

TOXO.
RABBIT
NEUTS.

JAN 9 1948

TOXO PLASMA NEUTRALIZATION TEST
(RABBIT)

TOXO PLASMA: 5 mice of Pan 362 sacrificed, all in good signs. Each brain smeared & stained & the four hemat infected pooled, ground & suspended in 16 cc Tyrodes. Allowed to settle for ca 15' & then 8 cc supernate withdrawn & used as the 1:10 susp.

Dilutions: Made in small flasks in Tyrodes as diluent. These made as follows:
(supernate)
1:10, 1:50, 1:500, 1:5000.

Procedure: Dilution of tox distributed to appropriate small dilution tubes in 0.15 cc amounts/tube. To these then added 0.15 cc of the appropriate serum. Well mixed & allowed to stand 30' @ room temp. Each set then inoc in 0.2 cc amounts intracutaneously into prepared, clean-shaven rabbits (Maxfield). Final tox concentrations = 1:20, 1:100, 1:1000, 1:10,000.

Rabbits numbered: 353, 354, 355, 356, 357, 358, 359.

Serial: Conducted at Eden Park, lab journal (ab initio pos & neg) & clinicals. See rabbit protocols for exact data.

TOXO. NEUT.

RAB # 353

JAN 9 1948

HEAD ↑

TOXO. DIL. (FINAL)

SERUM	DATE	DAY	DIS.	DAY	1:20	1:100	1:1000	1:10,000	
				13 JAN	PAP 10X10	SL. PAP - 5X5	—	—	
	24 July	22							PT.
				JAN 16	+++ N	+++ N	+	—	Equm
				13 JAN	PAP 10X10	PAP 10X10	JFT ERY.	—	
	25 Aug	55							Neg
				JAN 16	+++ N	+++ N	++ N	—	
				13 JAN	PAP 10X10	PAP 10X10	FT ERY 4mm.	ERY - 8x8	
TYRODE CONTROL	—	—							
				JAN 16	+++ N	+++ N	++ N	++ N	
				13 JAN	PAP 10X10	PAP 10X10	FT ERY	ERY - 8x8	
	29 Dec	184							Neg
				JAN 16	+++ N	+++ N	++ N	++ N	
				13 JAN	PAP 10X10	PAP 10X10	FT ERY	FT ERY	
	25 Aug	55							Neg
				JAN 16	+++ N	+++ N	++ N	+	

TOXO. NEUT.

PAB. # 354

JAN 9 1948

HEAD ↑

TOXO. DIL. (FINAL DIL.)

SERUM	DATE	Day Dis.	DAY	1:20	1:100	1:1000	1:10,000	
			4 th	PAP 10x10	PAP 10x10	PAP 5x5	-	
	6 Aug. 38							PT.
			JAN 16	+++ N	++ N	++ N	++ N	Neg
			4 th	PAP 10x10	ERY 5x5	-	-	
	25 Aug. 55							Equiv
			JAN 16	+++ N	++ N	±	-	
			4 th	PAP ^{SL NO. 12} 12x12	PAP 10x10	PAP 10x10	-	
TYRODE CONTROL	-	-						
			JAN 16	+++ N	+++ N	++ N	++ N	
			4 th	PAP 10x10	ERY 5x5	-	-	
	25 Aug. 53							
			JAN 16	+++ N	++ N	-	-	Pos.
			4 th	PAP 10x10	ERY 5x5	-	-	
			JAN 1	+++ N	++	-	-	Pos

TOXO. NEUT.

PAB. # 355

JAN 9 1948

HEAD. ↑

TOXO. DIL. (FINAL)

SERUM.	DATE	Day Dis.	1:20	1:100	1:1000	1:10,000	
		4 th	PAP 10x10	PAP 5x5	ERY 5x5	—	
		—					
LT.		JAN 16	+++ N	++	—	—	PT. Pos? Equiv
		4 th	PAP 7x7	PAP 6x6	ERY 5x5	—	
		—					
		JAN 16	++ N	+	—	—	Pos
		4 th	PAP 15x15 ^{NEC.}	PAP 10x10	ERY 5x5	—	
TYRODE CONTROL		—					
		JAN 16	+++ N	+++ N	++ N	±	
		4 th	PAP 6x6	ERY 5x5	ERY 5x5 (LEFT NECR.)	ERY 5x5	
	8 JAN.	—					
		JAN 16	++ N	—	—	—	Pos.
		4 th	PAP 6x6	ERY 5x5	ERY 5x5	ERY 5x5	
	6 JAN	—					
		JAN 16	++ N	—	—	—	Pos

JAN 17 1948

Rel. dead.

TOXO. NEUT.

PAB #356

JAN 9 1948

HEAD 7

TOXO. Dil. (Final)

SERUM

DATE

DAY
DIS.

JAY

1:20

1:100

1:1000

1:10,000

4th

PAP 12x12

PAP 8x8

ERY 5x5

—

24 Dec —

JAN 16 ~~+++~~
N

+++
N

++
N

+

Neg
RT.

4th

PAP 5x5

ERY - 2x2

—

—

24 Dec —

JAN 16 ++

++

?

—

Equiv

4th

PAP 10x10

PAP 8x8

ERY 5x5

—

TYRODE
CONTROL

—

JAN 16 ~~+++~~
N

+++
N

++
N

—

4th

PAP 11x11

PAP 8x8

ERY 5x5

—

—

JAN 16 ~~+++~~
N

+++
N

++
N

±

Neg

4th

PAP 10x10

PAP 8x8

ERY 5x5

—

—

JAN 16 ~~+++~~
N

+++
N

++
N

±

Neg

JAN 9 1948

TOXO. NEUT.

RAB. # 357

HEAD A

TOXO. DIL. (FINAL)

SERUM DATE

DAY
DIS.

DAY 1:20

1:100

1:1000

1:10,000

4th PAP 12X12

PAP 10X10

FT ERY

—

JAN 16 ~~+++~~
N~~+++~~
N~~+++~~
N~~+++~~
N

Neg.

4th PAP 12X12

PAP 8X8

FT ERY

—

RT.

JAN 16 ~~+++~~
N~~+++~~
N~~+++~~
N~~+++~~
N

Neg.

4th PAP 12X12

PAP 10X10

FT ERY

—

TYRODE
CONTROL.JAN 16 ~~+++~~
N~~+++~~
N~~+++~~
N~~+++~~
N4th PAP 12X12

PAP 10X10

FT ERY

—

22 Aug.

JAN 16 ~~+++~~
N~~+++~~
N~~+++~~
N~~+++~~
N

Neg.

4th PAP 12X12

PAP 10X10

FT ERY

—

9 Sept.

JAN 16 ~~+++~~
N~~+++~~
N~~+++~~
N~~+++~~
N

Neg.

JAN 17 1948

Rab. Dead.

TOXO. NEUT.

PAB. # 358

JAN 9 1948

HEAD 9

SERUM	DATE	DAY Dis.	TOXO. DIL. (FINAL)					
			DAY	1:20	1:100	1:1,000		1:10,000
			4 th	PAP 10X10	FT. PAP 5X5	ERY	—	
LT. [REDACTED]	25 Aug 57							RT.
			JAN 16	+++ N	+++ N	++ N	+	Neg
			4 th	PAP 10X10	FT PAP 5X5	ERY	—	
[REDACTED]	25 Aug 58							
			JAN 16	+++ N	+++ N	++ N	+	Neg
			4 th	PAP 10X10	PAP 10X10	FT. PAP 5X5	—	
TYRODE CONT.	—	—						
			JAN 16	+++ N	+++ N	+++ N	++ N	
			4 th	PAP 10X10	—	ERY	—	
[REDACTED]	25 Aug 57							
			JAN 16	+++ N	++ N	+	—	Eggs

JAN 17 1948

Rob. Dead.

Toxo. NEUT.

PAB. # 359

JAN 9 1948

HEAD A

SERUM	DATE	DAY DIS	Toxo. Dil. (FINAL)				
			1:20	1:100	1:1000	1:10,000	
		4 th	PAP 10X10	PAP 8X8	—	—	
	26 DEC.	—					
		JAN 16	+++ N	+++ N	—	—	Pos.
		4 th	PAP 8X8	FT PAP	—	ERY 5X5	
	6 JAN.	—					
		JAN 16	+++ N	+	—	—	Pos
		4 th	PAP 12X12	PAP 10X10	ERY 10X10	—	
TYRODE CONTROL	—	—					
		JAN 16	+++ N	+++ N	+++ N	—	
		4 th	PAP 10X10	PAP 10X10	ERY 5X5	—	
	8 JAN.	—					
		JAN 16	+++ N	+++ N	+++ N	± Pap	Neg
		4 th	PAP 8X8	VFF ERY	—	—	
	3 DEC.	—					
		JAN 16	+++ N	?	—	—	Pos

JAN 26 1948

TOXO PLASMA NEUTRALIZATION TEST - RABBIT

TOXOPLASMA: 2 mouse brains (Pan 366 - 6th day) pooled & suspended in 8.0 cc Tyrodes. Allowed to settle for 15 min. in ref'g. Then ~~4.2~~ 4.2 cc super removed & used as 1:10. These brains were very heavily infected (severe).

DILUTIONS: All made in Tyrodes from the 1:10 tube. $\rightarrow$ 1/50, 1/500, 1/5000.

PROCEDURE: Dilutions distributed to appropriate small tubes in 0.15 cc amounts. To these added 0.15 cc of appropriate sera, well-mixed, & after 30' incubation @ room temp. inoc. in 0.2 cc amounts into skins of rabbits. Inoc. time = 75 min. Tyrode control = low dil + equal amt Tyrode's sol. Final concentrations = 1:20, 10^{-2} , 10^{-3} , 10^{-4} .

Rabbits numbered: 394, 395, 396.

Sera: Clinicals & repeat Eden Park. See rabbit protocols & summary ~~sheet~~ table.

JAN 26 1948

TOXO. NEUT.

PAB. # 394

HEAD A

SERUM	DATE	DAY DIS.	TOXO. DIL. (FINAL)				
			1:20	1:100	1:1000	1:10000	
LT [REDACTED]	25 Jan		JAN 29 — JAN 31 PAP 5x5	JAN 29 — JAN 31 PAP 3x3	JAN 29 — JAN 31 —	JAN 29 — JAN 31 —	RT.
			+++	++	+	—	±
[REDACTED]	29 DEC.		JAN 29 ERY 3x3 JAN 31 PAP 10x10 NEC.	JAN 29 — JAN 31 PAP 3x3	JAN 29 — JAN 31 —	JAN 29 — JAN 31 —	
			+++	++	—	—	+
Tyrode CONT.			JAN 29 PAP 10x10 JAN 31 PAP 13x13 NEC.	JAN 29 PAP 8x8 JAN 31 PAP 12x12 NEC.	JAN 29 — JAN 31 PAP 8x8	JAN 29 — JAN 31 PAP 3x3	
			+++	+++	+++	+	
[REDACTED]	19 Jan		JAN 29 ERY 3x3 JAN 31 PAP 6x6 NEC.	JAN 29 — JAN 31 PAP 3x3	JAN 29 — JAN 31 —	JAN 29 — JAN 31 —	
			+++	++	—	—	+

DEAD 2/4/48

TOXO. NEG.

RAB. # 395

JAN 26 1948

HEAD ↑

SERUM	DATE	DAY DIS.	TOXO. DIL.			
			1:20	1:100	1:1000	1:10,000
[REDACTED]	29 DEC		JAN 29 —	JAN 29 ERY	JAN 29 ERY	JAN 29 ERY
			JAN 31 PAP 5X5	JAN 31 ERY	JAN 31 —	JAN 31 —
[REDACTED]	—	—	JAN 29 —	JAN 29 ERY	JAN 29 ERY	JAN 29 ERY
			JAN 31 —	JAN 31 —	JAN 31 —	JAN 31 —
Tyrode CONTROL	—	—	JAN 29 PAP 10X10	JAN 29 PAP 5X5	JAN 29 ERY	JAN 29 ERY
			JAN 31 PAP 12X12	JAN 31 PAP 8X8	JAN 31 —	JAN 31 —
[REDACTED]	25 Aug		JAN 29 ERY	JAN 29 ERY	JAN 29 ERY	JAN 29 ERY
			JAN 31 PAP 7X7	JAN 31 ERY 5X5	JAN 31 ERY	JAN 31 ERY
[REDACTED]	29 DEC.		JAN 29 ERY	JAN 29 ERY	JAN 29 ERY	JAN 29 ERY
			JAN 31 PAP 5X5	JAN 31 ERY 5X5	JAN 31 ERY	JAN 31 ERY

DEAD 2/4/48

JAN 26 1948

TOXO. NEUT.

PAB # 396

HEAD ↑

TOXO. DIL. (FINAL)

SERUM	DATE	Day Dis.	1:20	1:100	1:1000	1:10,000	
[REDACTED]	25 Aug		JAN 29 —	JAN 29 —	JAN 29 —	JAN 29 —	RF. 7
			JAN 31 PAP 9x9 NEC. 	JAN 31 ERY 3x3 ++	JAN 31 — +	JAN 31 — —	
				++	+	—	
			JAN 29 ERY	JAN 29 —	JAN 29 —	JAN 29 —	
[REDACTED]	29 Dec		JAN 31 PAP 10x10 NEC 	JAN 31 ERY 3x3 ++	JAN 31 — + v.s.	JAN 31 — —	7 Equiv Equiv.
				++	+	—	
			JAN 29 PAP 10x10	JAN 29 PAP 5x5	JAN 29 —	JAN 29 —	
			JAN 31 PAP 14x14 NEC 	JAN 31 PAP 12x12 NEC ++	JAN 31 PAP 4x4 ++	JAN 31 — + v.s.	
Tyrode CONTROL	—	—			++	+	+
			JAN 29 ERY	JAN 29 —	JAN 29 —	JAN 29 —	
			JAN 31 PAP 10x10 NEC 	JAN 31 PAP 3x3 +	JAN 31 — —	JAN 31 — —	
				++	—	—	

Dead Feb. 6

Neutralization Tests in Rabbits

FEB 25 1948

- Questions:
- 1) Can diluted toxo immune human serum (R. Black) which by itself is no longer, be rendered active by being diluted in "activator" (negative human serum - S. Schumacher)
 - 2) Is this "activator" heat-labile as for the in vitro phenomenon?
 - 3) Can human immune toxo serum which has been heated at 56°C be rendered active by the addition of large amount of "activator"?
 - 4) Neutralizing capacity of several sera used in other tests:
Hooper - strong in vitro + in mice; "clinical toxo"
Rainey - mother of proved "congenital toxo"
Gim - craniopharyngioma + cerebral calcific.
Peter Schloss - negative skin test - possible source of human "activator"

TOXOPLASMA USED - Peritoneal exudate obtained at 9:30 AM - kept in refrigerator. Used at 4 P.M. - same exudate used in mouse tests of same day. Dilutions made in 50% heated rabbit serum saline. The 1:10 dilution counted yielded 35 toxo per sq. mm.; $\therefore 35 \times 10 \times 1000 \times 10 = 3,500,000$ toxo per c.c. of exudate.
 $\therefore$ 0.2 cc of 1:20 has 35,000 toxo
" of 1:100 " 7,000 "
" " 1:1000 " 700 "
" " 1:10,000 " 70 "

For Rabbit 360 -

B [redacted] fresh
C [redacted] 1:5 in saline
D [redacted] Heated rabbit serum control
E [redacted] 1:5 in SS fresh
F [redacted] 1:5 in SS heated

head
1:20, 100, 1:1000, 1:10,000
(left to right)
tail

For Rabbit 361 -

G [redacted] heated 1:5 in SS fresh
H [redacted] " " " 1:5 in SS heated
I [redacted] Heated rabbit serum control
J [redacted]
K [redacted]

← 1:100, 1:20, 1:1000, 1:10,000
(this order by mistake for indicated mixture)
All others as on Rabbit 360

Note - [redacted] serum was obtained fresh to-day.
Portion inactivated at 56°C for 30 min to-day

Mixtures - 0.2 cc of indicated sera + 0.2 cc of 1:10, 1:50, 1:500, 1:5000
dilutions in rabbit serum-saline; Mixtures 4:32 4:45 P.M. inoculated in 1/2 hour

FEB 25 1948

Examination In Vitro (Cover-Slip + Methylene Blue)

of Mixtures Used for Inoculation of Rabbits

The 1:20 tubes which had been at room temperature for 1 hour (4:45 - 5:45 PM) were put in water bath at 37°C for an additional hour. 0.02cc + 2 Loops (5mm.) of methylene blue (pH 11)
Examined in order listed

Toxo 1:10 in heated 50% rabbit serum saline plus

A. [redacted] fresh (SS)(2/25)

B. [redacted] fresh 1:5 in saline

C. Undiluted heated rabbit serum

D. [redacted] fresh 1:5 in SS fresh

E. " " " 1:5 in SS heated

G. [redacted]

H. [redacted] 1:5 in SS fresh

I. " " " 1:5 in SS heated

J. [redacted]

K. [redacted]

F. [redacted]

Unstained
No.

Stained
No.

Unstained
%

32

18

64

18

7

72

0

25

0

20

5

80

17

8

68

20

0

100

47

3

94

0

25

0

8

2

80

35

15

70

17

8

68

- This may not have been the picture at time of inoculation after throat room Temp

(Toxo most difficult to find)

These very unexpected results led to various speculations.

- 1) Had [redacted] developed antibody since last bleeding?
- 2) Did [redacted] have antibody despite negative skin test?
- 3) Was to-days exudate unusually susceptible to action of fresh sera but not to heated sera?

OR

- 4) Did the heated rabbit serum contain in its pool the serum from a toxo-immune rabbit which was re-activated by the addition of any of the fresh sera?

The rabbit serum used was put in the refrigerator to be checked the next day. The peritoneal exudate used in the day's tests was also saved.

RABBIT 360

Final dilution of exudate

SERUM

1:20

1:100

1:1000

1:10,000

6

17x15 (N)

15x13 (N)

10x10 (N)

8x7

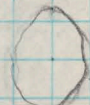
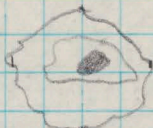
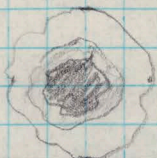
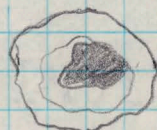
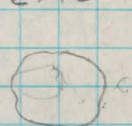
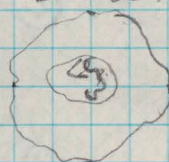
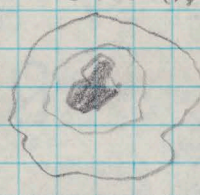
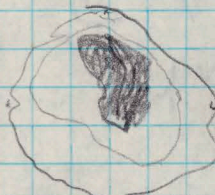
7

20x18 (N)

18x16 (N)

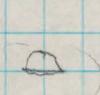
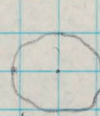
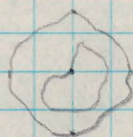
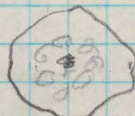
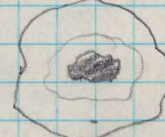
12x10 (N)

12x12

20x18 (N)
18x16 (N)17x17 (N)
18x15 (N)8x7
12x12 (N)—
3x324x23 (N)
27x2525x25 (N)
25x22 (N)20x20 (N)
22x20 (N)5x5
12x1016x16 (N)
17x17 (N)8x7
12x10

— 3x3?

—

17x17 (N)
20x17 (N)18x18 (N)
20x20 (N)14x13 (N)
16x15 (N)— 3x3?
10x10

6

10x8 (pale)

—

—

—

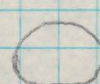
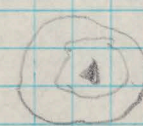
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16x15 (N)

12x8

3x3

—



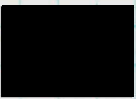
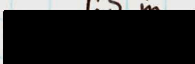
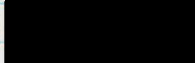
Bled 3-5-48

Dead 3-6-48

Stored 9-3

Rabbit 361

Final dilution of exudate

SERUM		Final dilution of exudate			
		1:20	1:100	1:1000	1:10,000
	6 days	18x17 (N)	15x13 (N)	10x8 (N)	8x5
	7 "	15x14 (N)	15x15 (N)	10x10	8x8
 heated 1:5 in  fresh	6	15x15 (N)	15x15	5x3 ??	?
	7	15x14 (N)	12x12	5x3	
 heated 1:5 in  heated	6	20x20 (N)	20x20 N	17x17 (N)	12x10 N
	7	20x18 (N)	20x18	17x17 (N)	12x10 (N)
Heated Rabbit Serum Control	6	25x22 (N)	23x23 (N)	20x18 (N)	3x3
	7	22x22 (N)	20x20 N	20x17 (N)	5x5
	6	18x18 (N)	13x12	5x4	—
	7	15x13 (N)	12x10	7x7	—
	6	23x23 (N)	20x20 (N)	13x13 (N)	13x10 (N)
	7	20x20	22x21 (N)	16x16 (N)	12x8

Died 3-5-48

Rabbit Neutralization Test.

5 April 48

Toxoplasma: Three mouse brains of I.C. Pass 384-A, containing numerous toxo on smear, ground & suspended in 12 cc 50% RSS (this RSS heated 50°C for 30' just prior to use.). Shortly thereafter filtered thru gauze & this heavy filtrate dil. in 50% RSS to make, in addition to the original 1:10, 1:50, 1:500, 1:5000.

Sera: Those used for previous *in vitro* tests except Brownie's which was freshly drawn (hyp. 1:10000). DeBunsh & Elliot Rg are Rackman's sera.

Test: 0.15 cc serum + 0.15 cc toxo dil. mixed, incubated $\frac{1}{2}$ hr at room temp & then 0.2 cc sm'ts injected into skin of rabbit.

Rabbit: #365 used. Had been neg. on *spectation* *in vitro* test on a previous test. However, rabbit rebled just prior to inoc.

APR 10 1948 Bled from ear for "5 day" serum.

APR 12 1948 Bled from ear for "7 day" serum. Direct smear of ear blood stained & Wright's = positive for toxo (free from cells.) Rabbit died later in the day.

(OVER)

TOXO. NEUT.

RAB. # 365

5 APRIL 48

(HEAD 1)

TOX. DIL. IN 50% PSS (FINAL)

SERUM DATE

1:10000

1:1000

1:100

1:20

ERY

PAP. ERY
NOD. 5X5

PAP 7X7
PAP 10X10 (NEC.)

PAP 8X8
PAP 10X10 (NEC.)

APR 8
APR 10

Apr.

+ PAP 5X5

+ PAP 11X11 (N)

+++ PAP 10X10 (N)

APR 11
AT.

Neg

ERY

- (?)

