TOTO.
COUNTS,
BUDGET
EFFORTS
ETC.

8 Dec '47

TOXOPLASMA – PERITONEAL EXUDATE COUNTS & IN VIVO TITRATION

Obj: This is a repetition of the 24 Nov expt in which toxo-containing peritoneal exudate will be counted & then titrated in mice, Icer & IP to check the validity of the count. Periodic counts of exudate undiluted & diluted in saline, saline-heparin, immune toxo serum & normal human sera – as well as simultaneous smears will also be examined to check on the previously-noted disappearance of toxo in mixtures with R. Black serum.

Peritoneal Exudate: Obtained by capillary from the bellies of 10 mice inoc on 3 Dec c̄ pan 354. These mice are all ill & must have enlarged bellies.

Procedure: Immediately after obtaining the pooled exudate, portions were added to prepared tubes as follows:

1. Undiluted – plain peritoneal exudate.
2. Per. Ex 0.4 cc + NaCl 1.6 cc. (1:5)
3. Per. Ex. 0.4 cc + NaCl 1.2 cc + Hep. 0.4 cc (same 1/500 previously used.)
4. Per. Ex 1/5 in NaCl – 1.0 cc + 1.0 cc R. Black ser. of 22 Oct. '47 (Ex = 1/10)
5. Per. Ex 1/5 in NaCl – 1.0 cc + 1.0 cc JONES SER. * (9 Oct. 47)
6. PER. Ex 1/5 in NaCl – 1.0 cc + 1.0 cc SARVER SER. * (16 Sept 47)

* Jones & Sarver sera obtained from Ruckman. Both had had neg. toxo neuts & have been kept frozen with same care used for toxoplasma studies.

In addition to the above, serial 10 fold dilutions (10^{-1} – 10^{-8}) in NaCl were also made from this exudate. This was titrated IP (0.25 ml/mouse) & Icer (0.03 ml/mouse) in CFW mice of 3-4 wks of age – 4 mice/dil. NB. These dil were made & injected between counts & thus stood for 60-75' in NaCl prior to injection.

Shortly after the above 6 tubes were set up (within a few moments), aliquots were removed in turn & a drop placed on each of two slides & in a WBC counting chamber. The sequence was always from #1 – #6 & counting was always performed in the same order. The same chamber was used for repeat counts of the same tube. This procedure was done @ the following times: Immediate,

1½ hrs, 3 hrs, 4½ hrs. & @ 28°. The last followed 5¾ hrs. at room temp. overnight in refrigerator & then 4 hr. @ room temp again. No gross phenomena were noted in any of the tubes except the formation of sediments of shreds that were difficult to resuspend.

Each tube's contents was mixed prior to each count by aspirating back & forth in a capillary. All smears of a given series were made on a single slide so as to get comparable staining.

Most striking was the appearance of the trypanosoma in R. Blackii serum. There seemed to be an immediate direct effect upon the parasites that made them baggy & indistinct. An occasional organism appeared normal but this was only noted during the immediate 1½ hr preparation & were rare as compared to the "injured" parasites. However, there was no complete lysis. This phenomenon will hamper the study. Neither of the 2 normal human sera exerted a similar effect.

-04-

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Specimen	Smears & Counts	Immediate	1½°	3°	4½°	24°
1 UNDIL. PER. EX.	Count. Chamber	38 32 40 28 30 26 26 33 30 26 26 39 34 29 29 24 [496] MANY POLYS - DIFF. TO COUNT.	25 35 22 30 33 28 35 33 40 27 32 28 35 32 30 23 [488] UNCHANGED	22 22 29 33 20 16 30 33 24 22 28 28 30 26 28 29 [425] FEWER LEUCOS? NORMAL.	16 19 10 14 18 18 13 16 18 21 17 18 14 19 18 17 [266] APPEAR TO BE DECREASING IN NUMBERS.	27 18 12 18 23 15 18 17 19 13 19 15 24 19 18 12 [288] NORMAL APP. LEUCOS OK.
	Wright's	1275/25 = 51/oil field	NOT COUNTED - VERY MANY/FIELD	238/6 = 40/F	235/6 = 40/F	196/6 = 32/F
2 PER. EX. 1:5 NaCl	Count. Chamb.	28 16 22 12 15 17 12 11 17 24 16 17 14 20 21 14 [276] EASIER COUNTING. MANY GROUPED.	20 18 16 15 17 23 21 18 [294] 17 23 19 17 20 17 15 18 EASY TO COUNT.	15 23 19 15 [1276] 17 14 15 14 22 15 22 16 20 14 16 21 DISTINCT. NORMAL	10 16 16 21 [253] 15 16 19 16 14 11 19 20 20 13 9 18 NORMAL	16 18 24 21 [249] 13 14 15 16 15 10 17 17 15 16 19 13 NORMAL TOXO. FEW LEUCOS
	Wright's	878/25 = 35/F	134/6 = 24/F	172/6 = 28.5/F	121/6 = 20.1/F	69/6 = 11.5/F
3 PER. EX. 1:5 HEP-NaCl	Count. Cham.	11 12 10 14 12 11 17 11 8 14 15 13 9 11 15 13 [196] SL. MORE DISPERSED THAN NaCl ALONE	18 20 14 21 [287] 14 17 28 23 14 14 17 20 12 19 18 18 EASY TO COUNT.	20 19 19 26 [303] 17 18 23 16 17 14 17 17 17 14 28 21 AS IN #2	14 14 18 14 [289] 16 17 14 18 20 21 19 23 19 22 18 22 NORMAL.	13 22 15 16 [204] 12 6 13 10 18 10 10 8 9 12 15 15 NORMAL
	Wright's	837/25 = 33.5/F	146/6 = 24.5/F	106/6 = 17.7/F 215/15 = 14.3/F	~~950 = 7.8/F~~ 152/6 = 25.3/F ~~875 = 0.65/F~~	119/6 = 20/F
4 PER. EX. 1/5 in NaCl 1:1 c R. BLACK SERUM.	Count. Chamb.	4 14 7 4 3 3 6 6 3 4 12 6 14 7 8 9 [110] APPEAR IMMOBILE. DIFF. TO MAKE OUT. "ABNORMAL"	6 8 6 6 [105] 6 7 6 7 7 9 8 6 9 7 6 4 DIFF. "GHOSTLIKE" INDISTINCT.	5 4 5 3 [76] 6 6 4 2 7 3 7 3 8 4 5 4 RARE "NORMAL" TOXO. OTHERWISE POOR. DEFIN.	4 6 7 3 [84] 9 8 7 5 4 4 6 5 7 3 3 3 SAME.	NOT COUNTED ALL "GHOSTS"
	Wright's	674/25 = 27/F	31/6 = 5.1/F	9/6 = 1.5/F 9/15 = 0.5/F	22/6 = 3.7/F	19/6 = 3.1/F
5 PER. EX. 1/5 in NaCl 1:1 c JONES SERUM.	Count. Cham.	25 36 22 22 22 18 24 20 19 22 23 20 21 19 21 19 [353] APPEAR "NORMAL" VERY DISTINCT.	19 20 19 21 [323] 23 18 18 19 26 18 26 19 20 20 17 22 "NORMAL" DISTINCT.	22 13 19 15 [305] 22 16 18 29 21 19 17 19 18 16 25 20 UNCHANGED	18 18 18 20 [346] 21 22 21 16 26 23 32 21 17 21 30 22 NORMAL	9 9 11 9 [177] 14 16 11 12 9 12 10 13 13 12 8 11 NORMAL.
	Wright's	899/25 = 36/F	194/6 = 32.5/F	106/6 = 17.7/F 75/15 = 5/F	185/6 = 30/F	134/6 = 22.3/F
6 PER. EX. 1/5 in NaCl 1:1 c SARVER SERUM.	Count. Chamb.	16 20 14 12 12 20 18 13 14 14 17 18 10 15 13 18 [244] APPEAR "NORMAL"	15 9 19 13 [220] 15 9 14 12 13 14 15 16 18 11 16 13 As in JONES.	13 14 8 14 [207] 15 13 12 19 12 10 14 18 15 11 10 9 UNCHANGED.	12 15 17 16 [233] 17 12 14 15 14 14 13 12 13 18 17 14 NORMAL.	7 13 7 6 [131] 5 11 4 12 9 6 8 10 9 9 8 7 NORMAL.
	Wright's	669/25 = 27/F	119/6 = 19.5/F	47/6 = 8.0/F 144/16 = 9/F	134/6 = 22.3/F	111/6 = 18.5/F

USING 276 as # TOXO @ 1:5 =
13,800,000/cc.

NONE OF THESE COUNTS SIGNIFICANT. APPEARS THAT TOTAL # OF TOXO, SAME BUT ALMOST ALL INDISTINCT, CRENATED, GHOSTLIKE. MEMBRANES (?) APPEAR INJURED. NUCLEI ALSO INDISTINCT. COUNTS APPROX.

N.B.:

111/6 = # TOXO/6 oil im fields

18.5/F = 18.5 toxo ave/oil im field

TOXOPLASMA (PER. EX.) CHAMBER COUNTS & SMEARS FOLLOWING INCUBATION WITH VARIOUS AGENTS

DEC 8 1947

TITRATION OF TOXOPLASMA (PER. EX.) I P - CORRELATION WITH PARASITE COUNTS.

McCE: CFU - ca 4 wks. All dil in Nacl from peritoneal exudate (used for other counting expts to-day.)
Remained in Nacl for 60-75' prior to inoc. May account for poor titer.

0.25 ml IP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	MORT.	# Par/dose
	–	–	–	–	CNS	D																								
10^{-3}	–	–	–	–	–	–	D																						4/4	3450
	–	–	–	–	–	–	D																							
	–	–	–	–	–	?	D																							
	–	–	–	–	–	?	?	→	–	–	–	–	–	–	–	–	–	–	–	–	D	Toxo Present in Brain								
10^{-4}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–							1/4	345
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
	–	–	–	–	–	–	–	D	?																				LD50 T.T.
10^{-5}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–							0/3	$10^{-3.7}$ 34.5
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
	–	–	–	–	–	–	–	RT	–	–	–	?	–	–	–	–	–	–	–	–	–	–								
10^{-6}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–							0/4	3.5
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
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	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
10^{-7}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–							0/4	0.3
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
10^{-8}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–							0/4	0.03
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								

PARASITE COUNTS: Young 276/sq mm @ 1:5

#PAR. EST. IN 0.25 ml dose @:
10^{-1} = 345,000
10^{-2} = 34,500
10^{-3} = 3,450
10^{-4} = 345
10^{-5} = 34.5
10^{-6} = 3.45
10^{-7} = 0.3
10^{-8}

∴ THEORETICAL LD_{50} TITER =

TOXO @ LD_{50} = 631

2760
5
13,800,000

DEC 8 1947

TITRATION OF TOXOPLASMA (PER. EX.) ICER – CORRELATION WITH PARASITE COUNTS.

MICE: CFW ca 4 wks old.
DOSE: 0.03 ml Icer.
DIL: All made in NaCl (same as for IP titration).

allowed to stand in NaCl for 60-75' & thus may account for poor titer

	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	ADOPT.	#Par/dose
10^{-3}	–		–	–	–	D																									4/4	418 ~~42~~
	–		–	–	–	CNS }																										
	–		–	–	–	CNS }																										
	–		–	–	–	–	D																									
10^{-4}	–		–	–	–	–	–	–	?	D																					1/4	42
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
10^{-5}	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–			0/4	LD50 TITER $10^{-3.7}$ 4.2
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
10^{-6}	–		–	–	–	–	–	–	RT	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–			0/4	0.4
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
10^{-7}	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–			0/4	0.04
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				
	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–		–	–	–		–	–		–				

PARASITE COUNTS:

PAR. ESTIM. in 0.03 ml dose @ – 10^{-1} = 41818.2
10^{-2} = 4182
10^{-3} = 418
10^{-4} = 41.8
10^{-5} = 4.2
10^{-6} = 0.42
10^{-7} = 0.04

∴ THEORETICAL LD_{50} TITER =

Dec. 11, 1947

Proposed Scheme for Determining Factors Influencing Determination of Number of Toxoplasma in Peritoneal Exudate.

1. Obtain 1 to 2 cc (ca 1.8cc (4 mice - 4½ dy)) of exudate 4 or 5 days after inocul.
2. Prepare 1:10 dilution in saline (0.2cc exud + 1.8cc saline)
   " 1:100 " " " (0.2cc of 1:10 + 1.8cc saline)
3. Fill chamber with 1:10 (10^{50}) and count after allowing settling for 2 minutes; (note time when chamber is filled)
4. Fill ~~other side of same~~ other chamber with 1:100 (10^{515}) and count after 2 minutes
5. After count is completed repeat (count at 30 and 60 minutes (11^{20}) after original filling time
6. If 1:100 dilution has 10 or fewer toxoplasma per square mm. — investigate counts in two-fold dilutions between 1:10 and 1:100, i.e. 1:20, 1:40, 1:80
7. If 1:100 has over 10 toxoplasma/sq. mm. (it will depend on whether it is 20, 30 or 40) count 1:50, 1:200, and depending on number 1:400 and 1:600

   All above counts to be made after 2 min. of settling
8. After range is found, determine dilutions at which proportionate counts begin to occur.
9. ~~After~~ Prepare tenfold dilutions in saline and inoculate 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} & if indicated 10^{-8} i.p. in doses of .25cc

Where are the data of this test

DEC 17 1947

The Enumeration of Toxoplasma In Peritoneal Exudate

Obj: To determine under what conditions it might be possible to enumerate the toxoplasma in mouse peritoneal exudate so that unit concentration will follow the dilution scheme.

Peritoneal Exudate: A pool of ca 1.9cc obtained from 4 mice - 4 dys after IP inoc c̄ Pass 355.

Dilution: All made in NaCl & counted shortly thereafter.

Counts: All made in Neubauer (blood) chambers filled from capillary pipettes. At least 2 minutes settling time in chamber prior to counting under high dry. All counts by ABS & HAF. A 1 sq mm area counted when concentration of parasites was high, otherwise 4 such squares enumerated.

Titration: This peritoneal fluid "OLD" pool, after 2 hrs @ room temp & 4° in the refrigerator was mixed & serially diluted in NaCl to 10^{-8}. 0.25 ml amts were immediately inoc IP into groups of 4 mice - 10^{-3} - 10^{-8}. Dil. 10^{-2} of this series was counted as a check on the persistence of parasites.

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Actual Counts of Toxoplasma in Peritoneal Exudate

All counts performed in Reichert chambers.

Dil	2 min.	#/mm²	30'	#/mm²	2' in chamber but 1 hr after 1/100 dil. made.	#/sq mm.
undil.	52 54 62 65 HAF 69 65 84 68	1034				
1:5	35 42 36 39 counted @ 10' 35 41 28 30 37 32 29 36 30 26 34 29	539	34 38 40 28 HAF 32 33 36 22 @ 3 hr. 29 35 40 33 33 32 38 30	533		
1:10	23 19 17 14 HAF 18 16 14 19 14 14 15 16 14 14 22 15	264	17 16 13 14 HAF 14 14 19 22 16 16 12 16 X 16 15 16	251.7 corrected		
1:50	6 3 5 4 / 2 1 1 4 ABS 12 4 3 1 / 1 2 2 3 2 3 2 7 (61) / 1 1 1 5 (45) 0 4 4 1 / 5 4 8 4 Ave. = 53.5 1 3 4 4 / 6 2 5 5 2 2 2 2 (53) / 7 1 2 5 (55) 3 11 6 2 / 2 2 4 5 5 2 2 2 / 2 4 1 2	53.5				
1:100	1 0 0 2 (LU) counted @ 18' 3 2 4 1 (34) 2 3 1 4 ABS 1 1 5 4 LL / LL @ 30' 3 0 0 3 / 3 0 0 3 1 1 0 1 (28) / 1 1 0 1 1 0 3 1 / 1 0 3 1 2 1 1 0 / 2 0 2 0	26	1 0 7 0 (RU) ABS 1 2 1 5 2 2 1 1 (23) 2 2 0 2 RL Ave.: 25 2 1 2 2 2 0 2 2 (27) 1 4 1 0 1 2 4 1	25	1 3 1 2 / 0 2 1 2 ABS 1 1 0 2 / 3 0 0 0 1 2 3 3 (29) / 3 4 2 1 (29) 4 2 3 0 / 2 4 1 2 Ave. = 30.5	30.5
1:200	0 1 4 1 / 0 0 0 0 ABS 2 1 1 4 / 0 0 1 1 0 0 1 0 (17) / 1 1 2 0 (17) 0 1 1 0 / 1 0 0 0 Ave. = 13.5 0 0 1 2 / 1 2 1 0 0 2 2 2 / 1 1 1 0 0 2 0 0 (16) / 1 1 0 1 (14) 0 3 1 1 / 0 1 1 2	13.5				
1:100 C mouse titration series - PE = 6 hrs old.	1 1 2 0 / 2 0 2 1 HAF 1 1 0 1 (20) / 2 2 3 2 (36) 4 1 2 0 / 6 1 0 3 2 0 3 1 / 2 2 4 4 Ave. = 31 5 0 2 1 / 0 4 2 1 3 2 1 1 (34) / 2 1 1 5 (34) 2 1 3 3 / 3 2 3 3 1 3 0 6 / 5 1 1 0	31				

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TITRATION OF PERITONEAL EXUDATE ("old") – 6 hours

Peritoneal Exudate: This was obtained as a pool from 4 mice in a.m. & used for the series of dilution-counts to determine the type of relationship between dilutions & counts. This tube of undiluted exudate remained at room temp for 2 hrs, then was placed in the refrigerator for 4 hrs. Following this it was diluted (ten-fold) in NaCl & immediately titrated in mice – 0.25 ml IP. Groups of 4 Ch. alb. ca 3-4 wks old.

No evidence of Fibrin Formation

Intraperitoneal Titration – 0.25cc

Dilutions in Normal Saline and Inoculated Immediately

Old NaCl titration

Final Dil.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Mort.	Estimated No. Toxope per dose
10^{-3}	–	–	–		?	?	CNS		D																				4/4	7,000
	–	–	–		?	?	CNS		D																					
	–	–	–		?	?	CNS		D																					
	–	–	–		–	–	?		D																					
10^{-4}	–	–	–		?	?	CNS		D																				4/4	700
	–	–	–		?	?	CNS		D																					
	–	–	–		?	?	CNS		D																					
	–	–	–		–	–	?		CNS	D																				
10^{-5}	–	–	–		–	–	–		?	D																			4/4	70
	–	–	–		–	–	–		–	D																				
	–	–	–		–	–	–		–	D																				
	–	–	–		–	–	–		–	CNS		D																		
10^{-6}	–	–	–		–	–	–		–	CNS		D																	1/4	7
	–	–	–		–	–	–		–	CNS		~~D~~	–	–		–	–		–	–	–	–	–	–	–	–	–	–		
	–	–	–		–	–	–		–	–		–	–	–		–	–		–	–	–	–	–	–	–	–	–	–		
	–	–	–		–	–	–		–	–		–	–	–		–	–		–	–	–	–	–	–	–	–	–	–		
10^{-7}	–	–	–		–	–	–		–	D – ?																			0/3	0.7
	–	–	–		–	–	–		–	–		–	–	–		–	–		–	–	–	–	–	–	–	–	–	–		
	–	–	–		–	–	–		–	–		–	–	–		–	–		–	–	–	–	–	–	–	–	–	–		
	–	–	–		–	–	–		–	–		–	–	–		–	–		–	–	–	–	–	–	–	–	–	–		
10^{-8}	–	–	–		–	–	–		–	–		–	–	–		–	–		–	–	–	–	–	–	–	–	–	–	0/4	0.07
	–	–	–		–	–	–		–	–		–	–	–		–	–		–	–	–	–	–	–	–	–	–	–		
	–	–	–		–	–	–		–	–		–	–	–		–	–		–	–	–	–	–	–	–	–	–	–		
	–	–	–		–	–	–		–	–		–	–	–		–	–		–	–	–	–	–	–	–	–	–	–		

LD50 TITER:
PREDICTED:
ATTAINED: $10^{-5.7}$

CHECK COUNT:
Immed. a.m. 10^{-2} – 260/cu.mm. or 1cc of undil. exudate has 26 million
at dilut. time 6 hrs. 10^{-2} – 310/cu.mm or 1cc " " 31 "

Average of both counts = 28.5 million /cc of exudate

17 Dec 47

Comparative LD_{50} Titers In Mice of A Toxoplasma-Infected Mouse Peritoneal Exudate Diluted In NaCl & 50% PSS.

Obj: Because a previous exp't (8 Dec.) suggests that NaCl may be toxic for toxoplasma, a fresh peritoneal exudate is to be diluted in NaCl & 50% PSS & then I Perito mice.

Peritoneal Exudate: "Fresh" - pool obtained from 2 mice this afternoon. These were of the same group used for this morning's counting experiments. This exudate also used for the exp't on the effect of different immune & non-immune sera on toxoplasma.

PE Counts: Made from the indicated dilution tube, shortly after completing the dilutions.

NaCl Series 1:100

2360	3107	
1122	2200	
0223 (28)	0541 (28)	
1214	0120	
Ave. = 28		
3112	1321	
1215	1053 (29)	
0103 (27)	1013	
3112	2222	

50% PSS Series 1:100

5012	1240	
1100	1121 (31)	
3803 (32)	3114	
2015	7120	
Ave. = 30		
2334	2215	
0001 (29)	3132 (29)	
2411	0212	
1203	1103	

Titration: About 30 45 minutes after obtaining the peritoneal exudate, a series of ten-fold dilutions were made in NaCl (10^{-1} - 10^{-8}). A similar series was then made in 50% PSS. Each was then inoculated into mice - 4/dil, 0.25 ml IP. Inoc. were made as quickly as possible after completion of the dilutions

DEC 17 1947

TITRATION OF TOXOPLASMA-INFECTED MOUSE PERITONEAL EXUDATE IN NaCl & 50% RSS.

PERITONEAL FLUID: FRESHLY OBTAINED. 4th DAY. POOLED FROM 2 MICE.

