stained & 58 were very poorly stained or unstained. The intra-cellular parasites were well-stained. These poorly-stained parasites were almost decolorized; what coloration was present, was distributed in a granular manner.

Serial ten-fold dilutions were then made in the same

(NAC.) 50% RSS used for the experiments of 12 March 48 & groups of Masfield mice inoculated IP (0.1 ml/mouse) as indicated in the protocols, immediately after dilution.

MAR 13 1948

SUMMARY OF TITRATION TO DETERMINE THE EFFECT
OF OVERNIGHT STORAGE IN NaCl UPON VIABILITY OF
TOXO PLASMA.

TEST	Final Dilutions of Toxoplasma							LD ₅₀
	-1	-2	-3	-4	-5	-6	-7	
Saline, Overnight	$\frac{5}{5}$	$\frac{4}{5}$	$\frac{4}{5}$	$\frac{6}{10}$	$\frac{1}{10}$	$\frac{0}{10}$	$\frac{0}{10}$	$10^{-4.1}$

EFFECT OF STORAGE IN NaCl

MAR 13 1948

DILUTIONS IN INAC. 50% PSS

INOC: 0.1cc IP

IMMEDIATELY AFTER DILUTION

Dilution	# M I C E	Days After Inoculation																					Total Toxo per Dose	Stained Toxo per Dose	Mort.	LD ₅₀
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
10 ⁻¹	1	-	-	-	?	Ase	Ase			D													172,500		5/5	
	2	-	-	-	?	Ase	Ase			D														72,450		
	3	-	-	-	-	Ase	Ase			D																
	4	-	-	-	-	Ase	Ase			D																
	5	-	-	-	-	?	Ase			D																
10 ⁻²	1	-	-	-	?	Ase	Ase			D													17,250		4/5	
	2	-	-	-	-	Ase	Ase			D														72,45		
	3	-	-	-	-	Ase	Ase			Ase	D															
	4	-	-	-	-	?	?			Ase	D															
	5	-	-	-	-	?	?			?	Ase	?	?	?	?	-	-	-	-	-	-	-				
Saline Omnite. 10 ⁻³	1	-	-	-	?	Ase	Ase			Ase	D												17,25		4/5	
	2	-	-	-	-	Ase	Ase			Ase	Ase	D												725		
	3	-	-	-	-	?	Ase			Ase	Ase	D														
	4	-	-	-	-	?	?			Ase	Ase	D														
	5	-	-	-	-	?	?			?	Ase	Ase	Ase	?	?	-	-	-	-	-	-	-				
10 ⁻⁴	1	-	-	-	-	Ase	Ase			Ase	D												173		6/10	
	2	-	-	-	-	Ase	Ase			Ase	Ase	D														
	3	-	-	-	-	?	Ase			Ase	Ase	D														
	4	-	-	-	-	?	?			?	Ase	?	?	?												
	5	-	-	-	-	?	?			?	?	-	-	-	-											
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	10	-	-	-	-	-	-			-	-	-	-	-	-											

Saline
Count.

10^{-4.1}

* died on 25th day - (2) both smears positive for toxo plasma

(over)

EFFECT OF STORAGE IN NaCl

MAR 13 1948

INOC: 0.1cc IP

Dilution	# M C E	Days After Inoculation																					Total Toxo pu Dose	Stained Toxo pu Dose	Mort	LD ₅₀
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
Saline <u>Oxynite</u> (con't)	10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.7	0.7	0/10	
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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MAR 12 1948

The Effect of Formalin Upon the Methylene Blue Affinity and Viability of Toxoplasma

Toxoplasma: the same PE used in all other experiments to-day.

This was made to 1/5 in 0.2% and 0.5% formalin as follows:

<u>Am't. of PE</u>	<u>Am't of Formalin 1% in NaCl.</u>	<u>Am't of NaCl</u>	<u>Final Conc. of Formalin</u>
0.2 cc	0.2 cc	0.6 cc	0.2%
0.2 cc	0.5 cc	0.3 cc	0.5%

The PE was added last to these mixtures.

Procedure: Following the making of the above mixtures, the two tubes were allowed to remain at room temperature (23°C) for 2 hrs., at which time differential counts were made in wet-films with 3 drops of meth. blue. with the following results:

Table:

NaCl (refig. for 7 hrs.) = 14/100 unstained.

0.2% formalin = 25/100 poorly stained.

0.5% formalin = 15/100 poorly stained.

Serial dilutions were then made in 50% ^{inoc.} RSS (same batch used to-day) + inoc. into mice as indicated in the protocols.

NB

Note: At 6⁰⁰ pm (4 hrs after 4 hrs. at room temp.) the original tubes were counted by AOS with the following results & notes:

0.2% formalin: Unstained = 0% Poorly stained = 63% Well stained 37%.

"Would be poor preparation for 'antigen'. Intracellulars same as extra-cellulars. Some well stained, others poor. Leucocyte nuclei as in 0.5% formalin."

0.5% Formalin: Unstained = 0% Poorly stained = 10%, Well stained = 90%

"have agglutinated. Intracellular trophoblasts deeply stained.
Leucocyte nuclei - pale, almost unstained. If present
state could be maintained, would be perfect "antigen"
for in vitro phenomenon."

SUMMARY OF TITRATIONS TO DETERMINE EFFECT

MAR 12 1948

OF FORMALIN UPON VIABILITY OF TOXOPLASMA.

Test	Final Dilutions of Toxo plasma							LD ₅₀
	1/5	-2	-3	-4	-5	-6	-7	
Control - no formalin	—	—	—	5/5	5/5	10/5	3/10	10 ^{-6.3}
0.2% Formalin	0/5	0/5	0/5	0/10	0/10	0/10	0/10	0
0.5% Formalin	0/5	0/4	0/5	0/10	0/10	0/10	0/10	0

EFFECT OF FORMALIN UPON VIABILITY

MAR 12 1948

DILUTIONS IN 2NAC. 50%RSS

INOC: 0.1cc IP IMMEDIATELY AFTER DILUTION

	Dilution	# MICE	Days After Inoculation																					Total Toxo per DOSE	Stained Toxo per DOSE	Mort.	LD. 50	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21					
Toxo 1:5 in Saline Dilutions in 50% NRS	10 ⁻⁴	1	-	-	-	-	?	Asc	Asc	Asc	Asc	D																
		2	-	-	-	-	?	Asc	Asc	Asc	Asc	D																
		3	-	-	-	-	?	?	Asc	Asc	Asc	D																
		4	-	-	-	-	-	?	Asc	Asc	Asc	D																
		5	-	-	-	-	-	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc
Control	10 ⁻⁵	1	-	-	-	-	?	?	Asc	Asc	Asc	D																
		2	-	-	-	-	?	?	Asc	Asc	Asc	D																
		3	-	-	-	-	-	?	Asc	Asc	Asc	D																
		4	-	-	-	-	-	?	Asc	Asc	Asc	D																
		5	-	-	-	-	-	-	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	
Star (F. ...)	10 ⁻⁶	1	-	-	-	-	?	Asc	Asc	Asc	Asc	D	toxo present in lung.															
		2	-	-	-	-	?	Asc	Asc	Asc	Asc	D	toxo present in lung															
		3	-	-	-	-	?	Asc	Asc	Asc	Asc	D	toxo present in lung															
		4	-	-	-	-	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	
		5	-	-	-	-	-	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	
		6	-	-	-	-	-	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	
		7	-	-	-	-	-	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	
		8	-	-	-	-	-	-	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	
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	10 ⁻⁷	1	-	-	-	-	?	Asc	Asc	Asc	Asc	D	Toxo PRESENT.															
		2	-	-	-	-	?	Asc	Asc	Asc	Asc	D	toxo present in lung															
		3	-	-	-	-	?	Asc	Asc	Asc	Asc	D	toxo present in lung															
		4	-	-	-	-	-	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	
		5	-	-	-	-	-	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	
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		10	-	-	-	-	-	-	-	-	-	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	

★ dead on 25th day - toxo ghosts seen: intense mono cytic reaction bacteria:

10^{6.3}

3/10

DILUTIONS IN 50% RSSINOC: 0.1cc IP

IMMEDIATELY AFTER DILUTION

	Dilution	# M I C E	Days After Inoculation																								Total Toxo per Dose	Stained Toxo per Dose	Mort.	LD ₅₀
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21							
Formalin 0.2% 2 hrs. at room temp	1/5	1	-	-	-	-	?	Asc	Asc	Asc	?	-	-	-	-	-	-	-	-	-	-	-	-	345,000		0/5				
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Formalin

0.2%

2 hrs.
at
room temp

0

EFFECT OF FORMALIN (CONT.)

MAR 12 1948

DILUTIONS IN 50% RSS

INOC.: 0.1cc IP IMMEDIATELY AFTER DILUTION

Dilution	# Mice E	Days After Inoculation																					Total Toxo pos Dose	Stained Toxo pos Dose	Mort.	L.D. 50
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
1/5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	345,000		0/5	
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		293,250		
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Formalin
0.5%

0

MAR 12 1948

Additional Studies With Formalized Toxoplasma

Toxoplasma: The pooled undiluted PE remaining from to-day's experiments was made to 1:5 in 0.5% formalin (in NaCl), the tube tightly stoppered & stored in the refrigerator.

MAR 13 1948

Examination in wet film = 3 drops of meth blue after

18 hrs in the refrigerator = 80 well-stained
19 poorly stained
1 unstained.

Intra-cellular parasites
well stained

In Vitro Test With Formalized Sanguin
of 12 March.

MAR 16 1948

after 4 day storage in the refrigerator as PE 1/5 in 0.5% formalized saline, a test was set up with R. Black's serum, both fresh & heated. 0.1 cc units used, tubes incubated one hour @ 37°, then in the refrigerator for 30 minutes. It was found necessary to use 6 drops of meth blue in order to get good staining.

R. Black (F) = 4/50 unstained

R. Black (H) = 4/50 unstained

NB. ∴ Formalin-killed toxoplasma are unable to "accept" antibody in this test.

SUMMARY OF TITRATIONS TO DETERMINE

RELATIVE EFFECTIVENESS OF VARIOUS

DILUENTS FOR SUSPENDING TOXOPLASMA.