PAP 3X3

PAP 7X7
PAP 10X10 (NEC.)

PAP 10X10 (N)
PAP 10X10 (NEC.)

APR 8
APR 10

T-PAP 3X3

T-PAP 5X5

+++ PAP 10X10 (N)

+++ PAP 12X12 (N)

APR 11

Neg

ERY

-

PAP 3X3

PAP 7X7
PAP 12X12 (NEC.)

PAP 8X10 (N)
PAP 13X13 (NEC.)

APR 8
APR 10

-

+ PAP 5X5

+++ PAP 10X10 (N)

+++ PAP 15X15 (N)

APR 11

Efu.

ERY

-

PAP(?) 2X2

ERY
PAP 5X5

PAP 6X7
PAP 10X10 (NEC.)

PAP 10X10 (N?)
PAP 16X16 (NEC.)

APR 8
APR 10

+ PAP 6X6

+++ PAP 10X10 (N)

+++ PAP 15X15 (N)

APR 11

Neg

PAP 10X10 (N?)
ERY

ERY
PAP 10X10

PAP 6X8
PAP 15X15 (NEC.)

PAP 10X12 (N)
PAP 16X16 (NEC.)

APR 8
APR 10

CONTROL
50% PSS

-

PAP 10X10
PAP 5X5

+ PAP 6X6 (N)

+++ PAP 15X15 (N)

+++ PAP 15X15 (N)

APR 11

Control

ERY

ERY

-

-

ERY

ERY

-

-

PAP 5X5
+ PAP 5X5

PAP 10X10
PAP 15X15 (NEC.)

APR 8
APR 10

+++ PAP 15X15 (N)

APR 11

Positive

ERY

ERY

-

-

ERY

ERY

-

-

ERY 10X10
PAP 11X11 (NEC.)
+ PAP 10X10 (N)

PAP 10X11
PAP 17X17 (NEC.)
+++ PAP 15X15 (N)

APR 8
APR 10
APR 11

Neg

ERY

ERY

-

-

ERY

ERY

-

-

ERY 10X10
PAP 13X13 (NEC.)
+++ PAP 14X14 (N)

APR 8 PAP 12X10
PAP 15X15 (NEC.)
+++ PAP 15X15 (N)

APR 10
APR 11

Neg

4 FEB.

Neg.					
Neg.					-
Equiv.	-				- sg.
Neg.					-
—					Cont.
++	-	-			+
Neg.					
Neg.					

Direct

Poor unreliable readings

TOXO. TITRATION RABBIT # 363

5 APRIL 48

RABBIT : #363 when tested by the in vitro test was found to have a highly reactantable serum ~~and~~ thus should be immune. Bled just before test.

Toxoplasma: The control dilutions used on Rab #365 were also used. in 0.2 cc amts ID across the back of this rabbit.

Inoc.: 1/20 on right, then 1/100, 1/1000, 1/10,000 on left.

DAY	HEAD ↑ FINAL DILUTIONS (IN SQUARES)				RT.
	1:10,000	1:1000	1:100	1:20	
8 April	Fr. Ery.	ERY	PAP 6x6	PAP 15x12	
10 April	PAP 9x9	PAP 13x13	PAP 20x20 Nec.	PAP 22x22 Nec.	
12 April					

APR 10 1948 Bled from ear for "5 day" serum. Serum stored in CO₂.

APR 13 1948 Bled from ear for "8 day" serum. Rabbit died shortly thereafter following a generalized convulsion.

RABBIT NOT IMMUNE TO TOXO

APR 14 1948

RABBIT NEUTRALIZATION TESTS

Toxo: Two buries of heavily infected mice of Toxo. See Par. 387, suspended in 8.0 cc 50% PSS. Fractured them gently & then diluted to 1/50, 1/500, 1/5000 in 50% PSS.

Sera: all fully thawed & distributed in 0.15 cc units per tube. (See protocol)

Test: 0.15 cc serum + 0.15 cc toxo diluti- mixed in small tubes, incubated $\frac{1}{2}$ hour at room temperature & then 0.2 cc units inoculated into the skins of the rabbits.

Rabbits numbered #362 & #364.

Controls = above two dilutions diluted in an equal amount of 50% PSS.

APR 19 1948

Both rabbits bled from ear veins. Serum stored. Both animals quite ill & lesions well advanced.

APR 20 1948

Both rabbits very ill. #362 jaundiced & in extremis. Lesions blanching & local spread.

APR 21 1948

Both rabbits dead.

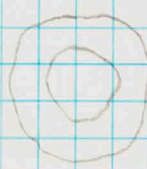
RAB. # 364

APR 14 1948

Serum	Date	Final Toxo. Dilution (in 50% R.S.S.)				
		1/20	1/100	1/1000	1/10,000	
LY. RH. # 142	APR 19 1 MAR	PAP 13x13 (N)	PAP 11x11 (N)	PAP 5x5	ERY	R+
	APR 20					-
RH. # 151	2 MAR.	PAP 13x13 (N)	PAP 10x10 (N)	PAP 3x3	ERY	-
						-
RH. 157	1 MAR	PAP 12x12 (N)	PAP 8x8 (N)	PAP 7x7	PAP?	-
						-
RH. # 158	1 MAR	PAP 16x16 (N)	PAP 12x12 (N)	PAP 4x4	PAP?	-
						-
CONTROL (50% R.S.S.)	-	PAP 15x15 (N)	PAP 13x13 (N)	PAP 11x11	PAP = ERY-small	-
						-
RAB. # 363	4 MAR	PAP 15x15 (N)	PAP 11x11 (N)	PAP 4x4	ERY	+
						+
RAB. # 349	23 DEC.	PAP 9x9 (N)	PAP 3x3	ERY	ERY	+
						+
RAB. # 398	24 DEC.	PAP 7x7 (N)	PAP 2x2	ERY	ERY	+
						+

RAB. #362

APR 14 1948

Serum.	Date	Final Toxo. Dilution				RT
		$\frac{1}{20}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{10,000}$	
LT. [REDACTED]	13 APR. APR 20	PAP 15x15 (M)	PAP 10x10	PAP 5x5	SL. PAP.	-
						-
RH. #152 (PRE-INOC.)	2 MAR.	PAP 15x15 (N)	PAP 10x10	PAP 5x5	PAP 3x3	-
						-
RH. #152 (26 th post. inoc. day)	29 MAR.	PAP 10x10	PAP 3x3	—	—	+
				o?	—	+
CONTROL (50% ASS)	—	PAP 15x15 (N)	PAP 11x11	PAP 2x9	ERY	—
						—
[REDACTED]	0 APRIL	PAP 10x10 (N)	PAP 3x3	—	—	+
					—	+
[REDACTED]	10 APRIL	PAP 10x10 (N)	PAP 3x3	—	—	+
					—	+
SP. "C"	22 FEB.	PAP 12x12 (N)	PAP 10x10	PAP 3x3	PAP ?	—
						—

RABBIT NEUTRALIZATION TEST

Toxo. : Two brains of heavily infected (3rd day) mice of Toxo. Iner.
 Pass. # 397. Suspended in 8.0 cc 2% RSS, immediately filtered
 thru gauze & then diluted in 2% RSS to 1/50, 1/500, 1/5000.

Rabbit : #380 (Mayfield) bled just before test & serum stored.

Sera : Distributed in 0.15 cc amts to each of 4 tubes, immediately after thawing.

A =

B =

C =

D =

E =

} Rec'd from Univ. of Utah, dated 15 April 48

- Rec'd from New Orleans - dated 23 April 48

- bled 13 May 48 - checked skin & in vitro test.

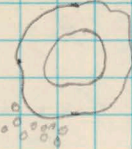
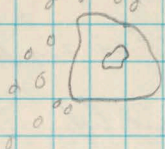
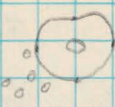
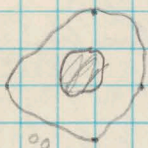
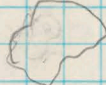
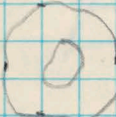
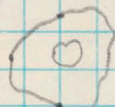
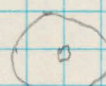
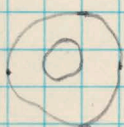
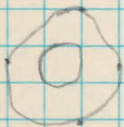
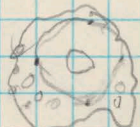
bled 5 April. Repeat check on neg. result of 5 April 48.

Test : 0.15 cc of each toxo dil & 0.15 cc of each serum mix. Ea 30' @ room temp
 & then 0.2 cc of each mix. into skin of Rab. #380. Control = toxo in 2% RSS.
 Final toxo dil. = 1/20, 1/100, 1/1000, 1/10,000

PAB. #380

MAY 14 1948

LT.

SERUM	DATE	FINAL DILUTION				
		1/20	1/100	1/1000	1/10,000	
[REDACTED]	15 Apr '48	PAP(N) 17x17 	PAP 10x10 N? 	PAP? 	—	MAY 18 RT. —
	15 Apr '48	PAP(N) 17x18 	PAP 9x9 	PAP? 	—	MAY 18 Neg
	23 Apr '48	PAP(N) 9x9 	— 	— 	—	MAY 18 +
CONTROL - 2% RSS	—	PAP(N) 17x17 	PAP(N) 14x15 	PAP 6x6 	ERY 	MAY 13
[REDACTED]	13 MAY '48	PAP(N) 17x18 	PAP(N) 9x9 	ERY 	ERY 	MAY 18 Neg
	5 Apr '48	PAP(N) 18x18 	PAP(N) 12x12 	ERY 	ERY 	MAY 18 Neg

MAY 21 1948 Final readings - 7th day

SEP 19 1947

TOXOPLASMA VS. PALUDRINE & SN-11,437

Exp I.

Obj.: To determine whether Paludrine &/or SN-11,437 administered as part of the diet will cure or suppress Toxoplasma infections of albino mice.
- whether Paludrine, intramuscularly, will also produce either effect.

1. Drugs: a) Paludrine - as 0.01% of powdered Laboratory Chow. Sole food source for 14 days beginning at the time of inoculation. Also, as an IM series to one group of mice.
b) SN-11,437 - as 1.0% of powdered Laboratory Chow. (As above)

2. Mice: CFU -18-20 gms. ^{Ave. of 12 = 18.6 gms.} 50 mice.
ALL MALES

3. Toxo: Fresh 10% MB Sump in NaCl Dose: 0.3 cc IP
Use 3 MB for the 10%. Prepared Dil: 10^{-1} - 10^{-5} (Tox. Cont. to 10^{-5})
in Ten Broek & filtered thru gauze as 10% sump. Smooth, even sump. Smears markedly heavy toxo. No bacteria.

4. TEST GROUPS:

- | | |
|--|----------------------|
| A. 0.01% paludrine - no toxoplasma | = Paludrine control |
| B. 1.0% SN-11,437 - " " | = SN-11,437 control |
| C. No DRUG + TOXOPLASMA | = Parasite control |
| D. 0.01% PALUDRINE + TOXOPLASMA | = Paludrine test. |
| E. 1.0% SN-11,437 + TOXOPLASMA | = SN-11,437 test. |
| F. 0.1 mg PALUDRINE ^{IM} BID + TOXOPLASMA | = PALUDRINE IM TEST. |

5. MOUSE GROUPS: 6/group except for 10^{-1} & 10^{-2} of the Paludrine & SN-11,437 test groups which will be of 8 mice.

6. Plan: Drugs to be ground with powdered chow ^{perina lab} to proper concentration. Untreated controls to receive plain, powdered chow. Drugs to be administered as part of diet for 14 days beginning immediately after inoculation. If significant numbers of the 10^{-1} & 10^{-2} drug groups are alive at 16 days then two each will be sacrificed & if the peritoneal cavity & viscera are

free of toxoplasma (on smear), a mixed pool will be injected to 5 mice each.

WGT'S AT BEGINNING OF EXPT. : GROUP A = 107 gms (6 mice)

GROUP B = 116 gms (6 mice)

WGT'S - OCT 2 1947

GROUP A = 123 gms (6 mice)

GROUP B = 98 gms (6 mice) ←

INOC. BEGUN @ 10^{-6} → 10^{-1} . SAME 1cc SYRINGE. INOC. ORDER = GR. F, E, D, & C.
COMPLETED AT 4²⁰ PM. 19 SEPT. '47. Last of live cult on BAP, caplast growth of from rods.

PROTOCOL

GROUP A (Paludine Control)

MOUSE

DAY

	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	29	30
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

GROUP B (11,437 CONT.)

MOUSE

DAY

	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	29	30
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

30 Sept - 5 of these mice have eyes closed - non-cloudy exudate. Appetites poor. Coats ruffled.
2 Oct - eyes of all involved. Appetites poor. Have lost wgt. Coats ruffled. No CNS signs. All 11,437 mice placed on regular pellet diet with but drug.

3 Oct - Mashed worm mt. Eyes clear in three
8 Oct - " " " almost clear in remaining three.
13 Oct - " " " - (open)

SEP 19 1947

TOXOPLASMA VS. PALUDRINE & SN-11437

EXP. I.
(CONT.)

GROUP C (PAR. CONTROL) — 0.3cc of indicated dilution i.p.

MOUSE		DAY																														
		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	29	30	
10 ⁻¹	1	-	-	-	-	D	} brain smear = too present. No hist.																									
	2	-	-	-	-	D																										
	3	-	-	-	-	CNS	D	} brain smear = too - no hist.																								
	4	-	-	-	-	CNS	D																									
	5	-	-	-	-	CNS	D																									
	6	-	-	-	-	CNS	CNS	D																								
10 ⁻²	1	-	-	-	-	-	D	} brain smear = too - no hist.																								
	2	-	-	-	-	-	CNS																									D
	3	-	-	-	-	-	CNS	D																								
	4	-	-	-	-	-	CNS	CNS	D																							
	5	-	-	-	-	-	CNS	CNS	D																							
	6	-	-	-	-	-	-	?	?	?	?	D																				
10 ⁻³	1	-	-	-	-	-	?	D	D																							
	2	-	-	-	-	-	-	?	D																							
	3	-	-	-	-	-	-	?	D																							
	4	-	-	-	-	-	-	-	D																							
	5	-	-	-	-	-	-	-	?	D																						
	6	-	-	-	-	-	-	-	?	D																						
10 ⁻⁴	1	-	-	-	-	-	-	-	?	D																						
	2	-	-	-	-	-	-	-	?	D																						
	3	-	-	-	-	-	-	-	?	D																						
	4	-	-	-	-	-	-	-	?	D																						
	5	-	-	-	-	-	-	-	?	D																						
	6	-	-	-	-	-	-	-	-	?	D																					
10 ⁻⁵	1	-	-	-	-	-	-	-	?	D																						
	2	-	-	-	-	-	-	-	?	?	D																					
	3	-	-	-	-	-	-	-	-	?	D																					
	4	-	-	-	-	-	-	-	-	?	D																					
	5	-	-	-	-	-	-	-	-	?	D																					
	6	-	-	-	-	-	-	-	-	?	-	?	?	±	±	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
10 ⁻⁶	1	-	-	-	-	-	-	-	-	D																						
	2	-	-	-	-	-	-	-	-	D																						
	3	-	-	-	-	-	-	-	-	-	?	D																				
	4	-	-	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

LD₅₀ 10^{-5.8}

6/6

6/6

6/6

6/6

5/6

3/6

GROUP D (PAL. TEST)

[illegible]

SEP 19 1947

TOXO PLASMA VS. PALUDRINE & SN-11,437

EXPT. I (CONT.)

GROUP E (11,437 TEST)

	MOUSE	DAY																													
		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	29	30
10 ⁻¹	1	-	-	-	-	-	CNS	D																							
	2	-	-	-	-	-	CNS	D																							
	3	-	-	-	-	-	CNS	D																							
	4	-	-	-	-	-	?	?	D																						
	5	-	-	-	-	-	?	?	D																						
	6	-	-	-	-	-	?	?	D																						
	7	-	-	-	-	-	?	CNS	D																						
	8	-	-	-	-	-	-	-	?	?	PR	PR	PR	PR	DIA	DIA	DIA	D													
		L. all better.																													
10 ⁻²	1	-	-	-	-	-	-	-	D																						
	2	-	-	-	-	-	-	-	D																						
	3	-	-	-	-	-	-	-	D																						
	4	-	-	-	-	-	-	-	?	D																					
	5	-	-	-	-	-	-	-	?	D																					
	6	-	-	-	-	-	-	-	?	PR	D																				
	7	-	-	-	-	-	-	-	?	PR	D																				
	8	-	-	-	-	-	-	-	?	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
10 ⁻³	1	-	-	-	-	-	-	-	?	?	D																				
	2	-	-	-	-	-	-	-	?	?	PR	D																			
	3	-	-	-	-	-	-	-	?	?	PR	PR	SACR	-	NO TOXO IN PERITONEAL CAVITY																
	4	-	-	-	-	-	-	-	?	PR	D																				
	5	-	-	-	-	-	-	-	?	PR	PR	PR	DIA	D																	
	6	-	-	-	-	-	-	-	?	PR	PR	PR	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA	DIA
10 ⁻⁴	1	-	-	-	-	-	-	-	?	?	PR	D																			
	2	-	-	-	-	-	-	-	?	PR	PR																				
	3	-	-	-	-	-	-	-	?	PR	PR	PR	D																		
	4	-	-	-	-	-	-	-	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
	5	-	-	-	-	-	-	-	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
	6	-	-	-	-	-	-	-	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
10 ⁻⁵	1	-	-	-	-	-	-	-	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
	2	-	-	-	-	-	-	-	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
	3	-	-	-	-	-	-	-	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
	4	-	-	-	-	-	-	-	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
	5	-	-	-	-	-	-	-	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
	6	-	-	-	-	-	-	-	?	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR

8/8

8/8

6/6

6/6

LD50 = 10⁻⁵+

4/5

OCT 2 1947 This mouse was in extremis, eyes shut & exudate similar to controls (dmg), having spontaneous convulsions, very weak. Sacrificed. Smear of brain = no toxo or bacteria. No peritoneal fluid. Spleen small & friable. Liver soft & friable & marked w many very dark spots which do not appear to be infarcts. Lungs soft, no areas of consolidation or hemorrhage. Portion of spleen, liver, kidneys (gross OK), lung, & 1/2 brain fixed in Ziehl-Neelsen 5% acetic. This may all be drug toxicity. Remainder of brain, kidney, spleen, one lung & portion of liver pooled, minced, ground = 0.6 gm. Inoculated in 6cc of 1% allowed to settle in ref for ca 1 1/2 hrs & then poured to 5 Ch alb - I ser. See added sheet.

Group F (PAL. IM TEST)

MOUSE		DAY																														
		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	29	30	
10 ⁻¹	1	-	-	-	D	disc. chn.																										
	2	-	-	-	D																											
	3	-	-	-	-	D	} brain smear = topo. - no bact.																									
	4	-	-	-	-	D																										
	5	-	-	-	-	PR D	} br smear = topo. No bact.																									
	6	-	-	-	-	"	D																									
	7	-	-	-	-	"	CAS D																									
	8	-	-	-	-	"	CAS D	BLOODY urine																								
10 ⁻²	1	-	-	(D)	disc. chn.																											
	2	-	-	-	D	} sm of brain = gran my rods. mod # topo. Peritoneal fluid loaded = gran - rods, occ topo. #2 of 10 ⁻¹																										
	3	-	-	-	-	?	D																									
	4	-	-	-	-	-	?	D																								
	5	-	-	-	-	-	?	D																								
	6	-	-	-	-	-	?	D																								
	7	-	-	-	-	-	?	PR D																								
	8	-	-	-	-	-	?	?	?	?	?	PR D																				
10 ⁻³	1	-	-	-	-	-	-	?	D	- peritoneal fluid loaded = topo. No bact.																						
	2	-	-	-	-	-	-	?	D																							
	3	-	-	-	-	-	-	?	D																							
	4	-	-	-	-	-	-	?	D																							
	5	-	-	-	-	-	-	?	D																							
	6	-	-	-	-	-	-	?	D																							
10 ⁻⁴	1	-	-	-	-	-	-	?	D																							
	2	-	-	-	-	-	-	?	D																							
	3	-	-	-	-	-	-	?	D																							
	4	-	-	-	-	-	-	?	D																							
	5	-	-	-	-	-	-	?	PR D																							
	6	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?		
10 ⁻⁵	1	-	-	-	-	-	-	-	D																							
	2	-	-	-	-	-	-	-	?	D																						
	3	-	-	-	-	-	-	-	?	D																						
	4	-	-	-	-	-	-	-	?	D																						
	5	-	-	-	-	-	-	-	?	D																						
	6	-	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?		

IM DOSAGE = 0.05 cc of 200 mg % SOL. ^{bid} PREPARED ON 15 SEPT. 47 IN STERILE DIST. H₂O. → IN BOILING H₂O BATH FOR 30', CULT = STERILE → STORED AT ROOM TEMP. WATER CLEAR. = 0.2 mg / dy or 11.1 mg / kg (initial wgt)

DOSES:

1 SEP 19 1947
SEP 20 1947

500 gm. AT HIND LEG.
900 gm. LT. " "

This procedure was continued thru 30 Sept - daily at ca 9⁰⁰ am. & 6⁰⁰ pm.
Discontinued on that date because of obvious ineffectivity.

OCT 2 1947

Panage From Group E, 10-4 (SN-11,437)

5 off all ca 16 days old. 0.02 ml Ice

Mouse

DAY

	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
1	-	-	-	-	Hy	?	?	?	?	?	?	?	?	?	?	RUF	RUF	RUF	RUF	RUF	RUF	RUF	RUF	RUF	RUF
2	-	-	-	-	Hy	?	?	?	?	?	?	?	?	?	?	RUF	RUF	RUF	RUF	RUF	RUF	RUF	"	"	"
3	-	-	-	-	-	-	-	?	-	-	-	-	?	?	?	RUF	RUF	RUF	RUF	RUF	RUF	"	"	"	"
4	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-	?	RUF	RUF	RUF	RUF	RUF	RUF	"	"	"
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	RUF	RUF	RUF	RUF	RUF	RUF	"	"	"
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	RUF	RUF	RUF	RUF	RUF	RUF	"	"	"

70% Titra.

Icer.

Test Date	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	LD ₅₀ # of fish @ endpoint	Theor LD ₅₀ @ endpoint
8 Dec.	—	—	4/4	1/4	0/4	0/4	3.7	10.5

TOXO TITRATIONS

check this fig.

← T.T. Def.