DIL. & INOC.: IN NaCl - TEN-FOLD. 0.25 ml AMOUNTS I.P. IMMEDIATE TO GROUPS OF 4 CH mice ca 3-4 wks.

NaCl. - FRESH - DILUTIONS IN NaCl - 0.25cc I.P. Inoculated Immediately

FINAL DIL.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	MORT.	Esimated No. Toxopl. in dose
10^{-3}	–	–	–	?	?	CNS	D																						4/4	7,000
	–	–	–	?	?	CNS	D																							
	–	–	–	–	–	CNS	D																							
	–	–	–	–	–	?	D																							
10^{-4}	–	–	–	?	?	CNS	D																						3/4	700
	–	–	–	?	?	CNS	D																							
	–	–	–	–	–	?	D																							
	–	–	–	–	–	?	CNS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
10^{-5}	–	–	–	–	–	–	D																						4/4	70
	–	–	–	–	–	–	D																							
	–	–	–	–	–	–	D																							
	–	–	–	–	–	–	D																							
10^{-6}	–	–	–	–	–	–	CNS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0/4	7
	–	–	–	–	–	–	CNS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
	–	–	–	–	–	–	CNS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
	–	–	–	–	–	–	CNS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
10^{-7}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0/4	0.7
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
10^{-8}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0/4	0.07
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		

~~LD50 TITER PREDICTED:~~

PERITONEAL FLUID COUNT:

PREDICTED TOXO. CONC. @ VARIOUS DILUTIONS:

0.25 ml @ 10^{-1}
" @ 10^{-2}
" @ 10^{-3}
" @ 10^{-4}
" @ 10^{-5}
" @ 10^{-6}
" @ 10^{-7}
" @ 10^{-8}

LD50 TITER OBTAINED:

$10^{-5.4}$

CHECK COUNT OF 10^{-2}:

DEC 17 1947

TITRATION IN 50% RSS.

PER. FL.: Fresh - same as used for NaCl (on other side of sheet.)

Dil: Ten-fold in 50% rabbit serum saline. — Inoculated Immediately

Inoc: 0.25 ml amounts IP into groups of 4 ch abt - 3-4 wks old. Inoc. immediately after dilutions.

FRESH 50% RSS

Fluid	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	No. of tox/dose	MORT.	
	–	–	–		?	?	CNS		D																						
10-3	–	–	–		?	?	D		D																				75,000	4/4	7,000
	–	–	–		?	?	WKL		D																						
	–	–	–		–	–	CNS		D																						
	–	–	–		?	?	CNS		D																						
10-4	–	–	–		?	?	CNS		D																				750	4/4	700
	–	–	–		–	–	?		D																						
	–	–	–		–	–	–		CNS D																						
	–	–	–		–	–	CNS		D																						
10-5	–	–	–		–	–	?		D																				750	4/4	70
	–	–	–		–	–	–		D																						
	–	–	–		–	–	–		D																						
	–	–	–		–	–	–		D																						
10-6	–	–	–		–	–	–		D																				7.5	4/4	7
	–	–	–		–	–	–		CNS D																						
	–	–	–		–	–	–		CNS CNS D																						
	–	–	–		–	–	–		WKL – D																						
10-7	–	–	–		–	–	–		– –		– –		– –		– –		– –		– –		– –		– –		– –		– –		0.75	1/4	0.7
	–	–	–		–	–	–		– –		– –		– –		– –		– –		– –		– –		– –		– –		– –				
	–	–	–		–	–	–		– –		– –		– –		– –		– –		– –		– –		– –		– –		– –				
	–	–	–		–	–	–		– –		– –		– –		– –		– –		– –		– –		– –		– –		– –				
10-8	–	–	–		–	–	–		– –		– –		– –		– –		– –		– –		– –		– –		– –		– –			0/4	0.07
	–	–	–		–	–	–		– –		– –		– –		– –		– –		– –		– –		– –		– –		– –				
	–	–	–		–	–	–		– –		– –		– –		– –		– –		– –		– –		– –		– –		– –				

CHECK COUNT @ 10^{-2}:

LD_{50} TITER:
PREDICTED:
ATTAINED: $10^{-6.7}$

0.25cc of undil = 5,000,000 LD_{50}
1cc " = 20,000,000 "

7

DEC 17 1948

The Effects of Toxoplasma Immune Sera From Three Different Species On Toxoplasma In Mouse Peritoneal Exudate.

Peritoneal Exudate: Pooled from 2 mice. The same exudate used for the comparative titrations in NaCl & 50% RSS to-day. Ave count = 29/sq mm @ 10-2

SERA : all freshly thawed or suspended from lyo. state (monkey). 0.2 cc serum mixed ± 0.2 cc of Exudate dil. in NaCl as follows:

Tube #	SERUM	EXUDATE
1	[redacted] (3 Dec 49)	1:5
2	" "	1:50
3	[redacted] normal Human)	1:5
4	[redacted] (lyo.)	1:50
5	" "	1:5
6	" "	1:50
7	[redacted] (Rh 934)	1:5
8	" "	1:50
9	Guinea Pig Immune (Warren)	1:5
10	" "	1:50
11	GP normal	1:5
12	" "	1:50

Exudate = same as used for comparative titration in NaCl & 50% RSS. to day.

Mixtures completed at 3^{30} pm. (exudate obtained @ 3^{10} pm.), smeared at room temp until 8^{45} pm. when blood drop slides were made of the 1:5 exudate of series. (5¼ hrs.) Tubes then placed in refrigerator until 11^{00} a.m. 18 Dec when all tubes were smeared again. In each instance, slides remained at room temp overnight for good drying to take place. In addition, immune & normal mate were stained on the same slide.

Freezing & Thawing: A portion of the same exudate (undil) was rapidly frozen & thawed in dry ice 2x. This exudate was dil to 1:100 & counted after each thawing. Slides for dissolution were made after the second thawing. (See protocol)

DEC 17 1948

The Effects of Toxoplasma Immune Sera on Toxoplasma In Mouse Peritoneal Exudate — In Vitro

TUBE #	MIXTURE (0.2 cc EACH) SERUM	PERITONEAL EXUDATE	SMEARS (#TOXO/#OIL IM. F.) 5 1/4 HRS.	18 1/2 HRS.	
1	[redacted] (Hu. IMM.)	1:5	28/30 (14/15) about 1/100	11/15 POS. FIELDS = 1, 2, 8.	
3	[redacted] (Hu. CONT.)	1:5	102/7	NUMEROUS	Counting Chamber UNSTAINED
2	[redacted]	1:50	———	9/19 — POS. FIELDS = 1, 1, 2, 2, 3.	Counted @ 3 hrs = 28, 29, 24, 25 AVE = 26.5 — all "ghosts"
4	[redacted]	1:50	———	26/19	Counted @ 3 hrs = 25, 31, 43, 28. AVE = 32 — normal.
5	[redacted] IMM.	1:5	NUMEROUS / FIELD. NOT COUNTED.	NUMEROUS	
7	[redacted] RM. Rh 934	1:5	21/10 (COUNTED 2X)	33/7	
6	[redacted] IMM.	1:50	———	68/14 ?	Counted @ 4 hrs = 38, 49, 41, 37 AVE = 41 — normal.
8	[redacted] NORM.	1:50	———	4/25 ?	Counted @ 4 hrs = 14, 20, 38, 29 Ave = 25 — many "ghosts"
9	G.P. IMM.	1:5	101/4	250/6	
11	G.P. ~~IMM.~~ NORMAL	1:5	101/4 (!)	67/6	
10	G.P. IMM.	1:50	———	20/17 POS FIELDS = 1, 4, 2, 4, 2, 3, 1, 1, 1, 1	
12	G.P. NORM.	1:50	———	3/22 POS. FIELDS = 1, 1, 1	

Effect of Freezing & Thawing — Slides made in usual fashion (heavy drops) & allowed to dry slowly.

Peritoneal Exudate — undil. & untreated = numerous parasites in smear

Frozen & Thawed 2X — 0 toxo in 15 oil imm fields. Leucos appear normal. There are many faint forms that might be residua of toxoplasma but no definite toxoplasma were noted.

Counts @ 1/100 after freezing & thawing 1X = 33, 31 — all "ghosts"

" " " " " " " 2X = 37, 39, 26, 41 — all "ghosts"

JAN 27 1948

The Direct Effect Upon Toxoplasma (PE) Of Positive And Negative Neutralizing Human Sera

Toxoplasma: Per. exudate pooled from 2 mice of IP Pass 366 (22 Jan 48). This was dil to 1:100 & counted immediately. Counts = 51/4 mm², 51/4 mm² = 12,750 000 tox/cc. Per. Ex. dil to 1:5 ± Na cl & then distributed in 0.15 cc amounts to small tubes. Then 0.15 cc of following sera added, mixed & allowed to remain at room temp (25°C) for 5 hrs when thick films & stained in chamber. Tubes then placed in refrig for overnight study.

SERUM	DATE	RAB. NEUT.	DIRECT EFFECT IN CHAMBER @ HRS.	STAINED FILM* @ 5 HRS.
[redacted]		+	5° - MOSTLY GHOSTS - NUMB BACT.	Numerous toxo. many groups & clumps
2 [redacted]	3 DEC.	+	5° ghosts - all	an occas'l toxo
3 [redacted]	JAN	+	5° - all ghosts	" " " (if anything, less than #2)
4 [redacted]	6 JAN	+	5° all ghosts.	an occ. TOXO.
5 [redacted]	26 Dec.	+	5 hrs - almost all ghosts	NO toxo markedly decreased but not as many as in #3 & 4.
6 [redacted]	6 JAN	+	5 hrs - " " "	an occ. toxo.
7 [redacted]	—	+	5 hrs. Refractile - OK	Numerous toxo
8 [redacted]	—	+	5 hrs - almost all ghosts	no marked reduction in # toxo - about as #5.
9 [redacted]	—	+	5 hrs. GHOSTS	Reduction in # toxo but not as much as #8
10 [redacted]	25 Aug	+	small clot @ 5° 5 hrs. - GHOSTS + Toxo seen	occ. toxo.
11 [redacted]	24 Dec	—	5 hrs - refractile O.K.	Red. in # toxo but more than #9 Numerous
12 [redacted]	22 Aug	—	5 hrs. - refractile OK	Numerous.
13 [redacted]		—	5° - ok "	Numerous
14 [redacted]	25 Aug	—	5° - ok "	Numerous.
15 [redacted]	29 Dec	—	5° " "	Numerous toxo - just like #1
16 [redacted]	25 Aug	—	5° " "	Numerous toxo - v many more than #2

* Thick films. Allowed to dry overnight & then stained ± Wrights.

14-15
24-16

DIRECT Effect — IN VITRO
Toxoplasma + Various Human Sera

JAN 27 1948

Serum mixed with equal parts of peritoneal exudate, diluted 1:5 in saline, left at room temperature 5 hours and big drop put on slide. Drop allowed to dry slowly overnight — stained with Wrights

No.	Name	Toxoplasma Neutr. Antib.	Counting Chamber 5 hrs	Smear	Interpret. re:
1	[redacted]	?	Some ghosts some OK.	Many toxo; stain poor	?
2		+	Ghosts	very few toxo; stain poor	+
3		+	Ghosts	" " " "	+
4		+	Ghosts	" " " "	+
5		+	Ghosts	" " " " (many shadows suggesting only chrom stain)	+
6		+	Ghosts	few toxo; chrom. shadows	+
7		± slight	Refractile OK.	Many toxo; stain	−?
8		+	Ghosts	few toxo; chrom. shad.	+
9		17/23 +	Ghosts	few tox; poor stain	+
10		+	(cost) Ghosts	few tox; poor stain	+
11		−	Refractile-OK	Many toxo; good st	−
12		−	"	" " ; " "	−
13		−	"	" " ; " "	−
14		−	"	" " ; very good st.	−
15		−	"	Innum. Toxo; stain good	−
16		−	"	Innum. toxo; stain good	−

JAN 28 1948 — The appearance of the thick smears of the immune mixtures, suggested that the many amorphous bits of blue-staining material represented the chromatin of the disintegrated toxoplasma. The way the normal toxoplasma stained in the thick smears, indicated that an alkaline reaction developed during the drying — since the chromatin did not stain reddish-purple. It appeared possible that in thin smears, the normal toxoplasma might stain well, ~~white~~ and

the appearance of the affected toxoplasma in the immune mixtures might, therefore, be judged more readily

Thick and thin smears (on the same slide) were accordingly prepared from mixtures 1, 2, 3, 12, 13 and 15, and stained with [redacted]

Note – On the same slide the toxo in the thin films stained well, i.e. reddish-purple chromatin and deep blue cytoplasm while in the thick films the staining was poor – all dark.

The important finding here was that in the immune mixtures (2 + 3) the affected toxoplasma showed the following changes in the staining:

Cytoplasm – usually very faint blue without inner granular structure seen in normal and cell outlines indefinite or distorted; frequently only the chromatin could be seen and almost no cytoplasm at all.

Chromatin – stained almost the same as in normal, but configuration more distorted.

Further Note of Importance

No. 1. – the [redacted] serum which presented a problem in the chamber and thick smears gave evidence of a partial effect in the thin smear – many of the toxoplasma showed the immune effect while at least an equal number and perhaps somewhat more appeared normal.

Direct Effect of Immune Serum on Toxoplasma

JAN 30 1948

TIME REQUIRED FOR PHENOMENON TO BECOME APPARENT
"STAINED SMEAR METHOD"

TOXOPLASMA – Perit. exudate from 2 mice inoc. 1/22 with 0.1cc of 10^{-4} 11,000,000 per cc. of undil. exud. as det. by counting 1:100 dilution in saline – immediately after draining

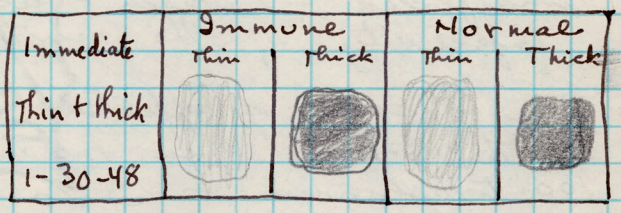
SERA – Immune – Ralph Black – frozen since 12/3/47

Normal – Susan Schumacher

A. MIXTURES PREPARED IN TUBES – SMEARS AT INDICATED INTERVALS

0.5cc of each serum + 0.5cc of exudate 1:5 in saline

SMEARS – Slide divided in 4 parts; ~~or~~ left for immune, right for normal. For thick smear, 0.02 cc was put on slide with 1cc calib. pipette and touched with loop to form approximate 1 sq.cm. For thin smear, the same pipette delivered a very tiny drop (by merely touching slide) which was then spread out over entire area @ 1.5×1cm. The thin drop dried within about 1 minute while the thick required about 30 min. for complete drying. The slides were stained with Wright's stain (4 min. stain, 3 min. water) about ~~2~~ 3 hours after preparation, except the 3 + 5½ hr. slides which were allowed to remain unstained overnight.



Results – The staining in all the thick smears was, as before, of the "alkaline type", i.e. the chromatin was unstained and the whole parasite was blue. In the thin smears, except for a few slightly thicker areas, the staining was of the "acid type", i.e. the chromatin stained red and in the normal series, the cytoplasm showed the usual inner structure and granularity.

In the immune series, the thin smears showed that the chromatin was unaffected (as noted on 1/28) while the cytoplasm was either so hazy that it merged into the background or could be distinguished as as an irregular "sky-blue" mass without any inner structure.

See other side for Data

Time after mixing Toxo + Serum	Normal		Immune	
	Thick Smear	Thin Smear	Thick Smear	Thin Smear
Immediate	++++	OK	+++	OK
15 minutes	+++	OK (beautiful)	++	Some OK. Many shadows
30 "	+++	OK "	+	Many shadows
1 hour	+++	OK	+	Shadows
2 hours	+++	OK	± (very few)	Shadows
3 hours	+++	OK	± very few	Shadows
5½ "	+++	OK	± very few	Shadows few good ones present

Examination in counting chamber of fresh material at 20 minutes showed large numbers of "ghosts".

NOTE! – 4 plus is intended to designate the relative number of toxoplasma seen. It appears therefore that while some of the toxoplasma are already affected at 15 minutes, as is evident by the reduction in numbers in the thick smear and the changed staining of some of the toxoplasma in the thin smear, the maximum effect occurred at 2 hours – beyond which no further change was seen.

As in previous tests, it was apparent that a certain proportion of the toxoplasma remain unaffected – which accounts for the residual infectivity of the mixture

A count of affected and unaffected parasites in the 3-hour thin smear showed that 5 of 100 toxoplasma – 5% remained unaffected.

B. Mixtures Prepared Directly on the Slide

This was to find out whether the phenomenon could take place on the slide during the period before the material dried

1. Thin smears – a) one loopful of serum + 1 loopful of exudate 1:10 Dried in 1 minute or less. – No effect – normal stain
b) one loopful serum + 1 loopful undil. exudate. Also no effect

Didn't work

2. Thick smears – a) 0.05cc of serum + 0.05cc exudate undil.
b) " " + " " 1:10

Drying time 30–35 min. – but no effect

Do changes during drying interfere with phenomenon?

JAN 30 1948

Effect of Immune Serum on Individual Toxoplasma Demonstrated with Methylene Blue in Fresh Preparations

Previous observations indicated that Toxoplasma affected by human immune serum exhibited a change in staining + appearance of the cytoplasm as seen in ~~fixed~~ dry thin smears stained with Wright's stain, or a disruption as evident by their disappearance in big drops allowed to dry slowly and then stained by Wright's. It was recalled that about 14 years back, ~~methylene~~ I observed that methylene blue added to peritoneal exudate in coverslip preparations stained the cytoplasm of the toxoplasma very deeply. It appeared interesting therefore to see whether there was any difference in the affinity for methylene blue in toxoplasma affected by human immune serum as compared with normal toxoplasma.

A. Serum + Exudate 1:5 – at room temperature for 3 hours

this is the ~~the~~ same mixture which was studied ~~periodically~~ at intervals by the "stained smear" method.

Normal Serum Mixture – toxoplasma stained a deep purple – areas occupied by chromatin appear clear

Immune Serum Mixture – with rare exceptions practically all toxoplasma have failed to take up the methylene blue. The important and interesting ~~additional~~ finding that all intracellular toxoplasma stained very deeply.

B. Serum + Exudate Undiluted – room temperature 3 hours

As above – here large numbers of intracellular toxoplasma were seen and in the immune serum mixture all the intracellular organisms were deeply stained showing that they were not affected by like the extracellular toxoplasma

JAN 30 1948

Is the Active Agent or Antibody in the Immune Serum Heat Stabile or Heat Labile?

The toxoplasma neutralizing antibody was previously found to become inactive on heating at 56°C for 30 min. and in the case of monkey immune serum, the addition of 1 part guinea pig complement to 3 parts of heated serum did not reactivate it.

	Meth. Blue Preparation	Counting Chamber
Human immune serum (Black) heated 56°C for 30 min + exudate 1:5 in saline	Toxo stained (∴ ser. inactive)	C.K. reparable
Heat inactivated Black serum + exudate 1:5 in normal fresh human serum (Schumacher)	Toxo not stained (∴ reactivated)	Ghosts
Heat inactiv. "Black" serum + (exudate 1:5 in) frozen guinea pig compl.	About 50-50 stained + unstained ∴ partially reactivated	50-50

These mixtures were incubated 1-2 hours before examination.

The g. pig serum by itself had no effect on the toxoplasma as seen in the chamber by H. Feldman } not quite correct however

Conclusions: 1) The effectiveness of immune serum is destroyed by heating it at 56°C for 30 min.

2) Reactivation is possible by the addition of a fresh human serum which in itself has no effect on the toxoplasma, indicating that the phenomenon requires the participation of 2 substances
   a) specific, heat stable
   b) nonspecific, heat labile (complement?)

3) Reactivation with guinea-pig complement (on a previous test a 0.3cc of 1:30 was 1 exact unit; ∴ at least about 10 units of complement contained in 0.1cc added to mixture) was only partial — since about 50% of the parasites took the stain

4) There is a suggestion that the heat-labile factor may be present in greater concentration in human serum than in guinea-pig serum — can it be the C1 component of complement?

JAN 30 1948

Effect of Freezing and Thawing on Affinity of TOXOPLASMA FOR METHYLENE BLUE

Procedure – Approximately 0.3cc of undiluted peritoneal exudate used same day in other studies was quickly frozen in thin layer in dry ice chest. Within few minutes it was thawed in water at 37°C and immediately refrozen in dry ice chest, and in turn thawed out.

The untreated exudate and the frozen thawed were examined simultaneously – 0.02cc of exudate + large loopful of alkaline methylene blue + cover slip Examined under 4SO magnification

Untreated exudate – toxoplasma deeply stained by methylene blue

Frozen and thawed 2x – none of the toxoplasma were stained ~~and~~ but looked otherwise intact.

Comment – Killing toxoplasma by freezing and thawing had same effect as treatment with human immune serum as regards effect of methylene blue.

Feb 8 – Check effect on nuclear chromatin with Heroin

JAN 30 1948

Can the Immune Phenomenon be Observed Directly in the Cover-slip Preparation when All Reagents are Added at Once?

0.1 cc. immune serum (Black) + 0.1cc toxoplasma suspension (~~either~~ undil. in one and 1:5 in another) + large loop of methylene blue on slide and coverslip put on.

Observed continuously for 2 hours — the toxoplasma took the stain immediately and did not lose it during the period of observation.

Conclusion —1) the antibody does not combine with the toxoplasma as rapidly as the methylene blue.

2) Once the methylene blue has combined with the toxoplasma — the in vitro immune phenomenon is not demonstrable.

Comment: Either the methylene blue has combined with the receptors ~~and~~ for the antibody

or the antibody may have combined ~~but~~ without releasing the methylene blue.

Questions 1) Are toxoplasma stained by methylene blue still infectious?

Titer stained and unstained peritoneal exudate in mice.

2) If they are infectious, can they still be neutralized by antibody?