MAR 22 1948

Diluent.	Final Dilutions of Toxoplasma							LD ₅₀
	-2	-3	-4	-5	-6	-7	-8	
50% R.S.S.	—	—	$\frac{4}{4}$	$\frac{1}{4}$	$\frac{3}{10}$	$\frac{0}{10}$	$\frac{0}{10}$	$10^{-5.2}$
10% R.S.S.	—	—	$\frac{4}{4}$	$\frac{2}{4}$	$\frac{0}{10}$	$\frac{0}{7}$	$\frac{0}{10}$	$10^{-5.0}$
2% R.S.S.	—	—	$\frac{5}{5}$	$\frac{3}{5}$	$\frac{2}{10}$ $\frac{1}{5}$	$\frac{0}{10}$	$\frac{0}{10}$	$10^{-5.4}$ 5.3
2% Bovine Alb.	—	—	$\frac{1}{3}$	$\frac{1}{5}$	$\frac{1}{10}$	$\frac{0}{10}$	$\frac{0}{10}$	$10^{-4.3}$
Tyrodex	$\frac{5}{5}$	$\frac{2}{5}$	$\frac{1}{9}$	$\frac{0}{10}$	$\frac{0}{10}$	$\frac{0}{10}$	—	$10^{-3.0}$
Saline	$\frac{5}{5}$	$\frac{4}{5}$	$\frac{0}{10}$	$\frac{0}{10}$	$\frac{0}{10}$	$\frac{0}{9}$	—	$10^{-3.4}$

$$\begin{array}{r} 5 \quad 0 \\ 3 \quad 2 \\ 1 \quad 8 \\ 0 \quad 10 \end{array} \quad \begin{array}{r} 9 \quad 0 \\ 4 \quad 2 \\ 1 \quad 10 \\ 0 \end{array} \quad \frac{\frac{4}{10}}{\frac{1}{10}} = \frac{66.7 - 50}{9} = \frac{16.7}{57.7} = 0.28$$

The Effectiveness of Various Diluents For Use In Toxoplasma Mouse Titations

Obj: To determine which of several diluents are less injurious to toxoplasma & thus more useful for titrations, etc.

Diluents Tested: A = 50% inoc. RS in NaCl.

B = 10% inoc. RS in NaCl

C = 2% inoc. RS in NaCl

D = 2% Bovine albumin in NaCl (Ammon Lot #47 stored in ref. as crystalline albumin) suspended & filtered just before use.)

E = Tyrode's solution

F = Saline solution.

Toxoplasma: PE pooled from 4, four-day mice (inoc. IP on 18 Mar '48). This was diluted $1/100$ in NaCl & counted in chamber = 41, 46, 35, 59 or $18\frac{1}{4} = 45.25 / \text{mm}^2$ or 45,250,000 / c.c. On this basis, some of the titrations were set up to run to 10^{-8} . See over for additional data.

Method: The undil. PE remained in the refrigerator for 2 hrs because of a shortage of NaCl. At that time, the various diluents were made from previously reused flasks. All of the RSS mixtures were made from one pool flask. Following the distribution of the diluents, the PE was thoroughly mixed & 0.1 cc units delivered from the same pipette to the 10^{-1} tube of each series. Serial ten-fold dilutions were then made (in order from A $\rightarrow$ F) & the tubes placed in the 37°C H_2O bath for 2 hrs. At that time, 0.1 cc units were inoc. IP into Max 8-10 gm mice. Inoculation order = A $\rightarrow$ F. Inoculation time = 90 minutes. Each series was refrigerated until inoculated. During the

This is the flicker?

time that the tubes were incubating, chamber counts were made of the 10^{-2} tube. The latter were refrigerated until counted except for the Tyrode & Leibers series which were in the H_2O bath for 10 minutes prior to counting. During this time also, some of the PE was made to 1/5 c Leibers serum, incubated for one hour & counted (see below).

Counts from titration: (10^{-2} tube)

<u>Series</u>	<u>Storage prior to count.</u>	<u># T₄₅₀/mm²</u>	<u># T₄₅₀/c.c. Unstained.</u>
A	2 hrs in refig.	32.25	32,250,000
B	" " "	28.25	28,250,000
C	75' in refig.	31	31,000,000
D	" " "	28.25	28,250,000
E	10' @ 37°C	29.5	29,500,000
F	" " "	35.5	35,500,000

EFFECT OF SCHLOSS SERUM ON EXUDATE

PE 1/5 in NaCl + 0.1cc Schloss. ONE HOUR @ 37°. 0.02 cc MINT.

plus 3 drops METH. BLUE = 62/100 UNSTAINED

4	4	0	8	0			
5	1	3	4	3	4/7	= 57.1	57-50 = 7
6	3	7	3	10	3/13	= 23	57-23 = 34
0	0	10	0	0			

TOXOPLASMA TITRATION IN 50% RSS

MAR 22 1959

Dilutions IN POOLED INACTIVATED RS MADE TO 50% IN NaCl.

INOC: 0.1cc IP AFTER INCUBATION FOR 2 HRS. @ 37°C.

Dilution	# Mice	Days After Inoculation																					Total Toxo per Dose	Mort.	LD ₅₀	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
10 ⁻⁴	1	-	-	-	-	-	-	-	Asc	Asc	Asc	D												323	4/4	
	2	-	-	-	-	-	-	-	Asc	Asc	Asc	D														
	3	-	-	-	-	-	-	-	Asc	Asc	Asc	Asc	D													
	4	-	-	-	-	-	-	-	Asc	?	Asc	Asc	Asc	D												
10 ⁻⁵	1	-	-	-	-	-	-	-	Asc	Asc	D													32	1/4	
	2	-	-	-	-	-	-	-	?	?	Asc	Asc	?													
	3	-	-	-	-	-	-	-	?	?	?	?	?													
	4	-	-	-	-	-	-	-	-	-	?	?	-													
10 ⁻⁶	1	-	-	-	-	-	-	-	Asc	Asc	Asc	Asc	D													
	2	-	-	-	-	-	-	-	?	?	Asc	Asc	Asc	D												
	3	-	-	-	-	-	-	-	?	?	Asc	Asc	?	?												
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"A"

50% RSS

10^{-5.2}

"A"

50% RSS

10^{-5.2}

* dead on 23rd P.I. day: - Toxo ghosts seen in lung smear.

TOXOPLASMA TITRATION IN 10% RSS

MAR 22 1948

DILUTIONS IN 10% RSS. INCUBATED 2 HRS. @ 37°C.

INOC: 0.1 cc IP

Dilution	# Mice	Days After Inoculation																					Total Toxo per Dose	Mort	LD ₅₀
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
10 ⁻⁴	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	283	4/4	
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
10 ⁻⁵	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28	2/4	
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
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10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.8	0/10	10-50
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
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"B"

10% RSS

10-50

EXAMINED 2 CONSECUTIVE DAYS.
MARKED BACTERIAL OVERGROWTH.
MANY MONOS. NO DEFINITE TOXO SEEN.
NO DIARRHEA.
NO TOXO. MANY POLYS. NO BACT.

TOXOPLASMA TITRATION IN 2% RSS

MAR 22 1948

DILUTIONS IN 2% RSS.

INCUBATED 2 HRS. @ 37°C.

INOC: 0.1cc IP

Dilution	# M E	Days After Inoculation																					Total Toxo per Dose	Mort.	LD ₅₀			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21						
10 ⁻⁴	1	-	-	-	-	-	-	?	Ase	Ase	D													3/10	5/5			
	2	-	-	-	-	-	-	?	Ase	Ase	D																	
	3	-	-	-	-	-	-	-	Ase	Ase	Ase	D																
	4	-	-	-	-	-	-	-	?	Ase	Ase	D																
	5	-	-	-	-	-	-	-	?	?	Ase	Ase	Ase	Ase	D													
10 ⁻⁵	1	-	-	-	-	-	-	?	?	Ase	Ase	D	Toxo present in lung.										3/1	3/5				
	2	-	-	-	-	-	-	-	?	Ase	Ase	D																
	3	-	-	-	-	-	-	-	-	?	?	Ase	Ase	Ase	Ase	D												
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	?	?	-	-	-	-	-	-	-					
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
10 ⁻⁶	1	-	-	-	-	-	-	Ⓚ	No toxo seen in lung!														3	2/10 1/9	10 ^{-5.4}			
	2	-	-	-	-	-	-	?	Ase	Ase	Ase	D	Toxo present in lung															
	3	-	-	-	-	-	-	?	?	?	?	?	?	?	-	-	-	-	-	-	-	-				-		
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10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	Ase	Ase	?											0.3	0/10			
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10^{-5.4}

TITRATION IN 2% BOVINE ALBUMIN

MAR 22 1948

DILUTIONS IN 2% BOVINE ALBUMIN, INCUBATED 2 HRS. @ 37°C.

INOC: 0.1cc IP

Dilution	# M	Days After Inoculation																					Total Toxo per Dose	Mort.	LD ₅₀	
	E	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
10 ⁻⁴	1	-	D	(brain passaged to 5 ch alb. mice) see added sheet																				283	1/3	
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
10 ⁻⁵	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28	1/5		
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.8	1/10	10-4.3	
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0/10		
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10 ⁻⁸	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	0/10		
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"D"

2%
BOVINE
ALBUMIN

10-43

TITRATION IN TYRODE'S SOLUTION

MAR 22 1948

DILUTIONS IN TYRODE'S.

INCUBATED 2 HRS. @ 37°C.

INOC: 0.1cc IP

Dilution	# M E	Days After Inoculation																					Total Toxo. per Dose	Mort.	LD ₅₀
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
10 ⁻²	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29,500	5/5	
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
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10 ⁻⁵	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29.5	0/10	
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(over)

10^{-3.0}

TYRODE TITRATION (CONT.)

MAR 22 1948,

Dilution	# M C E	Days After Inoculation																					Total Toxo per Dose	Mort.	LD ₅₀
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
"E" (con V)	10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0	0/10	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
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		7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
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		10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Tyrod's																									
	10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0/10		
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-
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TITRATION IN SALINE

MAR 22 1948

DILUTIONS IN SALINE.

INCUBATED 2 HRS. @ 37°C.

INOC: 0.1 cc I.P.