TCER

Expt Date	-1	-2	-3	-4	-5	-6	-7	LD ₅₀	Theor LD ₅₀	# ₆₄₀ LD ₅₀	# ₆₄₀ LD ₅₀	T.T. Def.	
8 Dec	-	-	4/4	1/4	0/4	9/4	0/4	3.7	5.8	79	0.5	2.1	Ice - NaCl - titration
27 Oct.	4/4	4/4	4/4	4/4	9/3	-	-	4.5	5.6	10	0.5	1.1	NRS - neut 20037
24 Nov.	4/4	3/3	4/4	4/4	0/4	0/3	-	4.5	5.1	1.9	0.5	0.6	" " " "
19 Dec	-	-	4/4	4/4	0/4	0/4	0/4	4.5	6.0	15	0.5	1.5	" " " "

IP

24 Nov.	4/4	4/4	4/4	4/4	4/4	2/4	-	6.0	6.5	¹⁰ 7.8	0.5	0.5	0.5 cc IP - NRS neut - 20037
8 Dec	-	-	4/4	1/4	0/3	0/4	0/4	3.7	6.9	631	0.5	3.2	0.25 cc " - NaCl tit.
17 Dec	-	-	4/4	4/4	4/4	1/4	0/3	5.7	7.3	14	0.5	1.6	" " - " " (cold)
	-	-	4/4	3/4	4/4	0/4	0/4	5.4	7.3	28	0.5	1.9	" " (pink)
	-	-	4/4	4/4	4/4	4/4	1/4	6.7	7.3	1.4	0.5	0.6	0.25 in 50% NRS - (pink)
12 JAN	-	-											

27 Oct

	-1	# tests	-2	# tests	-3	# tests	-4	# tests	-5	# tests	TCER	NEUTS (CR. BLACK)
27 Oct.	4/4	33000	4/4	3300	1/4	330						

TOXO. MOUSE
NEUTRALIZATION

EXPT. DATE	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	LD ₅₀	# TOXO LD ₅₀	NEUT. INDEX	ICER
27 OCT.	—	—	—	—	—	—	3.5	—	6	MB TOXO
27 OCT.	4/4	4/4	3/4	33	0/4	—	2.7	2300	60	PE TOXO BLACK
24 NOV.	4/4	3/4	1/4	63	0/3	0.06	2.5	315	100	PE " BLACK
24 NOV.	4/4	4/4	2/4	63	0/3	0.06	3.0	63	32	" " BLACK
19 DEC.	4/4	3/4	1/4	484	0/3	0.5	2.5	2420	100	PE TOXO BLACK
24 NOV.	3/3	4/4	4/4	1037	0/4	0/3	3.5	518	320	PE- 0.5% BLACK

IP

Numbers of TOXOPLASMA COUNTED in PERITONEAL EXUDATE ON DIFFERENT OCCASIONS

<u>DATE</u>	<u>Time after Inocul.</u> <u>DAYS</u>	<u>No. of Mice</u> <u>in Pool</u>	<u>No. of TOXOPLASMA</u> <u>per cc. of exudate</u> <u>Millions</u>	<u>No. Toxo</u> <u>per L.D₅₀</u>	<u>ROUTE</u>	<u>Condit</u>
10-27-47	4	2	11	10	i.c.	37°-2hrs
11-24-47	4	10	2	$\frac{2}{1}$	i.c. i.p.	" "
12-8-47	5	10	13.8			
12-17-47	4	4	28.5			
12-17-47	4	2	30	1.5	i.p.	Immed.
12-19-47	5	3	16	16	i.c.	37°-2hrs
1-12-48	5	2	13.2			
1-22-48	5	2	9.6			
1-23-48	6	3	30.			
1-27-48	5	2	12.75			

Eden Park

nitro

Rabbit Test

$$\pm (8/25)$$

+

$$\pm (8/25); -(8/25) \pm 8/25; \pm 12/25$$

$$\pm (8/25); -(8/25)$$

+

$$\pm; \pm (8/25); + (2/29)$$

+

$$- (8/25)$$

$$- (8/25)$$

$$- (12/29)$$

$$- (12/29)$$

$$= (8/25)$$

$$+ (8/25)$$

$$+ (8/25) \pm; \pm 12/29$$

In Vitro

Tests



7/24

8/6

8/25

8/25

8/25



Positives



100,000
10,000
1,000
100
10
1

1 2 3 4 5 6 7 8 9 10 11 12 13 14

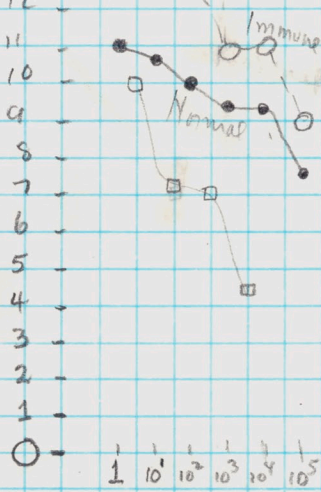
Avg. Day of Death

Immune

Normal

13
12
11
10
9
8
7
6
5
4
3
2
1
0

Avg.
Day
of
Death



Avg. Day of Death

No. of Toxopl.

Avg. Day of Death

Range

S

1

11

11, 11, 0, 0

S

10

10.7

10, 11, 11, 11

S

100

10

10, 10, 10, 10

10, 10, 11, 12 - 10.9

1000

9.2

9, 9, 9, 10

10, 10, 14, 14 - 11.0

10,000

9.2

7, 9, 9, 12

8, 9, 10 (9.0)

100,000

7.7

7, 7, 7, 10

No. of toxoplasma ip

at 24 hr. gen. cycle - 1 may be 10 in 1 day
and 10¹⁰ toxo liberated 100 in 2 days
per paratized cell 1000 in 3 days
10,000 in 4 days
100,000 in 5 "

Since difference between 1 and 100,000 is only 3 to 4 days
the generation cycle is either less than 24 hours
or the number per paratized cell is more than 10

Toxoplasma in Blood in Relation to Development of Antibody

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

RAT "Y"

Toxo in Blood

0 0

Left
Lunge

Antibody - 0

16 1024

RAT "R"

Toxo in Blood

1/3 0

0

0

0 ← lung positive (1/2)

Antibody - 0

64 1024

1024

256

1024

RAT "B"

Toxo in Blood

3/3 0

0

0

0

— lungs pos. 35 days

Antibody - 0

64 256

256

256

256

— 256 at 35 days.

Rhes. 152

Toxo in Blood

3/3 3/3 3/2

3/3

0

0

0 ← lungs 1/3
liver & spleen — 1/3
Brain — 4/4

Antibody 0

0 4 16

1024

4096

1024

CF

TOXOPLASMA INTRAPERITONEALLY IN GUINEA PIGS FEB 21 1948

Purpose - attempt to obtain large quantity of exudate ~~very~~ rich in toxoplasma.

GUINEA PIGS - Large - @ 400-500 gm. - two of 3 used for tests on presence of "antibody" in normal g. pigs - see tests on g. pigs A, B+C on 2-16-48 (A+B)

INOCULUM - 1cc of heparinized bloody mouse peritoneal exudate from 5-day mice given intraperitoneally to each.

	1	2	3	4	5	6	7	8	9	10	11
G. pig A	-	-	-	-	-	-	-	-	-	-	Sick
" B	-	-	-	-	-	-	-	-	-	-	2D - decomposed and stinking

FEB 26 1948 - abdomens not distended, but both g. pigs punctured with 18 g. needle but no exudate obtained

FEB 28 1948 - Spig. A appears sick to-day - doesn't right himself well. Killed with chloroform and abdominal cavity opened.

Very viscid ~~ropy~~ ^{thick} exudate present but only 6cc obtained and 1cc of heparin 1:1000 added to it.

1st washing - 25cc saline - cloudy with large pieces of fibrin

2nd washing - 30cc of saline used - also cloudy with fibrin

3rd washing - 30cc of saline - slightly blood tinged cloudy
All material filtered through gauze to remove fibrin

Examination for toxoplasma - since the original exudate was too thick to handle, it was mixed with the first washing to yield 30cc, i.e. 1:5, also final dilution of heparin 1:30,000

For Count - 2.5 million toxo per cc of diluted exudate

2nd washings - filtered through gauze

Count - 2.9 million toxo per cc.

The first and 2nd washings were pooled and tested with Schumacher serum and saline (see over)

FEB 28 1948

1. Guinea pig peritoneal exudate (1:20) + saline

2. " " " " " + S. Schumacher serum

37°C
water
bath for
1 hour

Examined with methylene blue immediately.

Saline mixture — $22/50 = 44\%$ unstainedSchumacher mixture — $40/50 = 80\%$ unstained

Since the same Schumacher serum used to-day with peritoneal exudate of 4-day mice failed to affect the toxoplasma (i.e. $49/50$ well stained), it would appear that in the 7-day guinea pig exudate, 44% of the toxoplasma ~~were~~ might already have been affected by the host's immune response and ~~in~~ since guinea pigs can be lower in "activator" content the addition of [REDACTED] serum yielded 80% unstained possibly because of more activator.

MAR 3 1948

— Guinea pig B found dead in advanced state of decomposition. Blood was clotted and only small amount of highly hemolyzed fluid could be obtained (see test of same day)

There was no free peritoneal exudate and the small drop that was obtained was so full of bacteria (postmortem invasion) that no toxoplasma could be seen.

Development of "Cytotoxic" Antibody Following Toxoplasmic Infection in Rats

Rats Used - Three full-grown albino rats (marked "R" red, "B" blue, and "Y" yellow) obtained from Warkany were used. (Weight -)

On Feb. 24 - each rat was bled from the heart (0.9 cc blood + 0.1 cc heparin 1:1000) and the fresh plasma was tested in vitro, and found to be negative in the heated specimens.

Inoculum - 1 cc of undiluted mouse peritoneal exudate (used in other tests same day)

	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	29	30	31	32
RAT "R" (red)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
"B" (blue)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
"Y" (yellow)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
"Antibody"	0																															

2 dead
35th dy.
(31 Mar 48)

MAR 1 1948 - All 3 bled; heparinized plasma stored - 9-8
Sediment + cells inoculated in mice

MAR 3 1948 - All 3 bled; heparinized plasma tested along with others
remained stored in - 9-8
Blood cells + sediment inoculated in mice

Rat "Y" - died during bleeding

No exudate in peritoneum.

Smears of spleen, liver & lungs - no toxoplasma found
Suspensions of viscera inoculated in mice.

MAR 10 1948 - Both Rat "B" & "R" bled 1.0 cc $\pm$ hep 1/10000. Ca 0.5 ml plasma separated & frozen. 9-8
Blood cells suspended in NaCl & passed to groups of 3 mice.

MAR 17 1948 - Both Rat "B" & "R" bled 0.9 cc $\pm$ hep 1/10000. Ca 0.4 cc plasma separated
Blood cells suspended in NaCl & passed to groups of 3 mice.

MAR 24 1948 Both rats bled 0.9 cc into 0.1 cc hep 1/1000 - from heart without difficulty. However,
Rat "Red" died suddenly some 15' after completely recovering from the anesthesia.
Chest opened shortly thereafter, lungs normal (3 lobes removed for parage) but
a large clot surrounded the heart. About 3.0 cc of blood were obtained from
the pericardial sac, heart, & pleural sacs, allowed to clot, serum removed,
(over)

& frozen in a lusteroid as: Rat "Red" 28th post-mortem serum 28 Mar. 48. ca 2 cc serum.

MAR 31 1948

Rat "Blue" died while being bled, quite evidently due to trauma. Chest opened immediately & 2 lobes of lung removed for paraffin (normal appearance). Then bled from heart. Bled heparinized (final conc. 1:10,000), spun & ca 2.0 cc serum frozen in lusteroid. This labelled "Rat Blue 35th post-mortem. day, 31 March 48.

Lung tissue suspended in ^{10cc} NaCl & after settling for 5'; 1.0 cc amts of supernate inoc. IP into each of 3 mice (see protocol)

Rat Blood Inoculations - [5th Day post-inoculation] with Tokoplasma

MAR 1 1948

5 DAY BLOOD

TOXO NEG

"Yellow" Rat

color yellow
mouse

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

best. overgrowth - no toxo seen
dead 34th day.

"Blue" Rat

color white
mouse

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PE loaded & Tokoplasma.

POS. TOXO

PE TAPPED - PE LOADED & TOXO.

tox present in lung smear

"Red" Rat

color red
mouse

Day	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-

Toxo PRESENT IN PE & LUNGS.

POS. TOXO.

Toxo PRESENT in tongue smear

Rat Blood Inoculations - [7th Day: post inoculation] with Tokoplasma

MAR 3 1948

7 DAY BLOOD

"Yellow" Rat

mouse

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

NEG TOXO

"Blue" Rat

mouse

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

lung smears (-) for toxo - loaded & bacteria

NEG TOXO

"Red" Rat

mouse

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5 on 85th day

NEG TOXO

MAR 3 1948

"Y" Rat Liver-Spleen Passage - I.P.

Mouse	Day																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-		-	-	-	-	-			-	-	-	-	-	-		-	-	-
2	-	-	-		-	-	-	-	-			-	-	-	-	-	-		-	-	-
3	-	-	-		-	-	-	-	-			-	-	-	-	-	-		-	-	-
4	-	-	-		-	-	-	-	-			-	-	-	-	-	-		-	-	-
5	-	-	-		-	-	-	-	-			-	-	-	-	-	-		-	-	-

"Y" Rat Lung Passage - I.P.

<u>mouse</u>	<u>Day</u>																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
1	-	-	-		-	-	-	-	-	D	}												toxo present in lung smear
2	-	-	-		-	-	-	-	-	D													
3	-	-	-		-	-	-	-	-	Asc	D												toxo present in lung smear
4	-	-	-		-	-	-	-	-	Asc													
5	-	-	-		-	-	-	-	-	-													

Pos Toxo

MAR 10 1948

Rat Blood Inoculations - [14th day - post-inoculation]
(with to 40 plasma)

14 day BLOOD

"Blue" Rat

[illegible]

"Red" Rat

<u>mouse</u>	<u>Day</u>																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	.	.	T	-	-	-	.		-	-	-
2	-	-	-		-	-	-	-	-	-		.	-	-	-	-	.		-	-	-
3	-	-	-		-	-	-	-	-	.		-	-	-	-	-	-		-	-	.

MAR 17 1948

Rat Blood Inoculations (Cont.)21st Post inoc. day - both "Blue" & "Red" Rat rbc diluted $\approx 0.5\%$ NaCl

4 each inoc. into 3 mice - 0.3 ml IP/mouse.

21 DAY BLOOD "Blue" Rat

<u>MOUSE</u>	<u>DAY</u>																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

"RED" RAT

<u>MOUSE</u>	<u>DAY</u>																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

MAR 24 1948

Rat Blood Inoculations 28th post inoc day."Blue" & "Red" rat rbc diluted $\approx 0.5\%$ NaCl - 0.2 cc given IP
- in each of 3 mice28 Day Blood. "Blue" Rat.

<u>Mouse</u>	<u>Day</u>																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

"Red" Rat.

<u>Mouse</u>	<u>Day</u>																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

MAR 24 1948

Passage From "Red" Lung.

3 lobes removed from lungs, normal appearance, minced, suspended in 5 cc NaCl, allowed to settle for ca 15' & then 0.5 cc units of supernate inoc. IP into each of 3 mice.

<u>Mouse</u>	<u>DAY</u>																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	?	D																			
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

no toxo seen on lung smear.

ase ase

tox present in lung smear.

Pos
Toxo

MAR 31 1948

Penicillin From Lung of Rat "Blue"Inoc:

2 lobes of lung, normal appearance, suspended in 10 cc. NaCl without abrasion.
 Allowed to settle for ca 5' & 1.0 cc. amts of super. inoc. IP into each of 3 mice.

MouseDAY

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	-	-	-	-	Asp	Asp	Asp	Asp	D											
2	-	-	-	-	-	Asp	Asp	Asp	Asp	Asp	D										
3	-	-	-	-	-	?	Asp	Asp	Asp	Asp	Asp	Asp	Asp	?	?						

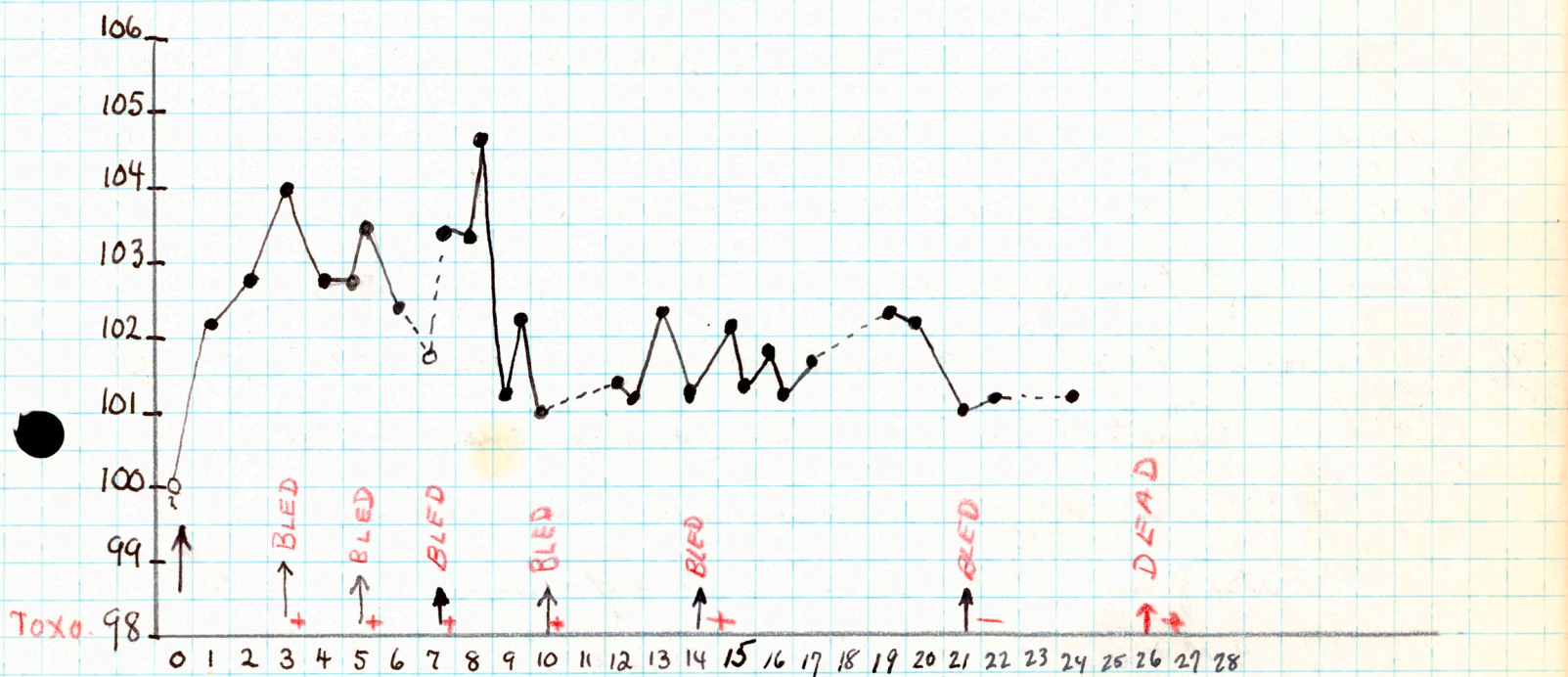
Toxo present in lung

Toxo ghosts present in lung (bact.)

Development of "Cytotoxic" Antibody in Rhesus Monkey Following Infection with Toxoplasma.

Rh 152 - Bled on March 2 and plasma tested same day showed no "antibody" in the heated specimen. **Stored 9-3**

Inoculation - 6 P.M. on March 3 - 0.25cc of undiluted peritoneal exudate (very rich in toxoplasma) used same day in in vitro tests - intracutaneously in each of two sites on abdomen; 5cc of 10% mouse brain suspension (settled 10min) freshly prepared of passage 375 given intraperitoneally.



MAR 4 1948 - Skin sites essentially neg.

MAR 5 1948 - Oedema 15x15 mm. - upper site; lower site - injured & clipped - also oedematous

MAR 6 1948 - Oedema & erythema 25x25 mm. upper; also dependent oedema - lower abdomen. 5cc of blood + 0.1cc of heparin (1:200) taken; plasma stored - 9-3
Blood cells & sediment inoculated into 3 mice

MAR 7 1948 - Marked oedema of abdomen

MAR 8 1948 - Bled 8.0cc + 0.1cc heparin (1/100) Plasma stored in **9-3** & cells & sediment into 3 alb. mice.

MAR 10 1948 - Bled 6.0cc + 0.05cc heparin 1/100. Plasma stored in **9-3** & cells & sediment into 3 alb mice.

Both skin sites oedema & erythema and beginning central necrosis - lesions about 25x20 mm.

MAR 11 1948 - Upper 40x25 mm; lower 30x25 mm.

MAR 12 1948 - Skin lesions bleaching

MAR 16 1948 - Skin lesions bleached & almost gone. Has skin infection at site of clipper with on belly wall.

MAR 17 1948

Bled 10 cc from left femoral with heparin 1/1000 to make 1/10000. Blood spun 20 min & ca 5 cc clear plasma removed & transferred to a custeroid. = 14 dy serum.
Rbc inoc in 0.5 cc amts IP to each of 3 mice. Monkey's lesions gone. Skin wound granulating & quite clean.

MAR 24 1948

Bled 10 cc from femoral = heparin to make 1/10000. Plasma transferred to custeroid & frozen. Animal quite well. Skin much practically healed. Two lesions gone.
RBC inoc. as 0.5 cc until RBC IP to each of 3 mice.

MAR 29 1948

Monkey found in extremis this a.m. Bled ca 40 cc from heart, during which animal died. The only external evidence of disease was the monkey's evident loss of weight. Skin lesions well-healed. Animal then placed in refrigerator for ca 2 hrs following which it was posted. No abnormalities were encountered in the viscera & CVS except for many lymph nodes along the upper GI tract which were shown to contain small worms (*Esophagostomum*?). Sections of lung, heart, liver, spleen, kidney, & the CVS were fixed in 10% formalin for sections.

A portion of lung, pool of liver & spleen, & a portion of cerebral cortex were each suspended in NaCl & inoc. IP into groups of mice for test. isolation.

Contact smear of lung stained c Wright's = many mms but no test.

Cause of death = ? Certainly not gross the or usual lesions of test.

MAR 6 1948

Test For Infectivity of RH #152 Blood Drawn Three Days after Inoculation with Toxoplasma

Inoc: Following centrifugation of the 5 cc of blood (heparinized) which was drawn for plasma to-day, the rbc were stored in the refrigerator for about 3 hrs in the original tube. At that time, 1.5 cc NaCl were added, the cells thoroughly suspended & then 1.0 cc amounts of this mixture were injected into each of 3 albino mice, I.P. thus there were slightly less than 2.0 cc of rbc.