JAN 30 1948

Test for Toxo Immune Phenomenon in Two Human Sera which ARRIVED in LABORATORY THIS AFTERNOON

[redacted] – arrived in frozen state from Syracuse. Clinically suspect of toxoplasmosis – see correspondence

[redacted] – arrived by ordinary mail from Hamilton, Ohio – nearby

Following story obtained the following morning from DR. B. J. LEHMAN, 309 S. 2nd St., Hamilton, O.

Serum taken JAN 30 1948 8 A.M. – separated + immediately mailed.

Patient – 26 year old woman, pregnant 6 times but always aborted or miscarried. Pregnant now and has toxemia of pregnancy – B.P. 170/110; albuminuria ++ Was seen at Duke and advised to have toxoplasma test

Both sera mixed with toxoplasma exudate 1:5 in saline at 4:15 P.M. Examined by methylene blue technique

3½ hours later at 7:45 P.M.

[redacted] – Not stained – Positive

– all stained – Negative

JAN 30 1948

EXPERIMENTS WITH HEATED R. BLACK SERUM IN THE IN VITRO TEST

Quest: Will heating for 30' @ 56° render R. Blacks serum inactive in the in vitro test and, if so, may the activity be renewed by the addition of fresh human &/or gp serum?

SERA: [redacted] 3 Dec 47 ampoule (used in all experiments conducted to day)
Susan Schumacher, frozen since 8 Jan 48. Rabbit sent neg.
ISP serum pool, frozen since 5 May 47. Used for comp in C-F tests but this was a fresh ampoule.

Toxoplasma: PE exudate employed in all of to-days tests & freshly diluted to 1/5 for these tests. Count @ 1:100 = 10, 10, 9, 15 = 44/4 mm² = 11,000,000 TOXO/cc.

Methods & RESULTS. Trial I: 0.15 cc R. Black sera & 0.15 cc Schumacher sera placed in separate Wassermann tubes & heated for 30' @ 56°. Cooled to room temps & 0.15 cc of toxo PE 1/5 in NaCl added to each tube. Mixtures completed @ 12 30 pm. Allowed to remain at room temps until 2 25 p.m. when examined in the chamber. Neither serum had any effect on the toxo although in other experiments conducted simultaneously, R. Blacks serum = very active & SSchumacher's = inactive with unheated algonists. Tubes remained at room temps until 10 30 pm when placed in the refrigerator. Reexamined (at 29°) in chamber & with no change in results.

Trial II: In view of results above, the experiment was redone using 0.1 cc units of freshly diluted PE (1:5 in the indicated sera or NaCl) + 0.1 cc units of freshly inactivated RB & SS serum & freshly thawed gp serum (frozen since 5 May '47).

Heated serum = 30' @ 56° C
Fresh serum = freshly thawed. See "sera" ↑ for duration of storage.

(over)

TEST SERUM	INAC. 30' @ 56°C.	TOXO PE 1:5 IN	CHAMBER	METH. BLUE.
[redacted]	+	Fresh Schumacher	POSITIVE @ 45 MIN.	
"	+	Fresh gp	POS. AT ONE HOUR BUT ca 30% refractile. Same at 29 hrs.	Def. activity @ 29 hrs. but many toxo c̄ slight blurring - like R. Black room temp expt of 31 Jan.
"	+	NaCl	NEG. AT 45' & at 29 hrs.	
"	+	[redacted]	POSITIVE @ 29 hrs.	
"	—	Fresh Schumacher	POSITIVE @ 1 hr.	
"	—	fresh gp.	POSITIVE @ 1 hr.	
[redacted] R	—	fresh gp.	NEGATIVE @ 1 hr & 29 hrs. ???	
"	+	fresh gp	NEGATIVE @ 1 hr & 29 hrs. ??	
"	+	NaCl	NEGATIVE @ 1 hr.	

N.B. + = inactivated, — = not inactivated.

INTERPRETATION:

1. [redacted] serum may be inactivated for the direct test by heating for 30' @ 56°C.
2. [redacted] serum (heated) may be "reactivated" by the addition of normal, fresh human serum in a concentration of 0.8 part normal to 1 part Black's serum.
3. This activity may also be renewed by the addition of fresh gp serum in the same concentration but such renewal seems to be only about 50% complete.

∴ The anti-toxoplasma activity of an immune human serum may be divided into two parts: a specific, heat stable (56°C) factor & a non-specific, heat-labile (56°) factor present in normal human &, to a lesser extent, in gp serum. (complement?).

JAN 30 1948

The Persistence of the Direct Anti-Toxoplasma Effect In Immune [redacted] Serum Stored At Room Temp.

1. Serum: Fresh ampoule of [redacted] serum obtained on 3 Dec 47 & stored on CO_2 since. Other ampoules from this lot have been consistently active, even as late as to-day.

2. Plan: Ampoule taped to wall at 3^{30} pm to-day. Whenever fresh PE is available, 0.1cc serum will be removed, ~~all~~ mixed with 0.1cc PE dil 1/5, allowed to stand for 2 hrs. at room temp & then examined in the chamber and with meth. blue for serum effect. When the latter disappears, fresh human serum will be added to R. Black's to determine whether its antitoxoplasma effect can be revitalized.

JAN 31 1948

Serum: 22 1/2 hrs. at room temp. 0.1cc RB mixed c̄ 0.1cc PE (18.3 mil/cc) 1/5.
2 hrs at room temp. Chamber Meth. Blue.

DATE	SERUM # HRS @ ROOM TEMP	TEST	INCUBATION	CHAMBER	METH. BLUE	
JAN 31	22 1/2	0.1cc SER + 0.1cc PE 1/5 (18.3 mil/cc undil)	2 HRS @ RT.	About 30% of TOXO appear to be refractile but not all are completely so - i.e., appear to be gradations. Remaining ca 70% are "ghosts."	Almost all toxo appear unstained as compared c̄ a control but many of these have slight gradations of "unstaining". Activity of serum dropping off.	
FEB 1	2 DAYS	as above together with other sera on 2/1/48	3 hrs.		94/100 unstained	
FEB 3	4 "	" " on 2/3/48	2 1/2 hrs		18/50 = 36% unstained	Fresh Black 41/50 = 82% unstain

30 JAN 48

Effects of Centrifugation & Hypotonic Solution upon Toxoplasma In the In Vitro Test

Toxoplasma : Same 1:5 in NaCl used in other experiments to-day.

Sera : [redacted] – used in other experiments to-day.

Centrifugation : 0.1cc of each serum & of PE 1:5 mixed in narrow, pointed tubes, allowed to remain at room temp for 4 hrs, then cent @ 2000 for 7 minutes. Then at room temp for four hours more. No gross difference between both tubes. Well mixed & examined in chamber. Both contained the usual picture of intact toxo but differing in reactivity

Was serum withdrawn & reconstituted in saline? No

∴ Centrifugation did not cause disruption of the "ghost-like" toxos.

Hypotonic Solution : From the two tubes (R. Black & Schumacher) set up for the activity time curve, 0.05cc was withdrawn after 8½ hrs at room temp & 0.1cc dist H_2O added to each. This reduces the salt content to 0.28%. Mixtures well shaken, allowed to stand for 30' & then examined in the chamber. The Schumacher chamber had an abundance of normal toxo while Black's had a similar number of "ghosts."

∴ Exposure to hypotonic sol. (0.28%) did not cause disruption of parasites in either Black's or Schumacher's serum. *

NB

* These trials were predicated upon the belief that the "ghosts" broke up & disappeared from thick films. However, in view of the thin film findings, it is apparent that there is no disruption of parasites in the dried films but rather a staining deficiency of the cytoplasma. Consequently, the findings in these trials should have been anticipated.

30 JAN 48

Titration of [redacted] um In the In Vitro Test.

Toxoplasma: Same 1/5 suspension used in other expts to-day.

Sera: R. Black & S. Schumacher used in other expts today.

Dilutions: Two-fold serum dilutions in NaCl (Final dil = 1/2, 1/4, 1/8, 1/16, 1/32)

Test: 0.1 cc of toxo 1/5 in NaCl added to each serum tube. Mixed & allowed to remain at room temps for 4 hrs when examined in chamber. At hours, they were examined c the meth blue procedure.

Results: In chamber: (Black's serum) 1/2 & 1/4 = usual amt of "ghosting." 1/8, 1/16, 1/32 all negative.

[redacted] serum = all normal (neg. test) toxo in each dilution.

∴ Titer = 1/4 by chamber test (Black's serum.)

However, the results of the meth. blue test were somewhat different, for while it agreed c the 1/4 chamber findings,

Examination of Various Dilutions of Immune Serum + Toxoplasma by Methylene Blue Cover-Slip Technique (8:40 PM.)

Final Dilution		Stained by Methylene Blue	Immune Phenomenon
1:2	UND	Neg. except for occasional one + intracellular	+
1:4	1:2	Neg. - perhaps 10% stained	+
1:8	1:4	Neg for about 40% - Pos. for ± 60%	± partial
1:16	1:8	Neg " " 20% - Pos. " 80%	± partial
1:32	1:16	Positive with rare exception	−

Question - is the limiting factor in this titration the specific heat stable component or the nonspecific heat-labile component?

30 Jan 48

The Effect of Varying The Number of Toxoplasma While Holding the Antibody Concentration Constant In the In Vitro Test.

Toxoplasma: Same PE used throughout to-day. Used in final dilutions of 1/2, 1/10, 1/100.

Sera: [redacted]

Method: 0.1 cc of serum, 1/5, & 1/50 toxoplasma in NaCl mixed ā 0.1 cc of each serum. Allowed to remain at room temps for 5 hrs when examined in the chamber. At 9 hrs, thick & thin films were made from each tube for differential counts.

Results: Chamber examination: At no concentration were any differences noted, ~~between~~ i.e., practically all of the toxo in each of the R. Black series were "ghosted" whereas none were in Schumbel's series.

Immune Phenomenon

Serum	+	Posit. Exudate	Chamber	Thin Smear No. Properly Stained	Thick Smear
Immune Undiluted	+	Undiluted	+	8/100	Few toxo
" "	+	1:5	+	5/100	very few toxo
" "	+	1:50	+	0/10 difficult to find	only 1 in 50 fields
[redacted] "	+	Undiluted	—	100/100	Many toxo
"	+	1:5	—	100/100	Many toxo
"	+	1:50	—	Difficult to find	Readily found

JAN 31 1948

The Effects of Treatment With Formalin and Phenol On the Functioning Ability of Toxoplasma In the In Vitro Test

Question: Is it possible to kill & preserve toxoplasma with formalin & phenol & have them function well in the in vitro test with serum-meth blue?

Toxoplasma: Peritoneal exudate pooled from the three surviving sick mice of the 10^{-3} & 10^{-4} dilutions of the "Immediate" titration of 22 Jan. '48.

Diluted 1:100 in NaCl count = 17, 20, 20, 16 = 73/4 qm. = 18.3 mil/cc.

Phenol & Formalin dil. fresh, from stock supplies.

Test Series:

a) Formalin

Conc. Formalin.	Amt.	Amt PE	Amt NaCl	Conc. PE.	% Formalin
1%	0.2 cc	0.2 cc	0.6 cc	1:5	0.2%
"	0.1 cc	0.2 cc	0.7 cc	1:5	0.1%
0.1%	0.5 cc	0.2 cc	0.3 cc	1:5	0.05%
0.1%	0.1 cc	0.2 cc	0.7 cc	1:5	0.01%

b) Phenol

Conc. Phenol	Amt.	Amt PE.	Amt NaCl	Conc PE	% Phenol
1%	0.2 cc	0.2 cc	0.6 cc	1:5	0.2%
0.1%	0.1 cc	0.2 cc	0.7 cc	1:5	0.01%

Tubes with mixtures remained at room temps for ca 1 hour, then placed in refrigerator. A simultaneous control of Peritoneal Exudate 1:5 in NaCl was also included.

JAN 31 1948

Follow-Up Examinations of Phenol-, Formalin-Toxo Mixtures. (Storage at 4°C)

DATE	MIXT. EXAMINED	METH. BLUE TEST.	
JAN 31 (2½ HRS)	NaCl Cont.	Normal toxo	
	0.2% Formalin	Normal toxo	
	0.2% Phenol	Normal toxo	
	0.01% Phenol	Normal toxo.	
FEB 1	NaCl	~~Normal toxo~~ TOXO STAINED QUITE WELL. LEUCOS	TOXO stained quite well. Leucos appear bluer than in fresh preparations - difficult to see toxo inside.
	0.2% Form.	~~Normal toxo~~	Toxo stain fairly well but not as well as in NaCl. Leucos appear as in NaCl. — NO ABS
	0.2% Phenol	~~Normal toxo~~	Poorest preparation of all but many toxo stained well enough so that could be used for test. — NO ABS
	0.01% Phenol	~~Normal toxo~~.	0.01% Phenol: almost like toxo control. Best of the treated preparations.

FEB 1 1948

Untreated EXUDATE — Parasites take methylene blue normally - deeply stained. Leukocytes, however, show a very marked change in that the nuclei now all stain a deep purple.

0.2% FORMALIN — Majority of toxoplasma stained, although not as intensely or diffusely as in the untreated exudate. The chromatin zone of the toxoplasma (under oil immersion) remains unstained. The nuclei of the leukocytes are not stained (except faint copper blue as in fresh leukocytes) as were those in the untreated exudate

0.2% Phenol — Toxoplasma unstained except for few granules. Leukocytes ~~unsta~~ nuclei unstained. These toxoplasma would not be suitable for test

0.01% Phenol — in fresh ~~hang~~ preparation most of the toxoplasma were well stained.

Note: some of these were re-examined 2-3 hours later and it was found that in the drying areas the toxoplasma were decolorized.

FEB 2 1948

Untreated Exudate — Most of toxoplasma stained well, although some had only few granules. Leukocytes same as 2/1/48

0.2% Phenol — same as on Feb. 1

0.01% Phenol — Toxoplasma stained well - only rare one unstained.

Effect of Various Chemicals on Preservation of Toxoplasma in Vitro (continued)

FEB 3 1948

Untreated – 45 stained well; 5 granules only (out of 50 counted)
Note!! – leukocytic nuclei not stained - only pale $CuSO_4$ blue

0.2% Formalin – Of 50 counted – 49 diffusely, definitely stained but not deep as in untreated. Leukoc. nuclei - pale

0.1% Formalin – 49 of 50 stained but not well; leukoc. nucl. pale

0.05% Formalin – of 50 – 3 stained well, 47 stained but poor; leuk pale

0.01% Formalin – of 50 – 25 well stained ± 25 poorly stained or not at all; leukocytic nuclei pale.

0.2% Phenol – of 50 counted – all poorly stained – almost all have some stained granules. Leuk. nuclei pale

0.01% Phenol – of 50, 36 stained well but more diffuse than untreated; 14 very poorly (occasional granule or not at all). Leukoc. nuclei pale.

Correlation between In Vitro Test for Toxoplasma ~~Ant~~ FEB 1 1948

Antibody and in vivo Neutralization Test on Rabbits

Procedure – The sera listed below were all tested 6 days before by the standard rabbit skin test. The frozen sera were thawed to-day and mixtures set up as follows:

0.1cc serum + 0.1cc of peri. exudate 1:5 in saline.

The exudate used had 18 million toxo per cc. and originated from a mouse inoculated 10 days before with 10^{-6} dilution of exudate.

Mixtures were left at room temperature and examinations begun two hours later – methylene blue added to 0.02cc on slide & cover slipped Because it was soon realized that a partial effect would have to be expressed quantitatively, 100 toxoplasma were counted at 450 magnification in each preparation and the number which had or had not taken up methylene blue noted. They were examined in the order noted below – so that some were not seen until 3 to 4 hours after preparation of the mixtures At 5 hours, thin smears were prepared of all mixtures – one large loop to 1/4 of the slide – and stained with Wright's stain

Name	Antibody by Rabbit Test	Methylene Blue Test No. Stained	Methylene Blue Test No. Unstained	Antibody in Vitro Test	Thin Smear Check
[redacted]	–	93	7	–	O.K.
	+	16	84	+	Ghosts
	+	15	85	+	Ghosts
	+	9	91	+	"
	+!	72	28	±	Some good " ghosts
	+	14	86	+	Ghosts
	+	10	90	+	"
	±	20	80	+	"
	±	6	94	+	"

Note: [redacted] 2 tests on 8/25/47 serum yielded equivocal results.
– previous test on 8/25/47 " " positive result
– 2 tests on 8/25/47 serum – one equivocal and one ~~positive~~ negative

FEB 3 1948

Influence of Prolonged Incubation in Refrigerator on Methylene Blue Test

The mixtures on the other side of page – prepared 2-1-48 – after 5 hours at room temp. were put in refrigerator at 4°C.

Purpose of Examination – Will equivocal serum (Latham) become positive? Will positive serum yield different results – will the affected toxoplasma regain affinity for methylene blue?

[redacted]

Result on 2/1/48	2/3/48
93% stained	72% well stained
84% unstained	100% unstained
72% stained	94% unstained

FEB 1 1948

Observations on Toxoplasma in Peritoneal Exudate of Dead Mice

Material – 4 dead mice from titration of 1-22-48 were used – 3 ~~&~~ had been inoculated i.p. with 10^{-6} & one $\geq 10^{-7}$. They had been dead not more than 24 hours and had already undergone some slight decomp. – however the intestines were still intact. They were ~~opened~~ with scissors and some of the exudate was examined with
a) methylene blue – cover slip
b) thin smear
c) thick smear.

Methylene blue – cover slip – There were innumerable toxoplasma seen but none was found stained by methylene blue. They looked exactly like those affected by immune serum or freezing and thawing

Thin smear – poor staining and disintegration of cytoplasm as in "immune" or frozen thawed

Thick Smear – almost none seen – except chromatin "debris"

Comment – It would appear that when mice are left at room temperature (70~~-75~~°F) after death, the toxoplasma in the exudate "die" rapidly – assuming that failure to take up methylene blue is indicative of death.

The Sad Events of FEB 4 1948

After all the tests had been set up it was discovered that the [redacted] alkaline methylene blue which had been used in all the previous tests no longer stained the toxoplasma a deep purple — instead the extracellular organisms took up a pink to reddish hue with some of the granules staining a bright red, while the intracellular toxoplasma stained a deep red. The slides, cover slips instruments, etc. were all checked (by using different ones) but the results were the same. The same also happened in mixtures with serum.

An old stock bottle of the stain (June, 1946) was then tried and gave the same result. Aqueous methylene blue (1%) didn't stain the toxoplasma at all and freshly prepared alkaline (alcoholic) methylene blue, according to [redacted] formula, as well as material from the hospital were practically no good at all. Some of the freshly prepared Loeffler's heated in a test tube for a few minutes gave a bluish stain to the toxoplasma — but not the deep purple seen before.

It was obvious that the whole question of what part of the stain the toxoplasma take up and under what conditions would have to be investigated before further work was possible.

FEB 4 1948

Effect of Monkey and Guinea pig Immune and other and Normal Sera on Toxoplasma In Vitro

a) Without activator – i.e. toxoplasma diluted in saline

b) With human serum as activator – toxoplasma in Human serum

Mixtures made 2:00 – 2:10 P.M.

NOTE – This is the day the "Meth. Blue" went bad and instead of examining these mixtures in cover-slip preparations – thin and thin smears were made.

INCUBATION TIME – Smears began 7½ P.M. – completed 8½ P.M. (1 to 20)

		Cytotoxic Effect
1	[redacted] Immune Monkey Serum + Periton. Exudate 1:5 in saline –	+ (24/25)
2	" " " " + " " 1:5 in SS serum –	+ (24/25)
3	Normal Monkey Serum (Rh 934) + P.E. 1:5 in saline	+ (24/25)
4	" " " " + P.E 1:5 in S.S serum	+ (23/25)
5	Frozen Immune Guinea Pig Serum 1037 + P.E. 1:5 in saline	–
6	" " " " + P.E 1:5 " SS serum	–
7	Normal Pooled Guinea Pig Serum (5/5/47) + P.E. 1:5 in saline	+?
8	" " " " + P.E 1:5 in SS serum.	+?
9	Frozen Rh Toxo rabbit ser. (398) + P.E. 1:5 in saline	–
10	" " " " " + P.E. 1:5 in SS serum	– ?
11	" P17 " rabbit " (349) + PE 1:5 in saline	–
12	" " " " " + " " in SS serum	–
13	Fresh Normal rabbit (351) " + " 1:5 in saline	+
14	" " " " + " 1:5 in SS serum	+
15	Frozen P17 pigeon serum + PE 1:5 in saline	–
16	" " " " + PE 1:5 in SS serum	–
17	Frozen P8 pigeon " + PE 1:5 in saline	–
18	" " " + PE 1:5 in SS serum	–
19	Frozen Rat #7 Toxo -5-9-47 serum + PE 1:5 in saline	+
20	" " " " + PE 1:5 in SS serum	+

~~Is the Limiting Factor in Human Immune Serum the Content of Specific Antibo~~

FEB 4 1948

Is the Content of Nonspecific Activator a Factor in Determining the Effective Dilution of Human Immune Serum

Mixtures prepared 2:45 PM.

		Cytotoxic Effect (See other sheet for fuller notes)
Human Immune Serum (Ralph Black) Dilutions of serum prepared in saline	undil. + P.E. 1:5 in saline	+ (50/50 = 100%)
	1:2 + " " "	+ (46/50 = 92%)
	1:4 + " " "	− (0/50 = 0%)
	1:8 + " " "	− (0/50)
	1:16 + " " "	− (0/50)
	1:32 + " " "	− (0/50)
	Undil. + PE 1:5 in S.S. serum	+ (50/50 = 100%)
	1:2 + " " " " "	+ (50/50 = 100%)
	1:4 + " " " " "	+ (50/50 = 100%)
	1:8 + " " " " "	+ (48/50 = 96%)
	1:16 + " " " " "	+ (46/50 = 92%)
	1:32 + " " " " "	± (45/100 = 45%)

Note - Fresh cover-slip preparations were not examined because the M.B. went "bad". Thin and thick smears were made.

Undil - saline and SS - smeared 8:20 PM
Others - 9:40 - 10:05 PM

Note - The peritoneal exudate used on FEB 4 1948 was obtained from 3 mice 5 days after i.p. inoculation.