Dilution	MICE	Days After Inoculation																					Total Toxo Avg Dose	Mort	LD ₅₀		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21					
F	10 ⁻²	1	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	35,500	5/5
		2	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
		3	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
		4	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
		5	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
	10 ⁻³	1	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	3,550	4/5
		2	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
		3	-	-	-	-	-	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
		4	-	-	-	-	-	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
		5	-	-	-	-	-	?	?	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	?	?	-	-	-	-	Asc		
SALINE	10 ⁻⁴	1	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	?	?	-	-	-	-	-	-	-	-	-	355	0/10
		2	-	-	-	-	-	Asc	Asc	?	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-		
		3	-	-	-	-	-	Asc	?	?	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-		
		4	-	-	-	-	-	Asc	?	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
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		6	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
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		10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	10 ⁻⁵	1	-	-	-	-	-	?	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35.5	0/10
		2	-	-	-	-	-	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
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		10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

10^{-3.4}

(over)

SALINE TITRATION (CONT.)

MAR 22 1948

	Dilution	# M / E	Days After Inoculation																					Total Toxo ppm Dose	Mort	LD50
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
"F" (100%)	10 ⁻⁶	1	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.6	0/10	
		2	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
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SALINE	10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	0.4	0/9	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
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* notocapsulae seen - marked bacterial overgrowth & fed.
neutrophilic reaction (lung smear)

Bleeding & Inoculation with Toxoplasma of 4 Mice.

MAR 24 1948

4 adult, male, Ch Alb were bled from the heart while under ether anesthesia. Since each survived, they were then given 0.25 cc of a PE $\frac{1}{4}$ I.P. This PE drawn from one survivor of the IPP an of 20 March.

The bled mice ~~then~~ were marked as follows:

- a) yellow Head (2) - ^{lgt} bloods pooled - 0.25 cc plasma frozen in buteroid. #1
- b) yellow Tail (1) - hep bl. ca. 0.1 cc plasma frozen #2
- c) yellow Head & Tail (1) - hep bl. ca 0.15 cc " " #3

Inoc.
IP.

Mouse.

DAY

	1	2	3	4	5	6
yel. Head.	-	-	-		D	
yel. Head.	-	-	-		D	
yel. Tail	-	-	-		D	
yel. Tail & Head.	-	-	-		Asc D	

In vitro tests on 24 May 48 = both fresh & heated sera negative.

APR 14 1948

Toxo Challenge Experiment

Obj: In view of the fact that all mice who scid tested & formalinized toxo. on 12 March survived, it appeared worthwhile to challenge each of these survivors with a single, moderately small dose of toxoplasma. As indicated in the protocols, various other mice were also challenged with the same dose.

TOXO PLASMA: PE pooled from 2 mice of 10 April passage (= 4 ch exudates) diluted to $1/100$ in NaCl & counted in chamber = 13, 12, 16, 18 or 14.5 tox/mm^2 or $14,500,000 / \text{cc}$. In order to achieve a challenge dose of 75-100 toxo, this undiluted exudate was diluted 1:1 in 50% RSS & this used as the undiluted tube for a series of ten-fold dilutions in 50% RSS to 10^{-7} . The 10^{-2} tubes of this titration was counted = 96, 96 = $7.5 / \text{mm}^2$ or $7,500,000 \text{ tox/cc}$. The 10^{-4} dilution was selected for challenge. Consequently the challenge dose was 75 toxo contained in 0.1 cc inoc.

Procedure: The control mice (10-8 survivors of the 12 Mar & 22 Mar deaths) were inoculated first. 10^{-4} dilution was made in a flask which was agitated by hand & by syringe with each filling of the latter. The challenge dose of 75 toxo. was contained in 0.1 cc which was administered I.P.

APR 14 1948

Control Titration for Challenge Experiment.Inoc: 0.1 cc IP.

Dilutions in 50% R.S.S.

0.1 cc IP

Dilution	# MICE	Days After Inoculation																					Total Toxo / Dose	Mort.	LD ₅₀
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
10 ⁻³	1	-	-	-	-	?	?	D																	
	2	-	-	-	-	?	?	Asc	Asc	Asc	D														
	3	-	-	-	-	-	-	Asc	Asc	Asc	Asc			D											
	4	-	-	-	-	-	-	Asc	Asc	Asc	Asc			D											
	5	-	-	-	-	-	-	-	Asc	Asc	Asc			Asc	Asc	D									
10 ⁻⁴	1	-	-	-	-	?	?	Asc	Asc	Asc	D														
	2	-	-	-	-	?	?	Asc	Asc	Asc	Asc			D											
	3	-	-	-	-	-	-	Asc	Asc	Asc	Asc			D											
	4	-	-	-	-	-	-	?	Asc	Asc	Asc			D											
	5	-	-	-	-	-	-	-	Asc	Asc	Asc			Asc	Asc	Asc	Asc	Asc	Asc	?	?	?			
10 ⁻⁵	1	-	-	-	-	-	-	Asc	?	Asc	D														
	2	-	-	-	-	-	-	?	?	Asc	Asc			D											
	3	-	-	-	-	-	-	?	?	Asc	Asc			D											
	4	-	-	-	-	-	-	?	?	Asc	Asc			D											
	5	-	-	-	-	-	-	-	-	Asc	Asc			D											
	6	-	-	-	-	-	-	-	-	Asc	Asc			D											
	7	-	-	-	-	-	-	-	-	Asc	Asc			D											
	8	-	-	-	-	-	-	-	-	?	Asc			D											
	9	-	-	-	-	-	-	-	-	?	Asc			D											
	10	-	-	-	-	-	-	-	-	?	?			D											
10 ⁻⁶	1	-	-	-	-	-	-	?	?	?	D														
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	6	-	-	-	-	-	-	-	-	-	?			Asc	?	?	?	?	?						
	7	-	-	-	-	-	-	-	-	-	?			?	?	?	?	?	?						
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10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	-														
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	10	-	-	-	-	-	-	-	-	-	-														

* dead on 23rd day: TOXO GHOSTS - OVERGROWN & BACT.

TOXO. CHALLENGE

APR 14 1948

INOC. 0.1cc IP of 10^{-4}

DOSE: 75 toxo. in 50% PSS.

Previously Inoculated				# MICE	Day After Challenge																				Mort.
Date	Test	Dil.	Dose Toxo		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
MAR 12	<u>Heat</u> <u>Expt</u>	10^{-4}	173	1	-	-	-	-	-	-	-	?	?	?	?	?	D								1/1
		10^{-5}	17.3	1	-	-	-	-	-	-	Asc	Asc	Asc	Asc	1	D									2/2
				2	-	-	-	-	-	-	?	?	Asc	Asc		D									
				3	-	-	-	-	-	-	?	?	Asc	Asc		D									
MAR 12	<u>Control</u>	10^{-6}	1.7	1	-	-	-	-	-	-	Asc	Asc	Asc	D											4/4
				2	-	-	-	-	-	-	Asc	Asc	Asc	Asc		D									
				3	-	-	-	-	-	-	?	?	Asc	Asc		D									
				4	-	-	-	-	-	-	-	-	Asc	Asc		D									
MAR 12 1948	<u>Heated</u> <u>15°</u> <u>50°C.</u>	10^{-1}	345,000	1	-	-	-	-	-	-	Asc	Asc	Asc	D											5/5
				2	-	-	-	-	-	-	Asc	Asc	Asc	D											
				3	-	-	-	-	-	-	Asc	Asc	Asc	D											
				4	-	-	-	-	-	-	Asc	Asc	Asc	Asc		D									
				5	-	-	-	-	-	-	Asc	Asc	Asc	Asc		D									
		10^{-2}	17,250	1	-	-	-	-	-	-	Asc	Asc	D												
				2	-	-	-	-	-	-	Asc	Asc	D												
				3	-	-	-	-	-	-	?	Asc	Asc	D											
				4	-	-	-	-	-	-	?	Asc	Asc	Asc		D									
				5	-	-	-	-	-	-	?	?	?	?		?	?	?	?	?	?	?	?	?	
		10^{-3}	1725	1	-	-	-	-	-	-	Asc	Asc	D												5/5
				2	-	-	-	-	-	-	Asc	Asc	Asc	D											
				3	-	-	-	-	-	-	Asc	Asc	Asc	D											
				4	-	-	-	-	-	-	Asc	Asc	Asc	D											
				5	-	-	-	-	-	-	Asc	Asc	Asc	D											
		10^{-4}	173	1	-	-	-	-	-	-	Asc	Asc	D												5/5
				2	-	-	-	-	-	-	Asc	Asc	D												
				3	-	-	-	-	-	-	Asc	Asc	Asc	D											
				4	-	-	-	-	-	-	?	Asc	Asc	Asc		D									
				5	-	-	-	-	-	-	?	?	Asc	Asc		D									
		10^{-5}	17.3	1	-	-	-	-	-	-	Asc	Asc	D												5/5
				2	-	-	-	-	-	-	Asc	Asc	Asc	D											
				3	-	-	-	-	-	-	?	Asc	Asc	D											
				4	-	-	-	-	-	-	?	Asc	Asc	D											
				5	-	-	-	-	-	-	?	?	Asc	Asc		Asc									

(over)

TOXO. CHALLENGE

APR 14 1948

DOSE: 75 TOXO IN 50% RSS.