3-DAY BLOOD

- POS. TOXO.

<u>MOUSE</u>	<u>Day</u>													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-	-	-	-	-	-	-	-	D	} toxo present in lung smears				
2	-	-	-	-	-	-	-	-	D					
3	-	-	-	-	-	-	-	-	Ascd					
										TOXO PRESENT IN LUNG.				

MAR 8 1948

Rh #152 was bled again on the fifth day after toxoplasma inoculation. The plasma was separated from the heparinized blood (by centrifugation) and the cells were resuspended again in 1.0 cc NaCl. The resuspended cells were injected I.P. into 3 albino mice (1 cc each) Plasma stored in 93

5-DAY BLOOD

- POS. TOXO.

<u>MOUSE</u>	<u>Day</u>														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	-	-	-	-	-	-	-	Ascd	D	Toxo					PRESENT IN LUNG
2	-	-	-	-	-	-	-	?	?	Ascd	D	Toxo		Present in lung	
3	-	-	-	-	-	-	-	-	?	?	D	Toxo Present in lung.			

MAR 10 1948

Rh #152: - blood of 7th day (heparinized, centrifuged, and plasma stored in 93. Remaining cells were resuspended in 1.0 cc NaCl and injected into 3 albino mice (1 cc each)

7 DAY BLOOD

POS. TOXO.

<u>mouse</u>	<u>Day</u>													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1							Ascd	D	} Toxo present in lung.					
2							-	-						
3							-	-						
										Ascd Toxo present in lung.				

MAR 13 1948

Rh 152 bled (heparinized) - blood centrifuged, plasma decanted and stored in 93 cels suspended in 1 cc saline and injected into 3 ch alb mice - 1 cc each

10-DAY BLOOD

Pos. Toxo

MiceDay
1 2 3 4 5 6 7 8 9 10 11

1	-	-	-	-	-	-	-	-	-	-	Asc D	Pos. For Toxo.
2	-	-	-	-	-	-	-	-	-	-	Asc Asc D	Pos. For Toxo.
3	-	-	-	-	-	-	-	-	-	-	? Asc D	Pos. For Toxo.

MAR 17 1948

Rh 152 bled (heparinized) blood centrifuged, plasma stored in cels injected (0.5 cc) into each of 3 white mice I.P.

14-DAY BLOOD

Pos. Toxo

Mouse

Day

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

1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Asc Asc D	Toxo in Lung.	BACT.
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	? ? Asc D	Toxo GHOSTS in Lung.	BACT.
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	? ?		

MAR 24 1948

Rh 152 bled (heparinized) blood spun, plasma decanted & stored. Cels (0.5 cc) injected into each of 3 ch alb. m. I.P.

21-day BloodMouse

Day

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

MAR 29 1948

Attempt To Isolate Toxo From Tissues Of RH #152

MAR 29 1948

Died 26 days after intracut. and i.p. inoculation

Lung: A piece of lung ca 1.5 cm x 1.5 cm. was ground in a mortar within 30 min after removal from body, suspended in 5.0 cc NaCl & after settling ca 15 min, 1.0 cc amts of supernate were inoculated IP into each of three mice.

Liver & Spleen: Pieces of liver & spleen were suspended in 5.0 cc NaCl (ca same amt of tissue as for lung), allowed to settle ca 20' & 1.0 cc amts of supernate inoc. IP into each of 3 mice.

Brain: A plug was removed from the left cerebral cortex - ca 1.5 x 1 x 1 cm suspended in 5.0 cc NaCl & allowed to settle for ca 15 min. 1.0 cc amts inoc. IP into each of 4 mice (239 needle.).

MOUSE

<u>HOUSE</u>	<u>DAY</u>												<u>LUNG</u>									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21 (yellow heads.)	
1	-	-	-	-	-	-	?	Asc	Asc	Asc	?	?	Toxo present in lung									
2	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	-	-	-	-	-	
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
																					<u>Pos. TOXO</u>	

POS. TOXO

MOUSE

	DAY										LIVER & SPLEEN									
	1	2	3	4	5	6	7	8	9	10										
1	-	-	-	-	-	-	?	Asc	Asc	Asc	?	?	?	?	?	?	?	?	?	?
2	-	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* dead 7^{th} day

(yellow tails)
pos. TOXO

* dead 22nd day

MOUSE

<u>DOSE</u>	<u>DAY</u>										<u>BRAIN</u> (white)	
	1	2	3	4	5	6	7	8	9	10		
1	-	-	-	-	-	-	Asc	Asc	?	?	toxo present in lung smears.	
2	-	-	-	-	-	-	?	Asc	Asc	?	toxo present in lung	
3	-	-	-	-	-	-	-	?	Asc	?	toxo present in lung	
4	-	-	-	-	-	-	-	-	Asc	Asc	?	toxo present in lung

POS. TOXO

POS. TOXO

Factors in Favor of In Vitro Cytotoxic Phenomenon Being Due to a Specific Toxoplasma Antibody Akin to that in Neutralization Test

1. Correlation between positive and negative human sera in rabbit test and in vitro with few exceptions however
2 sera neg. in rabbits — Pos. in vitro
1 of 2 " sl. pos. " " — neg. or irregular in vitro
2. Disappearance of in vitro phenomenon on heating of serum or long storage at room temp. and reactivation in presence of "large amounts" of activator present in human serum.
3. Failure of ^{heated} "normal" animal sera to be re-activated by addition of human activator
4. Positive effect exerted by pooled immune monkey serum and by rat immune serum — and reactiv. by human activator
5. Partial effect exerted by immune rabbit serum particularly in presence of activator

Factors not in Keeping with Assumption of Identity between Toxoplasma Neutralizing Antibody and in Vitro Phenomenon.

1. The negative results obtained with 2 of Ruchman's toxoplasma convalescent monkeys, except the sera obtained 1 week after inoculation which were positive

Most Important to Establish:

Relation between Recovery from Infection and Development of Specific In Vitro Phenomenon.

1. 3 rats — Test serum before — if not reactivable — ^{imm.} inoc. toxo i.p. Then test at 3 days, 5 days, 7 days, etc.
2. 2 rhesus monkeys — as with rats.
3. Repeat tests on ^{immune} monkey sera, ^{immune} G. pig serum, P₁₇ pigeon

Recheck human sera - posit. in vitro and neg. in rabbits

Test in rabbits capacity of reactivating heated neutr. antibody
or ineffective delated antibody

Test effect of temperature on in vitro phenomenon

Temp	<u>20 min</u>	<u>1 hour</u>	<u>2 hours</u>	<u>5 hrs</u>	<u>24 hrs</u>
② 0°C					
② 22°C					
② 30°C					
② 37°C					

Test fresh bovine ^{+ other} sera for reactivator in absence
of nonspecific cytotoxic effect

Check on nature of nonspecific "cytotoxic" effect of all
human fresh sera when tested in mice.

Is incubation at 37°C necessary for nonspecific
effect and not for immune effect?

(Rabbit neg. Cytox)
Normal serum mixture - no incubation - icer
i.p.

- 2 hrs at 37°C - icer
i.p.

Immune serum mixture - no incub. - icer
i.p.

2 hrs. at 37°C - icer
i.p.

Control - heated ~~normal~~ ^{normal} serum - no incub. icer
i.p.

2 hrs. at 37°C

Control 50% heated Rabb. ser - 2 hrs at 37°C - ic

Compare in single test effect of Nale, Tyrode's solution, 50% heat. rabb. ser.,
10% heated rabbit serum, 2% heated rabbit serum,

Outline of future work on "Cytoplasmic Immune" Phenomenon

I Question of Development of "Antibody" During Course of Infection

RATS : 1) Determine titer of antibody in 5-day, 7-day, 14-day, etc. sera. — all heated at 56°C for 30 min.
(Test 1:2, 1:4, 1:8, 1:16, 1:32 — prepare 1:64 + save in refrigerator; read 1:32 first — if at all positive set up further — always saving next detection in refrigerator until endpoint is obtained)
Repeat at 3 wks, 4 wks, etc. depending on results.

Rhesus :

1) Bleed 5th day, 7th day, 10th day, 14th day, 3 weeks, 4 weeks, etc.

Test for in vitro "antibody" — if present titrate as for rats

Also inoculate mice with sediment of heparin. plasma to test for circulating living toxoplasma

RABBITS — Note — rabbits do not die before 8th or 9th day and "antibody" has been found in surviving rats on 5th day. Does antibody form in rabbits which ultimately die.

Next rabbit neutralization test — pick 2 rabbits with known lack of antibody (heated) prior to inoculation, — and bleed at 5 and 7 days.

!! Preliminary test on Rabbit 360 (stored 9-3) — bled 9 days after inoculation (3-5-48) + died 10th days. (i.e. 3-6-48).

II Correlation between "Cytoplasmic Effect" and Cytotoxic Effect? Does lack of affinity of cytoplasm for dye necessarily mean death of organisms and vice versa?

Will have to be repeated MAR 12 1948

1) Perit. Ex. 1:5 in ^{bp} sal. + Black serum } 2 Rrs. at 37°C
" " " " + Rabb. 365 " } count no. unstained
" " " " + heated undil. rabbit serum }
No. of toxo in exudate to be counted before starting.
After 2 hrs. at 37°C — dilutions to be prepared in 50% heated rabbit serum, endpoints depending on count and 10 mice per dilution to be used in terminal range, i.e. if $10^{-3.7}$ is expected, 10^{-3} and 10^{-4} would have 10 mice and 10^{-5} , 10^{-6} , 10^{-5} mice

see attached

(see over)

Sketch of Proposed Test

On basis that 10^{-6} d.t. will have 0.5 - 0.7 Toxo per 0.1cc dose

	Heated undil. Rabb. serum (365)	Fresh undil. Rabb. ser. 365	R. Black fresh	
10^{-2}	—	5	5	
10^{-3}	—	5	5	
10^{-4}	5	10	10	Total mice needed
10^{-5}	5	10	10	<u>110</u>
10^{-6}	10	10	10	
10^{-7}	10	10	10	

- 2) Can toxo be killed by certain temperatures and have cytoplasm retain affinity for dye? yes

Perit. Exud. 1:5 in saline $\pm$ heparin final conc. 1:5,000

Minimum of ~~one to~~ 100 in sealed glass ampules which will be kept submerged in water bath $\pm$ weights.

Ampule 1 — 15 min. at 50°C } Examine with meth. blue.
Ampule 2 — 30 min " " }

If only amp. 1 is stained — titer only it
" both amps are " — titer both.

Titration in mice — dilutions in heated 50% rabbitser.

Control — Ex 1:5 kept in refrigerator 10^{-4} to 10^{-7} — dep. on number of toxo

Heated exudate — 1:5, 1:100, to 10^{-7} if necessary.

- 3) Can toxoplasma be killed by certain chemicals (formalin) without losing the cytoplasm losing its affinity for the dye?

Final Conc. Formalin	Am't of Formal. 1% in saline	Amount of saline	Amount of exudate
0.5%	0.5cc	0.3cc	0.2cc
0.2%	0.2cc	0.6cc	0.2cc

Leave at room temperature for 2 hours — examine with M.B.
Titrate in mice — one or both depending on staining affinities.

(continued)

- 3) "Toxic" effect of saline in relation to affinity of cytoplasm for dye?

Some of same peritoneal exudate used in 1) and 2) to be diluted 1:10 in saline and ^{after 1 hour at 37°C} stored in refrigerator for 24 hours. Next day examine with methylene blue, titrate in mice using 50% undiluted heated rabbit serum saline.

35

MAR 12 1948

III

Neutralization of toxoplasma in rabbits by

- a) Animal sera with "cytoplasmic" effect in fresh state which cannot be reactivated after heating

Rhesus sera — Rh 152 APR 14 1948
Rh 936

RABBIT sera — R 365
Norm. rabbit of 2-4-48

Sheep serum — frozen since 2-10-48 APR 5 1948

Guinea-pig — G. pig "C" frozen 2-12-48 APR 14 1948

- b) Animal sera whose in vitro cytoplasmic effect can be reactivated after heating by fresh human "activator" sera — does this represent true toxoplasma neutralizing antibody? NO

Rhesus sera — Rh 151 APR 14 1948
Rh 142 APR 14 1948
Rh 157 APR 14 1948
Rh 158 APR 14 1948

see list of 5/1/48 in vitro
all negative

Rabbit sera — R 363 ^{Surv.} to be challenged APR 14 1948
R 349 — challenged + resistant APR 14 1948
R 398 — " " " APR 14 1948

- c) Certain Human Sera

Akr

- 96% in vitro

- 44% " "

(each)

4)

30-48

APR 5 1948

IV - Neutralization of Toxoplasma in Mice

- in relation to results in rabbit skin test
- in vitro effect

- Preliminary determination of best diluent to use to get away from "spontaneous antibody" being activated by fresh sera.

Dilute exudate with counted no. of toxoplasma in

a) 50% heated rabbit serum saline - control

b) 10% " " " "

c) 2% " " " "

d) Tyrode's solution

e) Saline

f) 2% Boric acid in NaCl

Incubate dilutions in water bath at 37°C for 2 hours and inoculate mice - i.p. - o.i.c.

10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} - dilutions depend on count
↓ ↓ ↓ ↓ ↓
4 4, 5, 10, ? ← number of mice " " "

- After best diluent is known repeat test with several positive, equivocal, and negative sera using exudate in which toxoplasma yield at least a 90% stained preparation in presence of good activator

- Test effect of activator on immune serum
 - diluted in saline compared to dilution in activator
 - effect of activator on heated immune serum

Basic to this experiment is first finding the conditions under which activator per se is entirely or almost entirely without effect.

V The In Vitro Cytoplasmic Phenomenon

- Titer of antibody in several types of human sera
 - Inapparent infections - Sabin, Feldman
 - Mothers who gave birth at a known time to infants with proved congenital toxoplasmosis - Farber A47; Rainey
 - Adults with chorioretinitis - Mrs. White;
 - Infants with disease - during first year of life (select)

- Nature of blocking effect in certain animal sera - and is it present in some human sera?

a) ~~is~~ how far can the serum be diluted and still exhibit blocking phenomenon, i.e. Toxo + activator + blocking ser. in various dil. then add ~~imm~~ known immune serum

- 2) continued b) can blocking sera produce cytoplasmic effect when tested in greater dilution than that used thus far;
test 100, 500, 1000, 2000, 4000
i.e. g. pig or pigeon serum diluted in saline + Toxo in activator

- c) Do certain human sera (from children who may have toxoplasmosis) which gave negative in vitro tests possess blocking "antibody"?
Test Kenneth Butcher and Rudolph Schneider

- 3) Animal source of activator?

Test - chicken serum (1 = N.G.)
calf serum (1 = N.G.)
lamb serum (3 = N.G.)
Dog serum MAR 23 1948 2 sera. No good

- 4) Complete Eden Park series of tests on human sera -
titer several men on sera obtained early & late
- 5) Prior to publication test a series of dyes of different chemical composition to determine if possible the role of a specific "grouping" or linkage
- 6) Temperature "coefficient" of reaction - rate at
0°, 20°, 37°

Animal Bled

Date Drawn

Sera

Storage

Rabbit #365

4 March 48

2 amp. { 11 cc. each

9₃

Rabbit #363

4 March 48

1 amp - ca 10 cc

9₃

Rabbit #360

5 March 48

1 amp - ca 2.3 cc

9₃

The child was born in May, 1946. A few weeks after birth, progressive hydrocephalus, cerebral calcification, micro-ophthalmia and cloudy lens of the left eye, and chorioretinitis of the right eye found. Cerebrospinal fluid 4 weeks after birth was xanthochromic, but no toxoplasma were found on mouse inoculation.

August 28, 1947, lyophilized serum sent by air mail from Amsterdam arrived in Cincinnati, September 4, 1947. Stored in ordinary refrigerator until rabbits became available to permit performance of neutralization test.

December 30, 1947 -- complement fixation test on lyophilized sera -- complement fixation tests on both entirely negative beginning with 1:2 dilution of serum.

January 9, 1948 -- rabbit neutralization test -- [redacted] child and [redacted] entirely negative.

February 17, 1948 -- dye test in vitro -- child, [redacted] without activator, negative, with activator, 92% positive. [redacted] without activator, negative, with activator, 96% positive.

New sera requested from [redacted] on March 16, 1948. New serum ^{on Mrs. Bonsink only} sent April 5, 1948, in the fluid state, by air mail, arrived in Cincinnati in 4-5 days. Frozen in dry ice until tested by the in vitro dye test on April 30, 1948. Serum was tested undiluted and, while it was negative without activator, it was positive (96%) with activator. *Test 7 June 25, '48 = 1:256*

June 24, 1948 -- specimen of serum received by air mail from Professor [redacted], who brought it with him by plane from Amsterdam to New York. This specimen is labeled [redacted] This, presumably, is from the child born to the same mother about 9 months ago. *Tested June 25, '48 = 1:2*

R [REDACTED] age 6 months

Born, July, 1947 [REDACTED]

Baby admitted to Good Samaritan Hospital September 1, 1947, at the age of 3 months, because of poor feeding. The mother had no other complaints.

P. H. - premature infant - birth weight, 3 1/2 pounds - was kept in hospital for 2 1/2 months during which time a blood transfusion and ear paracentesis were done.

F. H. - no siblings; parents are first cousins.

Phys. - at age of 3 months (September 1, 1947)

Head - 15 in. Chest - 12 in. Stom. 11 in., length 20 in.

Head clearly pathologically enlarged with full, sl. bulging ant. and post. fontannels.

Chest- scattered coarse râls and rhonchi

Heart - rate rapid, with blowing systolic murmur at base

Abd. - neg.

Skin. - neg.

X-ray, chest - enlarged, globular heart, prominent thymic area and prominent hilar vascular markings

Ventriculogram - dilated ventricles

Progress - patient has had several episodes of pneumonic infiltrate. The size of the head has gradually enlarged. A ventriculogram (December 22, 1947) revealed greatly enlarged ventricles and questionable calcification along the inner table of the skull.

Dr.- 1. hydrocephalus with questionable calcification ✓

2. Cong. heart disease, acyanotic

No chorioretinitis

CSF - normal protein

normal cells

Blood obtained on mother and child on Dec. 27 '47
Immediately centrifuged - 2 amps. (2cc) each frozen
in H₃

To Dr. Zeldman
for Toxo note-book

Mother of child with proved congenital Toxoplasmosis

Age 28

First Child - Pregnancy onset about October 1, 1946. Lived in College Hill at time.
Child born prematurely in May, 1947.

Early in October, 1946, before knowing of pregnancy, took trip to Kentucky over week-end -- little country town beyond Lexington. Went to Loveland, Ohio, to visit parents-in-law.

During first pregnancy, lived in College Hill. Pigeons started to nest in apartment where they lived (not certain whether in fall of 1946 or spring of 1947 - at time when pigeons lay eggs). No pets of their own - but there was a dog next door whom she petted occasionally. Husband's people whom she visited had dog and cat. Used to go hiking to Mount Airy a lot for picnics. With exception of one day during last month of pregnancy when she vomited and was dizzy, she had no illness.

Pregnant again October - November, 1947.
Moved to East Mitchell Avenue in November 15, 1947.
Has not been ill since May, 1947.

in staff at Jewish Hospital, is present physician.

JUL 18 1948

Baby girl born to [redacted] at 10:15 a.m. to-day. Infant normal. Specimen of cord blood obtained at birth & refrigerated immediately. Venous blood obtained from mother 2 hours after birth & refrigerated. Placenta was also saved in the refrigerator. Specimens picked up in a.m. of 19 July 48 & sera separated & frozen in lusteroids. Portion of placenta also stored in dry ice.

Case of chorioretinitis referred [redacted] of Cincinnati, Ohio.

[redacted] - [redacted] First noticed difficulty with vision in the left eye in 1944 - 1945, during first year of high school. During the past year, [redacted]'s examination revealed chorioretinitis affecting the macular region of the left eye.

The patient, a 19-year-old girl in apparently otherwise perfect health, came to the laboratory on April 10, 1948, together with her mother, [redacted] who was born in 1907. The issue here was to determine whether or not evidence of toxoplasmic infection could be found in association with this chorioretinitis and, if it was present, to determine if possible whether it could have been congenital or acquired. For, if the mother would be toxoplasma negative, while the daughter was toxoplasma positive, it would be most unlikely that the infection was congenital. On the other hand, if they were both positive, it would not be possible to say one way or another since both the mother and the daughter could have acquired the toxoplasma infections at some time subsequent to the birth of the patient. For this reason, skin tests were done on both mother and patient and blood was obtained for serological tests.

Skin Tests:- the toxoplasma antigen, 1:100 and 1:1000 dilutions, was in each case put on the right forearm and the normal, chick embryo antigen in each case on the left forearm. The inoculations were made about 9:30 a.m. Twenty-four hours later, I was informed by telephone that the mother had a reaction of approximately 7x7 mm. at the toxoplasma 1:100 site, while the daughter had roughly a 10x10 mm. reaction at the same site. The 1:1000 sites were too slight to measure, while the normal, chick embryo sites showed nothing. Apparently the toxoplasma reactions increased, so that by late evening they were very much larger and itching. I saw both of them in the laboratory on April 12, 1948, fifty-five hours after the inoculations and both the mother and the patient showed strong, positive skin reactions to the toxoplasma antigen. The patient presented a marked, itching edema and erythema measuring 60x50 mm. with a central white area measuring approximately 15 mm. in diameter at the toxoplasma 1:100 site, and a similar lesion measuring 25x25 mm. at the toxoplasma 1:1000 site. There was no reaction whatever on the left forearm at the normal, chick embryo sites. The mother had similar lesions but of somewhat smaller size:

Toxoplasma — At the 1:100 site, the lesion measured 45x40 mm. and the toxoplasma 1:1000 site measured 12x10 mm. There was no reaction whatever at the normal, chick embryo antigen.

[redacted]

Immunization Protocol

G. P. #974

Recd 0.1 cc. I. Dermal of Toxo Mouse Br. #16 at 10-2	6-2-47
" " " " " " " " #18 at 10-1	6-20-47
Bled 7-2-47 , C.F. 1:128	7-2-47
Bled out, C.F. 1:128-1:256, Neutralizes complete at 1:100, partial at 1:20	7-24-47

G. P. #1037

Recd 0.1 cc. I. Dermally of Toxo #22 Mouse Brain at 10-3	7-2-47
" " " " " #28A " " " 10-1	8-1-47
" " " " " #30 " " " 10-1	8-8-47
Bled for serum, 5.0 cc. from heart.	10-8-47
Serum C.F. Titer, 1:256	
Bled out; serum to -20 deg.	10-10-47

Recd for Joel Wane

DEVELOPMENT OF C.F. ANTIBODY IN INFECTED GUINEA PIGS

G.Pig #	<i>Mouse brain</i> 10/29/47 0.1 cc I.DER.	11/13/47		<i>Mouse brain</i> 11/21/47 BOOSTER S.Q.	12/4/47 C.F.
		C.F.	LESION		
1113	1:100	0	0	0.25 cc -1110	64
1114	1:100	64	0	0.25 cc	128
1116	1:100	0	+	0.25 cc	Died 11/24/47
1117	1:100	0	+	0.25 cc	128
1118	1:100	0	+	0.25 cc	32
1119	1:10	0	+	Died 11/14/47	
1124	1:10	64	0	0.25 cc	128

all febrile
course.
These are
survivors.
of larger
number

Normal
1:4⁺ neg.