No. of toxoplasma per cc. undil. exudate =

Reactivation of Human Immune Serum Stored at Room Temperature

FEB 4 1948

These mixtures were left at room temp. for 5 hours before smears were made

[redacted] room temp. 5 days + Perit. Exud. 1:5 in saline

Thick smear – good, diffuse, – very many toxoplasma in each high dry field

Thin smear (oil) well stained – 48/50 or 96% OK.

[redacted] room temp 5 days + Perit. Exud. 1:5 in S. Schumacher serum

Thick smear – (one thick area) – only rare Toxo seen; "chromatin debris"

Thin smear (oil × 900) – good – only one/50 stained well

98% unstained

Conclusion: 1) Storage of an active serum at room temp. for 5 days deprives it completely of its "immune" ~~properdues~~ effect

2) Addition of a negative, normal human frozen serum re-activates the degraded immune serum

3) The loss of potency in toxoplasma immune sera on storage is due to inactivation of the nonspecific component.

Effect of "Negative" Human Serum on Titer of Human Immune Toxoplasma Serum

FEB 4 1948

	Thick	Thin
1:32 + Saline	Many well stained	50/50 = 100% beautifully stained
1:32 + S. Schumacher	Toxo present – but not many	55 very well stained 45 very poorly stained (It was difficult to count the poorly stained ones, because quite a number were encountered in which both chromatin & cytoplasm were poor – these were not counted at all)
1:16 + Saline	Very many – beautifully stained	50/50 = 100% beautifully stained
1:16 + Schumach. serum	Few; Much chromat. debris	46/50 = 92% only chromatin stained
1:8 + Saline	Very many; beautif. stained	50/50 = 100% well stained
1:8 + Schum. serum	Rare; much chromatin debris	48/50 = 96% only chromatin stained
1:4 + Saline	Many; well stained	50/50 = 100% well stained
1:4 + Schum. serum	Rare; much chromatin debris	50/50 = 100% only chromatin well stained
1:2 + Saline	Few toxo; chromatin debris	13/50 = 26% well stained 37/50 = 74% only chromatin
1:2 + Schumacher	Rare; chromatin	50/50 = 100% only chromatin
Undiluted + Peit Exud. in Saline	Almost all ghosts	4/50 = 8% well stained 46/50 = 92% only chromatin
Undil. + Schum. serum	All ghosts	50/50 = 100% chromatin

FEB 4 1948 Feb. 4

Observations on Toxoplasma Heated at 50°C for 15 minutes

About 10 years ago I found that exposure of toxoplasma to 50°C for 15 minutes was enough to destroy their infectivity.

Purpose of Test: 1) Will staining capacity be retained or will it disappear as after freezing and thawing

2) If staining capacity is retained, will it be possible to demonstrate the "immune effect" with the heated toxoplasma.

Procedure – The peritoneal exudate used in the other tests on the same day was diluted 1:5 in saline and ~~beds~~ put in a water bath at 50°C for 15 minutes

Since the methylene blue went bad that day a Wright-stained smear of the heated toxo and all were found to be beautifully stained – both cytoplasm & chromatin. Mixtures were then prepared with the Ralph Black immune serum and with Schumacher negative serum. After 5 hours at room temperature thin and thick smears were prepared, left overnight and stained with Wright's stain.

Heated Toxo + [redacted] serum – thick smear – few toxo seen; much chromatin debris

thin smear – 46/50 = 92% with poor or no cytoplasmic staining

Heated Toxo + [redacted] serum – thick smear – washed off

– thin smear – not very good but 40/40 = 100% well stained

These mixtures as well as the heated toxo were stored in the refrigerator since Feb. 4

Feb. 6 – Examination ~~of~~ of Cover-Slip Prep. with Methyl Violet ~~(1:5)~~ and Thionin ~~(1:5)~~

FEB 6 1948

Heated Toxo alone – well stained as in unheated controls

" " + [redacted] – " " " " " " "

" " + [redacted] – Only chromatin normally stained

Feb. 9 – Heated Toxo + Methylene Blue (pH 11) or with Thionin (1:5)

FEB 9 1948

Still stains well

FEB 16 1948

Feb. 16 – Heated Toxo + Methylene blue (pH 11) – Only chromatin normally stained

FEB 5 1948

Effectiveness of Stains Other Than Methylene Blue In The In Vitro Toxoplasma Test.

In view of the change in character of the methylene blue reaction encountered yesterday, and the lack of utility of freshly made Loeffler's, it was decided to try a number of new dyes in the reaction. The following were tested against good, (coverslip prep) fresh peritoneal exudate diluted 1:5 in NaCl & containing an average number of parasites:

a) Methylene blue – from stock reagents but with phosphate buffered water @ pH 7.0 substituted for the dilute KOH.

b) Methylene blue – as above but buffered to pH 8.0.

c) Thionin: A very old bottle obtained from Mrs Case & bearing the label:
"Thionin 1%
Phenol 1%
Saturated solution"

d) [redacted] Sulfate: obtained from Mrs Case & guaranteed to be more than 10 yrs. old. Labelled: Saturated aqueous.

e) [redacted] 1%, aqueous: also obtained from Mrs Case, vintage unknown.

Effects Noted:

a) Meth. blue pH 7.0 = almost all intra & extra cellular toxo stained blue-green in cytoplasm. Chromatin unstained. Most WBC also stained blue green.

b) Meth Blue pH 8.0 = as above except that color is bluer & therefore the toxoplasma appear better stained. Might be good enough for a test.

c) Nile Blue Sulfate: Set up in [redacted] undil + [redacted] serum & PE in Schumacher serum of 4 Feb (stored in refrig.) – all parasites in both preparations looked alike – very pale $CuSO_4$ blue. No good.

See other side

d) Thionin : let up with same sera as was Nile Blue.

The R. Black preparation was converted to a heavy, amorphous precipitate on admixture with dye. The rare toxo that was seen was heavily stained & visible thru the mass of precipitate. Best

On the other hand, the Schumacher preparation was useable. The parasites were stained an intense aquamarine blue. When counted, there were 44/50 toxo well stained = 88% stained, 12% unstained.

looked very green to me (ABS)

e) Toluidin Blue : added to PE, organisms like with Nile Blue - practically unstained, pale $CuSO_4$ blue. No good

See below

Comment: Of this group then, the thionin looks like the best possiblity & will be repeated ~~without phenol~~ with a freshly made stained not containing phenol.

N.B. Feb. 8 - Since my recollection of the results with the Nile Blue Sulphate and Toluidine blue were somewhat different - the tests were repeated with freshly obtained undiluted exudate and the above dyes from the same bottles used on Feb. 5

NILE BLUE - no precipitation - examined immediately after preparation the toxo look unstained except that most of them (not all) had one or more deep red granules - floating around (indicative of "neutralized" fatty acids). However on standing for more than 20 minutes and particularly at end of one hour, all the toxo had taken a deep blue granular coloration of the cytoplasm (seen very well under oil with bright light). Floating around in the nuclear space could be seen a very pale blue homogeneous structure - staining like the nuclei of the leukocytes. ∴ Time required for best results

The stain was tested with xylol - which turned red. It is quite possible that it had been boiled with H_2SO_4 for use in the Smith and Mair stain for fat in frozen sections

TOLUIDINE Blue - immediate intense precipitation - examination right after preparation - few toxo that could be seen outside of deeply stained meshes, looked practically unstained. However, 30-120 min later all toxoplasma beautifully stained - same as with aqueous (alcoh) thionin (i.e. without phenol

FEB 6 1948

The Usefulness of a Group of Freshly Dissolved Dyes In The In Vitro Toxoplasma Test

Dyes tested:

a) Methyl Violet 6B (C & Bell)

b) Thionin (Thionin Blue) (C & Bell)

c) Nile Blue Sulfate (C & Bell)

d) Cresyl Violet (Cresyl Echt Violet) - Natl Aniline Dye Content 97%

Formalin: Each of the above was dissolved according to the general formula:

Dye	0.1 gm.	Alc. added first, shaken, then dist H_2O.
95% alc	4.0 cc	
Dist H_2O	16 cc	

which was obtained from Conn, page 75, for a thionin stain "for general nuclear staining." Each dye was shaken a number of times during the hour immediately after mixing & just prior to use.

In addition to the above, a set of borate buffers were made & the media kitchen's stock saturated alcoholic methylene blue dissolved in the proportion of 1.5 cc meth blue to 5 cc buffered dist. H_2O. The pH's used: pH 8.0 (phosphate), 9.0, 10.0, 11.0. (Last three = borate buffers.)

Tests: Each of the above was tested in a wet preparation using 0.02 ml of PE #5 (of 5 hrs) & one loopful of dye.

Results of microscopic examinations: (monocular)

1. Methylene blue:

a) pH 8.0 = almost all toxo & leucos appear blue-green. Occ. red granules in toxo.

b) pH 9.0 = slightly bluer toxo & leucos than pH 8.0

c) pH 10.0 = as at pH 9.0.

d) pH 11.0 = definitely the bluest of all. Toxo well-stained & undistorted. Most of staining is in the cytoplasm.

2. Methyl Violet: Much stain ppt but toxo very densely stained, almost black. Leucos also dense.

3. Nile Blue SO_4: Much stain ppt. Toxos pale blue, same with red granules. Leucos pale blue, same with red granules.

4. Thionin: Toxos & leucos stained a deep blue but not as dense as with methyl violet. Much ppt.

5. Cresyl Violet: Toxo & leucos stained granules deep blue but there is much ppt.

Several of the above were reexamined after filtration & on the research scope.

1. Methyl violet: Using PE 1/5 of 5 Feb. Toxo densely stained. Under oil = very dark, almost black all membrane = red cytoplasm and chromatin (?). Latter frequently ringed in black.

2. Thionin: Strikingly different appearance: chromatin bright red & the cell margin is very dark (black?) giving the appearance of an endoplasmic inclusion body = magnification

6 Feb 48

Dyes (Continued)

Loeffler's: The Loeffler's made from stock reagents on 4 Feb & another batch made to-day were tried c̄ the same PE 1/5 of 5 Feb. Little immediate staining but in 30-60 minutes there is staining of the cell membrane & granulation of the cytoplasm. Could be used for a test.

Trials With Undiluted Methyl Violet (Filtered) & tests of 4 Feb 48 Which Have Been Stored in The Refrigerator. (Research Scope)

1. R. Black undil tube: toxo completely unstained.
2. PE 1/5 in NaCl : Toxo very deep red c̄ very dark cell border. Dye ppt in this mixture. Thus, perfect differentiation between these two but try diluted stain.
3. Heated toxo : toxo deep red c̄ very dark cell border (as in line, NaCl)
4. Heated toxo + Schumacher serum : deep red c̄ very dark border.
5. Heated toxo + R. Black : No dark borders but cytoplasm & chromatin red, altho much lighter shade than control.

Undil Filtered Thionin Used In Place of Methyl Violet Above.

1. R. Black, undil: toxo unstained but vivid red chromatin dot.
2. PE 1/5 in NaCl : Dark cell border c̄ granular dark material along inner cell wall, red chromatin (resembles herpetic inclusion).
3. Heated toxo : As in NaCl control ↑
4. Heated toxo + Schumacher serum : As in NaCl control ↑
5. Heated toxo + R. Black : Cell walls no longer dark, chromatin dot bright red & cytoplasm pink in place of colorless.

7 Feb 48

Diluted Methyl Violet & Thionin

Methyl Violet + PE of 5 Feb 48.

a) undil MV = Toxo very deeply stained, much pjt.

b) MV 1:5 in dist H_2O = toxo deeply stained. No pjt. ← use

b) MV 1:10 in dist H_2O = No pjt, toxo "very, very pale."

Thionin + PE of 5 Feb 48

a) undil = Toxo well stained. Some pjt.

b) 1:5 in dist H_2O = most toxo well-stained, some unstained. No pjt. ← use

c) 1:10 in dist H_2O = No pjt. Toxo as in 1:5

Incidental Observations

FEB 7 1948

Perit. Exudate 1:5 saline of Feb. 1 – in refrigerator
+1:5 methyl violet — Toxo well stained

Per Ex. 1:5 in saline of ~~Feb 1~~ + Schumacher serum – in refrigerator since Feb. 1.
(Methyl violet 1:5)
– toxoplasma unstained and many disintegrated

N. B. !! – this same mixture examined on Feb. 3 showed 76% of Toxo stained

Per-Exud. 1:5 + Bolles – in refrig. since Feb. 3. (methyl violet 1:5)
– Toxoplasma not stained

Mixtures of Feb. 4 (all ± methyl violet 1:5)

Normal Rabbit + Per. Ex. 1:5 in saline — Toxo unstained
" " " " " in Schumacher – " "

Normal Guinea Pig + Per-Exud. 1:5 in saline – Toxo unstained
" " + " " " in Schumacher " "

[redacted] (Room 5 days) + Per Exud. in saline — Toxo unstained
" + " " in Schumacher – " "
(Note: these same mixtures examined with Thionin showed that both the chromatin and cytoplasm have lost their affinity for the stain)

[redacted] (fresh 1:32 in sal.) + Per-Ex. 1:5 in saline — Toxo stained
" " " " + " " in Schumacher – unstained

Comment – There is a suggestion in these observations that toxoplasma stored in ~~fresh~~ negative sera may lose their affinities for the dye after 3 or more days — faster than toxo stored in saline.

To check whether this disintegration affects chromatin as well as cytoplasm two mixtures were re-examined with Thionin (1:5)

(see other side)

Thionin 1:5

FEB 7 1948

[redacted] #4 Penit expd. 1:5 - in refrigerator since Feb 1 -

Only one toxo seen with both cytoplasm + chromatin stained
~~Smears~~ Almost all other Toxo seen - neither chromatin nor cytoplasm stained
Rare toxo - only chromatin stained

Bolles serum mixture since Feb. 3

Many more toxo seen with both cytoplasm and chromatin stained. Majority of ~~those~~ others ~~unstained~~ - both chromatin and cytoplasm unstained.

N. B. - Since toxo affected by specific antibody retains capacity for staining chromatin with thionin as well as Wright's, it would appear that the disintegration described above may represent a non-specific effect.

FEB 9 1948

Observations on Methyl Violet -

a) Effect of concentration on Staining Effect

b) Difference between effect in presence of serum and in ordinary mouse peritoneal exudate undiluted or in saline.

Note - These observations are recorded because after we were all set up to use methyl violet in the day's tests, it suddenly didn't work and here is why.

Methyl violet 1:5 (2 loops) + Toxo in Schumacher serum - very light pink

Methyl violet 1:5 (1 loop) + Toxo in Perit Exud 1:5 saline - very light pink at first but quite deep ½ hour later

" " (3 loops) + " " saline - deep violet right away

Methyl violet undiluted - 2 loops + Toxo in S.S. serum - poor at first but good ½ hour later.

" " 1:2 - 2 loops + Toxo " " - poor all through 2½ hours of observation.

Conclusions - 1- Concentration of methyl violet used ~~is~~ has very narrow range between ineffectiveness and marked effectiveness

2. - Ordinary serum combines with or otherwise inhibits action of ~~cert~~ concentrations of dye which are effective in absence of serum.

FEB 9 1948

The Effects of Normal Sera From Several Species Upon Toxoplasma In the In Vitro Test

Toxoplasma: 4 day peritoneal exudate pooled from 2 mice this a.m. & diluted 1:5 with NaCl just prior to test. Used in 0.1 cc amts per tube.

Sera: All freshly thawed & used in 0.1 cc amts per tube.

Test: Serum - toxoplasma mixtures incubated in 37° H_2O bath for one hour. Because of difficulty in selecting dependable dye for the wet-film test, the tubes then removed at room temp for another 2 hrs before being mixed & examined.

Methylene Blue (pH 11.0) (made on 6 Feb)
Cover slip

SERUM:

- [redacted] (4 FEB) — all well stained — 23/25 = 92%
- SHEEP (4 FEB) — 36/50 = 72% unstained; 14/50 and intracellular ones well stained
- HORSE # 224 — 27/40 — well stained; 13/40 — poor or not at all (32.5% unst)
- HORSE # 226 — 23/25 = 92% unstained; intracellular stained
- HORSE # 229 — 29/50 = 58% well stained; others (42%) poor or not at all
- RHESUS # 934 — 98/100 = unstained; intracellular stained
- RHESUS # 936 — 83/100 — unstained; 17/100 + intracellular ones well stained
- RHESUS # 945 — 25/25 — unstained
- [redacted] (fresh) (positive control) — all unstained
- [redacted] room 10 days + PE in saline — 80% stained
  " — " + PE in Schuemacher — all extracellular unstained intracell. stained

Black - room + sheep — unstained
" 4 + Schuem — "

P_{17} toxo + saline — stained
" " + Black — unstained

9 Feb 1984

The Reactivability of [redacted] Serum stored For 10 Days At Room Temp.

Toxoplasma: Same PE used in animal serum tests to-day.

Sera: [redacted] (3 Dec) - at room temp since 30 Jan. '48.
- prepared to-day.
- frozen since 4 Feb 48.
Sheep " " " "

Test: Simultaneous with animal sera test of to-day. Same conditions.
Read: methylene blue pH 11.0

Protocol:

[redacted] (room temp 10 dys) + PE 1:5 in NaCl = 80% toxo stained

(room temp 10 dys) + PE 1:5 in Schumacher serum * = all extracellular unstained
Intracellular stained.

(room temp 10 dys) + PE 1:5 in sheep serum = " "
(sheep serum alone is active)

fresh, + PE 1:5 in NaCl = all unstained.

∴ Toxoplasma in mouse serum stored at room temp for 10 dys may be reactivated by the addition of normal, fresh, human serum.

* PE 1:5 in Schumacher serum = almost all stained.

Effect of Freezing and Thawing on "Chromatin" of TOXOPLASMA

FEB 10 1948
FEB 9 1948

Purpose – to determine whether or not the chromatin remains unaffected after freezing and thawing as is the case after action of "immune serum"

Procedure – 0.2cc of fresh undiluted peritoneal exudate put into pyrex tube ~~with~~ (100×10 mm) with cotton plug – exudate rolled around in thin layer and put into dry ice freezing box. One hour later it was thawed out by rolling in palm of hand and then put back in CO_2 box where it was left overnight. Thawed quickly in water bath at 37°C

The untreated peritoneal exudate was left in the regular refrigerator overnight

FEB 10 1948

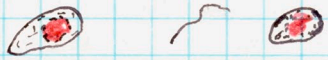
The thionin (G.D.) solution (diluted 1:5 in water) was used to demonstrate the effect on both the cytoplasm and the chromatin

To 0.02cc of exudate on slide were added 4 loops (5mm.) of the thionin, coverslip put and examined immediately and then again within an hour.

Control untreated exudate – cytoplasmic membrane of Toxoplasmas stained deep blue black and the chromatin a deep red

Frozen and thawed exudate – there are many both extracellular and intracellular toxoplasma in which only the chromatin is stained – thus

However, contrary to previous observations, there are quite a number of others in which there are varying degrees of dark cytoplasmic granulation, although only a few look like the untreated ones.



Effect of Methylene Blue (pH 11.0) – 2 loops to 0.02cc

Control untreated – here too the chromatin (less than with thionin) and the cytoplasm are stained, although the cytoplasmic staining is less on the periphery and more throughout as compared with the thionin

Frozen and thawed – same as with thionin, i.e. in some only chromatin is stained – in others varying degrees of cytoplasmic staining.

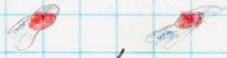
FEB 10 1948

Observations on thin films stained with Wright's stain

(See slides under date of Feb. 10)

Untreated control — very well stained —

Frozen and thawed — majority only chromatin stained well and cytoplasm indefinite & frayed



However, there were some that appeared well outlined and stained

Test for Infectivity of Frozen and Thawed Exudate

This was desirable just as a check because of the presence of a certain number of well staining toxoplasma.

The untreated as well as the frozen exudate were diluted 1:20 in saline and 0.1cc was injected intraperitoneally into each of 5 mice

	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
Untreated Control Exudate	1	–	–	–	–	Asc D																						
	2	–	–	–	–	Asc D																						
	3	–	–	–	–	Asc D																						
	4	–	–	–	–	–	Asc D																					
	5	–	–	–	–	–	Asc D																					
Frozen and thawed x 2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

NOTE – The most important difference between freezing and thawing and the effect of serum is that in the former – ℰ intracellular toxoplasma are also affected while with serum, the intracellular ones are not and stain normally

FEB 10 1948

The Reactivity & Reactibility of Various Heated Animal Sera In The In Vitro Toxoplasma Test

Toxoplasma: Peritoneal exudate pooled from 3 ~~five~~ day mice. Examined wet film = most fields positive 1.0k.

Test: 0.1cc amts serum & PE 1/5 mixed, incubated for one hour in 37° C H_2O bath & then 0.02 ml mixed ē 2 ~~drops~~ loopfuls (5mm.) of methylene blue pH 11.0 & examined.

Sera listed as "heated" below = 30 min. @ 56°C. H_2O bath. Those heated as "fresh" were rapidly thawed & kept refrigerated until used.