INOC: 0.1cc IP

Previously Inoculated				#	Day After Challenge																							Mort.
Date	Test	Dil.	Dose Toxo	MICE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
MAR 12 1948	Heated 15' 50°C (cont)	10 ⁻⁶	1.73	1	-	-	-	-	-	-	Asc	Asc	D															
				2	-	-	-	-	-	-	Asc	Asc	D															
				3	-	-	-	-	-	-	Asc	Asc	Asc	D														
				4	-	-	-	-	-	-	Asc	Asc	Asc	D														
				5	-	-	-	-	-	-	?	Asc	Asc	D														
				6	-	-	-	-	-	-	?	Asc	Asc	D														
				7	-	-	-	-	-	-	?	Asc	Asc	Asc	D													
				8	-	-	-	-	-	-	?	Asc	Asc	Asc	D													
				9	-	-	-	-	-	-	?	Asc	Asc	Asc	D													
				10	-	-	-	-	-	-	?	Asc	Asc	Asc				?	?	?	-	-	-	-	-	-		
	10 ⁻⁷	0.2	1	-	-	-	-	-	-	Asc	Asc	Asc	D															
			2	-	-	-	-	-	-	Asc	Asc	Asc	D															
			3	-	-	-	-	-	-	Asc	Asc	Asc	Asc	D														
			4	-	-	-	-	-	-	Asc	Asc	Asc	Asc	D														
			5	-	-	-	-	-	-	?	Asc	Asc	Asc	D														
			6	-	-	-	-	-	-	?	Asc	Asc	Asc	D														
			7	-	-	-	-	-	-	?	?	?	?				?	?										
MAR 12 1948	Heated 30' (50°C)	1/5	345,000	1	-	-	-	-	-	-	Asc	Asc	Asc	D														
				2	-	-	-	-	-	-	Asc	Asc	Asc	D														
				3	-	-	-	-	-	-	?	Asc	Asc	Asc	D													
				4	-	-	-	-	-	-	?	Asc	Asc	Asc	D													
				5	-	-	-	-	-	-	?	Asc	?	Asc	Asc	D												
		10 ⁻²	17,250	1	-	-	-	-	-	-	Asc	Asc	Asc	D														
				2	-	-	-	-	-	-	Asc	Asc	Asc	Asc	D													
				3	-	-	-	-	-	-	Asc	Asc	Asc	Asc	D													
		10 ⁻³	1725	1	-	-	-	-	-	-	Asc	Asc	D															
				2	-	-	-	-	-	-	Asc	Asc	Asc	D														
				3	-	-	-	-	-	-	Asc	Asc	Asc	D														
				4	-	-	-	-	-	-	?	Asc	Asc	Asc	D													
				5	-	-	-	-	-	-	?	Asc	Asc	Asc	D													
		10 ⁻⁴	173	1	-	-	-	-	-	-	Asc	Asc	Asc	D														
				2	-	-	-	-	-	-	Asc	Asc	Asc	D														
				3	-	-	-	-	-	-	Asc	Asc	Asc	D														
				4	-	-	-	-	-	-	Asc	Asc	Asc	Asc	D													
				5	-	-	-	-	-	-	?	?	Asc	Asc	D													
(next page please)																												

(next page please)

TOXO. CHALLENGE

APR 14 1948

DOSE: 75 TOXO IN 50% RSS.

INOC. : 0.1cc IP

Previously Inoculated				#	Day After Challenge																							
Dates	Test	Dil.	Dose Toxo	M/C	E	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
MAR 12 1948		10^{-5}	17.3	1	-	-	-	-	-	?	?	Asc	Asc	Asc	D													
				2	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
				3	-	-	-	-	-	-	-	Asc	Asc	Asc	Asc			D										
				4	-	-	-	-	-	-	-	?	Asc	Asc	Asc			Ans ? D										
				5	-	-	-	-	-	-	-	-	Asc	?	Asc			-	?	?	3	?	-	-	-	-	-	-
		10^{-6}	1.73	1	-	-	-	-	-	-	-	Asc	Asc	D														
				2	-	-	-	-	-	-	-	Asc	Asc	D														
				3	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
				4	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
				5	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
				6	-	-	-	-	-	-	-	?	Asc	Asc	Asc			D										
				7	-	-	-	-	-	-	-	?	?	Asc	Asc			D										
				8	-	-	-	-	-	-	-	?	?	?	Asc			?	?	?	?	?	-	-	-	-	-	-
		(con't)	0.2	1	-	-	-	-	-	-	-	Asc	Asc	D														
				2	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
				3	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
				4	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
				5	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
				6	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
				7	-	-	-	-	-	-	-	Asc	Asc	Asc	Asc			D										
				8	-	-	-	-	-	-	-	?	Asc	Asc	Asc			D										
				9	-	-	-	-	-	-	-	?	?	?	Asc			D										
				10	-	-	-	-	-	-	-	?	?	?	Asc			Asc	D									

10/10

TOXO. CHALLENGE

INOC: 0.1 cc IP

Previously Inoculated				Mort.	Day After Challenge																							
Date	Test	Dil.	Dose Toxo		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
MAR 12 1948	Formalin	10 ⁻⁶	1.7	1	-	-	-	-	-	Asc	Asc	Asc	D												5/5			
	2			-	-	-	-	-	Asc	Asc	Asc	D																
	3			-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	4			-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	5			-	-	-	-	-	?	Asc	Asc	Asc	D															
MAR 12 1948	Formalin	10 ⁻⁵	345,000	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo in lung.											5/5			
				2	-	-	-	-	-	Asc	Asc	Asc	D															
				3	-	-	-	-	-	Asc	Asc	Asc	Asc	D														
				4	-	-	-	-	-	Asc	Asc	Asc	Asc	D														
				5	-	-	-	-	-	Rt. Asc	Asc	Asc	Asc	Asc	D													
		10 ⁻²	17,250	1	-	-	-	-	-	Asc	Asc	Asc	D												4/4			
		2		-	-	-	-	-	Asc	Asc	Asc	Asc	D															
		3		-	-	-	-	-	?	Asc	Asc	Asc	D															
		4		-	-	-	-	-	Rt. Asc	Asc	Asc	Asc	Asc	Asc	D													
		10 ⁻³	1725	1	-	-	-	-	-	Asc	Asc	D												4/4				
	2	-		-	-	-	-	Asc	Asc	Asc	D																	
	3	-		-	-	-	-	Asc	Asc	Asc	D																	
	4	-		-	-	-	-	?	Asc	Asc	Asc	D																
	0.2%	10 ⁻⁴	173	1	-	-	-	-	-	Asc	Asc	Asc	Asc	D												5/5		
				2	-	-	-	-	-	Asc	Asc	Asc	Asc	D														
				3	-	-	-	-	-	Asc	Asc	Asc	Asc	D														
				4	-	-	-	-	-	Asc	Asc	Asc	Asc	D														
				5	-	-	-	-	-	?	Asc	Asc	Asc	D														
		10 ⁻⁵	17	1	-	-	-	-	-	Asc	Asc	Asc	D												5/5			
		2		-	-	-	-	-	Asc	Asc	Asc	Asc	D															
3		-		-	-	-	-	Asc	Asc	Asc	Asc	D																
4		-		-	-	-	-	Asc	Asc	Asc	Asc	D																
5		-		-	-	-	-	?	Asc	Asc	Asc	D																
10 ⁻⁶	1.7	1	-	-	-	-	-	Asc	Asc	D	D												5/5					
2		-	-	-	-	-	Asc	Asc	Asc	D																		
3		-	-	-	-	-	Asc	Asc	Asc	Asc	D																	
4		-	-	-	-	-	?	Asc	Asc	Asc	D																	
5		-	-	-	-	-	?	?	Asc	Asc	Asc	Asc	D															
10 ⁻⁷	0.2	1	-	-	-	-	-	Asc	Asc	Asc	D												3/3					
2		-	-	-	-	-	?	Asc	Asc	Asc	D																	
3		-	-	-	-	-	?	Asc	Asc	Asc	Asc	D																

TOXO. CHALLENGE

INOC: 0.1cc IP

Previously Inoculated

Day After Challenge

Mort.

MAR 12, 1948

Formalin

0.5%

Date	Test	Dil	Dose Toxo	# MICE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
		1/5	345,000	1	-	-	-	-	-	Asc	Asc	Asc	D													5/5
				2	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				3	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				4	-	-	-	-	-	?	Asc	Asc	Asc	D												
				5	-	-	-	-	-	?	?	Asc	Asc	Asc D												
		10 ⁻²	17,250	1	-	-	-	-	-	Asc	Asc	D														4/4
				2	-	-	-	-	-	Asc	Asc	Asc	D													
				3	-	-	-	-	-	?	Asc	Asc	Asc	D												
				4	-	-	-	-	-	?	?	Asc	Asc	D												
		10 ⁻³	17,25	1	-	-	-	-	-	Asc	Asc	D														5/5
				2	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				3	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				4	-	-	-	-	-	?	Asc	Asc	Asc	D												
				5	-	-	-	-	-	?	?	Asc	Asc	Asc D												
		10 ⁻⁴	173	1	-	-	-	-	-	Asc	Asc	D														
				2	-	-	-	-	-	Asc	Asc	Asc	D													
				3	-	-	-	-	-	Asc	Asc	Asc	D													
				4	-	-	-	-	-	Asc	Asc	Asc	D													
				5	-	-	-	-	-	?	Asc	Asc	Asc	Asc ?	?	?	?	?	?	?	?	?	?	?	?	
		10 ⁻⁵	17	1	-	-	-	-	-	Asc	Asc	Asc	Asc	D												5/5
				2	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				3	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				4	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				5	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc-Asc D												
		10 ⁻⁶	1.7	1	-	-	-	-	-	Asc	Asc	Asc	D													5/5
				2	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				3	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				4	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
				5	-	-	-	-	-	Asc	Asc	Asc	Asc	D												
		10 ⁻⁷	0.2	1	-	-	-	-	-	Asc	D															5/5
				2	-	-	-	-	-	Asc	D															
				3	-	-	-	-	-	Asc	Asc	Asc	D													
				4	-	-	-	-	-	?	Asc	Asc	Asc	D												
				5	-	-	-	-	-	?	?	Asc	Asc	D												

APR 14 1948

TOXO. CHALLENGE

DOSE: 75 TOXO IN 50% RSS.

INOC. 0.1cc IP

Previously Inoculated				# M I L E	Day After Challenge																					Mort.	
Date	Test	Dil.	Dose Toxo.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
MAR 22 1948	50% RSS.	10 ⁻⁵	32	1	-	-	-	?	?	Asc	Asc	D														3/3	
				2	-	-	-	?	?	Asc	Asc	Asc	D														
				3	-	-	-	?	?	Asc	Asc	Asc	Asc														
		10 ⁻⁶	3.2	1	-	-	-	?	?	Asc	Asc	Asc	D														
				2	-	-	-	?	?	Asc	Asc	Asc	D														
				3	-	-	-	-	?	Asc	Asc	Asc	Asc														
				4	-	-	-	-	-	?	Asc	Asc	Asc														
	(A)		5	-	-	-	-	-	?	Asc	Asc	Asc															
			6	-	-	-	-	-	-	Asc	Asc	Asc															
			7	-	-	-	-	-	-	Asc	Asc	Asc															
	MAR 22	10% RSS	10 ⁻⁵	28	1	-	-	-	?	?	Asc	Asc	D													2/2	
					2	-	-	-	-	-	Asc	Asc	Asc	D													
	MAR 22	2% RSS	10 ⁻⁵	31	1	-	-	-	-	-	?	Asc	Asc	Asc												2/2	
					2	-	-	-	-	-	?	Asc	Asc	Asc	D												
	MAR 22 1948	2% Douine Albumin	10 ⁻⁴	283	1	-	-	-	?	-	?	Asc	Asc	Asc												2/2	
					2	-	-	-	-	-	?	Asc	?	?													
10 ⁻⁵			28	1	-	-	-	?	-	Asc	Asc	Asc	Asc														
				2	-	-	-	-	-	?	Asc	Asc	Asc	D													
(D)			3	-	-	-	-	-	?	Asc	Asc	Asc	D												4/4		
			4	-	-	-	-	-	?	Asc	Asc	Asc	Asc	D													
MAR 22	Tyrodes	10 ⁻³	2950	1	-	-	-	?	?	Asc	Asc	Asc	Asc												3/3		
				2	-	-	-	?	?	Asc	Asc	Asc	Asc	D													
				3	-	-	-	-	-	?	Asc	Asc	Asc	Asc	D												
MAR 22	Saline	10 ⁻³	3550	1	-	-	-	-	-	Asc	Asc	D													1/1		

* dead on 23rd day: TOXO GHOSTS. NUMEROUS BACT.