See serial
29 May 48
for names

ELI LILLY AND COMPANY

INDIANAPOLIS 6, U.S.A.

April 20, 1948

Dear [REDACTED]

We are shipping to you by express a sample of fresh calf serum which you desired. This material will be shipped from Indianapolis early Wednesday morning and should reach Cincinnati sometime in the afternoon. It will be packed in dry ice as you requested.

The blood was drawn from a calf approximately eight months old on Monday forenoon, April 19. The blood was allowed to clot and the serum removed that afternoon and immediately frozen. No preservative was used.

We hope this serum will suit your needs, and we will be glad to supply additional amounts of this type of material without charge, if you so desire.

Very truly yours,
[REDACTED]

had/dm

*1 amp transferred to Lusteroid
stored in 98 other ampoules stored in C1*

Thanked 22 April

*Rec'd 21 April -
well frozen in 2 ampoules.*

WAYNE UNIVERSITY

BOARD OF EDUCATION

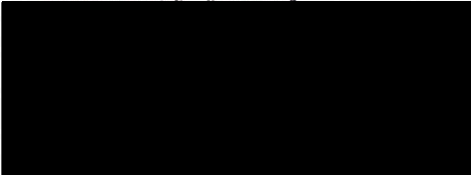
DETROIT 1, MICHIGAN

COLLEGE OF MEDICINE
1512 ST. ANTOINE
DETROIT 26, MICHIGAN
DEPARTMENT OF PHYSIOLOGY

May 14, 1948

Dear 

I am sorry we were not more explicit concerning our information on the potency of Ac-globulin we sent to you. The 1,000 units referred to the total quantity sent. The specific activity was 350 units per mg. of tyrosine.

Sincerely yours,


WHS:swm

Collection & Preparation of Lamb Sera

Slaughter House: S. Hall & Sons. 2121 Freeman Ave. CH-3946

This house slaughters lambs & sheep only & is very accommodating. Supply RBC to CSH.

Sample collection: Blood caught into heparin from jugular bleeding, immediately transferred to flask containing glass beads. Sample then defibrinated & returned to lab. immediately for separation & freezing of plasma. Blood was collected from three lambs (#1, 2, 3) in this fashion early this afternoon. These animals were said to be about 18 months old & weaned.

Sample Treatment: Portion of each specimen in 2 cent. tubes, plasma pooled, then one heparinoid & one 20 cc. amp (10 cc. plasma) filled, labelled: Lamb # $\frac{1}{3}$ Plasma 26 April & stored in 98

JUN 15 1948

TOXO. SERA FROM FORREST ADAMS (U. MINN.)

The following sera were rec'd via air mail this noon from Forrest Adams @ the Univ. of Minn. These were unfrozen & had been in garage for 24 hrs. Following arrival here, they were transferred to bacteroids & stored in 97

PATIENTSERUM
DATEDISEASE

23 MAR. 48 18 yrs. old. CONG. TOXO.

18 MAR 48 5 yrs. STILL'S DISEASE

— 5 yrs. " "

24 July 48 1/64
24 July 48 + 2 Aug 48 = 1/64
2. 24 July 48 = 1/64
+ 2 Aug 48.

UNIVERSITY OF MINNESOTA
THE MEDICAL SCHOOL
MINNEAPOLIS 14

DEPARTMENT OF PEDIATRICS

June 14, 1948

Dear [REDACTED]

This is just a note to thank you again for the kindness and consideration you showed me while I was in Cincinnati. Your ideas and suggestions will help me a great deal. I am sending ^{as you suggested} in a separate package 3 separate specimens on patients who have a positive rabbit test. Their names are:

[REDACTED] - congenital toxo.
Still's Disease
" "

I will be looking forward to your results and ideas.

Before leaving your fair city, I forget to return the enclosed meal tickets. Would you do that for me?

Sincerely
[REDACTED]

JUN 16 1948

TOXO. SERA FROM JOEL WARREN

The following sera were rec'd frozen from Joel Warren at the Ams to-day & were immediately stored in 102.

NAME	DATE	SOURCE	WARREN		C.H. R.F.
			C-F	RABBIT	IN VITRO TITER
	5-20-48	U.OF. TEXAS	1:64	N.D.	1:256
	2-11-48	CHILDREN'S HOSP.	1:16	Pos.	1:256
	3-14-45	FR. DR. PIN- COCK	1:128	Pos.	1:1024
	2-5-48	U.OF TEXAS	1:64	N.D.	1:256
	4-27-48	U.OF. WISCON- SIN	1:8	N.D.	1:16
	11-16-44	^{DETROIT} CHILDREN'S HOSP.	1:64	Pos.	1:1024
	13 July 48				1:256
	8-26-46	URGIA	1:8	NEG.	1:64
	11-26-47	JOHNS HOPKINS	1:8	N.D.	1:256
	5-8-48	AAF Hosp.	1:8	NEG.	1:256
	7-24-44	CORAL GABLES	1:8	EQU.	1:16

WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO

INSTITUTE OF PATHOLOGY
2085 ADELBERT ROAD

June 21, 1948

[REDACTED]

Dear [REDACTED]

I was greatly fascinated by the results you describe in your letter of June 16. This looks to be a very fruitful as well as fundamental problem.

I believe from your data that the activator is either complement or one of its heat labile components. I wouldn't be too disturbed about the apparent non-relationship of the hemolytic titers of the various serums used to their activating capacity. If you will review pages 201-207 in the textbook on Immunity by [REDACTED] you will note that it is a well known phenomenon that complement of certain species of animals does not always activate antibody produced by active immunization in other species. Off the record, we have obtained a similar phenomenon on the lysis of sensitized H. pertussis by various complements. We have noted that guinea pig complement does not activate anti-pertussis rabbit and human serums. We are further investigating this unexpected result. It may be valuable to determine similar relationships in your present study. Until you have carried this out, I cannot see how purified components of complement will be of much aid. In any event, the test would appear to be concerned only with the heat labile components (C'1 and/or C'2).

I may be able to furnish you some human C'1. The question arises as to how we could ship this substance to you since it is relatively unstable at room temperature. It may be necessary for you to pay us a visit to discuss this very fundamental problem more thoroughly. Please feel free to do so.

With best regards, I am,

Sincerely yours,
[REDACTED]

LP:ew

Human $C_1 + C_2$ — is it in greater con-
than in human serum

Mouse Serum (devoid of C_2) is inactive — Human fraction

C'_1

Fraction II + III

" III-1

C'_2

" IV-1

" IV-7

THE CHILDREN'S HOSPITAL RESEARCH FOUNDATION
ELLAND AVE. AND BETHESDA
CINCINNATI 29, OHIO

Toxo

June 24, 1948

Dear [REDACTED]

This letter concerns a piece of work which you published in the Journal of Immunology, Volume 46, 1943, under the heading, "The Complementary Activity of Mouse Serum." During the past few months, we have been studying an unusual immune phenomenon against toxoplasma and found that an accessory substance is required for the completion of the phenomenon. This accessory substance is thermolabile, is present in fresh, human serum in such small amounts that the activity disappears below the 50% end point at a dilution of 1:4, and guinea pig serum seems to have even less than human serum. Unfortunately, we are limited to the species of animals that can be used for these tests, because most other animals, with the exception of the mouse, yield sera with a non-specific effect in the fresh state. The mouse serum, however, has no activator effect at all.

I happened to be looking through the last edition of Topley and Wilson (1946, Volume 1, page 232) and there found the statement, "Mouse serum which has no complementary activity has neither C 1, C 2, nor C 4 (Brown, 1943)." However, when I read your original paper, it seemed as if this quotation from [REDACTED] was wrong in that you indicated that mouse serum was deficient in C 2 but had C 1, C 3 and C 4. I wonder if you would be good enough, if I am correct in what I think your conclusions are.

Previously, I was thinking that perhaps our accessory substance might be C 1 — first, because it was thermolabile and, second, because it apparently was present in greater concentration in human serum than in guinea pig serum. However, since mouse serum, which is supposed to have C 1, is completely devoid of our accessory factor, it seems to me to become a little difficult to be definite in ones suspicions in this respect.

Looking forward to hearing from you, I am,

Sincerely yours,

ABS:mjc

4 26

WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO

INSTITUTE OF PATHOLOGY
2085 ADELBERT ROAD

July 19, 1948

[REDACTED]

Dear [REDACTED]

I was glad to learn that the complement fractions arrived in good order. I hope that they will help to elucidate your findings.

As I told you over the phone, each of these fractions is concentrated 5 times over human serum. They are fully active and isotonic. The C¹₁ fraction contains only C¹₁ and is very pure. The C¹₂ fraction contains only C¹₂ activity but does contain albumin and certain alpha-globulin. Neither of these two fractions contain C¹₃.

If you desire any further information, please call upon me.

With best regards, I am,

Sincerely yours,
[REDACTED]

LP:ew

DEPARTMENT OF PHYSICAL CHEMISTRY
HARVARD MEDICAL SCHOOL
BOSTON 15, MASSACHUSETTS

July 24, 1948

[REDACTED]

Dear [REDACTED]

[REDACTED] has asked us to take care of the request for samples of human blood plasma fractions contained in your letter of July 11th. We were interested to hear about the accessory substance you have observed in your work on toxoplasma and are sending you samples of all the main plasma fractions to assay for this component. We have found that the best method for dissolving these powders is to take them up in 0.15 molar sodium chloride and adjust the solutions to around pH 7. Since the dried powders have been stored for some time (although at 0° to -2° C.) they may have lost some activity if the accessory substance is labile. We include a sample of whole plasma from which you could tell whether this is so, as you mention in your letter having tested both fresh and frozen plasma.

Some investigations of complement carried out here a few years ago indicated that C'-1 was in Fr. II+III, which might be of interest to you also.

We look forward to hearing from you again about your study and hope you will let us know if we can be of any further help to you.

Sincerely yours,
[REDACTED]

Longwood
6-2380

Should the original plasma be dissolved in dest. H_2O ?

Would 7.5% be equiv. to original? Yes

How about the citrate?

Can the others be dissolved to 7.5% conc.

pH before adjustment

What do they use for adjusting the pH.

— phosph. buffer 7.5%

J. Am. Chem. Soc.

Whole plasma

F_r. I — Fibrinogen

" II + III — γ + Beta glob — also lipid

" IV-1 — alpha + beta — lipid high

" IV-4 — alpha + beta + alb " low

" V — albumin

1946, 68
Cohn-Strong

Operator 6

WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO

INSTITUTE OF PATHOLOGY
2085 ADELBERT ROAD

September 1, 1948

[REDACTED]
Dear [REDACTED]

I am very sorry that I was not able to see you while I was in Cincinnati. Unforeseen circumstances prevented me from visiting your laboratory. I hope that I may have the same privilege on my next visit to Cincinnati.

I was extremely interested by your remarks on the effect of the components of complement on your antigen-antibody system. I would appreciate it very much if you could furnish me with the details. I shall be glad to give you my interpretations if you so desire.

With best regards to [REDACTED] and yourself.

Sincerely yours,
[REDACTED]

LP:ew

Toxo

October 8, 1948

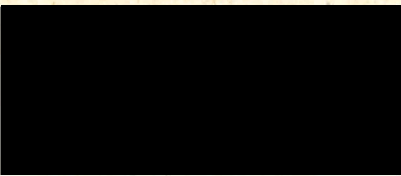
Dear [REDACTED]

Your letter of September 1 was deeply appreciated, and I regret that the pressure of other business has prevented me from writing to you earlier about the results we obtained with the concentrated human C'_1 and C'_2 fractions which you so kindly supplied.

Effect of Fractions in Toxoplasma Immunity Phenomenon

- | | |
|---|--|
| Source of toxoplasma antibody | - heated human serum in concentration of 5% in final mixture with toxoplasma. |
| Concentrated C'_1 and C'_2 used individually | - in concentration of 45% in final mixture with toxoplasma. |
| Combination of C'_1 and C'_2 | - each in a final concentration of 22.5%. |
| Source of heat-stable components of complement "accessory factor" | - human serum devoid of toxoplasma antibody heated at 56° C for 30 minutes in final concentration of 50% |

The "activating" capacity of these fractions alone or of the two together was tested by adding them to the heated immune serum in the concentrations indicated above, in the absence of heated normal human serum.



The capacity of these fractions to restore the "accessory factor" activity of a heated normal human serum (which exhibits this activity in the fresh state but loses it after heating) was tested in the presence of a 50% concentration of such normal heated serum. The results were entirely negative in both types of tests while the simultaneous control with the fresh normal human serum used in the test gave the expected positive result. You will recall that fresh mouse serum did not have the "accessory factor," and in the present test addition of C'_1 or C'_2 or both failed to activate it. Thus, it would appear that the heat-labile component of the "accessory factor" in fresh human serum needed for the toxoplasma immunity phenomenon was not present in the 5 times concentrated C'_1 and C'_2 fractions of human serum which you sent us.

Effect of Fractions in Hemolytic System

It was desirable, therefore, to determine the activity of these fractions in a hemolytic system both with the heated human serum that we used and with heated guinea pig serum which in the fresh state contained 100 - 120 units of complement per ml. The sensitized sheep erythrocytes consisted of a mixture of equal parts of 3% washed RBC and rabbit hemolysin diluted to contain 3 units per 0.25 ml. The guinea pig and mouse ^aserum were used in 0.1 ml. and the human serum in 0.2 ml. amounts; each fraction was used in 0.2 ml. amounts, constituting 40% of the total volume before addition of 0.5 ml. of the sensitized erythrocytes. The results were recorded after incubation in the water bath at 37° C for

30 minutes and 60 minutes, and after sedimentation in the refrigerator overnight; however, the final results were the same as those recorded after 30 minutes. They were as follows:

C₁ fraction (concentrated) + saline --- no hemolysis

" " " + fresh mouse serum --- no hemolysis

" " " + heated guinea pig serum --- 2-3 plus hemolysis

" " (1:5) + heated guinea pig serum --- no hemolysis

C₂ fraction (concentrated) + saline --- no hemolysis

" " " + heated guinea pig serum --- no hemolysis

!! " " " + fresh mouse serum --- no hemolysis

C₁ + C₂ (concentrated) + saline --- complete hemolysis

" " (1:5) + saline --- no hemolysis

" " (1:5) + heated guinea pig serum --- complete hemolysis

" " (1:25) + heated guinea pig serum --- 2 plus hemolysis

" " (1:5) + heated human serum --- complete hemolysis

" " (1:25) + heated human serum --- 1 plus hemolysis

!! " " (1:5) + fresh mouse serum --- no hemolysis

These data indicated that in the concentrated form these fractions were not pure, since hemolysis could be produced without addition of the heat-stable components of complement. However, diluting these fractions 1:5 got rid of the other components without loss of their capacity to activate either the heated human or guinea pig serum. Especially noteworthy is the fact that, contrary to expectations based on

[REDACTED]

paper on mouse serum, neither the concentrated C_2' fraction by itself nor $C_1' + C_2'$ (diluted 1:5) could impart complementary activity to fresh mouse serum.

These data would seem to suggest that fresh human serum (and perhaps to a lesser extent fresh guinea pig serum) contains a heat-labile component different from C_1' and C_2' , which is necessary for the consummation of the toxoplasma immunity phenomenon but not for hemolysis. These data also raise the question whether Gordon Brown's preparation which activated fresh mouse serum might have contained another component in addition to C_2' . Your comments on these observations would be greatly appreciated.

With kindest regards from [REDACTED] and myself,

Sincerely,
[REDACTED]

[REDACTED]

enc.

P.S. We have carried out similar tests with whole plasma and fractions I, II + III, IV-1, IV-4, and V which we obtained from Boston. The original plasma still had some complement (although in very low titer) when tested against sensitized RBC, but had no effect on the toxoplasma system. Neither the plasma nor any of its fractions, listed here, were capable of reactivating a heated normal human serum which lost its effectiveness in the toxoplasma system. There was, however, a partial effect with fraction IV-1 used as a 7.5% solution, but not with the 1% solution, which may suggest that the unknown component might perhaps be associated with this fraction.

Punch for
notebook

COPY

October 8, 1948

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[REDACTED]

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[REDACTED]

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" " (1:5) + heated guinea pig serum --- no hemolysis
C₂' fraction (concentrated) + saline --- no hemolysis
" " " + heated guinea pig serum --- no hemolysis
!! " " " + fresh mouse serum --- no hemolysis
C₁' + C₂' (concentrated) + saline --- complete hemolysis
" " (1:5) + saline --- no hemolysis
" " (1:5) + heated guinea pig serum --- complete hemolysis
" " (1:25) + heated guinea pig serum --- 2 plus hemolysis
" " (1:5) + heated human serum --- complete hemolysis
" " (1:25) + heated human serum --- 1 plus hemolysis
!! " " (1:5) + fresh mouse serum --- no hemolysis

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[REDACTED]

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Sincerely,

[REDACTED]

[REDACTED]

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THE CHILDREN'S HOSPITAL RESEARCH FOUNDATION
ELLAND AVE. AND BETHESDA
CINCINNATI 29, OHIO

Toxo

October 8, 1948

[REDACTED]

Dear [REDACTED]

I deeply regret that the pressure of other business has prevented me from letting you know the results of our tests for accessory factor in the toxoplasma immunity reaction in the whole plasma and fractions I, II + III, IV-1, IV-4, and V which you were good enough to send us. The original plasma still had some complement although in very low titer when tested with sensitized sheep erythrocytes but had no effect on the toxoplasma system. Neither the plasma nor any of its fractions were capable of reactivating a normal human serum which was deprived of its heat-labile components of the accessory factor by heating at 56° C for 30 minutes. It is perhaps noteworthy that a partial effect was observed with fraction IV-1 when it was used as a 7.5% solution, but not when it was used as a 1% solution. This may suggest that if the original plasma had been handled in a manner better to conserve the very heat-labile components of the accessory factor that they might perhaps be found to be associated with fraction IV-1.

With many thanks for your kindness in supplying us with this material,

Sincerely yours,

[REDACTED]

ABS:mjc
~~cc/ Dr. Pillemer~~

UNIVERSITY OF MICHIGAN
SCHOOL OF PUBLIC HEALTH
ANN ARBOR, MICHIGAN

DEPARTMENT OF EPIDEMIOLOGY
VIRUS LABORATORY

October 11, 1948

Dear [REDACTED]

Thank you for the copy of your letter to [REDACTED]

Needless to say, I was disturbed to find you had not been able to repeat my old results with mouse serum. The first thing that occurs to me as a possible explanation is the question as to whether [REDACTED] fractionation of C-2 and mine employed the same technique. As you saw in my paper I followed the procedures of [REDACTED] and [REDACTED] in which the endpiece (E-1, containing C-2 and C-4) was separated from fresh whole complement by treatment with CO₂. Treatment of one portion of this endpiece at 56°C. left the thermo-stabile C-4 and treatment with NH₄OH left the thermolabile, ammonia-resistant C-2. If [REDACTED] uses a different method of fractionation, we might possibly have a partial answer.

My observation that the endpiece (C-2 + C-4) restored the complementary activity of mouse serum was not original since [REDACTED] in 1911 described that phenomenon. It is of course possible that some C-4 remained in my C-2 preparation but that is unlikely if the ammonia treatment was satisfactory. You might play with C-4 a bit and see what comes out.

Another point, although I honestly don't think it important, is the fact that all my tests were made on sera from six genetically pure, inbred strains of mice from Bar Harbor, and on the wild mouse Mus musculus. Could it be that the ordinary white Swiss mouse is different?

Your phenomenon sounds very interesting. Since I know nothing about it I am presuming you have an in vitro test which you hope will replace the neutralization technique you described in 1942. Have you considered the possibility of some heterogenetic factor such as the Forssman reaction? And here is a real "flier". Why don't you try a completely new hemolytic system such as guinea pig or dog cells plus anti-guinea pig or anti-dog cell rabbit serum? I know it sounds crazy and your answer will probably be found in a study of the serum.

Please keep me informed of the progress of this work. I'm becoming very interested.

Best personal regards,
[REDACTED]

P.S. As you have probably heard, or will soon, our

GCB:nc

Miners virus gave only titers 10-3.16

es.

WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO

INSTITUTE OF PATHOLOGY
2085 ADELBERT ROAD

October 28, 1948

[REDACTED]

Dear [REDACTED]

I reviewed the information contained in your letter of October 8 (for which I am deeply grateful) and agree with your interpretations entirely. It would appear from your findings that another component in fresh human serum is necessary for the full toxoplasma phenomenon. Your results with the Cohn fraction also seem to bear out this hypothesis. It is, however, well known that the components of certain species are not mutually supplementary. I refer you to the paper by Hagedus and Griener, Z. Immunitats, 92, 1, 1938.

If I can be of any other help to you, please let me know.

With best regards to [REDACTED] and yourself, I am,

Sincerely yours,
[REDACTED]

LP:ew

DEPARTMENT OF PHYSICAL CHEMISTRY
HARVARD MEDICAL SCHOOL
BOSTON 15, MASSACHUSETTS

[REDACTED] 8, 1948

Dear [REDACTED]

Thank you for your letter of October 8th containing the report of your investigations for the toxoplasma antibody's "accessory factor" among the plasma fractions we sent you last summer.

We think you may be interested to hear that we have recently developed a new fractionation procedure for human blood plasma, in the interest of better preservation of the more labile components, in which category your report indicates the "accessory factor" belongs.

This new method is very different from our previous one. The time for the whole process is greatly reduced. The fibrinogen and most of the globulins and lipoproteins are precipitated in the initial step and maintained in the solid state until fractionally extracted. During the main separations the pH never goes below 5.6 or above the physiological range and the highest alcohol concentration is just under 20% at -5°C .

We will soon have prepared sufficient quantities of the major fractions so that aliquots of them can be made available for investigation. These aliquots represent all the initial components of the plasma. Later we will also have new subfractions for distribution.

If you wish to carry out investigations of these new fractions and will let us know, we will make every effort to provide you with material for assay.