Tube #	SERUM		PE 1:5 in	Cytotoxic Effect	Wet Film (Meth. Blue.)
I	[redacted]	fresh	NaCl	+	Unstained (except intracellular)
II		heated	NaCl	−	All beautifully stained
III		heated	[redacted]	fresh +	Unstained (except intracellular)
IV		heated		heated −	All beautifully stained
V	Sheep	heated	NaCl	−	" " "
VI	Sheep	heated	[redacted]	fresh −	45/50 = 90% " "
VII	HORSE #226	heated	NaCl	−	All beautifully "
VIII	Horse #226	heated	[redacted]	fresh −?	41/50 = 82% ? unstained
IX	Rhesus #936	heated	NaCl	−	All beautifully stained
X	Rhesus #936	heated	[redacted]	fresh −	24/25 = 96% " "
XI	Rhesus #945	heated	NaCl	−	All " "
XII	Rhesus #945	heated	[redacted]	fresh +	22/25 = 88% unstained
XIII	RAB. #351	heated	NaCl	−	All beautifully stained
XIV	Rab. #351	heated	[redacted]	fresh −	25/25 " "
XV	Rab #352	fresh	NaCl	+	48/50 = 96% unstained
XVI	Rab #352	heated	NaCl	−	25/25 beautifully stained
XVII	Rab #352	heated	[redacted]	fresh. −	All beautifully stained

Check

(over)

Tube #	SERUM	TOXO 1:5 in	Wet Film (Meth Blue)
* XVIII	GP pool (5 MAY) fresh	± NaCl	Toxo - deeply stained - clumped around leukocytes / Very few away from cells; 10/15 free - stained
XIX	GP pool " heated	− NaCl	All deeply stained
XX	GP pool " heated	− Schumacher, fresh.	" " " 24/25 = 96% unstained; intracellular stained
XXI	NORM. RAB. (4 FEB) fresh	+ NaCl	All deeply stained and agglutinated mostly around leukocytes
XXII	NORM RAB. (4 FEB) heated	− NaCl	
XXIII	NORM RAB (4 FEB) heated	− Schumacher, fresh.	All deeply stained

new cover-slip preparations made and counted

XVIII – G. pig – normal fresh ± NaCl – Many more toxo free
56/100 deeply stained
44/100 unstained

XXI – Normal rabbit + " – 98/100 unstained

Note: In view of the above unexpected results, new mixtures were prepared as follows, incubated 37°C for 1 hour and immediately examined.

Fresh sheep serum + P.E. 1:5 in saline – 35/50 = 70% unstained +
" " " + " " in fresh Schum – 25/50 = 50% unstained ±
Fresh rabbit 351 + " " in saline – almost all unstained +
" Rhesus 936 + " " " " – most toxo unstained +

FEB 10 1948

Do the various active animal sera ~~have~~ behave the same as regards the toxoplasmic chromatin as the human immune sera?

Purpose - attempt to find some difference in behavior between animal (probably - although not definitely nonspecific) and human immune?

Mixtures Used - were prepared and studied on Feb. 9. Remained in refrigerator overnight.

Thionin 1:5 - 4 loops to 0.02cc on slide - cover slip.

[redacted] + P.E. 1:5 in saline — Chromatin + cytoplasm well stained

[redacted] (immune) fresh + " " " — only chromatin stained in extracell org. Both cytoplasm + chromatin stained in intracellular ones.

Sheep (fresh) + " " " — like R. Block

Horse 226 + " " " — " " "

Rhesus 936 + " " " — " " "

Rhesus 945 + " " " — " " "

FEB 11 1948

The Activity & Reactivibility of Various Immune Human & Animal Sera In the Toxoplasma In Vitro Test

Toxoplasma: Pooled PE from 2, five day IP mice (Pass of 6 Feb). Many toxo at 1/10.

Test: "Fresh" sera = freshly thawed, no further treatment.

"Heated" sera = 30 min. @ 56° C.

ACTIVATOR = SS (Schumacher serum of 4 Feb 48)

Serum - toxo mixtures (0.1 ml of each) incubated one hour in 37°C H_2O bath, then 0.02 ml mixed ⊂ 2 loops (5 mm) of meth. blue, pH 11.0 in a cover slip preparation.

TUBE #	SERUM	PE 1:5 in	Cytotoxic Effect	
1	[redacted] heated	NaCl	−	Almost all toxo deeply stained
2	heated	SS	+	" " " unstained
3	fresh	NaCl	+	" " " "
4	heated	NaCl	−	" " " deeply stained
5	heated	SS	+	24/30 (79%) unstained
6	fresh	NaCl	+	Almost all unstained
7	heated	NaCl	−	" " deeply stained
8	heated	SS	+	Almost all (@ 90%) unstained
9	fresh	NaCl	+	Almost all unstained
10	heated	NaCl	−	" " deeply stained
11	heated	SS	+	" " (88%) unstained
12	fresh	NaCl	+	70/100 = 70% unstained
13	heated	NaCl	−	Almost all deeply stained
14	heated	SS	+	36/50 = 72% unstained
15	fresh	NaCl	+	23/25 = 92% unstained
16	heated	NaCl	−	Almost all deeply stained
17	heated	SS	+	23/25 = 92% unstained

Tube #	SERUM		PE 1:5 in	Cytotoxic Effect	
18	Im. Monk.	fresh	NaCl	+	49/50 = 98% unstained
19	Im. Monk	heated	NaCl	−	Almost all deeply stained
20	Im. Monk	heated	SS	+	37/50 = 74% unstained
21	Rat #7	fresh	NaCl	+	99/100 = 99% unstained
22	Rat #7	heated	NaCl	−	Almost all deeply stained
23	Rat #7	heated	SS	+	45/50 = 90% unstained
24	Rab #398	fresh	NaCl	±	Out of 100 — 64% deeply stained, 36% unstained
25	Rab #398	heated	NaCl	−	99/100 = 99% deeply stained
26	Rab #398	heated	SS	±	Out of 100 — 52% deeply stained, 48% unstained
27	Rab #349	fresh	NaCl	±	38/50 = 76 well stained; 12/50 = 24 unstained; 68/100 - stained *, 32/100 - unstained
28	Rab #349	heated	NaCl	−	97/100 = 97% deeply stained *
29	Rab #349	heated	SS	+	60/100 = 60% unstained * ; 40/100 = 40% stained

* check by Feldman
32/50 = 64% unstained
18/50 = 36% stained

* These were in refrigerator 2½ hours after 1 hr. at 37°C when ~~th~~ fresh slides were prepared. The older preparations had dried ~~out~~ partially and toxo were agglutinated. The note in pencil on No. 27 is the count on the older preparation – 1 hr. at 37°C — the question appears whether further incubation might have helped this weak serum

Note – In all above mixtures the intracellular toxo always stained.

FEB 12 1948

Toxoplasma In Vitro Tests With & Without "Activator" On A Group of Clinical Samples.

Toxoplasma: PE pooled from 4 mice (IP Pass of 8 Feb). Many tox.

Test: 0.1 cc serum + 0.1 cc PE 1/5, in 37°C H_2O bath one hour. Wet films as yesterday with methylene blue, pH 11.0. 0.02 ml serum-PE mixt + 2 loops (5 mm.) methylene blue.

SERA: Beginning with #11, all the follow sera from Ruckman for test to check with his rabbit tests. "H" = heated 56° C for 30'. "F" = fresh serum. SS = Schumacher sera of 4 Feb.

Tube #	SERUM	PE 1:5 in	Cytotoxic Effect	
1	[redacted]	NaCl	−	Almost all toxo deeply stained
2		SS	+	44/50 = 82% unstained
3		NaCl omitted		
4		SS	+	44/50 = 88% unstained
5		NaCl	± ?	35/50 = 70% st; 30% unst.
6		SS	+	37/50 = 74% unstained
7		NaCl	−	44/50 = 88% stained
8		SS	±	35/50 = 70% stained; 30% unst.
9		NaCl	−	80/100 stained
10		SS	− ?	72% " (36/50)
11 (R Ruck.)		NaCl	−	Almost all stained
12		SS	−	" " "
13		NaCl	−	" " "
14		SS	−	" " "
15		NaCl	+	46/50 = 92% unstained
16 (Post Equiv.)		SS	+	Almost all unstained
17		NaCl	+	47/50 = 94% unstained
18		SS	+	44/50 = 88% "

Choriocarcinoma

Rack	TUBE #	SERUM	PE 1:5 IN	Cytotoxic Effect	
+	19	[redacted] (F) 1-21-47	NaCl	+	Almost all unstained
	20	(4)	SS	+	" " "
	21	4-1-47	NaCl	−	18/100 unstained; 82% stained
Equiv.	22		SS	+ sl.	57/100 unstained
	23	1-47	NaCl	+	43/50 = 86% "
+	24		SS	+	41/50 = 82% "
	25	2-19-47	NaCl	+	41/50 = 82% "
+	26		SS	+	40/50 = 80% "
	27	2-10-47	NaCl	−	53/100 = 53% stained 43/100 = 43% unst.
−	28		SS	−	Most stained
	29	28-47	NaCl	+	Most unst.
+	30		SS	+	" "
	31	5-47	NaCl	−	37/50 = 74% stained
− checked w/BS	32		SS	−	43/50 = 84% "
	33	5-47	NaCl	+	83% unstained
Equiv.	34		SS	+	82% "
	35	15-47	NaCl	±	53/100 = 53% stained 47/100 = 47% unstained
Neg	36		SS	±	63/100 = 63% stained 37/100 = 37% unstained
	37	5-47	NaCl	+	Almost all unstained
+	38		SS	+	" " "
	39	6/3/47	NaCl	+	" " "
Equiv	40		SS	+	" " "

FEB 13 1948

Occurrence of Toxoplasma with Poor Affinity for Stain in Peritoneal Exudates of Moribund Mice

Material – Exudate was obtained from 3 mice inoculated 10 days before – ~~with~~ one with 10^{-4} and ≠2 with 10^{-5}. The exudates were pooled. 1cc + 4cc saline – 1:5 – used for count and examination.

1:5 in chamber yielded 126 toxo per sq. mm.
∴ 1260 per cu. mm. or 1,260,000 per cc of 1:5 or 6.3 million toxo per cc of undiluted exudate

Observations – Coverslip + Meth. Blue pH 11 (1:5 in saline)

First examination indicated that while most of the toxoplasma stained very well, there were an appreciable number that did not. When the examination was repeated it was found that 41/50 or 82% stained well and 18% did not stain

Examination of the undiluted exudate yielded 80% well stained and 20% unstained

1:5 dilution in Susan Schumacher Serum – incubated at 37°C in water bath for 10 minutes

first count 31/50 = 62% well stained
19/50 = 38% unstained or very poor

continued count 63/100 = 63% ~~un~~stained well
37/100 = 37% unstained or very poor

New Material – 3 additional mice from Feb. 3 experiment – inc. ē 10^{-3}
Exudate collected separately – diluted 1:5 in saline and examined.

Mouse 1 – 45/50 = 90% well stained – many toxo in preparation

Mouse 2 – 50/50 = 100% well stained – not as many toxo as in No. 1

Mouse 3 – Only two toxo found in @ 50 high dry fields.

Conclusion – Certain exudates (perhaps particularly those of moribund animals) may have too large a percentage of poor staining toxoplasma to be useful in tests in which 50% results (staining or non-staining) are looked for

FEB 13 1948

Toxoplasma In Vitro Tests With & Without "Activator" On A Group of Normal GP & Human Clinical Sera

Toxoplasma: Pooled PE from 2 mice of "K" 10^{-3} of 3 Feb ment. 1/10 dil contains fewer top than we usually use but appear to be plentiful enough for a test.

Sera: GP fresh bled normals on 12 Feb. Others obtained from Ruch. - begin ± #12.

Test: "H" = heated 30' @ 56°C just before test. "F" fresh sera. SS = Schumacher pooled serum.

0.1 cc serum + 0.1 cc PE 1/5 in NaCl or SS. In 37° H_2O bath 1 hour, then usual ~~hanging~~ dark preparation of 0.02 ml serum-PE + 2 loops (5 mm) of meth blue, pH 11.0.

TUBE #	SERUM	PE 1:5 in	Cytotoxic Effect	
1	[redacted]	NaCl	−	92% (23/25) stained
2		SS	+	92% (23/25) unstained
3	GP "A" (F)	NaCl	+	92% (23/25) unstained
4	GP "A" (H)	NaCl	−	100% (30/30) stained
5	GP "A" (H)	SS	−	80% (16/20) ~~unstained~~ stained; 7:10 PM 64% (16/25) stained
6	GP "B" (F)	NaCl	+	76% (19/25) unstained
7	GP "B" (H)	NaCl	−	100% (50/50) deeply stained; checked — toxo — not too diffic. to find; 6:30 PM
8	GP "B" (H)	SS	± ?	68% (34/50) unstained; (6:30 P.M.)
9	GP "C" (F)	NaCl	+	95% (19/20) unstained
10	GP "C" (H)	NaCl	−	96% (24/25) deeply stained; 6:50 PM.
11	GP "C" (H)	SS	± ?	72% (18/25) unstained; 6:50 PM
↓ RUCH. 12	[redacted]	NaCl	+ ?	76% (19/25) unstained
13		SS	+ ?	80% (20/25) "
14		NaCl	+	92% (23/25) "
Equiv 15		SS	+	88% (22/25) "
16		NaCl	+ ?	72% (18/25) "
17		SS		

TUBE #	SERUM	PE 1:5 IN	Cytotoxic Effect	
18	[redacted]	NaCl	+?	88% (22/25) unstained
19	[redacted]	SS	+?	72% (18/25) "
20	[redacted]	NaCl	±?	67% (20/30) "
21	[redacted]	SS	±?	64% (16/25) "
22	[redacted]	NaCl	+?	88% (22/25) "
23	[redacted]	SS		
24	[redacted]	NaCl	±?	64% (16/25) unstained
25	[redacted]	SS		
26	[redacted]	NaCl	+?	80% (12/15) unstained
27	[redacted]	SS		
28	[redacted]	NaCl		
29	[redacted]	SS		
30	[redacted]	NaCl		
31	[redacted]	SS		
32	[redacted]	NaCl		
33	[redacted]	SS		
34	[redacted]	NaCl		
35	[redacted]	SS		
36	[redacted]	NaCl		
37	[redacted]	SS		
38	[redacted]	NaCl		
39	[redacted]	SS		
40.	[redacted]	NaCl		
41	[redacted]	SS		
42	[redacted]	NaCl		
43	[redacted]	SS		

+

Neg Equiv

Neg

Equiv

Neg on stests

FEB 8 1948

Saline 1:10 = 26/26

FEB 13 1948

TUBE #	SERUM	PE 1:5 IN
44	[redacted]	NaCl)
45		SS
46		NaCl
47		SS
48		NaCl
49		SS
50		NaCl
51		SS

Peculiar Susceptibility of Toxoplasma to Fresh, Neg. Activator Serum

N.B. – Because so much difficulty was encountered in finding toxoplasma and so many of those found were unstained, the original 1:5 exudate dilutions prepared at 1 P.M. and kept in the refrigerator or at room temperature were reexamined at 5:30 P.M. An equal volume of saline was added to each to make the comparable 1:10 dilution.

5:30 P.M. Exudate in saline (refrigerator) — 26/26 = 100% well stained. Toxoplasma readily found.

" in Schumacher serum (room temp.) — 12/25 = 48% unstained !!!

6:30 P.M. Heated G. pig serum (No. 7) + PE in saline — 50/50 = 100% deeply stained.

6:50 " " " " " (No. 10) + " " — 24/25 = 96% " "

Conclusion: Since the ~~pig~~ toxoplasma in the original exudate diluted in saline or mixed with heated sera retained the capacity to stain well, while @ 48% of those mixed with the usually inactive fresh Schumacher serum failed to stain, it would appear that the toxoplasma used to-day were peculiarly susceptible to the action of fresh serum.

FEB 14 1948
FEB 14 1948

Influence of Concentration of Serum and Temperature of Incubation on Effect of a "Negative Serum" on Toxoplasma

Purpose -1) to determine whether the serum used as "activator" on Feb. 13 and before can continue to be so used
2) Best conditions for determining whether a given toxoplasma suspension in a given "activator" serum is suitable for use
3) May it be better to add "activator" to heated serum rather than making up toxoplasma suspension in "activator" serum, i.e. does 80% of "activator" serum affect more toxoplasma nonspecifically than 50% "activator" serum

Tube No.	Serum	Perit. Exud 1:5 in	Incubation	Per cent (out of 25) deeply stained at 15 min	30 min	60 min.	2/15 24 hrs.
1	[redacted]	SS (fresh)	Room	92	100	100	72 (18/25)
2	" "	" "	37°C	96	96	96 (84)	80 (20/25)
3	" (fresh)	Saline	Room	92	100	100	80 (20/25)
4	" "	"	37°C	88	92	88 (86) 43/50	72 (18/25)

SS = [redacted]

Pencil - observations by HF
Ink - " " AS

3 days (2/17/48)

No. 1	28%
No. 2	0%
No. 3	16%
No. 4	4%

Procedure used Above: Perit. exudate obtained by puncture from two $D\ 10^{-3}$ mice of test of Feb. 3.
Mixtures - 0.2cc serum + 0.2cc PE 1:5

Conclusions - 1) [redacted] serum by itself has no effect on toxoplasma of 2/14/48 in either 80% or 50% concentration at room temp, but 10-15% may lose staining capacity at 37°C

2/17 Comment on 3 day Examination - With few exceptions the chromatin and cytoplasm remained unstained. The undiluted peritoneal exudate stored at the same time - had a coagulated sediment but of the toxoplasma seen free 80% (20/25) were still deeply stained

(over)

It is apparent, again, that toxoplasma stored in fresh serums lose their staining capacity much faster than when stored by itself in the exudate or diluted with saline

FEB 16 1948

Repetition of Toxoplasma In Vitro Tests of Feb 13, 1948 With Normal GP & Human Clinical Sera.

Toxoplasma: PE pooled from 3 mice of I Passage of 12 Feb 48. Moderately rich in toxoplasma when dil 1:10 with NaCl & SS serum. Well-staining ⊂ meth. blue. 1:5 dilution in saline or in fresh SS (activator) serum used in test

Sera: Same as used on 13 Feb except that Robt. Olivier replaces Mrs. Louise Smith (gms.), #19815

Test: "H" = heated 56°C for 30'. "F" = freshly thawed. SS = Schumacher fresh serum of 4 Feb.

0.1 cc serum + 0.1 cc PE 1/5. Room temp for 2 hrs then 0.02 ml ser + 0.02 ml PE ⊂ 2 loops (5 mm.) of meth. blue pH 11.0 in a wet film.

CONTROL Observations: Per. Exud. 1:5 in fresh SS serum – 50/50 deeply stained; toxo easily found

[redacted] serum (heated) + Per. Ex 1:5 in SS – 10 minutes – 75/75 deeply stained
(kept at room temperature) – 3 hours – 97/100 " "
– 8½ " – 23/25 = 92% " "

CONTROL 3 97 3% –

TUBE #	NAME	PE 1/5 IN	EFFECT # UNSTAIN	EFFECT # STAINED	EFFECT % UNST.	CYTOTOXIC EFFECT	REMARKS
1	[redacted]	NaCl	0	25	0	–	Ruch
2		SS	20	5	80	+	
3	GP "A" (F)	NaCl	20	5	80	+	
4	GP "A" (H)	NaCl	1	24	4	–	
5	GP "A" (H)	SS	11	39	22	–	
6	GP "B" (F)	NaCl	17	8	68	+	
7	GP "B" (H)	NaCl	1	24	4	–	
8	GP "B" (H)	SS	6	19	24	–	
9	GP "C" (F)	NaCl	22	3	88	+	
10	GP "C" (H)	NaCl	0	25	0	–	2:40 PM
11	GP "C" (H)	SS	4	21	16	–	
12	[redacted]	NaCl	6	19	24	–	RUCHMAN
13		SS	20	5	80	+	+ 2'55

* = # UNSTAINED, % UNSTAINED.

FEB 16 1948

TUBE #	NAME	PE'S IN	RESULT # UNST.	# STAINED	% UNST.	CYTOTOXIC EFFECT	REMARKS Ruch
14	[redacted]	NaCl	2	23	8	−	
15		SS	17	33	34	−?	3:10 −
16	[redacted]	NaCl	5	20	20	−	3:20
17		SS	38	12	76	+	+
18	[redacted]	NaCl	18	7	72	+	3:45
19		SS	20	5	80	+	+
20	[redacted]	NaCl	4	21	16	−	Equivoc once
21		SS	11	14	44	?	neg. once
22	[redacted]	NaCl	21	4	84	+	
23		SS	18	7	72	+	−
24	[redacted]	NaCl	20	5	80	+	
25		SS	24	1	96	+	Equiv. Choroiditis 4:20
26	[redacted]	NaCl	21	9	70	+	
29		SS	21	4	84	+	Neg - 5 times
28	[redacted]	NaCl	1	24	4	−	4:55
29		SS	2	23	8	−	Equiv. - then negative
30	[redacted]	NaCl	25	0	100	+	
31		SS	24	1	96	+	Equivocal
32	[redacted]	NaCl	1	24	4	−	5:15
33		SS	3	22	12	−	−
34	[redacted]	NaCl	25	0	100	+	5:05
35		SS	20	5	80	+	Equivocal

8

FEB 16 1948

IN VITRO GP & CLINICAL TESTS (CONT.)

TUBE #	NAME	PE/S IN	RESULT # UNST.	# STAINED	% UNST.	CYTOTOXIC EFFECT	REMARKS Ruch
36	[redacted]	NaCl	21	4	84	+	+
37		SS	21	4	84	+	
38		NaCl	5	20	20	−	−
39		SS	4	21	16	−	
40		NaCl	47	3	94	+	+
41		SS	22	3	88	+	
42		NaCl	20	5	80	+	Equivocal
43		SS	18	7	72	+	
44		NaCl	2	23	8	−	−
45		SS	3	22	12	−	
46		NaCl	4	21	16	−	−
47		SS	3	22	12	−	
48		NaCl	0	25	0	−	−
49		SS	1	24	4	−	
50		NaCl	18	7	72	+	+
51		SS	22	3	88	+	

FEB 17 1948

Peritoneal Exudate Used — 1 mouse of 2-12-48 } @ 4cc obtained
2 mice of 2-13-48 }
Many toxoplasma stained well with 2 loops of MB pH 11 (bottle stoppered was used before)

Exudate 1:5 in fresh SS serum — clotted in 5 to 10 min. at room temp.