TOXO. CHALLENGE

INOC: 0.1cc IP

Previously Inoculated				#	Day After Challenge																					Mort.
Date	Test	Dil.	Dose Toxo.	MICE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
MAR 12		10 ⁻³	1725 86.3	1	-	-	-	-	-	-	Asc	Asc	Asc	D												2/2
				2	-	-	-	-	-	-	Asc	Asc	Asc	D												

APR 19 1948

The Correlation Of Toxoplasma Counts With Mouse Pathogenicity

Obj: To repeat previous tests in which toxoplasma were titrated in mice to test the predictability of mouse pathogenicity on the basis of chamber counts.

Toxoplasma: PE obtained from one mouse, four days after IP passage.

Procedure: The fresh PE was dil to $1/100$ in NaCl & counted in the chamber.

Counts equalled: 23, 28, 36, 34 = 30.25 toxo/mm^2 or $30,250,000/\text{cc}$.

A titration scheme was then set up on the basis of this count.

Titration "A": Immediately after the above count, a series of ten-fold dilutions were made in 50% RSS (10^{-1} - 10^{-8}). The 10^{-2} tube of this series was counted = 37, 29, 34, 28 = 32 toxo/mm^2 or $32,000,000 \text{ toxo/cc}$. Immediately thereafter, 0.1 cc units were inoculated IP into Maxfield 8-10 gm mice as indicated in the protocol.

Titration "B": The original PE was stored in the refrigerator for $1\frac{1}{2}$ hrs. & then the entire procedure repeated. 10^{-2} counts = 31, 31, 31, 31 or 31 toxo/mm^2 = $31,000,000 \text{ toxo/cc}$. See protocols for mouse inoculations.

TOXO PLASMA TITRATION

APR 19 1948

INOC. : 0.1cc IP

ALL DILUTIONS IN 50% PSS.

MAX. 8-10gm.

NO INCUBATION

Test	Dilution	# MICE	Day After Inoculation																							Total Toxo Dose	Mort.	LD ₅₀
			E	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
Titration "A"	10 ⁻³	1	-	-	Asc	?	?		D																	3,200	5/5	
		2	-	-	Asc	?	?		Asc	D																		
		3	-	-	?	?	?		Asc	D																		
		4	-	-	-	-	-		Asc	D																		
		5	-	-	-	-	-		Asc	Asc	D																	
	10 ⁻⁴	1	-	-	?	?	?		D																	320	5/5	
		2	-	-	-	?	?		Asc	D																		
		3	-	-	-	-	?		Asc	D																		
		4	-	-	-	-	-		Asc	D																		
		5	-	-	-	-	-		?	Asc	Asc	Asc	D															
	10 ⁻⁵	1	-	-	-	?	?		Asc	D																32	10/10	
		2	-	-	-	-	-		Asc	Asc	D																	
		3	-	-	-	-	-		Asc	Asc	D																	
		4	-	-	-	-	-		Asc	Asc	D																	
		5	-	-	-	-	-		Asc	Asc	D																	
		6	-	-	-	-	-		Asc	Asc	D																	
		7	-	-	-	-	-		?	Asc	D																	
		8	-	-	-	-	-		?	Asc	Asc	Asc	D															
		9	-	-	-	-	-		?	Asc	Asc	Asc	Asc	D														
		10	-	-	-	-	-		-	?	Asc	Asc	Asc	D														
	10 ⁻⁶	1	-	-	-	-	-		Asc	Asc	D															3.2		
		2	-	-	-	-	-		Asc	Asc	Asc	D																
		3	-	-	-	-	-		Asc	Asc	Asc	D																
		4	-	-	-	-	-		Asc	Asc	Asc	Asc	D															
		5	-	-	-	-	-		Asc	Asc	Asc	Asc	D															
		6	-	-	-	-	-		?	Asc	Asc	Asc	D															
		7	-	-	-	-	-		?	Asc	Asc	Asc	D															
		8	-	-	-	-	-		?	Asc	Asc	Asc	?	Asc	?	?	-	-	-	-	-	-	-	-	-			
		9	-	-	-	-	-		-	?	Asc	?	?	?	?	?	-	-	-	-	-	-	-	-	-			
		10	-	-	-	-	-		-	?	?	?	-	?	?	?	-	-	-	-	-	-	-	-	-			
10 ⁻⁷	1	-	-	-	-	-		Asc	?	Asc	D														0.32			
	2	-	-	-	-	-		?	?	Asc	Asc	Asc	D															
	3	-	-	-	-	-		?	-	Asc	?	?	-															
	4	-	-	-	-	-		-	-	Asc	?	-	-															
	5	-	-	-	-	-		-	-	?	-	-	-															
	6	-	-	-	-	-		-	-	-	-	-	-															
	7	-	-	-	-	-		-	-	-	-	-	-															
	8	-	-	-	-	-		-	-	-	-	-	-															
	9	-	-	-	-	-		-	-	-	-	-	-															
	10	-	-	-	-	-		-	-	-	-	-	-															

* COMPLETELY EATEN EXCEPT FOR SKIN

TOXOPLASMA TITRATIONS (CONT.)

APR 19 1948

INOC: 0.1cc IP

ALL DILUTIONS IN 50% RSS.

MAX. 8-10 gms.

NO INCUBATION

[illegible]

		1	- - Ax ? ?	D					
		2	- - ? ? ?	Asc D					
	10 ⁻³	3	- - ? ? ?	Asc D				3,100	5/5
		4	- - - ? -	Asc D					
		5	- - - - -	Asc D					
Titrations		1	Died shortly after inoculation						
	10 ⁻⁴	2	- - - ? ?	Asc Asc D				310	4/4
		3	- - - ? ?	Asc Asc D					
		4	- - - - -	Asc Asc D					
		5	- - - - -	Asc Asc M Asc Asc Asc D					
B		1	- - - D	No toxo seen: - bacteria seen in smear.					
	10 ⁻⁵	2	- - - ? ?	Asc Asc D					
		3	- - - - -	Asc Asc D				31	9/9
		4	- - - - -	Asc Asc D					
		5	- - - - -	Asc Asc D					
		6	- - - - -	Asc Asc D					
		7	- - - - -	Asc Asc D					
		8	- - - - -	Asc Asc D					
		9	- - - - -	Asc M D	Toxo present in lung				
		10	- - - - -	Asc M Asc D					
		1	- - - - -	Asc Asc Asc D	Toxo present in lung				
		2	- - - - -	Asc Asc Asc D	u	u	u	u	
		3	- - - - -	Asc Asc Asc D	u	u	u	u	
		4	- - - - -	Asc Asc Asc D	u	u	u	u	
	10 ⁻⁶	5	- - - - -	Asc ? Asc Asc D	u	u	u	u	
		6	- - - - -	? ? Asc Asc D	u	u	u	u	
		7	- - - - -	? - Asc Asc Asc D	u	u	u	u	
		8	- - - - -	- - Asc Asc Asc Asc D	Toxo in lung				
		9	- - - - -	- - ? Asc Asc Asc D	Toxo in lung				
		10	- - - - -	- - ? Asc Asc Asc D	Toxo in lung				

TOXOPLASMA TITRATION (CONT.)

INOC: 0.1cc IP

ALL DILUTIONS IN 50% RIS

MAX. 8-10 g/m.

NO INCUBATION

Test	Dilution	# M C E	Day After Inoculation																					Total Toxo/ Dose	Mort.	LD ₅₀	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
Titration "B" (crit)	10 ⁻⁷	1	-	-	-	-	-	Asc	Asc	Asc	Asc	D	Toxo present in lung										0.3				
		2	-	-	-	-	-	?	Asc	Asc	Asc	D	"	"	"	"	"	"	"	"	"	"				"	
		3	-	-	-	-	-	-	-	Asc	Asc	Asc	?	?	?	-	-	-	-	-	-	-				-	
		4	-	-	-	-	-	-	-	Asc	?	?	?	?	-	-	-	-	-	-	-	-				-	
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	10 ⁻⁸	1	-	-	-	-	-	-	D	No Toxo in Lung. MANY BACT. NO ASC.												0.03					
		2	-	-	-	-	-	-	-	?	-	-	D	No Toxo seen													
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-	-	
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MAY 11 1948

The Effectiveness of 50% RSS, 2% RSS & Tyrodes Solution As Diluents For Toxoplasma Titration

Obj: This is, essentially, a repetition of the exp't of 22 March 48 except that fewer diluents are used. It is expected to gather additional data on the relation between chamber counts (br. plasma concentrations) and mouse pathogenicity as well as to check on the previous finding that 2% RSS was as good as 50% RSS for dilution & that both were considerably better than Tyrode's.

Toxoplasma: PE harvested from each of 2, four day mice. Since neither fluid was plentiful, & both contained many free parasites they were pooled & used as one. Harvested @ 9:45 a.m. they were diluted ^{in saline} & counted @ 10^{-2} @ 10^{-4} a.m. = 28, 24, 32, 35 = 29.7 toxo/mm² or 30,000,000/c.c. !!

Rabbit serum: Pooled serum ^{from 6 rabbits} prepared & inactivated by the Media Room on 10 April 48. This serum was reactivated for 30' @ 56°C this morning, just prior to use.