Sincerely yours,
[REDACTED]

THE CHILDREN'S HOSPITAL RESEARCH FOUNDATION
ELLAND AVE. AND BETHESDA
CINCINNATI 29, OHIO

November 11, 1948

[REDACTED]

Dear [REDACTED]

Thank you very much for letting me know of your new methods for fractionation of plasma. Would it be possible for you to send me some of the original plasma from which the new fractions are being prepared? Should the original plasma be as inactive as the last preparation we had, there would not be much use in our testing any of its components. If you have the original plasma in the lyophilized state, may I suggest that it be sent to us by air mail, special delivery, rather than by parcel post, the way it came last time. However, if you still have it in the frozen state, I would like to suggest that it should be sent to us packed in dry ice, by air express.

Sincerely yours,

[REDACTED]

ABS:mjc

Punch for note book.

DEPARTMENT OF PHYSICAL CHEMISTRY
HARVARD MEDICAL SCHOOL
BOSTON 15, MASSACHUSETTS

November 23, 1948

[REDACTED]

Dear [REDACTED]

In reply to your letter of November 11th, we wonder whether the following might be the easiest way to answer your question about the "accessory factor" activity in our initial plasma, as collected for the new fractionation procedure.

Rather than have us freeze or lyophilize an aliquot of this plasma and attempt to send it to you refrigerated, could you yourself draw a small amount, using conditions similar to those we now use, and then assay it for "accessory factor"?

The procedure by the new method is to collect 450 cc. of blood in a bottle containing 75 cc. of acid-citrate-dextrose solution. The blood is then cooled immediately to 5° C. and centrifuged within two hours of the time at which it was drawn. Immediately following centrifugation the first fractionation step is started and is complete within six hours of the time of bleeding. Therefore, if you could draw perhaps 6 or 12 cc. of blood into 1 or 2 cc. of ACD, centrifuge and assay, you would be able to determine whether an appreciable amount of "accessory factor" activity was lost even before the first step of the new process.

If you decide to do this, find the majority of the activity still present in the plasma, and wish then to assay the fractions, we will be glad to provide you with them.

As we mentioned before, we hope by the new procedure to preserve the more labile components of the plasma better and therefore we would be particularly interested to hear what you find, in the meantime assuring you of our good wishes.

Sincerely yours,
[REDACTED]

THE CHILDREN'S HOSPITAL RESEARCH FOUNDATION
ELLAND AVE. AND BETHESDA
CINCINNATI 29, OHIO

For notebook

December 1, 1948

[REDACTED]

Dear [REDACTED]

Many thanks for your letter of November 23, and I regret the unavoidable delay in replying to it. From your description of the procedure used in your new method of processing plasma for unstable components, I can tell without testing that our toxoplasma accessory factor would be preserved during the early stages of preparation if present in the original plasma. However, it would still be necessary to test the original plasma to make certain that it is free of specific toxoplasma antibodies, for if such antibodies are present we can not carry out the tests for accessory factor. We know from some recent studies that the blood of many normal adults may contain toxoplasma antibodies and, as a matter of fact, one sample of gamma globulin that we had tested recently contained this antibody in a titer of 1:256.

If you do not have any facilities in your department for shipping plasma in dry ice, I am sure that Dr. Eaton, of the Department of Bacteriology, would be good enough to oblige by sending off the specimen to me.

With many thanks,

Sincerely yours,

[REDACTED]

ABS:mjc

Punch

DEPARTMENT OF PHYSICAL CHEMISTRY
HARVARD MEDICAL SCHOOL
BOSTON 15, MASSACHUSETTS

January 5, 1949

[REDACTED]

Dear [REDACTED]

I wish to apologize for our delay in writing you regarding the dried plasma which was sent to you on December 20th. Although we did get the material off to you, the intervention of Christmas delayed me in writing to inform you that it had been sent.

We would like to explain that the aliquot was dried instead of frozen because even freezing produces the lipid denaturation mentioned in a previous letter, drying does no further harm as far as the lipids are concerned and for shipping purposes drying is more satisfactory.

At least this will enable you to determine whether the activity loss you observed was the result of a long storage period or is due to some other factor, since the first plasma we sent, as you know, had been stored for quite a long time at 0° C.

We hope the new material reached you promptly, in spite of the delay of this letter, and we look forward to hearing from you again when you have had a chance to assay it.

With best wishes for a happy new year,

Sincerely yours,
[REDACTED]

Pumot

January 18, 1949

[REDACTED]
Dear [REDACTED]

I wish to apologize for having delayed so long in letting you know the results of our tests on the sample of lyophilized plasma which you were good enough to send us. Our tests on this sample brought out several points of interest as follows:

- 1) Whatever treatment this plasma had had prior to the time of lyophilization was apparently sufficient to destroy the very labile accessory factor required for the toxoplasma immunity phenomenon.
- 2) Although it was devoid of this accessory factor, it still had enough complement for the lysis of sensitized sheep erythrocytes to yield a 50% hemolytic dose of 0.02 cc. You will realize, however, that this is approximately one-tenth of the amount of complement usually present in fresh or well preserved human serum.
- 3) This sample of plasma (and I do not know how large a pool it represents) had toxoplasma antibody in a titer of 1:16.

In view of these findings there would not be very much point in testing any of the fractions that might have been derived from this sample for accessory factor. It may indicate to you, however, that your starting material already had lost certain very labile components.

Sincerely yours,

[REDACTED]
ABS:mjc

DEPARTMENT OF PHYSICAL CHEMISTRY
HARVARD MEDICAL SCHOOL
BOSTON 15, MASSACHUSETTS

*Punch
Notebook*

January 24, 1949

[REDACTED]

Dear [REDACTED]

Thank you very much for your letter of January 18th containing the report regarding your toxoplasma accessory factor assay of the lyophilized plasma we sent you.

We wondered whether your previous studies of this factor have given any indication that the lyophilizing process itself may be injurious to this factor and would be interested in any information you could give us on this point.

May we express our appreciation of your help in testing the toxoplasma accessory factor, (and also complement, in relation to the former's activity loss) for us and the hope that, as our work continues, we may be able to determine better conditions for the preservation of this labile component.

Sincerely yours,
[REDACTED]

January 28, 1949

[REDACTED]

Dear [REDACTED]

In reply to your letter of January 24, I might say that we know that the toxoplasma accessory factor holds up quite well when stored in the frozen state in an insulated box containing solid CO₂. We also know that it can be frozen and thawed at least three to four times without losing its activity. We have also found that certain lyophilized specimens of serum (we haven't tested many, however) which were lyophilized by the process in which the serum is first frozen in dry ice, were active as regards the toxoplasma accessory factor. You probably also know that the titer of ordinary complement is well preserved in serum that has been properly stored in the frozen state prior to lyophilization.

Sincerely yours,

[REDACTED]

ABS:mjc

Punch for notebook

DEPARTMENT OF PHYSICAL CHEMISTRY
HARVARD MEDICAL SCHOOL
BOSTON 15, MASSACHUSETTS

February 3, 1949

[REDACTED]

Dear [REDACTED]

Thank you for your letter of January 28th, containing further information regarding the stability of the toxoplasma accessory factor. If, as our work continues, we obtain material under conditions which we think should have better preserved this component, we would like to write you again and hope you might consider assaying an aliquot or two for us then. In the meantime, may we thank you again for your help.

I recently read a report by [REDACTED] on her work in Japan with the Ekiri Commission and was interested to learn that it was your initial observations of this disease which gave the impetus to their work.

With best wishes,

Sincerely yours,
[REDACTED]

JUL 20 1948

RELATION OF C'_1 and C'_2 TO ACCESSORY FACTOR REQUIRED FOR IMMUNE PHENOMENON AFFECTING TOXOPLASMA

REAGENTS - C'_1 and C'_2 received in frozen state from Dr. Pillemer of Cleveland. They are said to have been derived from pools of human sera and to be in a concentration 5x that ordinarily present in human serum.

Toxoplasma - PE from one three day mouse. Rich in tox.

SERA - IMMUNE - MRS. Kope (5-28-48)

Normal - EB. - Buescher serum used as activator

TUBE	Heated Immune SERUM	DILUENT	Toxo in 0.2cc SALINE	No. unstained	Per cent unstained
1	None	0.2cc saline		2/50	4
2	0.02cc	0.18cc C'_1	" 2 ⁰¹	1/100 unit.	1
3	"	" C'_2	" 2 ²⁰	6/100	6
4	"	0.09cc C'_1 + 0.09cc C'_2	" "	4/100	4
5	"	0.18cc saline	" 2 ³⁰	mult ppt but overall look stained	0
6	"	" "	Heated EB serum 2 ³⁰	0/50	0
7	"	" fresh EB	"	95/100	95
8	"	" C'_1	" 2 ³⁰	1/52	2
9	"	" C'_2	" "	4/50	8
10	"	0.09cc C'_1 + 0.09cc C'_2	" "	2/100	2
11	None	0.2cc saline	" 2 ⁴⁵	1/50	2
12	"	0.2cc "	Fresh mouse serum "	0/50	0
13	0.02cc	0.18cc saline	" "	2/50	4
14	"	" C'_1	" "	0/62	0
15	"	" C'_2	" "	13/100	13
16	"	0.09cc C'_1 + 0.09cc C'_2	" "	9/100	7

9/100 *
20/100 *

* These tubes (#14 & #15) stored in refig. for 2 hrs. & then incubated @ 37°C again for 30 min. & then these counts were obtained.

Additional Data For Tube #7:

When the contents of this tube were examined after one hour's incubation, it was found that all the trophoblasts were well-stained. Since it was most likely that the heated immune serum had been omitted, 0.02 cc of the heated Kope serum was added & the tube reincubated. (37°C)
Examined @ 30' = 75/100 unstained, at one hour, 95/100 unstained.
This demonstrated:

- a) That a good immune serum was used for to-day's test.
- b) "Antirator" will withstand incubation @ 37°C for one hour without appreciable loss of activity.

Activity of PILEMER C_1' and C_2' purified fractions Used in TOXOPLASMA TESTS AS REGARDS Hemolysis of Sensitized ERYTHROCYTES BY COMPLEMENT

REAGENTS - 3% washed sheep RBC in saline
+ 3 units of hemolysin

GUINEA PIG COMPLEMENT - Lot of ...
used on several occasions
0.5cc of 1:50 to 1:60 = 1 unit

MOUSE SERUM and C_1' and C_2' fractions - same as
used in toxoplasma test

TUBE	Material added to 0.5cc sensitized RBC	RESULT - Hemolysis		
		37°C 1/2 hr	37°C 1 hour	Remarks
1	0.5cc saline	0	0	0
1052	0.1cc <u>fresh</u> g.pig serum + 0.4cc saline	4	4	4
3	0.1cc <u>heated</u> " " + 0.4cc "	0	0	0
1054	0.1cc " " " + 0.2cc C_1' + 0.2cc saline	2	3	2-3
5	0.1cc " " " + 0.2cc C_2' + 0.2cc "	0	0	0
6	0.1cc " " " + 0.2cc C_1' + 0.2cc C_2'	4-?	4-?	4
7	0.1cc saline + " " + " "	4-?	4-?	3.5
8	0.1cc <u>fresh</u> mouse serum + 0.4cc saline	0	0	0
9	0.1cc " " " + 0.2cc C_1' + 0.2cc saline	0	0	0
10	0.1cc " " " + 0.2cc C_2' + 0.2cc "	0	0	0
11	0.1cc " " " + 0.2cc C_1' + 0.2cc C_2'	4-?	4-?	4
12	0.1cc <u>heated</u> " " + 0.2cc C_2' + 0.2cc saline	0	0	0
13	0.1cc " " " + 0.2cc C_1' + 0.2cc C_2'	4-?	4-?	4-

Heat at 56°C for 30 min. - 0.6cc g.pig serum pool
- 0.3cc mouse serum

3540
23 1948

Since the C'_1 and C'_2 fractions did not behave as expected, particularly the combination produced hemolysis by itself, it was decided to repeat the following tests using $1/5$ the concentration of these fractions, i.e. 1:5 dilution in saline.

<u>Tube</u>		37°C 1/2 hr.	37°C 1 hr	Overnight refrig.
14	0.1 cc saline + 0.2 cc C'_1 (1:5) + 0.2 cc C'_2 (1:5)	0?	0?	tr
15	0.1 cc heated g.pig. + " " " + " " "	4	4	4
16	0.1 cc fresh mouse + " " " + " " "	0	0?	tr
17	0.1 cc heated " + " " " + " " "	0	0?	tr
18	0.5 cc saline	0	0	0
19	0.1 cc heated g.pig + 0.2 cc C'_1 (1:5) + 0.2 cc saline	0	0	0
20	0.2 cc C'_1 (undil) + 0.3 cc saline	0	0	0
21	0.2 cc C'_2 (1:5) + 0.3 cc "	0	0	0

To complete these observations it was deemed desirable to test the reactivating power of the C'_1 and C'_2 fractions on the human serum used in the toxoplasma tests

<u>Tube</u>		37°C 1/2 hr	37°C 1 hr	
22	0.2 cc fresh E.B. serum + 0.4 cc saline	4	4	4
23	0.2 cc heated " " + 0.2 cc C'_1 (1:5) + 0.2 cc C'_2 (1:5)	3.5	3.5	4-
24	0.2 cc " " " + 0.2 cc C'_1 (1:25) + 0.2 cc C'_2 (1:25)	0?	0?	1
25	0.2 cc " G.pig " + 0.2 cc C'_1 (1:25) + 0.2 cc C'_2 (1:25)	1?	1?	2
26	0.2 cc " " " + 0.4 cc saline	0	0	0

JUL 8 1948

Family - Toxoplasma Study

Patient: [redacted] ⁻²²⁻⁴⁸ (age 4 mos.) - well until about 3 months of age when infant began to have repeated episodes of ~~seizures~~ seizures which are described as not being typically tonic. These occurred at frequent intervals up to & during admission to Ch. Hosp. Baby appears to be in extremis to-day. Clinical diag.: Trabec?
Bled this afternoon & serum separated & stored in CO_2 ice.

Mother: [redacted] ^{gr.} ana i, gravid i. Had uneventful pregnancy & is well now. Bled this afternoon & serum stored in dry ice.

Post-mortem:

9 July 48

Infant died during the night & was "posted" this a.m. Gross examination unrevealing so no tissue diagnosis. Portion of right testes removed & portion frozen in CO_2 while another piece made to 20% ~~testis~~ suspension in NaCl (ground slowly without abrasives), allowed to settle for ca 5' & super proc Icr & IP to 6 ch alb. 0.3 ml IP & 0.03 ml Icr.

MOUSE

DAY

	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	29	30
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

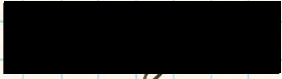
10 July 48

Mother & child negative at until 4/6/48 in toxoplasma in vitro tests.

JUL 14 1948

Serum Specimens From Zuelger

1.



serum obt. 13 July 48, rec'd here, unfrozen, 18 July 48.
This patient delivered a proven toxoplasma-infected infant
in October 1944. Cent. & stored in:

2.



sera obtained 16 July 48 &
rec'd here 17 July 48 unfrozen.
Cent. & stored in:

3.

4.

JUL 17 1948

JUL 16 1948

Complement Fractions From Pillemer

Two frozen tubes labelled "C₁ & C₂" rec'd to-day from Pillemer. When he called earlier this afternoon he stated that these were concentrated from human serum pools & should be used ~~as~~ in 1:5 dilution in NaCl to equal their normal concentration in normal human serum. Should be kept frozen when not in use. Each tube contains about 5.0 cc serum.

Stored in 84

DEC 28 1948

Mouse Peritoneal Exudate Antigen For Toxoplasma Skin TestAntigen: Made by ABS & labelled "Tox. Mouse Peritoneal Exudate

'B-1' 11-18-48 Cent. again 13000 rpm.

This was made from pooled mouse peritoneal exudate & back represented the supernate of two one hour spins at 13000 rpm. Stored in the ordinary refrigerator since with merthiolate 1:10,000.

On 22 Dec 48 this antigen was tested for sterility by seeding 0.1 cc airts on each of two BAP's & into two tubes of BHI broth & freshly boiled thioglycollate broth. In addition 0.03 cc airts were inoc. Icen into each of 4 Chall mice 2-3 wk old.

All media remained sterile for 5 days & mice well.

When used in the C-F tests this antigen contained ~~to~~ titrated to 1:64.

Test: In order to bring the C-F antigen content to a level with that used when CAM antigen was used for skin test (1:4), the Per. Ex. Antigen was first diluted to 1:16 & then this diluted to 1:160 ($\approx$ 1:100 egg) & 1:1600 ($\approx$ 1:1000 egg). 0.1 cc airts of the 1:160 & 1:1600 dil. injected into flexor surfaces of forearm of ABS & HAF with the 1:1600 distal.

Readings: No immediate erythema or wheals.

SABIN

8 hrs.
160: 15x10
raised red.

1600: 0

24 hrs.

28x26 } No pain
raised $\uparrow$ } No pruritus
red. &
15x10

32 hrs.

40x40 } itchy
both } &
raised & red. } painful
25x18
Head aches, flushed. T: 98.6°
oral.

48 hrs.

55x55 E 80
Both itchy
No pain
30x25 E 40

FELDMAN

160 10x10
ERY

1600 5x5

28x28 } SORE
raised, red } PRURITUS
18x13 } SORE

36 hrs.

30x40 E 40 } Pruritus
25x30 E 40 } &
renewed malaise } painful

65x55 E 40

45x40 E 40

see over

HOURS
AFTER
INOC.

SARIN

FELDMAN

1:160

1:1600

1:160

1:1600

8 HRS.

15X10 E40

0

10X10 E

5X5 E

24 HRS.

28X26 E40

15X10 E40

28X28 E40

18X13 E40

NO PAIN OR PRURITUS

SORE, PRURITUS

SORE

32 HRS (ABS)

40X40 E40

25X18 E40

30X40 E40

25X30 E40

36 " (HAF)

SLIGHTLY PAINFUL & ITCHY.

PRURITUS & PAINFUL

BEGINNING HEADACHE,
FLUSHED, FEVERISH BUT
ORAL TEMP. = 98.6°

MARKED MALAISE, THROAT
SL. SORE, NO FEVER.

48 HRS.

55X55 E40

30X25 E40

65X55 E40

45X40 E40

↑ PRURITUS →

PAINFUL

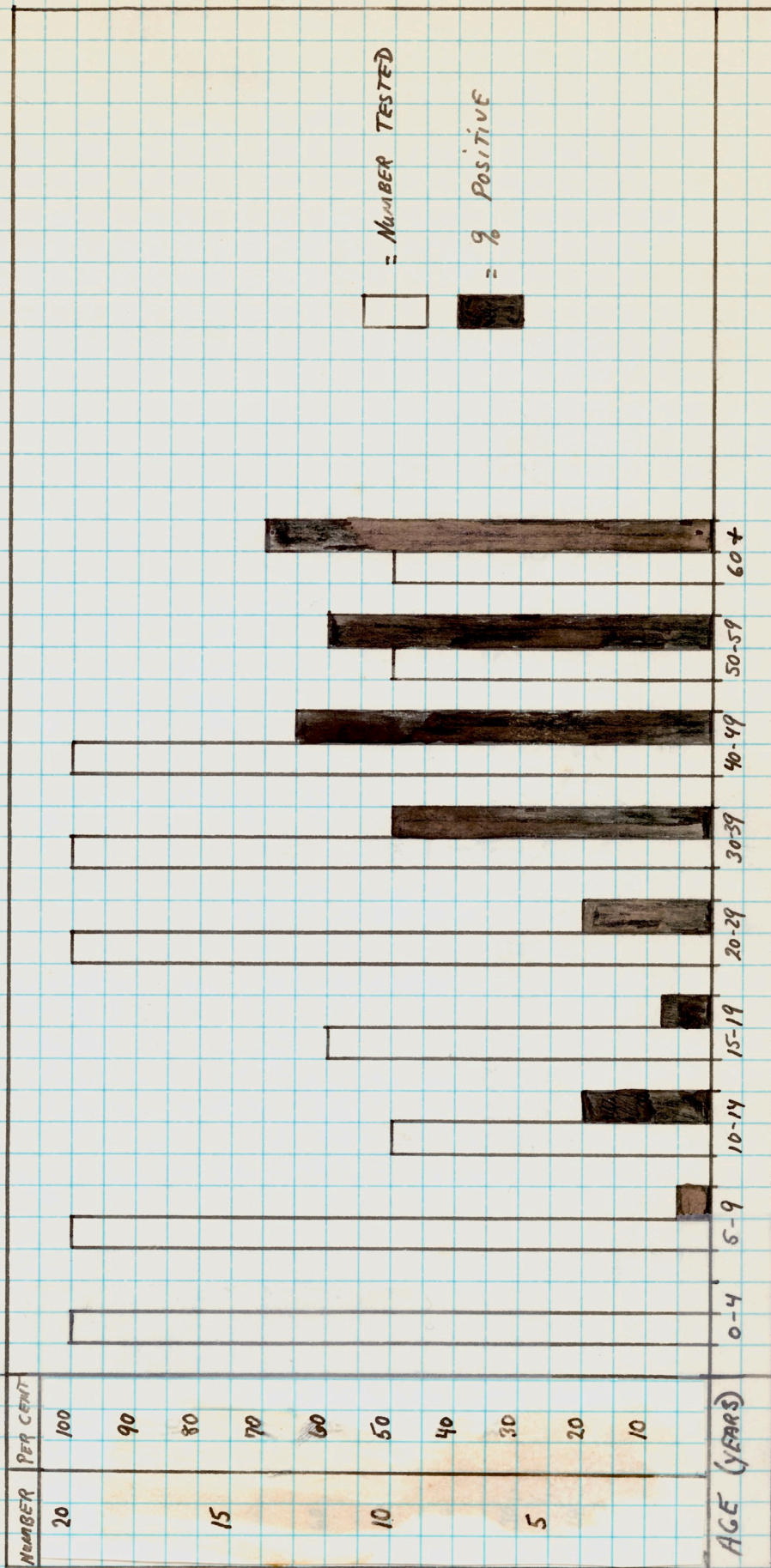
NO SYSTEMIC
SYMPTOMS

PAINFUL SL. PRURITUS

• SEVERE MALAISE

SYSTEMIC SYMPTOMS LET
UP AT ~~54~~ 54 HRS.

INCIDENCE AND AGE DISTRIBUTION OF POSITIVE REACTORS AMONG 142 RESIDENTS OF THE CINCINNATI AREA SKIN TESTED WITH TOXOPLASMA ANTIGEN (EGG).



DEC 12 1947

TOXO PLASMA SKIN TESTS (WARREN ANTIGEN)

ANTIGENS: TOXOPLASMA - rec'd frozen from Joel Warren & stored as such until this a.m. when clarified by spinning (after rapid thawing) & transferred to fresh bottles.