Exudate (0.1cc) + Heparin (0.05cc of 1:1000) + SS serum (0.35cc) prepared at 11:10
Immediate examination — 2 loops MB (11.0) — toxo stained poorly
4 " " " — 50/50 deeply stained
40 min. at room temp — 4 " " " — 100/100 " "

Mixtures made at 2 PM

TUBE #	NAME	PE % IN	EFFECT No. UNSTAINED	EFFECT No. STAINED	EFFECT % UNSTAINED	Cytotoxic Effect	Result of Rabbit Test		
1	[redacted]	NaCl	0	25	0	−			
2		SS	16	9	64	+		3:00	
			14	6	70			3:35	
			17	3	85			7:50	
3		NaCl	1	24	4	−			
4		SS	12	13	48	±		3:30	
			24	1	96	+		7:50	
5		NaCl	0	25	0	−		4:15	
6		SS	23	2	92	+		4:20	
7		NaCl	22	3	88	+	+		Discrepancy E & F
8		SS	30	20	60	+			
9		NaCl	2	23	8	−	−	4:32	
10		SS	0	25	0	−			
11		NaCl	21	4	84	+	Equiv. (2 tests)	4:45	
12		SS	20	5	80	+			
13		NaCl	1	24	4	−	−		clear very easy to count
14		SS	0	25	0	−			as above
15		NaCl	17	3	85	+	=		Diffic. to find toxo; cloudy granular serum
16		SS	10	15	40	?			

Tube #	Name		Pe/s in	Effect			Cytotoxic Effect	Result of Rabbit Test	
				No. unstained	No. stained	% unstained			
17	[redacted]		No Cl	8	3			+	Bad serum diff. to find tox
18			SS	7	10				
19	RH. #1 PRE-INOC. (12-13-46)	(F)	No Cl	10	15	40	±		6PM + 4 hrs
20	RH #1 PRE-INOC	(H)	SS	3	22	12	−		
21	RH. #1 1 wk. (12-24-46)	(F)	No Cl	50	0	100	+		
22	RH. #1 1 wk.	(H)	SS	22	3	88	+		
23	RH. #1 2 wks. (12-31-46)	(F)	No Cl	20	0	100	+		Difficult to find
24	RH #1 2 wks.	(H)	SS	4	21	16	−		" " " Pink ghosts instead of leukocytes
25	RH #1 3 wks (1-7-47)	(F)	No Cl	25	0	100	+		
26	RH. #1 3 wks	(H)	SS	5	25	17	−		" " "
27	RH #1 4 wks (1-14-47)	(F)	No Cl	28	22	56	+		
28	RH #1 4 wks.	(H)	SS	1	24	4	−		just as difficult
29	RH #2 PRE-INOC. (4-2-47)	(F)	No Cl	5	1				Can find no toxo or leukocytes
30	RH #2 PRE-INOC.	(H)	SS	2	18	10	−		" " — difficult 7:07
31	RH #2 1 WK (4-11-47)	(F)	No Cl	23	2	92	+		
32	RH #2 1 wk	(H)	SS	23	2	92	+		
33	RH. #2 2 wks (4-18-47)	(F)	No Cl	19	1	95	+		
34	RH #2 2 wks	(H)	SS	4	21	16	−		Diff. to find
35	RH. #2 3 wks. (4-25-47)	(F)	No Cl	22	3	88	+		
36	RH #2 3 wks	(H)	SS	1	24	4	−		
37	RH #2 4 wks.	(F)	No Cl	10	5	67	+		
38	RH #2 4 wks	(H)	SS	3	22	12	−		7:50
39	[redacted]		SS	0	25	0	−		7:20 Both leukocytes and toxo readily found + well stained

FEB 17 1948

TUBE #	NAME	PE's IN	RESULT NO. UNSTAINED	NO. STAINED	% UNSTAINED	CYTOTOXIC EFFECT	REMARKS
40	[redacted]	NaCl	1	24	4%	—	clean - very easy to count.
41		SS	2	23	8	—	as above.
42		NaCl	1	24	4	—	as above.
43		SS	2	23	8	—	as above.

[redacted]

neg

FEB 18 1948

Is there a Prozone in Early Monkey Convalescent Sera

Purpose - In each of two monkeys tested the serum, negative prior to infection, became positive 1 week after inoculation and then negative again 2, 3 and 4 weeks after inoculation (test of 2-17-48). Our pooled monkey convalescent serum (@ 6 weeks after inoculation) was shown to be positive in vitro. Is the negative effect exhibited by the 2, 3 and 4 week sera due to too much of the antibody (a prozone)?

Rhesus 1 - inoculated with mouse brain tissues-inf. toxoplasma (IP) - Ruchman

Rhesus 2 - " " chick embryo toxoplasma (IP) - "

Toxoplasma: PE pooled from three mice (2 = 4 dy + 1 = 5 dy s after IP inoc.). This exudate was extremely rich in toxo so that the various tubes could be examined @ great facility. Used throughout today's experiments as PE 1/5 @ heparin 1:5000

Tests: Today's tests = Serum 0.1cc + toxo 0.1cc (PE 1/5 + hep 1:5000). Two hrs @ room temp. Examined @ 4 days of methylene blue

Mixtures made 1:15 P.M. - at 23-24 °C

Tube No.	SERUM	PE 1:5 in	Effect Unstained No.	Stained No.	Unstained %	Cytotoxic Effect	
1	Rh. 2 - 1:4 fresh 3 weeks	NaCl	2	48	4		4 20
2	" " - 1:4 heated	SS	0	50	0		
3	" " - 1:16 fresh	NaCl	0	50	0		4 28
4	" " - 1:16 heated	SS	1	49	2		
5	" " - 1:64 fresh	NaCl	3	47	6		Toxo shrunken - large numbers present 4:40
6	" " - 1:64 heated	SS	0	100	0		Toxo shrunken
7	" " - 1:256 fresh	NaCl	0	50	0		4 45
8	" " - 1:256 heated	SS	0	50	0		
9	Rh 2 - 8 wks - undil. - heat.	SS	NBS 4 / HF 3	46 / 47	7		4 PM. Very large numbers of leukocytes destroyed
10	Rh 1 - " - " - "	SS	NBS 2 / HF 2	98 / 48	2		

6/18/48 Invalid "Bad" tubes

Note - Rh serum diluted in saline prior to heating at 56 °C

FEB 18 1948

Concentration of Nonspecific Substance in Normal Guinea pig Sera Exerting "Cytotoxic Effect."

Purpose – To correlate number of units of complement with
1) Nonspecific "Cytotoxic Effect"
2) Content of "activator" for specific cytotoxic effect

Dilutions of g.pig sera (thawed from frozen state) prepared in saline
Mixtures – 0.1cc of serum + 0.1cc of exudate 1:5
Prepared at 12:45 P.M – at 23-24°C

Tube No.	SERUM	PE 1:5 in	Effect UNSTAINED No.	STAINED No.	UNSTAINED %	Cytotoxic Effect	
11	G.p. pool of 5/5/47 – undil.	NaCl	3	97	3		2:45
12	" " " – 1:2	"	4	96	4		These toxoplasma are large and not shrunken
13	" " " – 1:4	"	1	49	2		2:50
14	" " " – 1:8	"	0	50	0		
15	" " " – 1:16	"	4	96	4		2:55
16	G.p. B of 2/12/48 – undil	"	0	50	0		
17	" " " – 1:2	"	5	95	5		Deeply stained but almost
18	" " " – 1:4	"	6	94	6		3:20 all look narrow + shrunken
19	" " " – 1:8	"	5	95	5		
20	" " " – 1:16	"	2	48	4		3:40 Shrunken + narrow

Since previous tests on the pooled g.pig serum yielded 44% unstained (2/10/48) and on G-pig B – 68% unstained (2-16/48) – it was desirable to determine whether or not the added heparin, used to-day, made a difference

G.pig B + Toxo 1:5 in saline (without heparin) – 2 hrs. 24°C – 94/100 stained – not shrunken – looks well
(HF) 0/50

" No. 16 – repeated + heparin – 4½ hrs 24°C – 94/100 stained – not shrunken
(HF) 49/50 – stained

2-19-48 – Tubes stored in refrigerator overnight

11:20 A.M. – Tube 11 – 24/50 (48%) unstained – mostly agglutinated – but only unagglutinated ones counted – not shrunken

Tube 16 – 22/50 (44%) unstained – " " " "

Periton. Exud 1:5 in Hep. Saline (by itself) – 22/100 = 22% unstained

FEB 18 1948

1) Concentration of ~~Base~~ Activator in S. Schumacher Serum.

2) Activating Effect of diluted guinea pig serum

3) " " of 20% bovine albumin (crystallized)

Toxoplasma Suspension – as in other tests of same day

Mixtures ~~made~~ made 1:25 P.M. – 23-24°C

Tube No.								Unst. No.	Stain No.	Unst. %	Cytotoxic Effect
								45	55	45	(2:25)
21	1:2.5	[redacted]	0.1cc	+	PE (1:5) in SS serum (0.1cc)			75	25	75	(4:05)
								43	7	86	(5:25)
22	1:4	[redacted]	+ SS. undil. (0.1cc)	+	PE 1:5 in NaCl (0.2cc)		4:55	4	96		
23	1:8	" "	" + " 1:2 "	+	" " " "			1	99		
24	1:16	" "	" + " 1:4 "	+	" " " "		4:55	1	49		
25	1:32	" "	" + " 1:8 "	+	" " " "			1	49		
26		" "	" + G.p. pool 1:2 "	+	" " " "			3	97		
27		" "	" + " " 1:4 "	+	" " " "		5:05	4	96		
28	~~Guck~~	~~" "~~	" + " " 1:8 "	+	" " " "						
29		" "	" + 20% bov. Alb. "	+	" " " "			4	96		
30		Saline	" " + " " "	+	" " " "		5:30	1 See	1 below		

21 – 1 hour – very many toxo } 4:05 – 36/50 = 72% HF
4:05 – fewer toxo } 5:25 – 37/50 = 74% HF

22, 23, 26, 27 – many toxo – many shrunken 29-HF-5:17 – 1/50 unst.

29 – many shrunken – stained somewhat more translucent
30 – many " and greenish hue; some unstained
some poorly stained

5:45 PM
PE 1:5 in saline + heparin (used in test for all tubes) kept at room temp all the time – many toxo – all beautifully stained
none shrunken !!!

(over)

2-19-48 Tubes put in refrigerator after 4½ hours at room temp

Reexamination of tube 22 — 12/50 = 24% unstained

PE 1:5 in Kep. saline/per se — 22/100 = 22% "

~~PE~~

FEB 18 1948

Tests on Patients' Sera

Patient Gim - Children's Hosp. - craniopharyngioma with cerebral calcification
Age 8

Toxoplasma suspension - as in other tests of same day.

Mixtures made at 1:20 P.M. - 23 to 24°C

1:20 !!!

Tube No	Serum.		PE 1:5 in	Effect			Cytotoxic Effect	Result of Previous Rabbit Test	*Re-examination 2-19 - 1:30PM. 24 hours			
				UNSTAINED No.	STAINED No.	UNSTAINED %			UNSTAIN NO.	Stain No.	UNSTAIN %	Cytotoxic Effect
31	[redacted]	fresh	Nacl	0	50	0	-	not done	~~35~~	15	70	+
32	" "	heated	SS	5	45	10	-		~~48~~	2	96	+
33	[redacted]	fresh	Nacl	~~51~~	49	2	-	-	22	28	44	?
34	" "	heated	SS	0	50	0	-		1 Diff. to agglutinated	24 count	4	- - Ruch
35	[redacted]	fresh	Nacl	13	87	13	-	+	34	16	68	+
36	" " "	heated	SS	4	46	8	-		49	1	98	+ Ruch

Note: Serum [redacted] fatty - at 2½ hours - the leukocytes were apparently destroyed, pink amorphous masses being seen in their place; toxoplasma also fewer than in other mixtures

*FEB 19 1948 - The mixtures were stored in the refrigerator after they had been at room temperature for 4½ hours

Reexam. of PE, 1:5 in SS serum per se - 15/50 = 30% unstained
PE 1:5 in saline " " - 22/100 = 22% "

The results obtained in tubes 31, 32, 35 & 36 would appear to be significant therefore and would suggest that with the toxoplasma used at 23-24°C more time was required for the cytotoxic effect

FEB 19 1948

Toxo Suspension – Exudate from 3 mice (5th day) – 18,000,000 toxo per cc.
UNDILUTED – used without heparin
1:5 in NaCl – 1 part heparin 1:1000
1 part exudate
3 parts saline

Effect of Normal and Immune Serum in Mixtures with Undiluted Exudate.

0.1cc serum + 0.1cc undiluted exudate – 12:40 P.M. – Room Temp -28°C

	1 3/4 hrs	4 1/2 Hours
[redacted]	42/50 (84%) unstained	~~96/100~~ = 96% unstained
[redacted] (norm.)	5/50 = 10% unst.	30/100 = 30% "

Note – The 30% unstained ones with the normal serum at 4 1/2 hours is higher than at any time before and somewhat higher than in the same exudate diluted 1:5 in ~~Schumacher serum~~ heparinized saline and mixed with Schumacher serum (see below

Percentage "Cytotoxic" Effect" at Different Times after Preparation of Mixtures

0.1cc of serum + 0.1cc exud. 1:5 in heparinized NaCl – 12:40 PM 28°C

	Percentage of unstained toxo at indicated intervals			
	20 min.	1 hour	2 hours	4 1/2 hours
[redacted]	40% 40/100	62% 31/50	86% 43/50	96% 48/50
[redacted]	4% 2/50	0% 0/50	2% 1/50	12% 6/50

(over)

FEB 19 1948

Effect of Various Normal Guinea-Pig Sera on Toxo

This was repeated to-day because the day before 2/18 the g. pig sera had no appreciable nonspecific "cytotoxic effect".

The same tubes were used as on 2/18 – in the case of the pool of 5/5/47 it had been frozen and thawed 3x

0.1cc ser. + 0.1cc exud. in heparin. NaCl – 12:40 PM. – 28°C

Serum	Per cent unstained toxo at	
	2 hours	4 1/2 hours
Guinea pig "A"		52% 26/50
" " "B"	20% 10/50	44% 22/50
" " "C"		80% 24/30
G. pig pool of 5/5/47	6% 3/50	16% 8/50

Concentration of Nonspecific Activator in [redacted] Serum and Effect of Heating on Specific Antibody in [redacted] Serum.

FEB 20 1948

Toxoplasma Suspension - Exudate from 3, four day mice (16 Feb)

1:5 dilution in serum or saline - 1cc exud. + 0.05cc heparin (1%) + 3.95cc saline or serum

Mixtures completed 2:05 P.M. - Left at room temp. - 28°C

Note: 1) more conc. heparin used
2) Sera diluted in saline
3) Heating at 56°C for ½ hr

Tube No.	Serum	PE 1:5 in	Time	Effect Unstained No.	Effect Stained No.	Unstain. %	Cytotoxic Effect	Tubes in refrigerator Reexamination on 2/21/48 6:30 PM (28 hours)
1.	[redacted] fresh - undil	NaCl	4:07	18	7	72	+	diff. to find
2	" " 1:2 sal.	"	4:35	8	17	32	±	
3	" " 1:4 "	"	4:55	0	25	0	−	
4	" " 1:8 "	"	4:50	0	32	0	−	
5	" " 1:16 "	"	5:05	0	25	0	−	
6	" " undil	SS	4:07	21	4	84	+	
7	" " 1:2 sal	"	4:35	21	4	84	+	
8	" " 1:4 "	"	4:55	18	7	72	+	
9	" " 1:8 "	"	4:50 5:25	14 26	11 4	86	+	
10	" " 1:16 "	"	5:05	14	11	56	+	
11	" " 1:32 "	"	5:20	5	20	20	±	21/25 = 84% unstained
12	" " 1:64 "	"	5:20	0	50	0	−	12/25 = 48% "
13	[redacted] heated - undil	NaCl	5:40	1	49	2	−	
14	" " - "	SS	"	18	7	72	+	
15	" " - 1:2 sal	"	5:55	18	7	72	+	
16	" " - 1:4 "	"	"	20	5	80	+	
17	" " - 1:8 "	"	6:00	15	10	60	+	
18	" " - 1:16 "	"	"	6	19	24	±	
19	" " - 1:32 "	"	6:16	0	25	0	−	
20	" " - 1:64 "	"	"	0	25	0	−	8/25 = 32% unstained

Dil. from heated undil (1:5)

Note: [redacted] Serum heated undiluted; dilutions made from heated sample.

FEB 20 1948

Tube No	SERUM	Exud. 1:5 in	TIME	Effect Unstained No.	Effect Stained No.	Effect Unstain. %	Cytotoxic Effect	
21	[redacted] heated (0.1cc) + 5S. fresh (0.1 cc) undil	NaCl 0.2cc	6:25	9	16	36	±	
22	" " " + " " " 1:2 Sal	"	"	5	20	20	±	
23	" " " + " " " 1:4 Sal	"	6:30	1	24	4	−	
24	[redacted] heated - undil. (0.1cc)	fresh G.p. pool 0.1cc	6:30	10	15	40	±	
25	" - "	"	6:40	6	19	24	±	
26	heated (0.1cc) + G.p. pool (fresh) undil 0.1cc	NaCl 0.2cc	"	7	18	28	±	
27	" " + G.p. " (heated) " 0.1cc	"	6:50	0	25	0	−	
28	heated - undil.	heat. g.p. pool	7:05	2	23	8	−	
29	" - "	"	6:58	2	23	8	−	
30	" - undil.	SS	6:58	0	25	0	−	7/25 = 28% unst. on 2/29 after 24 hrs. in refriger.

Conclusions

1. Activator in fresh [redacted] serum absent in dilution of 1:4 and only small in amount in 1:2 dilution
2. "Specific antibody" in [redacted] serum reduced in half by heating undiluted serum at 56°C for ½ hour.
3. [redacted] fresh serum in original 1:2 dilution (tube 21) only 36% effective same as [redacted] 1:2 (32% effective) — indicating very low titer of "activator" in fresh human serum
4. Concentration of activator in undiluted g. pig serum equivalent to that of human serum dil. 1:2 or perhaps even less.

TESTS ON SERA of Certain Patients

FEB 20 1948

Same exudate used as in other tests of same day.

[redacted] - in test of Feb. 18 it was neg. at 2½ hrs. and positive overnight

Tommy Miller (patient) and Mrs. Miller (mother) of Akron, Ohio

Cortical atrophy, convulsions - no cerebral calcification
no chorioretinitis

[redacted] - age 5 months.

Pate - 4 age -

serum taken 1/26/48

Sent from Kansas City by [redacted]

Mixtures Made at 3 P.M. - Room temp - 28°C.

0.1cc. serum + 0.01cc of 1% heparin + 0.09cc undil. exudate

Tube No.	Mixture	Time	Effect			Cytotoxic Effect
			Unstain No.	Stain No	Unstain %	
35	[redacted] 1:5 in SS	7:00PM	20	5	80	+
36	undiluted exudate	"	7	43	14	-?
37	+ undil. exudate	7:23	0	50	0	-
38	+ " "	"	19	31	38	?
39	+ undil. exud.	7:35	2	48	4	-
40	" + PE 1:5 in SS	"	2	23	8	-

FEB 21 1918

Further Observations on Effect of Heating Toxoplasma on ~~Their~~ Affinity for Certain Dyes.

Purpose – Previous tests showed that heating at 50°C for 15 minutes did not deprive the toxoplasma of their affinity for "methylene blue" methyl violet or thionin. While the heated toxo, ~~when~~ on storage in the refrigerator retained this capacity for at least 5 days, the cytoplasm no longer combined with these dyes after 12 days.

Questions – Will heating at higher temperatures and longer periods also destroy the "autolytic or other enzymes" which ultimately change the toxo on storage without affecting the combining power for the dyes?

Toxoplasma used – Peritoneal exudate from 3 mice inoculated 5 days earlier. Abundant toxo seen in coverslip preparation.

To avoid possibility of coagulation, which sometimes occurs, heparin was added as follows to the 2 concentrations of Toxoplasma studied:

Exudate 1:2 – 1 part exudate
1 part heparin in saline (0.1% – 1:1000)

Exudate 1:5 – 1 part exudate
1 " hep. in saline (1:1000)
3 parts saline

Heating at 56°C for 30 minutes

Exudate 1:2 (1.3cc) and exudate 1:5 (2cc) in 100×10mm. pyrex tubes with cotton stoppers, submerged about 2cms. below level of fluid in tubes kept in water bath for 30 min. – temp. varied between 55.5°C and 56.5°C.

Methylene Blue (pH 11) – 0.02cc exudate + 2 loops (5mm) of dye

Exudate 1:2

Unheated control – at least 25 well stained toxo in each high dry power field and no unstained seen ~~in~~ among 300 counted. Most leukocytes sl. purplish

56°C for 30 min – Practically all toxo fail to stain, some granular and others with slight blue hue. Perhaps 1% stained but even in these appearance is different from control. Occasional intracellular toxo seen staining deep purple. Leukocytes are unstained.

Exudate 1:5 – Toxo less numerous but effect is same. (over)

VII Effect of 56°C for 30 min – continued

Thionin 1:5 – 2 loops – controls poorly stained and chromatin unstained
Not reliable therefore.

Scarlet Red (Sudan IV) – 0.01cc. exudate + 2 loops dye.

Exudate 1:2 – control and heated examined simultaneously.

The red-staining minute vacuoles (neutral fat) appeared both larger and more numerous in the heated toxoplasma than in the unheated control. The heated leukocytes also appeared to have more material with affinity for the scarlet red.

Nile Blue Sulfate (Miss Case's bottle - old - marked aqueous - contains the oxazones by the xylol test)
0.01cc + 2 loops

Exudate 1:2 – Control – the cytoplasm stains a faint blue (fatty acids) and contains red "vacuoles" (one or two – rarely three) – neutral fat

56°C-30 min – the cytoplasm stains a somewhat deeper blue and the "red vacuoles" seem to be larger and more numerous than in the control (as in the scarlet red prep).

Effect of [redacted] – 0.01cc + 1 loop

Exudate 1:2 – Control – toxo bright red hue

56°C – toxo appear to be deeper stained than in the unheated preparation

FEB 22 1948

Neutral Red – 1% Aqueous – 0.02cc exud. + 1 loop
Quick ppt. and "jelling"

Exud 1:2 – control – under high dry most toxo seemed to have a light greenish hue; however, under oil immersion it was found that most toxo had very many deep reddish granules – some very minute – others larger – against pale yellow or orange background in the cytoplasm. A few toxo had taken up nothing. The leukocytes were full of deep red granules + large droplets.