Procedure: The entire dilution & inoculation procedure was performed by A.B.S. All dilutions were made in large tubes with 2 cc volumes of mixtures. Each series was made to 10^{-1} & then diluted out completely, & placed in the water bath for incubation before the next series was begun.

Order of dilution: 1) Tyrodes - began @ 10^{30} a.m. into H₂O bath @ 10^{32} a.m.
2) 2% RSS - began @ 10^{40} a.m. into H₂O bath @ 10^{48} a.m.
3) 50% RSS - began @ 10^{50} a.m. into H₂O bath @ 10^{59} a.m.

All tubes incubated for 2 hrs in 37°C H₂O bath & inoc.

in 0.1 cc amts IP into Mex 8-10 gm mice.

Inoc. order: Tyrodes (12^{38}), 2% RSS (12^{50}), 50% RSS (ca. 1^{05}).

(OVER)

Each series (when necessary) was kept in the refrigerator from the time of removal from the water bath until inoculated.

TOXO PLASMA COUNTS:

a) Prelim. on PE $1/100$ in NaCl = 28, 24, 32, 35 = $29.7/\text{mm}^2$

b) 10^{-2} in 50% PSS* = 31, 30, 34, 43 = $34.5/\text{mm}^2$

c) 10^{-2} in 29% PSS* = 40, 36, 34, 32 = $36/\text{mm}^2$

d) 10^{-2} in Tyrodes** = 23, 35, 42, 32 = $33/\text{mm}^2$

* Counted after 15-20 min. @ room temp.

** Counted after 1 hr in 37°C H_2O bath (approx. 20% toxo. are "ghosts").

On review of the above counts, $35 \text{ toxo}/\text{mm}^2$ of $1/100$ selected as base-line figure for calculating dosage: $35,000,000 \text{ toxo}/\text{cc undil.}$

MAY 11 1948

SUMMARY OF COMPARATIVE TOXOPLASMA TITRATIONS
USING TYRODES, 50% RSS & 2% RSS AS DILUENTS

INOC.: 0.1cc IP AFTER 2 HRS. AT 37°C.

Diluent.	Dilution of Toxoplasma							LD ₅₀
	-2	-3	-4	-5	-6	-7	-8	
Tyrodé's	5/5	5/5	2/5	0/5	0/5	0/5		3.8
50% RSS.	5/5		5/5	4/5	4/10	1/10	0/10	6.0
2% RSS.			5/5	3/5	1/10	0/10	0/10	5.3

TOXO PLASMA TITRATION

MAY 11 1948

0.1cc IP

AFTER 2 HRS @ 37° C.

MAX. 8-10 gms.

Diluent	Dilution	# M C E	Days After Inoculation																								Total Toxo ppx Dose	Mort.	LD ₅₀	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21							
Tyrode's	10 ⁻²	1	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	35,000	5/5		
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	Toxo present in lung																													
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		5	-	-	-	-	-	-	?	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc				
	Toxo present in lung																													
	10 ⁻⁴	1	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	350		
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	Toxo present in lung																													
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TOXOPLASMA TITRATION

MAY 11 1948

0.1 cc IP

AFTER 2 HRS @ 37°C

MAX. 8-10 gms.

[illegible]

TOXO PLASMA TITRATION

MAY 11 1948

0.1cc IP

AFTER 2 HRS @ 37°C

MAXFIELD

8-10 GMS.

DILUENT	Dilution	# M E	Days After Inoculation																					Total Toxo per dose	Mort.	LD ₅₀
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
	10 ⁻⁴	1	-	-	-	-	Asc	Asc	Asc	D																
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		3	-	-	-	-	Asc	Asc	Asc	Asc	D															
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	10 ⁻⁵	1	-	-	-	-	Asc	Asc	Asc	Asc	Asc	D														
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		4	-	-	-	-	-	-	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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2% R.S.S.	10 ⁻⁶	1	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	D													
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		10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* No toxo on lung smear.

11 MAY 48

In Vitro Activity of Toxoplasma In Various Diluents Used For Titrations To-day.

Toxoplasma Preparations: The 10⁻¹ Tubes of the 50% RSS, 2% RSS, and Tyrodes preparations used in the titration & stored in the refrigerator since about 11⁰⁰ a.m. were set up with & without [redacted] serum as indicated below.

Test: 0.1cc of the toxoplasma suspension with & without 0.1cc Buescher serum mixed & placed in 37°C H₂O bath @ 12⁵⁵ (2 hrs. in refg.) Incubated for one hour & then ~~plac~~ examined in wet-film with 1 loop of meth. blue.

<u>Toxoplasma 1/10 in</u>	<u>Serum</u>	<u>Result</u>	<u>% unstained</u>
50% RSS	None	13/50 unstained	26
50% RSS	[redacted]	48/100 "	48
2% RSS	None	7/50 "	14
2% RSS	[redacted]	33/50 "	66
Tyrodes	None	10/50 "	20
Tyrodes	[redacted]	30/50 "	60

Toxoplasma Mouse Neutralization Tests With Negative Human And Some Rabbit & Monkey Sera.

- Obj.:
- 1) To determine the neutralization indices (in the mouse test.) of a group of human sera which are negative in the rabbit neutralization & in vitro toxoplasma tests.
 - 2) To determine the neutralization indices of rabbit & monkey sera which can & cannot be reactivated in the in vitro test.

Toxoplasma: PE pooled from 2 mice three days after I.P. inoc. (10⁹ i.l.). This was obtained @ 11³⁵ a.m., immediately diluted to 10⁻² with NaCl & counted in the chamber @ 11⁴⁵ a.m. = 43, 36, 49, 53 = 45/mm² or 45,000,000 toxo/cc undil.

Dilutions: Immediately after the initial count, stock toxoplasma dilutions were made in large tubes, using 2% RSS as the diluent. NRS = same undil. pool reactivated yesterday. First tube = 1/5 & then tenfold so that final dil. in test would equal 10⁻⁷. The 1/50 tube of this series was dil. to 1/100 after 1 hr in the refrigerator & counted in the chamber = 46, 34, 48, 53 = 45/mm² = 45,000,000 toxo/cc undil. All toxo nicely refractile.

Test: 0.3 cc of the toxo dil. & 0.3 cc amits serum, mixed, immediately after above & placed in the 37°C H₂O bath @ 12⁵⁰ p.m. for 2 hrs. incubation. Then removed & placed in ice water bath & 0.1 cc of amits inoc. into each of 5 mice (max 8-10 gm.) I.P. for each dilution. Total inoc. time = 80 min. Inoc. scheduled from "A" to "K".

SUMMARY

MAY 13 1948

TOXOPLASMA MOUSE NEUTRALIZATION TESTS WITH NORMAL
HUMAN & SOME RABBIT & MONKEY SERA.

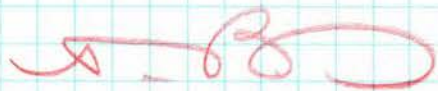
0.1cc IP

2% RSS DILUENT

2 HRS. @ 37°C.

MAX. 8-10gm MICE

This whole test doesn't make
sense for some reason not clear
to me.



MAY 13 1948

TOXO MOUSE NEUT.

0.1cc IP

2% RSS Diluent

2 HRS @ 37°C

Serum	Dilution	# Mice	Day After Inoculation																					Mort.	LD ₅₀	Neut Index								
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21											
"A"	10 ⁻⁴	1	-	-	-	-	?	Asc	Asc	Asc	Asc	D	Toxo present w/ lung										5/5											
		2	-	-	-	-	?	Asc	Asc	Asc	Asc	D																						
		3	-	-	-	-	-	?	Asc	Asc	Asc	D																						
		4	-	-	-	-	-	?	-	-	-	D																						
		5	-	-	-	-	-	-	-	-	-	D																						
	10 ⁻⁵	1	-	-	-	-	Asc	Asc	Asc	Asc	Asc	D	Toxo present w/ lung																					
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	10 ⁻⁶	1	-	-	-	-	?	?	-	-	-	-	D	Toxo present w/ lung																				
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Saline Control
2% PSS
CONTROL

Note
w/ serum
leaking

Saline
Control
2% RSS
CONTROL

Note

und.
serum
lackm

10 ⁻³	1	-	-	-	-	?	Asc	Asc	Asc	D	Toxo present in lung.										5/5		
	2	-	-	-	-	-	Asc	Asc	Asc	D													
	3	-	-	-	-	-	Asc	Asc	Asc	D													
	4	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	D											
	5	-	-	-	-	-	?	-	Asc	Asc	Asc	D											
10 ⁻⁴	1	-	-	-	-	?	Asc	Asc	Asc	D	Toxo present w/ lung										5/5		
	2	-	-	-	-	-	Asc	Asc	Asc	D													
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	5	-	-	-	-	-	?	Asc	Asc	Asc	Asc	D											
10 ⁻⁵	1	-	-	-	-	-	Asc	Asc	Asc	D	(chewed - only skin (sft.))										5/5		
	2	-	-	-	-	-	?	-	Asc	Asc	D	Toxo present w/ lung											
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Neg. neut.
Alg. in vitro★ dead on 22nd day: - Toxo present w/ lung

2 HRS. AT 37°C

[illegible]

TOXO. MOUSE NEUT.

MAY 13 1948

0.1cc IP

290 RSS DILUENT

2 HRS. AT 37°C

[illegible]

TOXO. MOUSE NEUT.

MAY 13 1948

0.1 cc IP

270 RSS. DILUENT

2 HRS @ 37°C

Serum	FINAL Dilution	# M C E	Day After Inoculation																					Mort	LD ₅₀	Neut Index
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
"G"	10 ⁻³	1	-	-	-	-	-	?	Asc	Asc	Asc	D														
		2	-	-	-	-	-	-	Asc	Asc	Asc	D														
		3	-	-	-	-	-	-	-	Asc	Asc	D														
		4	-	-	-	-	-	-	-	-	-	Asc	Asc	D	Toxo present w lung											
		5	-	-	-	-	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
"G"	10 ⁻⁴	1	-	-	-	D	No tws.																			
		2	-	-	-	-	-	-	Asc	Asc	Asc	D														
		3	-	-	-	-	-	-	Asc	Asc	Asc	D	Toxo													
		4	-	-	-	-	-	-	-	-	-	Asc	Asc	D	Toxo present w lung											
		5	-	-	-	-	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
NEG. RAB.	10 ⁻⁵	1	-	-	-	-	-	-	-	-	-	x	D	Toxo present w lung												
		2	-	-	-	-	-	-	-	-	-	x	Asc	D	"	"	"	"	"	"	"	"	"	"		
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
"G"	10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

NEG. RAB.