Toxo antigen label: TOXO E66 #3A CAM ANTIGEN 20% susp 3000 rpm
MERTHIO LATE 1:10,000 10-20-47.
 Dil made in N/NaCl.

NORMAL CAM ANTIGEN #2 10% susp. 160g unit 3000 rpm/15 min
10-13-47 NO MERTHIO LATE.

SUBJECTS:

-CONU. TOXO. (CONG.)

TAY-SACHS. HAS HYDROCEPHALUS. VERY SIMILAR APP
 TO RALPH BLACK ALTHO ~~GR~~ MORE MARKED INANITION

SITES: TOXO: LEFT FOREARMNORMAL: RIGHT FOREARM.

SUBJ.	DIL.	ANTIGEN	80'	6°	24°	48°	96°
	1:100	TOXO	—	E 10x10 mm.	E 40 15x10 mm. (VSL EDEMA)	10x10 mm. E 40 (VSL. ED)	—
		NCAM	—	E 10x10 mm.	9x5 mm. discolored BUT NOT RED	—	—
1:1000		TOXO	—	E 10x10 mm.	—	—	—
		NCAM	—	—	6x5 mm. discolor BUT NOT RED	—	—
1:10000		TOXO	—	E 10x10 mm.	—	—	—
		NCAM	—	—	—	—	—
1:100		TOXO	—	—	—	DIED	—
		NCAM	—	—	—	2.5 Q.M.	—
1:1000		TOXO	—	—	—	14 DEC 47	—
		NCAM	—	—	—	—	—
1:10000		TOXO	—	—	—	—	—
		NCAM	—	—	—	—	—

R. BLACK -
 POSITIVE
 @ 1/100

— = NO REACTION
 E = ERYTHEMA
 O = EDEMA

CHILDREN'S HOSP.

RESIDENT PHYSICIANS

TOXO ANTIGEN

FEB 2 1948

NORMAL ANTIGEN

1:100

1:1000

1:100

1:1000

put less
than

30

14x12 eryth

3x3 er.

8x8 er.

○

○

○

8x8 "

○

32 1/2

14x12 er.

4x4 er.

○

○

35x25 o+E

13x12 o+E

○

○

33

12x12 er.

35x15 er

12x12 er.

5x5 er

20x20 er.

15x13 er.

30x20 "

10x7 "

28

○

○

○

○

○

○

○

○

35 1/2

15x11 er.

3x3 er.

○

○

○

6x6 ft. er.

○

5x5 ft. er.

27

○

○

○

○

○

○

○

○

E 27

○

○

○

○

7x7 er.

○

7x7 er.

○

29

○

○

○

○

○

○

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○

34-35

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32 1/2

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○

10 April 1948

TOXOPLASMA SKIN TEST - [REDACTED]

The same toxo CAM & namul CAM dilutions used for testing Mrs. Mabel Cann & Shirley Cann this a.m. were also used to test Dr. Buescher. 0.1 cc units of each inoculated intradermally as follows:

Left forearm: 1:1000 distal & 1:100 proximal of toxo antigen.

Right forearm: 1:1000 distal & 1:100 proximal of namul antigen.

Readings:

a) at 24 hrs: 1:1000 = both negative

1:100 = both control & toxo sites have areas of light erythema (ca 5x5 mm.).

b) at 48 hrs: All sites free of reaction.

Conclusion: Negative skin test.

13 April 48: Bled 70 cc & serum frozen.

TOXOPLASMA SKIN TESTS (WARREN ANTIGEN)

JUN 14 1948

ANTIGENS: TOXO. EGG #3A CAM ANT. 20% MERTH. 1:10,000 10-20-47

NORM CAM ANTIGEN #4 16 dy EMB. 10% Susp. MERTH. 1:10,000 2-18-48

SEE BELOW

NAME	AGE	SEX	COLOR	DIAGNOSIS	TOXO ANTIGEN				CONTROL			
					1/100		1/1000		1/100		1/1000	
					24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H
	5	F	W	F. U. O	—	—	—	—	—	—	—	—
	6	M	W	cont. Intst. Obst.	12x12 FT. ERY.	—	—	—	—	—	—	—
	6	M	C	Tbe spine	—	—	—	—	—	—	—	—
	8	F	W	Chron. Osteo.	—	—	—	—	—	—	—	—
	8	M	W	Post Polio	—	—	—	—	—	—	—	—
	8	M	Y	Tbe Spine	—	—	—	—	—	—	—	—
	9	M	W	Hydrocystus	—	—	—	—	—	—	—	—
	9	M	W	Pulm. Tbe	—	—	—	—	—	—	—	—
	9	F	C	Sarcoidosis	—	—	—	—	—	—	—	—
	12	F	W.	? Rheum. Arthr. ^{F40}	15x15 FT. ERY.	30x30 ERY.	—	20x15	5x5 FT. ERY.	—	—	—
	10	F	W	Chorea	15x12 ERY.	10x15 V. FT. ERY. ^{OVER #}	—	—	—	—	—	—
	13	F	W	Chorea	—	—	—	—	—	—	—	—
	21	F	W	Normal-Nurse	—	—	—	—	—	—	—	—
	21	F	W	Normal-Nurse	—	—	—	—	—	—	—	—
	22	F	W	Normal-Nurse	10x10 V. FT. ERY.	—	—	—	—	—	—	—
	26	M	W	REPEAT NORMAL	15x5 FT. ERY.	8x10? V. FT. ERY.	5x5 FT. ERY.	—	15x5 FT. ERY.	8x10? V. FT. ERY.	5x5 FT. ERY.	—
	24	M	W	NORMAL (LAB.)	20x20 ERY. 5x5 DE. PRURITUS	15x50 ERY. 5x5 DE. PRURITUS	20x20 ERY.	10x15 ERY.	15x15 ERY.	—	15x20 ERY.	—
RAB. # 349	—	—	—	TOXO. IMMUNE	12x12 ERY.	10x12 ERY. SL. RAISED	8x8 ERY.	U.S.L. ERY.	—	—	—	—
RAB. # 398	—	—	—	TOXO IMMUNE	9x6 ERY.	5x5 SL. ERY.	—	—	—	—	—	—

Antigens: These were brought frozen by J. Warren on 30 May & were stored as such until 13 June. After thawing on that date each contained a great deal of floccular precipitate. Ten c.c. portions were removed & after spinning at 2000 for 15', the clear supernates were removed, cult. & stored @ 4°C. BAP 000

Cultures being neg. to day, dilutions for testing were made in No. 2 this a.m., & the remainder of the diluted material discarded after tests were completed. The original vials were refrigerated ^{cent.} while the cleared antigens are stored @ 40 C.

Tests: 0.1 cc units of each dilution were used. The $1/1000$ dilutions are distal on the flexa surfaces of the forearm & the $1/100$ proximal. The two antigens are on the left forearm & the controls on the right.

All patients are on the wards @ Children's Hosp & in good nutritional state. The nurses are ambulant & working.

Frambel: Has FUO for about 1 year. Looks & feels well. Began SD & streptomycin about 8 days ago & has been afebrile since except for temp of 102° about 20 hrs after skin test applied. Fever lasted several hours.

[redacted] readmitted in Oct. 1948. Abdominal laparotomy performed & terminal ileum & ^{cecum} ascending colon removed — diagnosis = regional enteritis.

Etiology unknown. Hist. note: sarcinaceous of this tissue = well after 30 days obs.

JUL 13 1948

TOXOPLASMA SKIN TESTS - G.G.H.Antigens: (From Warren)

- 1) Toxo: Toxo Egg 3A CAM ANT. 20% MERTH. 1:10,000 10-20-47 Spec 13 June 48
 2) NCAM: #4 16dy Emb. 10% susp. MERTH. 1:10,000 2-18-48 Spec 13 June 48 x
 Refriger. since. x
 refriger. since.

Both antigens diluted to 1/100 & 1/1000 in NaCl just before test.

0.1cc emuls. in. ID in toxo on left forearm & NCAM in right. 1/1000 placed distal & 1/100 proximal.

NAME	SEX	AGE	TOXO. 1/100		TOXO. 1/1000		NCAM 1/100		NCAM 1/1000		P.E.
		YRS	24°	48°	24°	48°	24°	48°	24°	48°	
[REDACTED]	M	W	17	0	0	0	0	0	0	0	F.U.O.
[REDACTED]	M	W	21	10x10 E	40x30 E 5x5-O	0	10x10 E	0	0	0	Ac. RH. FEV.
[REDACTED]	M	W	28	0	0	0	0	0	0	0	SBE
[REDACTED]	M	N	43	20x30 E	35x50 E 10x10-O	5x5 E	10x10 E	0	0	0	TBC - PN. EMP.
[REDACTED]	M	W	46	0	0	0	0	0	0	0	DIABETES
[REDACTED]	M	W	48	0	0	0	0	0	0	0	HEPATOMA HAND.
[REDACTED]	M	W	53	5x5 E	5x5 E	0	0	0	0	0	G.I. HEM. (+)
[REDACTED]	M	W	58	15x15 E	30x30 E 10x10-O	0	0	0	0	0	MYO. INF.
[REDACTED]	M	W	80	20x20 E	20x50 E	5x5 E	10x10 E	0	0	0	CONG. LUES PNEU.

JUL 14 1948

Toxoplasma Skin Tests - children's Hosp.Antigens: [redacted] from Wavlen

1. Toxoplasma: Toxo. EGG. #3A CAM ANT. 20% MERTH. 1:10,000
10-20-47. Spun 13 June 48 (Refrig. since.)
2. Control: Norm CAM ANTIGEN #4 16dy emb. 10% susp.
MERTH. 1:10,000 2-18-48 Spun 13 June 48 (refrig. since.).

Patients:

1. [redacted] - born 1-16-45 - patient of Sherry
admitted for diagnostic studies: has microcephaly,
retarded mental development, one suspicious area of calcification
in the skull, & one suspicious area of choroiditis in one
eye. This child is the 3rd child of normal parents. Older two
children well & normal. Pregnancy normal. Dt. died 13 July 48
2. [redacted]: born 7-31-47. Poor ~~physical~~ mental
& motor development. Has convergent squint. Eye-grounds normal.
Diag: Cerebral damage following anoxia @ birth.

PATIENT	TOXO 1/100		TOXO 1/1000		NCAM 1/100		NCAM 1/1000	
	24°	48°	24°	48°	24°	48°	24°	48°
[redacted]	5X5 Ery.	10X10 Ery.	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

+ TOXO 1/100

NEG.

→ SERUM OF 13 JULY 48 = 1:64 (POS) IN INVITRO TEST OF 21 JULY 48

(OVER)

Toxoplasma skin tests OF Wolmer Family &
Sven Gard.

26 July 48

Antigens: same refrigerated, fluid antigens used on 14 July 48.

SUBJECT	TOXO. 1/100		TOXO. 1/1000		NCAM 1/100		NCAM 1/1000		
	240	480*	240	480*	240	480	240	480	
[REDACTED]	16X16 E+0	28X30 faded ery	8X8 E+0	15X15 faded ery	0	0	0	0	Mother, age 28 yrs. +
	0	0	0	0	0	0	0	0	father age 35 yrs.
	0	0	0	0	0	0	0	0	son 11 Dec 42 = 5 1/2 yrs.
	0	0	0	0	0	0	0	0	" 7 Nov 43 = 4 1/2 yrs.
	20X25 E+0	56X39 ery	15X16 E	32X29 ery	7X7 E	0	0	0	grand, 40 yrs. +

* [REDACTED] reactions had definitely faded @ 480 altho they could still be made out. She stated that the peak reaction had occurred @ 360.

[REDACTED] 480 readings were taken @ 360 when, he stated in a letter, the peak reaction was noted. These "had definitely decreased" at 48 hrs.

JUL 16 1948

TOXOPLASMA SKIN TESTS - GENERAL HOSP.WARD IV (PSYCHIATRY)ANTIGENS USED: (From Warren)1) TOXO: TOXO, EGG #3A CAM ANT. 20% MERTH. 1:10,000 10-20-47.2) NORM: NORM. CAM. ANT. #4 16dy emb. 10% susp. MERTH. 1:10,000 2-18-48

Antigens thawed from frozen state, cent 20' @ 2000 to clear of ppt.

& then made to $\frac{1}{100}$ & $\frac{1}{1000}$ with fresh NaCl just prior to test. 0.1ccamts live ID = $\frac{1}{1000}$ distal & $\frac{1}{100}$ proximal on forearm. Toxo in left fore-arm & NCAM. in right.

NAME	SEX	AGE YRS.	TOXO $\frac{1}{100}$		TOXO $\frac{1}{1000}$		NCAM $\frac{1}{100}$		NCAM $\frac{1}{1000}$		DIAG.
			240	480	240	480	240	480	240	480	
FC 15			0	0	0	0	0	0	0	0	SCHIZO. -
FW 19			0	0	0	0	0	0	0	0	NORM. ST. NURSE -
FW 19			0	0	0	0	0	0	0	0	" -
MC 22			0	0	0	0	0	0	0	0	EPILEP. -
FW 29			10x25 E	50x50 F 10x10 O	12x12 E	30x30 E 7x9 O	0	0	0	0	DEPRESSION +
MC 35			10x10 E 10	13x13 0x E	13x13 3x4 E 10	4x4 E 10	5x5 E	0	5x5 E	0	MENT. DEFICIENCY +
MW 38			10x10 E 10	20x10 O 20x20 E	5x5 E	10x10 E 10	0	0	0	0	HYPOMANIA +
FC 38			0	0	0	0	0	0	0	0	DEPRESSION -
FC 38			15x20 E	10x12 E	10x5 E	6x6 E	0	0	0	0	PARANOID +
MW 45			12x12 E	20x20 E 5x5 O	0	5x5 0x E	0	0	0	0	DEPRESSION +
MC 48			15x20 0x E	15x20 0x E	8x10 0x E	8x8 0x E	0	0	0	0	+
MW 51			15x20 0x E	30x40 0x E	5x5 E	15x15 E	0	0	0	0	ALCOHOLIC +
FW 51			15x15 E	30x30 E 5x5 O	5x5 E	6x6 E	0	0	0	0	DEPRESSION +
MW 56			30x30 0x E	40x50 E 10x10 O	13x15 0x E	15x15 E	4x5 E	0	0	0	DEPRESSION +
MW 59			10x15 E 10	0	5x5 E	0	10x10 E	0	0	0	" -?
MC 59			12x12 0x E	10x12 0x E	10x10 0x E	8x10 0x E	0	0	0	0	Hyp. Br. Dis +

(OVER)

JUL 16 1948

NAME	SEX	COLOR	AGE YRS.	Toxo 1/100		Toxo 1/1000		NCAM 1/100		NCAM 1/1000		
				240	480	240	480	240	480	240	480	
	F	C	60?	10X10 04E	10X10 04E	10X12 04E	10X10 04E	0	0	0	0	ORG. BR. DIS. +
	M	W	68	0	0	0	0	0	0	0	0	DEPR. ESS. -
	M	W	71	14X14 04E	20X25E 8X8 0	10X10 E40	10X10 E40	0	0	0	0	SEN. +
	M	W	81	0	0	0	0	0	0	0	0	SEN. -

JUL 18 1948

Bledings: All patients marked = ✓ bled to-day.
Blood stored in refrigerator until 19 July when serum separated,
transferred to lusteroid, labelled " —, Psycho. 7-18-48" &
frozen.

JUL 19 1948

Three more patients (✓) bled to-day. Serum separated
20 July & treated as above.

TOXOPLASMIN SKIN TESTS

Inoc. 12/29/47

Name	NORMAL - Left				TOXO - Right			
	1:100		1:1000		1:100		1:1000	
	24	43	24	43	24	43	24	43
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	14x12 E	0	12x10 E	0	7x5 E	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	15x10 (ft.)	12x8 al.	?	faint	faint?	faint	?	?
	7x7	6x6	4x3	?	6x6	5x5	0	0
	0	0	0	0	0	0	0	0
	ft?	0 0	0	0 0	25x15 O+E	32x22 O+E	22x22 O+ft?	18x13 O+E
	0	0	0	0	0	0	0	23x20 O+E
	0	0	0	0	0	0	0	10x10 O+ft?
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	7x4 se.
	0	0	0	0	0	0	0	0
	0	0	0	0	0	13x13	35x23	10x10
	0	0	0	0	0	20x18	0	20x17
	ft?	0	ft?	0	ft?	0	0	9x7 mm
20x20	0	16x14	0	30x30 O+E	60x50 (20x20)	25x20 O+E	40x40 (15x15)	

22 Rows - Toxo - oedematous very red - sore
Normi - erythema - flat

TOXOPLASMA SKIN TESTS.

SEP 23 1948

ANTIGENS: TOX EGG #3A CAM 20% MERTH. 1/10,000 10-20-47
 NCAM ANT. #4 16dy EMB. 10% MSUSP. MERTH 1/10,000 2-18-48. *

NAME	AGE	SEX	COL. R.	DIAG.	Toxo 1/100		NCAM 1/100	
					24°	48°	24°	48°
[REDACTED]	4 1/12	♀	W	CYST. FIBROSIS	○	○	○	○
	6	♂	C	RL HT DIS	○	○	○	○
	7	♂	W	THE HIP	○	○	○	○
	6	♀	W	APLECTASIS	○	○	○	○
	8	♂	C	amp. finger	○	○	○	○
	9	♂	C	BRON-HI CL.	○	○	○	○
	10	♂	W	Eng Bredis	○	○	○	○
	11	♂	C	THE SPINE	○	○	○	□
	11	♂	C	SICKLE CELL ANEM.	○	○	○	○
	11	♀	W	DIABETES	○	○	○	○
	12	♂	W	RH. HT. DIS.	○	○	○	○
	13	♂	W	OSTEO. CYSTITIS	○	○	○	○
	19 1/12	♀	W	NORM.	○	○	○	○
	35	♂	W	"	20x20 E	E=40x60 O=12x14	○	○
	34	♂	W	"	E=22x22 O=12x12	E=55x30 O=12x12	E=10x10	○

* Antigen thawed from frozen state & 5.0cc quantities clarified by centrifuging before test. These then diluted to 1/100 in NaCl & used as follows:

a) TOXO: 0.1cc 1/100 intradermally, left forearm.

b) NCAM: " " " " right " "

all subjects have negative history of egg allergy.

Toxoplasma Shiri Tests At Cin. Gen. Hosp.

OCT 4 1948

Antigens: same lot given for test of 23 Sept 48 & refrigerated to date. Diluted & saline just before test.

Test: 0.1 cc intradermally. Toxo in left forearm & NCAM in right.

NAME	AGE	S E X	C O D	DIAG.	TOXO /100		NCAM /100		
					24°	48°	24°	48°	
	14	♀	C	M.H. Pul. Tbc.	0	0	0	0	-
	16	♀	C	ARTH.	0	0	0	0	-
	17	♂	W	Dermato-mycosis?	0	0	0	0	-
	17	♀	W	DIABETIC	0	0	0	0	-
	17	♀	C	Thy. ? BRONCH.	0+2 10x12	E=20 20x30	0	0	+
	17	♀	W	CONV. PYL.-NEPH.	0	0	0	0	-
	34	♀	C	CONV. NEPHRITIS	0	0	0	0	-
	35	♀	W	MULTIPLE SCLEROSIS	E=15x15	E=20x20 PRURITUS	E=5x5	0	+
	35	♂	W	PEP. ULCER.	E=15x15 NO D. PRUR.	OOO=8x9 FADING RAY.	0	0	+
	37	♀	C	Pylor-neph.	0+E=12x25	0+E=15x12	0	0	+ fading.
	39	♀	C	CONV. TBC EFFUSION	0	0	0	0	-
	40	♂	C	? Tbc.	E=30x30 O=7x7	almost gone has puncta	0	0	+ Reg. fading fast this a.m.
	41	♂	C	Pul Tbc	0	0	0	0	-
	42	♀	C	CORD TUMOR	0+E=30x30	E=20x40 PRURITUS	0	0	+ fading
	43	♂	C	? Tbc.	0+E=15x15	0+E=10x10 PRU.	0	0	+
	43	♀	C		discharged from hosp.				
	44	♂	W	Cong HT. F. C.	E=6x6	disch.	0	disch.	omit
	45	♂	C	THYROTOXIOSIS	0	0	0	0	-
	46	♂	W	PEP. ULCER	E=18x12	O=8x10 O=10x10 PRU. AITUS	0	0	+

NAME	AGE	SEX	C/O	DIAG.	TOXO /100		NCAM /100		
					240	480	240	480	
	46	♂	C	EXTRA-DURAL GRANULOMA	0+ 15x15	E 20x20 FADING	0	0	+
	47	♀	C	A.S. HT. DIS.	0	0	0	0	-
	49	♂	W	CNS Les	E=20x20	E=25x30 SL. PRUR.	0	0	+
	65	♂	W	MALNUT. CLIPANOSIS	E=35x25	E=20x22 FADING. ^{deg. palp. reduced}	0	0	+
	69	♂	W	P.A. RElop	E=10x10	E=7x10	0	0	+
	71	♂	W	herm. Tum		manic benton Opt/pea-verapuntae			
	73	♀	W	REC. CA. BREAST.	0	E 40 10x11	0	0	+
	79	♂	W	MEDIAST. MASS.	2 15x15	E=22x22 D=10x10	0	0	+

TOXO PLASMA SKIN TESTS

OCT 28 1948

ANTIGENS: TOXO. EGG #3A CAM. ANT. 20% $\pm$ MERTH. 1:10,000.
NORMAL CAM ANT. #4 10% MERTH. 1:10,000.

Both thawed & clarified by spinning for 10' @ 2000 on 10-19-48.
Stored in refrig. since. Diluted to 1/100 in fresh NaCl fluid
prior to test this a.m.

Test: 0.1cc of toxo 1/100 in left forearm & 0.1cc of NCAM 1/100 in right
forearm - both intradermal.

NAME	AGE (YRS.)	SEX	COLOR	DIAG.	TOXO. 1/100		NCAM 1/100		
					24°	45°	24°	45°	
	15	0+	C	RHEU. ARTHR.	0	0	0	0	-
	49	0+	W	CARCINOMA	E=12x12 540 13x10 ↓	E=0 12x10	0	0	+
	41	0 ⁿ	W	NONE		E=12x20	0	0	+
	46	0 ⁿ	W	ENCLUES	0	E=0 10x9	0	0	+
	65	0 ⁿ	W	BL INF.	0	0	0	0	-
	5 1/2	0 ⁿ	W	ATYP. PN.	0	0	0	0	-
	23	0+	W	TECH. NORM.	0	0	0	0	-
	23	0+	W	" "	15x15 240	ca 25x25 E=0 pt. measured.	0	0	+
		0 ⁿ	W	" "	ca 30x15 240	E=20x15 E=20x10		0	+

↑ these approx. by M.H. Still visible on 2 Nov.