56°C – under high dry – almost same as control but under oil – none of the toxo had any red granules or any color whatever. The leukocytes – full of "droplets" but none stained red or any color

FEB 21 1948

Repetition of Observations on Toxoplasma Heated at 50°C for 15 minutes.

Purpose – Since heating at 56°C for 30 min. deprived the toxo of its affinity for the "methylene blue complex", it was desirable to determine on the same exudate:

a) whether 50°C for 15 min. would not do it (as before)

b) whether 50°C for 15 min. under the conditions of this test would actually kill the toxoplasma.

Procedure – Since it was important for the pathogenicity test that no toxo escape the heating, the exudate was put in glass ampules which were sealed in the flame and completely submerged in the water bath. 0.2cc each of exudate 1:2 and exudate 1:5 were used. The temperature varied from 49.5 to 50.5°C ___ maintained for 15 min.

Methylene Blue (pH 11) – 0.02cc + 2 loops.

The exudate 1:2 as well as 1:5 – heated at 50°C for 15 minutes stained deeply with the "methylene blue complex" as before although a greater "smoothness or translucency or lack of granularity" was apparent in the heated than in the control.

FEB 21 1948 **Test for pathogenicity** – the unheated as well as heated 1:5 exudate were further diluted 1:5 in saline and 0.1cc was injected i.p. into each of 5 mice

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

Unheated Control	1	–	–	–	z	D																						
	2	–	–	–	z	Asc	D																					
	3	–	–	–	–	Asc	Asc	D																				
	4	–	–	–	–	Asc	Asc	D																				
	5	–	–	–	–	Asc	Asc	Asc	D																			
50°C for 15 min.	1	–	–	–	–	–	–	–	–	D	[TOXOPLASMA SEEN IN LUNG SMEAR]																	
	2	–	–	–	–	–	–	–	–	S	D																	
	3	–	–	–	–	–	–	–	–	S	D																	
	4	–	–	–	–	–	–	–	–	S	S	D	– Toxo present in lungs.															
	5	–	–	–	–	–	–	–	–	S	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

FEB 28 1948 – see over for examination of exudates stored in refrigerator

FEB 28 1948 — The unheated and heated (50°C) which had been stored in the refrigerator were reexamined with methylene blue - p14 11

Unstained

Unheated 1:2 — 99/100 = 99%

" 1:5 — 90/100 = 90%

50°C 1:2 — 99/100 = 99%

50°C 1:5 — 95/100 = 95%

The changes which ultimately deprive the toxoplasma of their affinity for the methylene blue complex, also occcer in exudate heated at 50°C for ½ 15 min. Such heated exudate is not suitable for use as an "antigen" for the in vitro test.

FEB 23 1948

Observations on Fresh Serum of [redacted]

Mother of Proved Case of Congenital Toxoplasmosis

Note – In May 1947, [redacted] (age 28) gave birth to a premature infant who died shortly thereafter and in whose brain toxoplasma were found.

Blood taken at 10:30 A.M. – Allowed to clot at room temperature (22°C). Centrifuged 1:45 P.M. Some of serum used fresh – remainder frozen.

Toxoplasma Suspension – obtained by puncture from 4-day mouse. Very many cells (loaded with toxo) and relatively few toxo seen in immediate cover-slip preparation

Mixtures set up:

0.1cc of Exudate 1:5 in heparin. saline + 0.1cc [redacted] serum } 2:05 P.M. 22°C
" " " " " + " saline }

1 hour – Saline – 0/25 = 0% unstained } relatively few toxo in field
[redacted] – 1/50 = 2% unstained }

2 hours – Saline – 0/35 = 0% unstained } still relatively few
[redacted] – 6/25 = 24% " }

Because it was thought that the 22°C temp. might be slowing up the process the tubes were put in the water bath at 37°C for 1 hour

2 hrs at 22°C + 1 hr. at 37°C { Saline – 0/100 – unstained – very many toxo in each field now – apparently many of the leukocytes must have ruptured

Rainey – 36/100 – 36% unstained – very many toxo as above; also many only partly stained & therefore difficult to count

Note: Since toxoplasma continued to come out of the cells, one could not be certain how long they were exposed to the action of the serum. Tubes put in refrigerator over night to be re-examined

FEB 24 1948 – Reexamined 9 A.M. – 2 hrs at 22°C, 1 hr. at 37°C, and 16 hrs at 4-5°C

Saline – 5/100 unstained

[redacted] – 74/100 unstained

(over)

Observations on Mixture with Undiluted Exudate

Because so few extracellular toxoplasma were present in the exudate, some of it (0.1cc) was mixed undiluted (without heparin) with 0.1cc of [redacted] serum. – room temp – 22°C

The mixture was found to be a solid clot at 1 hour, which was broken up and shaken – .

1½ hours – Rainey ++ – very large numbers of extracellular toxoplasma now present – cells must have ruptured during clotting or breaking up of clot

20/100 = 20% unstained

Exudate per se – still few extrac. toxo – 0/50 – unstained

2 hours (22°C) – [redacted] – 15/100 – unstained

Exud. – 0/50 – "

IN VITRO TOXOPLASMA TESTS WITH NORMAL RAT SERA AND HUMAN CLINICAL SERA.

FEB 24 1948

Toxoplasma: PE pooled from 3 mice (IP passage of 19 FEB.)
Exudate small in amount.

Preliminary Test with SS serum – 0.2cc exudate + 0.1cc of heparin 1:1000 + 0.7cc. SS serum (last bit in tube used for several days)

After 10 min at 37°C - water bath – 99/100 deeply stained.
Kept in refrigerator until tubes set up & same tube used

Incubation – 3:20–4:20 P.M. at 37°C in water bath – then at room temperature 25–26°C

RAT SERA – Normal – bled to-day – 0.1cc heparin 1:1000 + 0.9cc blood.

TUBE #	SERUM	PE 1/5 IN	EFFECT # UNSTAINED NO.	EFFECT # LO. STAINED	EFFECT % UNSTAINED	CYTOTOXIC EFFECT	TIME	REMARKS
1	[redacted] (H)	SS	20	5	80	+	4:28	
2	RAT "R" (F)	NaCl	16	34	32	±		
3	RAT "R" (H)	SS	0	25	0	–	4:50	
4	RAT "y" (F)	NaCl	11	14	44	±		
5	RAT "y" (H)	SS	1	24	4	–	5:10	
6	RAT "B" (F)	NaCl	11	14	44	±		
7	RAT "B" (H)	SS	1	24	4	–	5:25	
8	[redacted]	NaCl	29	21	58	+		
9		SS	40	10	80	+	5:40	
10	) AKRON (BAYER)	NaCl	48	2	96	+	~~6:00~~	
11	) "	SS	48	2	96	+	6:00	
12	(F) "	NaCl	26	24	52	±		
13	(H) "	SS	18	32	36	±	6:55	Difficult to see unstained ones because of fat
			15	15	50	→	6:20	
PE 1:5 in SS serum			23	77	23			
Repeat			9	41	18	—	7:12	– not as many as in saline
PE 1:5 in saline	"		1	99	1		6:55	– Very easy to count numerous in each field

Check on Peculiar Results Obtained on FEB 25 1948

FEB 26 1948

1. If there was something peculiar about the exudate of 2/25, and not ~~it~~ about the rabbit serum that was used, similar results should be obtained with the exudate of 2/25 diluted in saline, and perhaps not with exudate from other mice obtained to-day.

2. If on the other hand, Schumacher and Peter Schloss have "antibodies" for toxo, the same result should be obtained with another exudate (diluted in saline).

TOXOPLASMA USED – EXUDATE "A" – FEB 25 1948 stored undiluted in refrigerator

Exudate "B" – obtained to-day from two 5-day mice

Mixtures – 0.2cc of frozen sera + 0.1cc exudate 1:5 in saline
In water bath at 37°C for 1 hour at 10 A.M.

	Unstain No.	Stain No.	Unstained %
Saline + exudate of 2/25 1:5 in saline	8	17	32
[redacted] + " " " "	36	14	72
[redacted] + " " " "	28	22	56
Saline + exudate of 2/26 1:5 in saline	3	47	6
[redacted] + " " " "	5	45	10
[redacted] + " " " "	3	47	6

Conclusions – 1) [redacted] and [redacted] sera do not have "antibodies" according to test with exudate of 2/26

2) The exudate of 2/25 "deteriorated" by storage in the refrigerator overnight, and the greater number of unstained toxoplasma in the mixtures with the sera may indicate that at a certain stage toxoplasma are more readily affected by any fresh serum.

3) It is still necessary to determine whether the heated rabbit serum used on 2/25 can be activated by the [redacted] and [redacted] sera, using to-day's good exudate.

(see over)

FEB 26 1948

Tests on Rabbit Serum Used on Feb. 25, 1948

The 50% rabbit serum saline was prepared on 2/25 from the same batch of heated rabbit serum which was used undiluted in the tests; both were stored in the refrigerator over night.

Toxoplasma - same as that used in earlier test of same day. No heparin used.

Mixtures (~~equal~~ 0.1cc of serum + indicated exudates) - 37°C water bath one hour from 12:15 PM to 1:15 PM and then in refrigerator

	~~Ser~~ Serum	Exudate 1:5 in	Unstained No.	Stained No.	Unstained %	Time	Remarks
11	[redacted]	NaCl	4	46	8	2 PM	Many toxo
9	"	Undil. Rabb. heated	20	30	40	1:45	
10	"	50% Rabb. heated	27	23	54	"	
14	[redacted]	NaCl	2	48	4	2:16	Clotted Toxo diff. to find
12	"	Undil. Rabb Heated	8	32	20	2:00	"
13	"	50% Rabb. heated	3	22	12	2:16	"
7	Heated Rabbit - undil.	Und. Rabb. heated	1	49	2	1:25	Many toxo
8	" " - 50% in sal.	50% Rabb. heated	3	47	6	"	

Conclusions:

1) The heated rabbit serum per se was without effect

2) The fresh human sera per se were without effect on toxoplasma in saline

3) [redacted] serum + heated rabbit serum - definite "activating" effect of the order of magnitude seen with ~~as~~ heated sera from immune rabbits (see test of Feb. 11)

4) Activating effect of [redacted] serum - suggestive but not definite - has clotting anything to do with it?

5) Tentatively the rabbit serum used in the tests of 2-25-48 must be regarded as probably "immune"

Program for → FEB 26 1948

1. Determine whether or not [redacted] serum of 2-25-48 exerts "cytotoxic effect" against exudate of 2-25 diluted in saline (instead of heated rabbit serum) and new exudate of 2-26. Same for serum of Peter Schloss

   Nothing can be done, until this is settled

2. If either [redacted] or [redacted] serum is <u>without</u> effect against toxo in saline, then bleed monkeys and set up their serum fresh against toxo in saline and heated against toxo in activator

3. Obtain guinea pig exudate by paracentesis and count toxoplasma

27 Feb 48

Toxoplasma In Vitro Tests With Normal and Stored Immune Monkey Sera.

Toxoplasma: PE pooled from 3 mice (IP inoc 6 dy on 21 Feb 48). Many tox.

Sera: Monkeys #157 & 158 recently aquired & unused. Bled this a.m. & serum separated & used in this test. Rh 409 = 1 cc lyo & stored in refrigerator. Originally obtained 14 weeks after infection of aquired. Reconstituted = dist H_2O just prior to test. Rh Pool - obtained in 1935 & Seitz-filtered in 1939. Stored in refrig. since. Has a great deal of ppt, much of which is caked. This was cleared by centrifugation just prior to test.

Test: 0.1 ml serum + 0.1 ml PE 1/5 in phys SS or NaCl. Incubated one hour at 37° (H_2O bath) & then placed in refrigerator before readings. Wet films = 0.02 ml test mixture + 2 loops methyl bl ph 11.0. Test out of H_2O bath @ 3:10 pm.

Tube #	SERUM	PE 1/5 IN	TIME	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	REMARKS
			30' @ 37°	34	16	68	NB This is a 6 dy PE & is no good. Test to be repeated, as well as to determine differences between 4 & 5 day & older exudates.
1	NONE - CONTROL	SS	1 HR @ 37°	41	9	82	
2	NONE - CONTROL	NaCl	1 HR @ 37°	10	62	14	
3	RH 157 (F)	NaCl					not examined
4	RH 157 (H)	SS					" "
5	RH 158 (F)	NaCl	3:40	23	2	92	
6	RH 158 (H)	SS					not examined
7	RH 409 (F)	NaCl					not examined
8	RH 409 (H)	SS					not examined.
9	RH Pool (F)	NaCl	4:00	26	0	100	
10	RH Pool (H)	NaCl	4:00	25	0	100	

(over)

Tube #	SERUM	PE% IN	
11	RH Pool (H)	SS	not examd
12	[redacted] (H)	SS	not examd.

NB since this is another instance of an exudate harvested beyond the 5th/6th day after IP inoc. being no good for this test, the question of whether such older exudates are useless because of *in vivo* sensitization by mouse antibody & subsequent activation by the addition of normal, fresh serum is to be investigated.

FEB 28 1948

Toxoplasma In Vitro Tests With Normal Monkey And Rabbit Sera & Immune Monkey Sera.

Toxoplasma: PE pooled from 3 mice (inoc 24 Feb IP with 10% MB), rich in parasites

SS control: PE made 1/5 in fresh SS serum, inc. 10' in 37°C H_2O bath. Counted in wet-film, 50/50 stained c̄ meth blue. ok (clotted so heparin used in test)

Sera: Rh 157 & 158, normals bled ~~27~~ 27 Feb, serum frozen overnight. Rabbit sera obtained 27 Feb (ear bleeding), serum rapidly removed & frozen overnight. These are from normal rabbits. Rh #409 - reconstituted from lyo. ampoule on 27 Feb. Originally obtained 14 weeks after ~~two~~ inoc. Frozen overnight. RH pool = An immune pool obtained in 1935 (heat-stable neutralizer), Seitz-filtered in 1939, refrigerated since. Heavy ppt removed by centrifugation 27 Feb & frozen overnight. PPt = needle-like crystals.

TIME IN WATER BATH AT 37°C (OVER)

TUBE #	SERUM	PE 1/5 IN	TIME	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	CYTOTOXIC EFFECT	REMARKS.
1	None- CONTROL	SS	12^{40}	0	25	0	−	
			4:15	1	49	2	—	Just a re-check to see how it held
2	None- CONTROL	NaCl	12^{40}	1	24	4	−	
			4:15	1	49	2	—	
3	[redacted] (H)	SS	1^{00}	23	2	92	+	diff to ct - much ~~[illegible]~~ "much" IC: ok
4	RH. 157 (F)	NaCl	1^{00}	24	1	96	+	ok
5	RH. 157 (H)	SS	1^{20}	23	2	92	+	some much - IC ok
6	RH. 158 (F)	NaCl	1^{20}	23	2	92	+	ok IC - ok
7	RH. 158 (H)	SS	1^{40}	22	3	88	+	ok - IC ok some much
8	RH 409 (F)	NaCl	1^{40}	7	18	28	−	ok
9	RH. 409 (H)	SS	2^{09}	26	24	52	±	ok - some much
10	RH. Pool (F)	NaCl	2^{09}	25	0	100	+	ok - many crystals
11	RH. Pool (H)	NaCl	2^{35}	25	0	100	+	ok - many crystals - leucos also unstained
12	RH. Pool (H)	SS	2^{35}	19	6	76	+	v diff - cells have some stain - protective effect of SS?
13	RAB. # 362 (F)	NaCl	3^{10}	23	2	92	+	ok
14	Rab # 362 (H)	SS	3^{10}	9	16	36	?±	ok

TUBE #	SERUM	PE 1/5 IN	TIME	RESULTS # UNSTAINED	RESULTS # STAINED	RESULTS % UNSTAINED	CYTOTOXIC EFFECT	REMARKS
15	RAB. #363 (F)	NaCl	5⁰⁰	25	0	100	+	ok
16	RAB. #363 (H)	SS	5⁰⁰	21	4	84	+	ok
17	RAB. #364 (F)	NaCl	5:23	24	1	96	+	
18	RAB #364 (H)	SS		7	18	28	−	
19	RAB #365 (F)	NaCl	5:40	25	0	100	+	
20	RAB #365 (H)	SS		3	47	6	−	

The mixtures were kept in the refrigerator ~~except~~ after the 1 hour at 37°C.

TEST CONDITIONS: "F" = fresh serum. "H" = heated 56° for 30'. 0.1 cc serum + 0.1 cc PE 1/5 in NaCl or fresh SS serum, both containing heparin 1/10000, incubated in 37° C H_2O bath for one hour, then into refrigerator until each wet film was made & examined. Wet films = 0.02 ml of test mixt + 2 loops (5 mm) of meth blue, pH 11.0.

MAR 1 1948

Comparison Of The Effect Of Normal Fresh Human Serum Upon 4 and 6 Day Mouse Retained Exudates.

Toxoplasma: 4 day: exudates I & II harvested this a.m. from 2 mice inoc. IP with PE on ~~25 Jan~~. 26 Feb. '48

6 day: PE harvested this a.m. from a mouse inoc. on 24 Feb. IP with 10% MB.

Preparations of each of these undil + meth blue = many toxo.

Serum: [redacted] fresh.

Test: Each of the three exudates made to 1:5 as follows:

PE = 0.05 cc
Hep 1/1000 = 0.05 cc
Saline = 0.15 cc

0.1 cc SS + 0.1 cc PE 1/5 incubated 1 hour in 37° C H_2O bath & then staining in wet film with meth blue. pH 11.0.
Tests refrigerated until read but this was completed within an hour after incubation.

Tube #	TEST	RESULT # UNSTAINED	# STAINED	% UNSTAIN	AFFECTED by SS	
—	4 dy PE # I 1/5 in NaCl	4	96	4		Innumerable parasites
1	0.1 cc SS + 0.1 cc 4 dy PE #I 1/5 in NaCl.	25 13	75 (F) 87 (S)	19	±	" "
—	4 dy PE #II 1/5 in NaCl	7	93	7		" "
2	0.1 cc SS + 0.1 cc 4 dy PE #II 1/5 in NaCl	24 23	76 77	23	±	" "
—	6 dy PE 1/5 in NaCl	1	49	2		Numerous toxo but about 1/3 of the 2, 4 dy exudates. This exudate spun & supernatant removed & frozen
3	0.1 cc SS + 0.1 cc 6 dy PE 1/5 in NaCl.	47	53	47	+	

PEA (fresh) + PE in Sal.
" " + PE in SS.
PEA (heated) + PE in Sal
PEA (") + PE in SS.

MAR 2 1948

In Vitro Tests With Normal Monkey, Immune GP, And Clinical Human & Pigeon Sera As Well As Of Peritoneal Fluid.

Toxoplasma: PE pooled from 3 mice (IP inoc. 27 Feb) - very rich in toxoplasma.

SS control: 1/5 in SS & 10' @ 37° = jellying, 6/200 unstained = 3% unst. ok use hep.

Sera: Rh 142 & 143 obtained from normal, recently arrived monkeys, bled 1 Mar '48 & sera rapidly removed & frozen overnight.

Immune GP # 974 obtained from [redacted] frozen to date.

P-17 serum & V. Gim = retests [redacted] pos. skin test. bled next day & serum frozen to date.

"PE": Obtained 1 Mar 48 as supernate from PE of harvested 6 days after IP inoc. & which had about 50% of its toxo unstained when SS was added. Super. frozen overnight.

Test: 0.1 cc serum + 0.1 cc PE 1/5 in NaCl or SS (c̄ hep 1/5000), incubated one hour in 37° C H_2O bath, then refrigerated until read. c̄ meth bl p H 11.0 in wet film.

"F" = fresh "H" = heated for 30' @ 56° C. Out of 37° C H_2O bath at 12^{30} pm.

Tube #	SERUM	PE 1/5 IN	TIME	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	CYTOTOXIC EFFECT	REMARKS
1	NONE - CONTROL	SS	12^{30}	9	91	9	—	Numerous toxo.
2	NONE - CONTROL	NaCl	12^{30}	6	94	6	—	Numerous toxo.
3	[redacted] (H)	SS	12^{30}	96	4	96	+	Numerous toxo, Intra cell. ok.
4	RH. 142 (F)	NaCl	12^{55}	95	5	95	+	Numerous toxo
5	RH. 142 (H)	SS	12^{55}	42	8	84	+	Numerous toxo. Intracell ok.
6	RH. 143 (F)	NaCl	12^{55}	47	3	94	+	" "
7	RH. 143 (H)	SS	1255 1:45	48 29 14	42 71 36	48 (F) 29 }5 28	+ ? -	Numerous toxo. Intracell ok.
8	PE-6dy (F)	NaCl	1^{20}	2	48	4	—	Numerous.
9	PE-6dy (F) + PE in NaCl +	SS	1^{20}	5	45	10	— ?	By error, this PE dil 1:1 c̄ PE 1/5 in NaCl, then 0.1cc of this mixed c̄ 0.1cc PE 1/5 in SS.
10	PE-6dy (H) 0.7 + 0.7 PE 1/5 (Heated in 1 tube, then divided)	NaCl	1^{20}	1	49	2	—	Numerous toxo. Intra. ok.
11	PE-6dy (H) 0.7cc + 0.7cc PE 1/5	SS	1^{20}	5	45	10	—	Numerous toxo. Intra. ok.