"H"	10 ⁻³	1	-	-	-	-	-	-	Asc	Asc	D	Toxo present w lung	5/5	
		2	-	-	-	-	-	-	Asc	Asc	D			
		3	-	-	-	-	-	-	-	-	D			
		4	-	-	-	-	-	-	-	-	Asc D			
		5	-	-	-	-	-	-	-	-	Asc Asc D			
"H"	10 ⁻⁴	1	-	-	-	-	-	-	x	D	Toxo present w lung			
		2	-	-	-	-	-	-	-	-		Asc Asc		
		3	-	-	-	-	-	-	-	-		x		
		4	-	-	-	-	-	-	-	-		x		
		5	-	-	-	-	-	-	-	-		-		
Rabbit #365	10 ⁻⁵	1	-	-	-	-	-	-	x	D	Toxo present w lung			
		2	-	-	-	-	-	-	-	-		-		
		3	-	-	-	-	-	-	-	-		-		
		4	-	-	-	-	-	-	-	-		-		
		5	-	-	-	-	-	-	-	-		-		
NOT REACTI- VATED.	10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	-	
		2	-	-	-	-	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-	-	-	-	-	-
		4	-	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-	-

5/5

MAY 13 1948

2% RSS Deficient

2 HPS @ 3700

REACTIVATED
IN VITRO
TEST.

NOT REACTIVE
VARIABLE

Toxoplasma present in lung smear.

Nature of Limiting Factor in Effectiveness of Neutralizing Antibody Upon Dilution of Serum (Mouse Toxo. Neut.)

Toxoplasma: PE pooled from 4, four day mice (IP pan of 15 May). Immediately dil to 10^{-2} with saline & counted in chamber = 23, 28, 31, 34 = 29,000,000/cc.

Test Sera: a) Human immune = Feldman (from def. bl. of 18 May 48)
b) Human inactivated = Ruescher (from def. bl. of 17 May 48)

Dilutions: All heated sera inactivated for 30' @ 56°C just prior to test. Both fresh & heated sera diluted as indicated & stored in refrigerator until used about 90' later.

All toxo dilutions made = 2% RSS in small flasks immediately after initial count. First dilution = 1 cc PE + 4.0 cc 2% RSS, then ten-fold dilutions (0.6 + 5.4 cc). Immediately after completing dilutions, 0.3 cc amounts distributed to appropriate tubes.

Test: 0.3 cc of toxo dil + 0.3 cc serum mixed, incubated for 2 hrs. @ 37°C (H_2O bath) then each placed in ice-water bath of 0.1 cc amts inoc. IP into groups of 5 Max 10 gm mice.

Final Counts: The 1:50 dil was diluted 1:1 in NaCl after one hour in refrigerator & this 1/100 dilution counted in chamber = 24, 22, 18, 27 = 23 toxo/mm² = 23,000,000 toxo/cc undil.

Time schedule: PE obtained @ 11⁰⁵ a.m. Count completed @ 11²⁰ a.m.

Dilutions & toxo-serum mixtures begun immediately thereafter. Test into 37°C H_2O bath @ 12³⁵ p.m. Out @ 2³⁵ p.m. Inoc. begun immediately thereafter. Inoc. completed @ 5⁰⁰ p.m.

(OVER)

MAY 19 1948

Mixtures, dilutions, identification:

- A = undil rabbit (misc.) serum control.
B = human immune [redacted] undiluted & unheated.
C = [redacted] unheated 1:4 in NaCl
D = " " 1:16 " "
E = " " 1:64 " "
F = " " 1:4 in human activated (Buescher)
G = " " 1:16 " " "
H = " " 1:64 " " "
I = human active [redacted] undiluted & unheated.
K = [redacted] undiluted & heated 56°C for 30'.
L = " , heated, 1:4 in fresh Buescher serum
M = " " 1:16 " " " "
N = " " 1:64 " " " "
O = " " 1:4 " Buescher heated 56°C for 30'.

Inoculation Order: From A $\rightarrow$ O

There is also something dead
wrong in this test

MAY 19 1948

SUMMARY TOXOPLASMA MOUSE NEUTRALIZATION TESTS

TO DETERMINE NATURE OF LIMITING FACTOR IN EFFECTIVENESS
OF NEUTRALIZING ANTIBODY UPON DILUTION OF SERUM.

INOC: 0.1 cc IPMICE: MAX 10 gms.DILUENT: 2% RSSINCUBATION: 2 HRS. @ 37° C.

CODE	SERUM			FINAL DILUTION						LD ₅₀	NEUT. INDEX
	SOURCE	DILUTION	DILUENT	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷		
A	RAB. SER. (H)	UNDIL.	—	—	—					6.4	
B	██████ (F)	UNDIL.	—						—	4.2	
C	██████ (F)	1:4	NaCl							4.6	
D	██████ (F)	1:16	NaCl							4.6	
E	██████ (F)	1:64	NaCl							5.3	
F	██████ (F)	1:4	██████							4.5	
G	██████	1:16	██████							4.7	
H	██████	1:64	██████							4.5	
I	██████	UNDIL.	—							5.5	
K	██████	UNDIL.	—	—	—					5.2	
L	██████	1:4	██████							4.4	
M	██████	1:16	██████							4.4	
N	██████	1:64	██████							3.8	
O	██████	1:4	██████	—	—					5.5	

F = FRESH

H = HEATED 56° C for 30'

SERUM:

① — HUMAN IMMUNE
 ② — HUMAN ACTIVATOR

TOXO. NEUT.

MAY 19 1948

0.1cc IP

2 HRS. @ 37°C

290 RSS DILUENT

Strain	Dilution	# Mice	Day After Inoculation																					Mort.	LD ₅₀	Nesb. Index
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
A	10 ⁻⁴	1	-	-	-	✓	-	Asc	Asc	Asc	D	Toxo present in lung														
		2	-	-	-	✓	-	Asc	Asc	Asc	D															
		3	-	-	-	-	-	Asc	Asc	Asc	D															
		4	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc			
		5	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
	10 ⁻⁵	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung														
		2	-	-	-	-	-	Asc	Asc	Asc	D															
		3	-	-	-	-	-	-	Asc	Asc	Asc	D														
		4	-	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
		5	-	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		
	10 ⁻⁶	1	-	-	-	-	-	-	Asc	Asc	Asc	D														
		2	-	-	-	-	-	-	Asc	Asc	Asc	D														
		3	-	-	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung												
		4	-	-	-	-	-	-	-	Asc	Asc	Asc	D													
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

UNDIL. RS CONTROL

TOXO. NEUT.

MAY 19 1948

0.1cc IP 290R55 DILUENT

2 HRR @ 37°C

[illegible]

0.1cc IP

2% RSS DILUENT

2 HRS @ 39°C

Serum	Dilution	# M E	Day After Inoculation																					Mort.	LD ₅₀	Nest Index
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
C (con't)	10 ⁻⁵	1	-	-	-	-	-	-	-	-	-	-	-	D	✓	Toxopresent in lung										
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
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D	10 ⁻²	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
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	10 ⁻³	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
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	10 ⁻⁴	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
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		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	10 ⁻⁵	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
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		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

MEMORANDUM

0.1 cc IP 2% RSS DILUENT

2 HRS @ 37°C

Sera	Dilution	Day After Inoculation																					Mort	LD ₅₀	Neut Index
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
D (cont'd)	10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	0.23	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

E	10 ⁻² 23,000	1	-	-	-	-	Asc	Asc	Asc	D	Toxo present w/ lung											5/5					
		2	-	-	-	-	Asc	Asc	Asc	Asc	D																
		3	-	-	-	-	Asc	Asc	Asc	Asc	D																
		4	-	-	-	-	Asc	Asc	Asc	Asc	Asc	D															
		5	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	10 ⁻³ 2,300	1	-	-	-	-	Asc	Asc	Asc	Asc	D	Toxo present w/ lung															
		2	-	-	-	-	Asc	Asc	Asc	Asc	D																
		3	-	-	-	-	Asc	Asc	Asc	Asc	D																
		4	-	-	-	-	Asc	Asc	Asc	Asc	D																
		5	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc						
	10 ⁻⁴ 230	1	-	-	-	-	Asc	Asc	Asc	Asc	D											5/5					
		2	-	-	-	-	Asc	Asc	Asc	Asc	D																
		3	-	-	-	-	Asc	Asc	Asc	Asc	D																
		4	-	-	-	-	Asc	Asc	Asc	Asc	D																
		5	-	-	-	-	Asc	Asc	Asc	Asc	D																
	10 ⁻⁵ 23	1	-	-	-	-	Asc	Asc	Asc	Asc	D	Toxo present w/ lung															
		2	-	-	-	-	Asc	Asc	Asc	Asc	D																
		3	-	-	-	-	Asc	Asc	Asc	Asc	Asc	D															
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
10 ⁻⁶ 2.3	1	-	-	-	-	-	X	-	-	-	D	Toxo present w/ lung															
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
10 ⁻⁷ 0.23	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							

Toxo. NEUT.