TOXOPLASMA SKIN TESTS

0-4 yrs.

NAME	AGE YRS.	TOXO.	CONTROL.
1	3/12	0	0
2	4/12	0	0
3	3/12	0	0
4	5/12	0	0
5	5/12	0	0
6	4/12	0	0
7	7/12	0	0
8	11/12	0	0
9	12/12	0	0 - neg dye test
10	13/12	0	0
11	13/12	0	0
12	13/12	0	0 +
13	1 5/12	0	0
14	2 9/12	0	0
15	3 6/12	0	0
16	3 7/12	0	0
17	4 3/12	0	0
18	4 8/12	0	0
19	4 9/12	0	0
20	5	0	0
21	4 10/12	0	0

12 M.
8

100% NEG

TOXOPLASMA SKIN TESTS

6-10 YRS.
5-9

#	NAME	AGE YRS.	TOXO.		NCAM	
			1/100	1/1000	1/100	1/1000
1		6	0	0	0	0
2		6	0	0	0	0
3		5 1/2	0	0	0	0
4		6	0	0	0	0
5		6	0	0	0	0
6		8	0	0	0	0
7		8	0	0	0	0
8		8	0	0	0	0
9		8 1/2	+	+	0	0
10		8 1/2	0	0	0	0
11		9	0	0	0	0
12		8 1/2	0	0	0	0
13		10	+	0	0	0
14		8	240 4x3 480 10x6 870 only	0	0	0
15		5	0	0	0	0
16		6	0	-	0	-
17		7	0	-	0	-
18		6	0	-	0	-
19		8	0	-	0	-
20		9	0	-	0	-
		5 1/2	0	-	0	-

1/10 ✓

neg. both in Dec. 47 & June '48.

down

No edema again at any time. Considered negative

Toxoplasma SKIN TESTS10-14 YRS.

#	NAME	AGE	TOXO		NCAM			
			1/100	1/1000	1/100	1/1000		
1		10	<u>+</u>	0	0	0	1/16	✓
2		12	<u>+</u>	<u>+</u>	0	0	1/16	✓
3		13	0	0	0	0		
4		10	0	-	0	-		
5		11	0	-	0	-		
6		11	0	-	0	-		
7		11	0	-	0	-		
8		12	0	-	0	-		
9		13	0	-	0	-		
10		14	0	-	0	-		

TOXOPLASMA SKIN TESTS

15-19 yrs.

#	NAME	AGE	TOXO		NCAM	
			1/100	1/1000	1/100	1/1000
1		15	0	0	0	0
2		19	0	0	0	0
3		19	0	0	0	0
4		19	0	0	0	0
5		17	0	0	0	0
6		19 1/2	0	-	0	-
7		16	0	-	0	-
8		17	0	-	0	-
9		17	0	-	0	-
10		17	<u>+</u>	-	0	-
11		17	0	-	0	-
12		15	0	-	0	-

neg.

✓

TOXOPLASMA SKIN TESTS

20-29 YRS.

#	NAME	AGE	TOXO.		NCAM		
		YRS.	1/100	1/1000	1/100	1/1000	
1		21	0	0	0	0	until 72 ⁺ - 1:4 neg tit ₀ - neg
2		21	<u>+</u>	<u>+</u>	0	0	sh 144 in
3		21	0	0	0	0	
4		21	0	0	0	0	
5		22	0	0	0	0	
6		22	0	0	0	0	
7		22	<u>+</u>	<u>+</u>	0	0	1/16 ✓
8		24	<u>+</u>	<u>+</u>	0	0	1/16 ✓
9		22	0	0	0	0	0
10		26	+	0	+	0	0 neg test. Control & toxo equal.
11		28	0	0	0	0	
12		27	0	0	0	0	
13		27	0	0	0	0	
14		-	0	0	0	0	
15		-	0	0	0	0	
16		29	0	0	0	0	
17		^{ca} 25	0	0	0	0	0
18		28	0	0	0	0	
19		29	<u>+</u>	<u>+</u>	0	0	1/4 ✓
20		29	0	0	0	0	0
21		30	0	0	0	0	

20% Pos.

20% Pos.

TOXOPLASMA SKIN TESTS

30-40 YRS.
39

#	NAME	AGE (YRS.)	TOXO.		NCAM	
			1/100	1/1000	1/100	1/1000
1		31 1/2	+	+	0	0
2		32 1/2	0	0	0	0
3		33	+	+	+	+
4		34	0	0	0	0
5		35 1/2	0	0	0	0
6		35	0	0	0	0
7		35	+	+	0	0
8		38	+	+	0	0
9		38	0	0	0	0
10		38	+	+	0	0
11		33	+	+	0	0
12		35	0	0	0	0
13		32	0	0	0	0
14		3-	+	+	0	0
15		38	0	0	0	0
16		39?	+	+	0	0
17		30	0	0	0	0
18		35	+	-	0	-
19		34	+	-	0	-
20		34	0	-	0	-
21		35	+	-	0	-
22		35	+	-	0	-
23		37	+	-	0	-
24		39	0	-	0	-

1/4 ← ✓

omit

ca same degree in all.

omit

1/16 ✓

✓

neg.

1/256 ✓

1/64 ✓

20

1/64 ✓

1/256 ←

1/16 ✓

omit for survey

omit for survey

10/20 = 50% Pos.

15x15 @ 2vln. (Nod. + Run) @ 40°-0 = 8x9
fold neg.

TOXO PLASMA SKIN TESTS

40-48 YRS.

#	NAME	AGE (YRS.)	TOXO.		NCAM	
			1/100	1/1000	1/100	1/1000
1		41	<u>+</u>	<u>+</u>	0	0
2		45	<u>+</u>	<u>+</u>	0	0
3		48	<u>+</u>	<u>+</u>	0	0
4		43	<u>+</u>	<u>+</u>	0	0
5		46	0	0	0	0
6		48	0	0	0	0
7		43	0	0	0	0
8		46	<u>+</u>	0	0	0
9		49	0	0	0	0
10		40	<u>+</u>	-	0	-
11		41	0	-	0	-
12		42	<u>+</u>	-	0	-
13		43	<u>+</u>	-	0	-
14		44	-	-	-	-
15		45	0	-	0	-
16		46	<u>+</u>	-	0	-
17		46	<u>+</u>	-	0	-
18		47	0	-	0	-
19		49	<u>+</u>	-	0	-
20		41	<u>+</u>	-	0	-
21		46	<u>+</u>	-	0	-
22		49	<u>+</u>	-	0	-

omit
omit

1/16 ✓

1/16 ✓

✓

1/1024 ✓

1/4 ←

Q 240 =
E = 30x30 0 = 7x7 @ 450:
Abundant. Residual primitive

disch. Scap. reading.

Q 240 =
E = 18x12 @ 450
0 = 8x10 E = faded. Primitive

1:16 CF - 0

240 = 0
450 = 0 + E 10x7

TOXOPLASMA SKIN TESTS

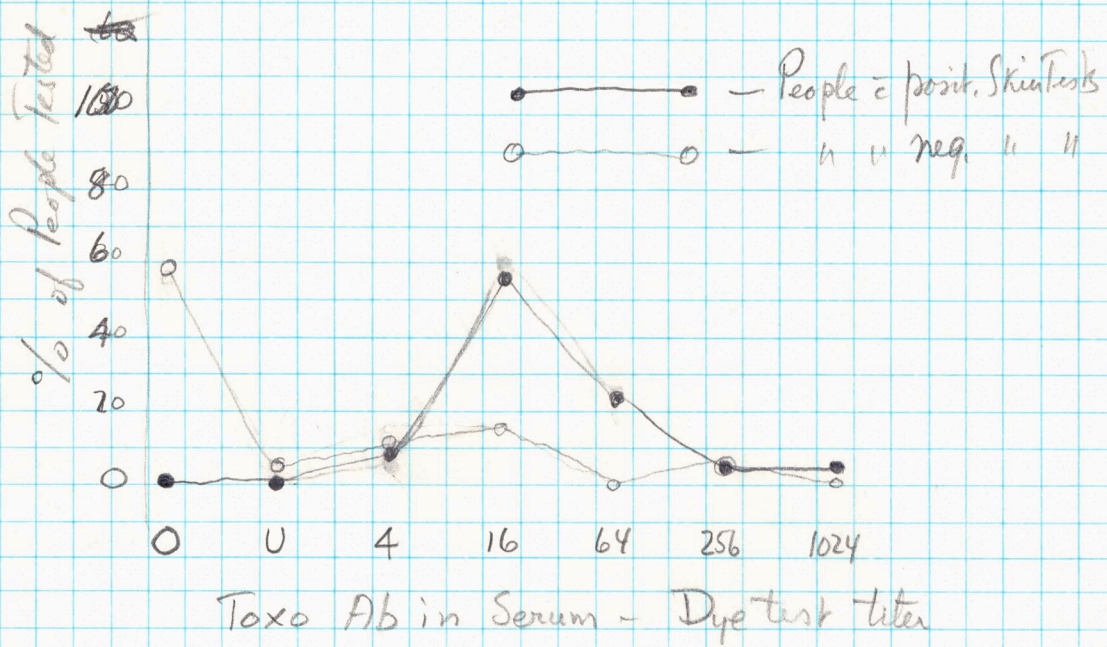
59
50-60 YRS.

#	NAME	AGE (YRS)	TOXO		NCAM		
			1/100	1/1000	1/100	1/1000	
1		51	<u>+</u>	<u>+</u>	0	0	1/16 ✓
2		51	<u>+</u>	<u>+</u>	0	0	1/16 ✓
3		53	(+)	0	0	0	N.D. ?
4		53	0	0	0	0	1/16
5		53	0	0	0	0	1/16
6		58	<u>+</u>	0	0	0	1/16 ✓
7		58	<u>+</u>	0	0	0	N.D. ✓
8		56	<u>+</u>	<u>+</u>	0	0	1/16 ✓
9		59	0	0	0	0	1/16 ? (Pos. @ 24°, neg. @ 48°) ←
10		59	<u>+</u>	<u>+</u>	0	0	1/64 ✓
#		60?	<u>+</u>	<u>+</u>	0	0	

← not counted as
Pos. for
tuberc.

@ 24° + 48°
= 5x5 E 1/1,000 + controls;
neg.

7090 Pos.



TOXOPLASMA SKIN TESTS

60- 788

#	NAME	AGE (YRS.)	TOXO.		NCAM	
			1/100	1/1000	1/100	1/1000
1		68	0	0	0	0
2		71	<u>+</u>	<u>+</u>	0	0
3		80	<u>+</u>	<u>+</u>	0	0
4		81	0	0	0	0
5		60?	<u>+</u>	<u>+</u>	0	0
6		65	<u>+</u>	-	0	-
7		69	<u>+</u>	-	0	-
8		73	<u>+</u>	-	0	-
9		79	<u>+</u>	-	0	-
10		65	0	-	0	-

Neg.

1/64 ✓

✓

1/4 ←

✓

✓

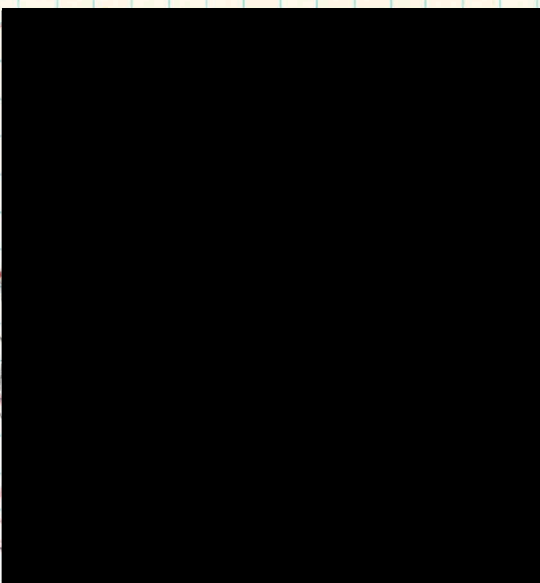
✓ ? $24^{\circ} = E = 10 \times 10$ $48^{\circ} = E = 10 \times 7$

✓

✓

Human Sera to be tested

✓ 2
✓ 3
✓ Neg
✓ to 1:64



AMS

✓ 4
✓ 5
✓ 1



(CF 1:128)
(CF 1:16)

✓ - 2 1/2 yrs. old. - Cerebr. calcif., chorioretin.

(CF 1:64)

2 1/2 yrs. old - microceph., chorioret., cerebral calcif., spastic

(CF 1:32)

11-16-44

25 days old - Proved fatal case

✓ 6

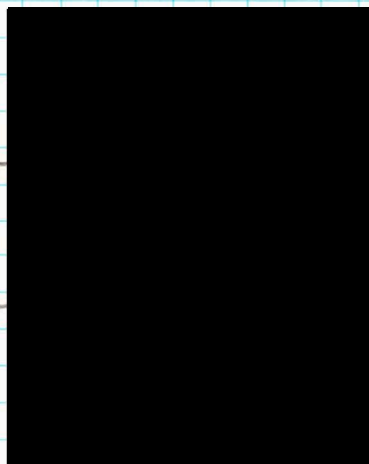


(CF 1:64)

Mother
of

2 yrs. old. - Microceph., chorioret., strabismus,

9
~~10~~
11
~~12~~
13



(CF 1:8)

Age 53 yrs

Active pneumonia
Died - toxo

(CF 1:8)

26 yrs.

Bilater. choroid.

(CF 1:8)

30 yrs.

Old chorioretinitis

(CF 1:8)

22 yrs.

Chorioretin.

(CF 1:8)

30 yrs.

- gave birth to child - cong. cataracts - nothing to suggest toxo

Effectiveness of Various Fresh Animal Sera in Reactivating Heated Immune Human Toxoplasma Serum

Count 100
toxoplasma

Fresh human immune	+	P.E. 1:5 in saline
Heated " "	+	P.E. " " "
" " "	+	P.E. 1:5 in fresh hum. normal
" " "	+	P.E. 1:5 " " rabbit "
" " "	+	P.E. 1:5 " " monkey "
" " "	+	P.E. 1:5 " " sheep "
" " "	+	P.E. 1:5 " " guinea pig "
" " "	+	P.E. 1:5 " " horse "
" " "	+	P.E. 1:5 " heated human "

P.E. 1:5 in fresh human normal per ~~se~~

" " " "	rabbit	" " "
" " " "	monkey	" " "
" " " "	sheep	" " "
" " " "	guinea pig	" " "
" " " "	horse	" " "

$$55-115 = 2.4$$

$$NaCl = 2.7$$

Can Partially Positive Human Sera be "Fortified" by
Addition of Fresh Normal Human Serum or
other ACTIVATOR

§

[redacted] serum + PE 1:5 in saline

" + PE 1:5 in fresh hum. normal

[redacted] serum + PE 1:5 in saline

" " + PE 1:5 in fresh human normal

[redacted] + PE 1:5 in saline

+ " " in fresh hum. normal

+ PE 1:5 in saline

+ PE 1:5 in fresh human normal

$$Nad = 2.0 = 2.0 + 0.5 \text{ PE}$$

$$SS = 2.0 = 2.0 + 0.5 \text{ PE}$$

$$12 - 6$$

$$13 - 6$$

$$14 - 10$$

Arrived July 1, 1948 - in Dry Ice
Toxo. Guinea Pig Serum

Stored

B-8-3

C-F

✓ # 2463 O ($\leq 1:2$) Pre-infect. 5-18-48
" O ($\leq 1:4$) 2 wks. after I.D. 6-1-48
" 1:128+? 34 days post infection 6-21-48

✓ 2464 O ($\leq 1:2$) Pre-infect. 5-18
" O ($\leq 1:4$) 2 wks after ID 6-1

✓ 2467 O ($\leq 1:2$) Pre-infect 5-18
" 1:16 2 wks after ID 6-1
" 1:64 34 days post infection 6-21

✓ 2468 O ($\leq 1:2$) Pre-infect. 5-18
" O ($\leq 1:4$) 2 wks after I.D. 6-1
" 1:64 34 days post infection 6-21

✓ 2470 O Pre-infect. 5-18
" O 2 wks after ID. 6-1

✓ 2472 O Pre-infect 5-18
" O ($\leq 1:4$) 2 wks after ID. 6-1
" 1:128+? 34 days post infect 6-21

✓ 2473 O Pre-infect. 5-18
" O 2 wks. after ID 6-1
2473 1:64 34 days post infect. 6-21

✓ [redacted] (CF 1:8) 6-16-48 1 cc.
11 years old - Brester. chorion. first noted at age 7 (1944) - serum of mother and child then said to be pos.

From [redacted]

Army Med School

AUG 16 1948

SERUM	DATE	
[REDACTED]	3 June '48	8 ₁
Horn - All Variola Material [REDACTED] mother & child mother & child from Van Bevelde)	Sera Rec'd 24 Aug 48 Sera Rec'd 27 Aug 48 Rec'd 7 Sept 48	No Names " " contain,

AUG 16 1948

SERUM	DATE	
SERA: G. Pig 974 " 2463 pre-inoc. " " 2 wk. " " 34 days " 2464 pre-inoc. " " 2 wk. " 2467 pre-inoc. " " 2 wk. " " 34 days " 2468 pre-inoc. " " 2 wk. " " 34 days " 2470 pre-inoc. " " 2 wk. " 2472 pre-inoc. " " 2 wk. " " 34 days " 2473 pre-inoc. " " 2 wk. " " 34 days		83
All of these Tubes placed in a cool - barrel kept & transferred to 1st large can Oct 11/48	6-16-48	

SERUM

DATE

8
3

7 OCT '48
30 Sept. '48

1 OCT. '48

6 OCT. '48

7 OCT. '48
10-14-48
10-14-48

AUG 16 1948

SERUM

DATE

Fraction C,
Fraction C₂

8₄

cho. SKIN TESTS

(INFANT. ENGLAND)

8-10-48

Spinal fluid
Serum

(mother)

"
"

"

8₅

26 July

13 July

23 Feb.

7-18-48

~~7-18-48~~

8 July

8 July

9 July

7-23

7-24-48

13 July-48

~~11-16-44~~

23 July

27 July

30 Aug. 48

10-16-48

43 dy follow-up.

AUG 16 1948

SERUM

DATE

9₁

22 Aug. '47
24 Dec. '47
24 Dec. '47

19 Jan. '48
5 Apr. '48
5 Feb. '48
11-24-47
6-10-48
6-10-48

9₂

8 Jan. '48
30 Jan. '48
26 Dec. '47
6 Jan. '48
17 Feb. '48
30 Jan. '48

23 Dec. '47
10 Apr. '48
10 Apr. '48
~~15 Apr. '48~~
15 Apr. '48
23 Apr. '48
5 May '48
28 May '48
28 May '48

depleted

one tube $\approx$ ca 0.5 cc. *unintended*
8 Sept '48

SERUM

DATE

Toxo. Immune Rh.

Rh. 152 pre-inoc
 " " 3rd day
 " " 5th day
 " " 7th day
 " " 10th day
 " " 14th day
 " " 21st day
 " " 26th day

4 Feb. '48
 2 Mar. '48
 6 Mar. '48
 8 Mar. '48
 10 Mar. '48
 13 Mar. '48
 17 Mar. '48
 24 Mar. '48
 29 Mar. '48

93

Rabbit # 377

" # 368

" # 349

" # 349

" # 360

" # 363

" # 363

" # 363

" # 365

" # 365

" # 365

" # 369

" # 370

" # 371

" # 376

" # 380

" # 398

" # 398

Normal Sheep

pre-inoc.

5th day8th day ?

pre-inoc., 2 tubes

5th day7th day

pre-inoc.

15 Mar. '48

15 Mar. '48

23 Dec. '47

12 June '48

13

4 Mar. '48

10 Apr. '48

13 Apr. '48

4 Mar. '48

10 Apr. '48

12 Apr. '48

15 Mar. '48

15 Mar. '48

15 Mar. '48

15 Mar. '48

14 May. '47

24 Dec. '47

12 June '47

94

SERUM

DATE

Rat, Yellow, 28 day
Rat Blue 35 day

24 Mar. '48
31 Mar. '48
4-15-46
3-18-46
5-18-48
6-9-48
6-9-48

3-March

7-6-48
7-6-48
5 Aug.

Some tubes also stored in 99

95

9 Feb. '48
8 Jan. '48
4 Feb. '48
25 Feb. '48
2 Dec. '47
4 Mar. '48

13 Mar. '48
4-12-48
17 May '48
14 May '48
5-22
5-22
27 April
27 April
6-17-48
12-01-48

96

SERUM

DATE

Normal Rabbit Serum

Rabbit #351
 Rabbit #352 pre-inoc.
 " #362 5 day
 " #364 5 day
 Rhesus #142
 " #143
 " #151
 " #157
 " #158

Dog, Brown + Black

Dog, Nellie

American Horse #229

" " #224

" " #226

Lamb I

" II

" III

Mouse Serum, 5th day Pool, Post-infer.

Chick

23 Dec. '47

24 Dec. '47

19 Apr. '48

19 Apr. '48

1 Mar. '48

1 Mar. '48

3 Mar. '48

1 Mar. '48

1 Mar. '48

22 Mar. '48

97

Normal Calf

G. Pig A

" " B

" " C

P-2

P-4

P-5

P-6

P-7

P-9

P-10

P-11

P-12

P-13

P-16

P-17

P-18

P-20

P-21

P-22

P-23

P-?

P-14

P-8

P-3

12 feb.

12 feb.

12 feb.

98

All pages sera
 placed in cardboard box
 & transferred to first car
 box. 11 Oct 48.

5 Aug.

SERUM

DATE

25 Aug. '48
 29 Dec. '47
 29 Aug. '47
 25 Aug. '47
 25 Aug. '47
 25 Aug. '47
 29 Dec. '47
 25 Aug. '47
 6 Aug. '47
 25 Aug. '47
 29 Dec. '47
 25 ~~Aug.~~ '47
 25 Aug. '47
 29 Dec. '47
 10 July '47
 29 Dec. '47
 29 July '47
 29 Dec. '47
 29 Dec. '47
 29 Dec. '47
 9 July '47
 29 Dec. '47

all transferred to
 card-board boxes
 & placed in "A" to
 storage box.

SERUM

DATE

98

HORN CA - III
" CA - IV
" MB Pass III
" MB " IV
" MB " V
" MB " VI

27 Sept. '48
29 " "
25 " "
2 Oct. '48
5 " "
7 " "

SERUM

DATE

9/9

Discarded Sera

Rec'd 5 Mar. 1948

" " "

" "

" "

10^m 18-48