MAY 19 1948

0.1 cc IP

2% RSS DILUENT

2 HRS @ 37°C

Serial	Dose (cc)	# Mice 6 E	Day After Inoculation																					Mort	LD ₅₀	Survival Index
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
F	10 ⁻²	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung														
		2	-	-	-	-	-	Asc	Asc	Asc	D															
		3	-	-	-	-	-	Asc	Asc	Asc	D															
		4	-	-	-	-	-	Asc	Asc	Asc	Asc															
		5	-	-	-	-	-	-	-	-	Asc	Asc Asc Asc - - - - -														
23,000	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung															
	2	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	3	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	4	-	-	-	-	-	Asc	Asc	Asc	D																
	5	-	-	-	-	-	-	-	-	Asc	Asc	Asc D.														
10 ⁻³	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung															
	2	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	3	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	4	-	-	-	-	-	Asc	Asc	Asc	D																
	5	-	-	-	-	-	-	-	-	Asc	Asc	Asc D.														
2,300	1	-	-	-	-	-	Asc	Asc	D	Toxo present in lung																
	2	-	-	-	-	-	Asc	Asc	D																	
	3	-	-	-	-	-	Asc	Asc	Asc D																	
	4	-	-	-	-	-	Asc																			
	5	-	-	-	-	-	-	-	-	-																
10 ⁻⁴	1	-	-	-	-	-	Asc	Asc	D	Toxo present in lung																
	2	-	-	-	-	-	Asc	Asc	D																	
	3	-	-	-	-	-	Asc	Asc	Asc D																	
	4	-	-	-	-	-	Asc																			
	5	-	-	-	-	-	-	-	-	-																
230	1	-	-	-	-	-	Asc	Asc	D	Toxo present in lung																
	2	-	-	-	-	-	Asc	Asc	D																	
	3	-	-	-	-	-	Asc	Asc	D																	
	4	-	-	-	-	-	Asc																			
	5	-	-	-	-	-	-	-	-	-																
10 ⁻⁵	1	-	-	-	-	-	Asc	Asc	D	Toxo present in lung																
	2	-	-	-	-	-	Asc	Asc	D																	
	3	-	-	-	-	-	Asc	Asc	D																	
	4	-	-	-	-	-	Asc																			
	5	-	-	-	-	-	-	-	-	-																
23	1	-	-	-	-	-	Asc	Asc	D	Toxo present in lung																
	2	-	-	-	-	-	Asc	Asc	D																	
	3	-	-	-	-	-	Asc	Asc	D																	
	4	-	-	-	-	-	Asc																			
	5	-	-	-	-	-	-	-	-	-																
10 ⁻⁶	1	-	-	-	-	-	Asc	Asc	D	Toxo present in lung																
	2	-	-	-	-	-	Asc	Asc	D																	
	3	-	-	-	-	-	Asc	Asc	D																	
	4	-	-	-	-	-	Asc																			
	5	-	-	-	-	-	-	-	-	-																
2.3	1	-	-	-	-	-	Asc	Asc	D	Toxo present in lung																
	2	-	-	-	-	-	Asc	Asc	D																	
	3	-	-	-	-	-	Asc	Asc	D																	
	4	-	-	-	-	-	Asc																			
	5	-	-	-	-	-	-	-	-	-																
10 ⁻⁷	1	-	-	-	-	-	Asc	Asc	D	No toxo seen.																
	2	-	-	-	-	-	Asc	Asc	D																	
	3	-	-	-	-	-	Asc	Asc	D																	
	4	-	-	-	-	-	Asc																			
	5	-	-	-	-	-	-	-	-	-																
0.23	1	-	-	-	-	-	Asc	Asc	D	Toxo present in lung																
	2	-	-	-	-	-	Asc	Asc	D																	
	3	-	-	-	-	-	Asc	Asc	D																	
	4	-	-	-	-	-	Asc	Asc	D																	
	5	-	-	-	-	-	-	-	-	-																
G	10 ⁻²	1	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung															
		2	-	-	-	-	Asc	Asc	Asc	D																
		3	-	-	-	-	Asc	Asc	Asc	D																
		4	-	-	-	-	Asc	Asc	Asc	Asc	D															
		5	-	-	-	-	-	Asc	Asc	Asc	Asc	D														
23,000	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung															
	2	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	3	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	4	-	-	-	-	-	-	-	Asc	Asc	D															
	5	-	-	-	-	-	-	-	-	Asc	D															
10 ⁻³	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung															
	2	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	3	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	4	-	-	-	-	-	-	-	Asc	Asc	D															
	5	-	-	-	-	-	-	-	-	Asc	D															
2,300	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung															
	2	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	3	-	-	-	-	-	Asc	Asc	Asc	Asc	D															
	4	-	-	-	-	-	-	-	Asc	Asc	D															
	5	-	-	-	-	-	-	-	-	Asc	D															

Toxo. NEUT.

0.1 cc IP

2% RSS DILUENT

2 HRS @ 37°C

MAY 19 1948

Strain	Dilution	H M C E	Day After Inoculation																					Mort	Liso	Neut Index
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
"G" (em'4)	10 ⁻⁴	1	-	-	-	-	-	-	Asc	Asc	D	Toxo present in lung														
		2	-	-	-	-	-	-	-	Asc	Asc	D	Toxo present in lung													
		3	-	-	-	-	-	-	-	x	Asc	D	Toxo present in lung													
		4	-	-	-	-	-	-	-	x	Asc	D	Toxo present in lung													
		5	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
	10 ⁻⁵	1	-	-	-	-	-	-	-	x	-	-	Toxo present in lung													
		2	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		3	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		4	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		5	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
	10 ⁻⁶	1	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		2	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		3	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		4	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		5	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
	10 ⁻⁷	1	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		2	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		3	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		4	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		5	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
"H"	10 ⁻²	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung														
		2	-	-	-	-	-	Asc	Asc	Asc	Asc	D	Toxo present in lung													
		3	-	-	-	-	-	Asc	Asc	Asc	Asc	D	Toxo present in lung													
		4	-	-	-	-	-	Asc	Asc	Asc	Asc	D	Toxo present in lung													
		5	-	-	-	-	-	-	-	Asc	Asc	D	Toxo present in lung													
	10 ⁻³	1	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung														
		2	-	-	-	-	-	Asc	Asc	Asc	Asc	D	Toxo present in lung													
		3	-	-	-	-	-	Asc	Asc	Asc	Asc	D	Toxo present in lung													
		4	-	-	-	-	-	-	-	Asc	Asc	D	Toxo present in lung													
		5	-	-	-	-	-	-	-	Asc	Asc	D	Toxo present in lung													
	10 ⁻⁴	1	-	-	-	-	-	-	Asc	Asc	D	Toxo present in lung														
		2	-	-	-	-	-	-	Asc	Asc	D	Toxo present in lung														
		3	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		4	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		5	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
	10 ⁻⁵	1	-	-	-	-	-	-	-	x	-	-	Toxo present in lung													
		2	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		3	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		4	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													
		5	-	-	-	-	-	-	-	-	-	-	Toxo present in lung													

5/5

TOXO. NEUT.

MAY 19 1948

0.1cc IP

2% RES DILUENT

2 hrs @ 37°C

Serum	Dilution	# M C	Day After Inoculation																				Mort	LD ₅₀	Neut Index
			E	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19			
H (cont)	10 ⁻⁶	1	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
		2	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
		3	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
		4	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
		5	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
	2.3	1	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
		2	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
		3	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
		4	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
		5	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		
10 ⁻⁷	1	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-			
	2	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-			
	3	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-			
	4	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-			
	5	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-			
0.23	1	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-			
	2	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-		-	-	-			

Conc.	1	2	3	4	5	Notes
10^{-2}	1	-	-	-	-	asc asc D Toxo present in lung
	2	-	-	-	-	asc asc D
	3	-	-	-	-	asc asc D
	4	-	-	-	-	asc asc D
	5	-	-	-	-	asc asc asc asc D
23,000						
10^{-3}	1	-	-	-	-	asc asc D Toxo present in lung
	2	-	-	-	-	asc asc asc D
	3	-	-	-	-	asc asc asc D
	4	-	-	-	-	asc asc asc asc D
	5	-	-	-	-	asc asc asc asc asc D
2300						
10^{-4}	1	-	-	-	-	asc asc D Toxo present in lung
	2	-	-	-	-	asc asc asc D
	3	-	-	-	-	asc asc asc D
	4	-	-	-	-	asc asc asc asc D
	5	-	-	-	-	asc asc asc asc asc D
230						
10^{-5}	1	-	-	-	-	asc asc D Toxo present in lung
	2	-	-	-	-	asc asc asc D
	3	-	-	-	-	asc asc asc D
	4	-	-	-	-	asc asc asc asc D
	5	-	-	-	-	asc asc asc asc asc D
23						
10^{-6}	1	-	-	-	-	No Toxo seen in lung smear.
	2	-	-	-	-	D
	3	-	-	-	-	D
	4	-	-	-	-	D
	5	-	-	-	-	D ✓ Toxo present in lung
2.3						
10^{-7}	1	-	-	-	-	-
	2	-	-	-	-	-
	3	-	-	-	-	-
	4	-	-	-	-	-
	5	-	-	-	-	-
0.23						

TOXO. NEUT.

MAY 19 1948

0.1 cc IP

2% RES DILUENT

2 HRS @ 37°C

Serum	Dilution	# of 6 E	Day After Inoculation																					Mort	LD ₅₀	Neut Index
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
"K"	10 ⁻⁴	1	-	-	-	-	-	-	Asc	Asc	D	100% present in lung.											5/5			
		2	-	-	-	-	-	-	Asc	Asc	D															
		3	-	-	-	-	-	-	Asc	Asc	D															
		4	-	-	-	-	-	-	Asc	Asc	D															
		5	-	-	-	-	-	-	Asc	Asc	Asc	D														
	10 ⁻⁵	1	-	-	-	-	-	-	D	No toxo seen																
		2	-	-	-	-	-	-	Asc	Asc	Asc	D	100% present in lung													
		3	-	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	D	Toxo present										
		4	-	-	-	-	-	-	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc	Asc		Asc	Asc		Asc	
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"L"	10 ⁻²	1	-	-	-	-	-	-	Asc	Asc	Asc	D	Toxo present in lung.											5/5		
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	10 ⁻³	1	-	-	-	-	-	-	-	Asc	Asc	D	Toxo present in lung											5/5		
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Тожд. нечт.

MAY 19 1948

0.1cc IP

29. RSS DILUENT

2 HRS @ 3700

Serum	Dilution	# M C E	Day After Inoculation																					Mort	LD ₅₀	Neut index
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[illegible]

Toxo. NEUT.

MAY 19 1948

0.1cc IP

2% RSS DILUENT

2 HRS @ 37°C

Sera	Dilution	# 1 2 3 4 5	Day After Inoculation																					Mort.	LD ₅₀	Neut Index																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
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* dead 24th day - no toxo seed.

(over)

Toxo. NEUT.

MAY 19 1948

once IP

2% RSS D, LUENT

2 HRS @ 370 €

Strain	Dilution	# MICE	Day After Inoculation																					Mort	LD ₅₀	Nesb Index
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
"O" (Con't)	10 ⁻⁶ 2.3	1	-	-	-		-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-			
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	10 ⁻⁷ 0.23	1	-	-	-		-	-	-	-	-				-	-	-	-	-	-	-	-	-			
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