Tube #	Serum			PE's in	Time	Result. Unstained No	Stained No	Unst. %	Cytotoxic Effect	
12	P-17	(F)		NaCl	1:58	4	96	4	—	Very many toxoplasma
13	P-17	(H)		SS		12	88	12	—	"
14	GIM	(F)		NaCl	2:27	5	95	5	—	"
15	GIM	(H)		SS		5	95	5	—	"
16	GP #974	(F)	Imm.	NaCl	2:44	2	98	2	—	"
17	GP #974	(H)	Imm.	SS		5	95	5	—	"
18	[redacted]	(F)		NaCl	3:07	94	6	94	+	
19	[redacted]	(H)		SS		90	10	90	+	

In water bath at 37° C at 3:45 PM

Tube 13 (P17-H SS Toxo) 0.1 cc + R. Black (H) + 0.1 cc — 4:55 — 29 — 71 — 29

Tube 17 (974-H + SS Toxo) + R. Black (H) — " — 28 — 72 — 28

20	Rh 151 (plasma)	F	NaCl	5:16	47	~~53~~	94	Intracellular deeply stained
21	" 151	" (H)	SS	"	44	~~56~~	88	
22	Rh 152	" F	NaCl	5:33	44	6	88	
23	" 152	" (H)	SS	"	3	47	6	
24	GP 974-F	1:4 saline	SS	5:45	5	45	10	
25	"	"-F 1:8 "	SS		5	45	10	
	—		NaCl	5:45	3	47	6	
	—		SS		6	44	12	

A. Development of "~~&~~" "Cytotoxic" Antibody in Rats Following Infection with Toxoplasma

MAR 3 1948

B. "Antibody" in "blood" of guinea pig dead 11 days after infection

C. Is there a "blocking" substance or antibody in immune guinea pig and pigeon sera which are negative in in vitro tests

RAT PLASMA USED – 0.1cc of heparin 1:1000 in saline + 0.9cc heart's blood – centrifuged and plasma used.
Pre-inoculation specimens (first tested on 2-24-48) and 5day specimens (3-1-48) kept frozen in CO_2 box
7-day specimen obtained to-day & used fresh.

Dead guinea pig – inoculated Feb. 21 – found dead this A.M. quite decomposed and stinking. Blood clotted – difficult to obtain – mixed with some thoracic fluid – very deep red – hemolyzed – bacteria present.
(Peri. exud. could not be used – because it was almost a solid bacterial culture)

"Blocking" substance? – G. pig 974 (obtained from Warren) – described as hyperimmune with high complement fixing titer. Test of 3-2-48 – negative in vitro.
Pigeon 17 – naturally infected – toxoplasma recovered from viscera – and serum neutralized toxo in rabbit.
Test of 3-2-48 in vitro negative

Plan – Incubate above sera + toxoplasma in exud. in saline if neg. result confirmed
Add equal volume of fresh (frozen) R. Black ~~&~~ immune serum (to supply both known antibody and adequate amount of activator) to determine if toxo as is still susceptible to "cytotoxic" phenomenon.

Toxoplasma Used – Exudate of 3 four-day mice – too few toxoplasma
" " 3 five-day " – very rich in toxo.
(IP Pan of 27 Feb 48)

PE 1/5 in SS = PE 0.3cc, Hep 1/1000 = 0.03cc, SS = 1.2cc } for test
PE 1/5 in NaCl = PE 0.25cc, Hep 1/1000 = 0.25cc, NaCl = 0.95cc } for test

Result of Preliminary Test with S. Schumacher (S.S.) serum – PE 1/5 in fresh SS. 10' @ 37° = 2/98 unstained OK

(see over)

Mixtures in water bath at 37°C for 1 hour between 12[15] + 1[15] PM. – Otherwise in refrigerator

Tube No.	Serum	Exud. 1:5 in	Time	Result: Unstained No.	Result: Stained No.	Result: Unstained %	Cytotoxic Effect	Comments
1	None - Control	SS	1:20	7	93	7		
2	" "	NaCl	"	10	90	10		
3	[redacted] Positive Control	SS	1:20	90	10	90		
4	Rat "R" (red) - Pre-inoc. - (H)	SS	"	0	100	0		
5	" - 5 day (F)	NaCl	1:40	50	0	100		Intracellular toxo stained
6	" - " (H)	SS	1:40	39 86	11 14	(HF) 78 (PB) 86		
7	" - 7 day (F)	NaCl	2:00	96	4	96		
8	" - " (H)	SS	"	77	23	77		
9	Rat "Y" (yellow) - Preinoc. (H)	SS	2:02	2	48	4		
10	" - 5 day (F)	NaCl	"	50	0	100		
11	" - " (H)	SS	2:25	64	36	64		
12	" - 7 day (F)	NaCl	"	83	17	83		
13	" - " (H)	SS	2:45	55	45	55		
14	Rat "B" (blue) - Preinoc (H)	SS	"	1	99	1		
15	" - 5 day (F)	NaCl	3:35	98	2	98		
16	" - " (H)	SS	"	36	14	72		Amorphous clot - might have formed during heating
17	" - 7 day (F)	NaCl	3:45	72	28	72		
18	" - " (H)	SS	"	75	25	75		
19	G.pig - dead 11 days (F)	NaCl	4:00	mostly stained poor preparation				2 loops M.B. - insufficient to stain cells or MB; 4 loops +0.01cc
20	" " " (H)	SS	"	8	42	16		Precipitated; needed 4 loops M.B. to 0.01cc to stain
21	G.pig 974 (F)	NaCl	1:40	10	90	10		
22	Pigeon 17 (F)	"	"	6	94	6		
*	G.p. 974 (F) + Exud. NaCl + Black (F)	- - -	3:03	48	52	48		Antibody blocked partially
	Pigeon 17 (F) + " + " "	- - -	"	17	83	17		" " almost entirely

0.1cc from tube 21 + 0.1cc Black (frozen) } in water bath at 37°C for 1 hour
" " " 22 + " " } between 1:55 and 2:55

MAR 8 1948

The Reactivating Power of Peter Schloss' Serum In The In Vitro Toxo. Test.

See Eight 16 mm for repeat of this one.

Toxoplasma: PE pooled from 3 mice (IP inoc. 3 Mar 48). 5 day. Moderately rich in toxo.

SS control: PE dil 1/5 in fresh SS & incubated 10' @ 37°. Then examined c meth blue c 1/50 unstained. OK for use. — See over

Test: "F" = fresh. "H" or heated = 30 min. @ 56° C. Except for tube #7 other tubes made up of 0.1 cc serum & 0.1 cc of PE 1/5 (as noted.) Incubated for one hour in 37° C H_2O bath (out at 5 20 pm.) & then read in wet film c 3 loops of meth blue. Refrigerated until read.

SERA: SS = SCHUMACHER PS = PETER SCHLOSS OF 4 MARCH 48

TUBE #	SERUM	PE 1/5 IN	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	CYTOPLASMIC EFFECT	REMARKS Time
1	NONE - CONTROL	SS	10	40	20		5 20
2	NONE - CONTROL	NaCl	6	44	12		5 20
3	[redacted] (F)	NaCl	42 38	58 62	42 38		6:35 5:20
4	[redacted] (H)	SS	almost all		∅95		5 20
5	[redacted] (H)	PS	all unst.		∅95		5 50
6	[redacted] (H)	PS HEATED	all 1	49	2		
7	[redacted] + [redacted] 0.1 cc	NaCl 0.2 cc	43	7	86		

6:10 P.M. — When the Schloss serum was found to be so active, the 1:5 exudate in P.S. (Schloss) fresh serum was examined — it had been at room temperature for 2 hours.

10/50 or 20% unstained

Conclusion: [redacted] serum may have more activator than Schumacher. Test again but on 4-day exudate to see whether essentially neg. result can be obtained and whether it can be used.

Additional Notes Regarding PE Used:

In view of what occurred during the test, it is of especial interest to record additional experience re the exudate employed.

After 10' @ 37° - exudate counted as follows:

1/5 in SS = 1/50 unstained 1/4 in NaCl = 0/50 unstained.

However, the staining intensity of the SS preparation was not too good so I recounted the same slide, which had now stood for some 30 min. Count now was as follows:

1/5 in SS = 10/50 unstained 1/4 in NaCl = 5/50 unstained

A fresh preparation was then made (ca 35 min. after making the 1/5 in SS). This was not counted but it was suggested by [redacted] that more meth blue be used in the test & thus probably circumvent the poor staining. Consequently, 3 loops of meth bl were used for the actual tests; this did help to produce better-stained parasites for counting but the additional observations noted above help to point up the fact that this was not a very satisfactory exudate to begin with & that if [redacted] serum is more active than SS, then the "sensitivity" of the exudate was overestimated in the test.

MAR 16 1948

The Reactivating Power Of [redacted] Serum In The In Vitro Test - Repetition Of Expt. of 8 Mar 48.

Toxoplasma: PE pooled from 2 mice (IP inoc of 12 Mar. = 4 day). Very rich in top. Exudate dil 1/5 in fresh SS & PS & examined with 3 drops of meth bl after 10 minutes @ 37°C. In each instance 100/100 counted parasites were well-stained. Thus test was set up.

SERA. [redacted]

"H" = heated 56°C fr 30' "F" = freshly drawn.

TEST: Except as noted in #7, 0.1cc serum + 0.1 cc PE 1/5 incubated one hour in 37°C H_2O bath. Then read in wet film + 3 drops of meth blue. Tubes refrigerated ~~until~~ read. Out of H_2O bath @ 1 15 pm.

TUBE #	SERUM	PE 1/5 IN	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	TIME	CYTO-EFFECT	REMARKS
1	NONE - CONTROL	SS	4	96	4	1 15 pm	—	Many top
2	NONE - CONTROL	NaCl	6	94	6	1 15 pm	—	" "
3	[redacted] (F)	NaCl	2	98	2	1 15 pm.	—	" "
4	[redacted] (H)	SS	82	18	82	1 15 pm.	+	" "
5	[redacted] (H)	PS	85	15	85	2 45 pm.	+	" "
6	[redacted] (H)	PS HEATED	5	95	5	2 45 pm.	—	" "
7	0.1cc [redacted] (H) + 0.1c [redacted] (F)	NaCl 0.2cc	49	51	49	2 45 pm.	±	" "

Interpretation: [redacted] serum behaves as does [redacted] both as a "reactivator" & as a negative serum with a properly selected exudate. The titer of reactivating substance in [redacted] serum is about 1/64 that of [redacted]

MAR 17 1948

Additional Tests For The Development Of Antibody In The Rat and Monkey

Toxoplasma: PE pooled from 4 mice (IP inoc. of 13 March + 8 of incubate.) Very rich in toxoplasma, this PE was tested by making a 1:5 dil. in fresh SS & incubating 10' @ 37°C & examined in wet film ± 3 drops of meth. blue = 4/200 unstained. Numerous toxo / field. OK for use.

Test: 0.1cc serum + 0.1cc PE 1/5 inoc. for 1 hr in 37° C H_2O bath. Tubes refrigerated until read. "F" = fresh. "H" = heated 56° C for 30'. Wet films made ± 3 drops of meth. blue.

Tube #	SERUM	PE 1/5 IN	TIME	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	EFFECT	REMARKS
1	NONE - CONTROL	NaCl	2 00 pm.	6	94	6	—	NUMEROUS PARASITES IN ALL PREPARATIONS.
2	NONE - CONTROL	SS	2 00 pm	50	150	25	—	→ SEE OVER
3	[redacted] (H)	SS	2 00 pm	84	16	84	+	IN VIEW OF THE 25% UNSTAINED
4	RAT "RED" (H) 14th dy	SS	2 00 pm.	48	52	48	+-	PARASITES ± SS, TEST TO BE
5	RAT "RED" (H) 21st dy	SS	2 35	56	44	56	+-	REPEATED IN ENTIRETY.
6	RAT "BLUE" (H) 14th dy	SS	2 35	45	55	45	+-	
7	RAT "BLUE" (H) 21st dy	SS	2 35	74	26	74	+	
8	RH #152 (H) PRE-INOC.	SS	2 35	38	62	38	—	
9	RH #152 (H) 3rd dy	SS	3 00	55	45	55	+-	
10	RH #152 (H) 5th dy	SS	3 00	69	31	69	+	
11	RH #152 (H) 7th dy	SS	3 00	83	17	83	+	
12	RH #152 (H) 10th dy	SS	3 00	75	25	75	+	
13	RH #152 (H) 14th dy	SS	3 25	71	29	71	+	
14	RH #152 (F) 14th dy	NaCl	3 25	98	1	99	+	(OVER)

Additional Studies With The PE 1/5 in SS

In view of the discrepancy between the 10 minute preliminary test & the one hour final test results ∈ PE 1/5 in SS, the following data were obtained:

a) PE 1/5 in SS, same tubes used in a.m. for the 10' test, had been sitting on the bench for 5 hrs in a warm room = 28/100 unstained.

b) Tube #2 recounted at completion of test = 29/100 unstained.

c) PE 1/5 in SS made up for the test, had been stored in the refrigerator for 2 3/4 hrs. = 4/100 unstained.
after 20' @ 37°C = 15/100 unstained
after 50' @ 37°C = 23/100 unstained.

∴ It is quite evident that 10' preliminary incubation is not sufficient. 1) DO ONE HOUR INCUBATION IN ACTIVATOR TO SELECT USABLE PE. DO THIS AT 1/10 TO SIMULATE TEST.

2) TEST INDIVIDUAL PE'S BEFORE POOLING FOR DAY. (SEE EXP'T OF MAR 23 1948 FOR ADDITIONAL DATA.)

MAR 23 1948

The Effect of Normal Fresh Human Serum Upon Four Individual Mouse Peritoneal Exudates.

Toxoplasma: Peritoneal exudates were harvested from four mice & kept as individual specimens for use as described below.

Procedure: 0.02 ml units of each PE diluted 1:5 ≡ 0.08 cc of Peter Schloss' fresh thawed serum, incubated for one hour in the 37°C H_2O bath & then examined in wet films using three drops of methylene blue.

TUBE #	FROM MICE INOC. WITH	MICE INOC. ON	AGE OF PE TODAY	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	SPONTANEOUSLY SUSCP. TO IN SCHLOSS SERUM.	REMARKS
1	5dy PE UNDIL. 0.2cc/MOUSE IP	20 MAR.	3 dys	40	160	20	+-	MANY TOXO.
2		20 MAR.	3 dys	2	98	2	−	MANY TOXO.
3	0.5cc 10% MB IP	19 MAR.	4 dys	25	275	8	−	NUMEROUS TOXO.
4		19 MAR.	4 dys	75	25	75	+	MANY TOXO. (JELLED)

Nos. 2 & 3 pooled for tests conducted on 23 March 48.

MAR 23 1948

In Vitro Toxoplasma Tests: Titration of A Human Serum (Inapparent Infection), Two Dog Sera, & A PE Supernate

Toxoplasma: A pool of one three day & one 4 day exudates (Tubes 2 & 3 of individual PE tests performed to-day) – numerous toxo & quite unaffected by Schlass serum.

Sera: [redacted] frozen since 6 Jan 48 = inapparent infection.

Dog sera: normal dogs obtained from chemistry = [redacted] a collie maintained at CHRF for months. Bled on 20 Mar & hep serum quickly frozen; [redacted] O_+, recently arrived at CHRF, unused for experiments, bled 22 March & hep. plasma rapidly frozen.

PE Supernate – Tube #4 (4 dy PE, 75% unstained) of to-day's tests, cent for 20 minutes @ 2000 & super withdrawn & used shortly thereafter.

Test: 0.1cc amts of serum & PE 1/5 incubated one hour @ 37°C. Then read c̄ 3 drops meth blue. (Refrig. until read.) "F" = fresh. "H" inactivated for 30 min. @ 56°C. Test out of H_2O bath @ 4^{00} pm.

PS = [redacted] SERUM.

Tube #	SERUM	PE 1/5 IN	TIME	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	EFFECT	REMARKS.
1	NONE-CONTROL	NaCl	4^{00}	6	94	6	–	
2	NONE-CONTROL	PS	4^{00}	42	158	21	–	
			5^{55}	24	76	24	–	
3	[redacted] UNDIL.	NaCl	4^{10}	93	7	93	+	This serum contains much ppt, which lessens c̄ dilution.
4	" 1:2	NaCl	4^{10}	86	14	86	+	
5	" 1:4	NaCl	4^{10}	76	24	76	+	
6	" 1:8	NaCl	4^{10}	63	54	54	+-	
7	[redacted] UNDIL.	PS	4^{30}	91	9	91	+	
8	" 1:2	PS	4^{30}	91	9	91	+	

(OVER)

MAR 23 1948

TUBE #	SERUM		PE 1/5 IN	TIME	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	EFFECT	REMARKS.
9	[redacted]	1:4	PS	4 30	92	8	92	+	
10	"	1:8	PS	"	88	12	88	+	
11	"	1:16	PS	4 50	69	31	69	+	
12	"	1:32	PS	"	44	56	44	+-	
13	"	1:64	PS	"	35	65	35	-	
14	[redacted]	UNDIL.	NaCl	"	8	92	8	-	
15	[redacted]	UNDIL.	PS	5 00	44	6	88	+	
16	"	1:2	"	"	44	6	88	+	
17	"	1:4	"	5 10	45	5	90	+	
18	"	1:8	"	"	46	4	92	+	
19	"	1:16	"	5 20	38	12	76	+	
20	"	1:32	"	"	56	44	56	+-	
21	"	1:64	"	5 29	42	58	42	+-	
22	PE SUPER. (F)		NaCl	"	10	90	10	-	SUPERNATE OF A PER. EX. VERY ACTIVE IN NORMAL FRESH HUMAN SERUM HAS NO DEMONSTRABLE ANTIBODY
23	PE SUPER (F)		PS	5 39	11	89	11	-	
24	PE SUPER (H)		NaCl	"	8	92	8	-	
25	PE SUPER (H)		PS	"	17	83	17	-	
26	DOG "NELLIE" (F)		NaCl	5 40	50	0	100	+	PPT IN SERUM. FRESH DOG SERUM ALSO NON-SPECIFICALLY ACTIVE. NOT SUITABLE FOR ACTIVATOR.
27	" "	(H)	PS	"	7	93	7	-	
28	DOG BR. & BL. O$_4$ (F)		NaCl	5 50	32	18	64	+	NEITHER DOG HAS ANTIBODY
29	" "	(H)	PS	5 55	8	42	16	-	

PE 1/5 USED ABOVE MADE AS FOLLOWS:

IN NaCl = PE 0.3cc
Hep 1/1000 0.3cc
NaCl 0.9cc

IN PS = PE 0.5cc
Hep. 1/200 0.1cc
PS = 1.9 cc

MAR 29 1948

In Vitro Tests With Serial Rat & RH #152 Sera

PE: Pooled from 2 three day mice. Tested against Peter Schloss serum (PE 0.02 cc + PS 0.08 cc) ~~after~~ for one hour @ 37°, one of which yielded 2/100 unstained & the other 4/100. Organisms were numerous. A third fluid tested at the same time yielded 21/100 unstained & was discarded.

Test: 0.1 cc serum + 0.1 cc PE 1/5 in PS (with hep 1/5000, added as 0.04 cc 1/100 hep) incubated one hour in 37° C H_2O bath & then examined in sequence ~ 3 ~~loops~~ of meth. blue. Organisms were quite numerous & thus readily counted. Test went into H_2O bath @ 12 30 p.m. whereas the preliminary test was completed & read at 10 30 a.m. The two PE's pooled for the test were not pooled until just prior to addition to the PS & test tubes. Tubes in rpig. until read. Last tube completed @ 3 45 p.m.

Tube #	SERUM	PE 1/5 IN	RESULT # UNSTAINED	# STAINED	% UNSTAINED	EFFECT	REMARKS
1	CONTROL	NaCl	13	87	13	−	
2	CONT. 0.1 cc NaCl + 0.1 cc PE 1/5 in	PS	19	81	19	−	
3	[redacted]	PS	86	14	86	+	
4	RAT. "RED" (H) 7dy	PS	79	21	79	+	
5	" " (H) 14dy	PS	53	47	53	+−	
6	" " (H) 21dy.	PS	48	52	48	+−	
7	" " (H) 28dy	PS	84	16	84	+	
8	RAT. "BLUE" (H) 7dy	PS	65	35	65	+	
9	" (H) 14dy	PS	61	39	61	+	
10	" (H) 21dy	PS	59	41	59	+	
11	" (H) 28dy	PS	68	32	68	+	

(OVER)

TUBE #	SERUM	PC'S IN	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	EFFECT	REMARKS
12	RH #152 (H) PRE-INOC	PS	23	77	23	−	
13	" (H) 3 dy	PS	38	62	38	−?	
14	" (H) 5 dy.	PS	83	17	83	+	
15	" (H) 7 dy	PS	78	22	78	+	
16	" (H) 10 dy	PS	86	14	86	+	
17	" (H) 14 dy	PS	59	41	59	+	
18	" (H) 21 dy	PS	78	22	78	+	

MAR 30 1948

Sensitivity Studies With Three Day Exudates

Problem: During the past three experiments, exudates have been selected on the basis of their good staining abilities after 1 hrs. incubation [redacted] serum only to find that the number of unstained has increased to ca 20-25% when the actual test is run. Is this due to a change in the organisms as they stand for 1-3 hrs in the refrigerator, to the fact that larger quantities of PS are made for the test & thus there is more of the serum "activator", or is it due to some change produced by diluting the mixing of several, individual PE's.

Procedure: PE's harvested from 8 mice, three days after inoculation were labelled from A-H & placed in individual tubes. Following preliminary examination as wet films & meth blue, all except E, G, & H were discarded because of cellular exudates. The three retained had large numbers of extracellular (predominant) tetr. These were then set up as follows in the protocol (about one hour after withdrawal from mouse) incubated in 37°C H_2O bath for one hour & then examined in wet film & 3 drops of meth. blue.

(over)

TUBE #	EXUDATE	TUBE MIXTURE cc PE	cc PS	EFFECT # UNSTAINED	# STAINED	% UNSTAINED	REMARKS.
1	E	0.02	0.08	10	90	10	[Neither varying the amts of the reagents,
2	E	0.1	0.4	10	90	10	or mixing exudates has any effect upon the
3	G	0.02	0.08	14	86	14	ratio of unstained to stained tetr.]
4	G	0.1	0.4	19	81	19	
5	H	0.02	0.08	39	61	39	
6	H	0.1	0.4	37	63	37	
7	MIXT.	0.02 cc E, G, H.	0.36	39	161	19.5	

(over)

Procedure. (Cont.) The original exudate tubes used for the first series (see other side) were returned to the refrigerator immediately after use & stored thus until late in the afternoon — 6 hrs. later when they were again set up & incubated for one hr @ 37°C & again examined in the wet film ± 3 drops of meth. blue. Since the morning exam had indicated little difference between the various quantities used & since little remained of each exudate only one set was made of each.

TUBE #	EXUDATE	TUBE MIXTURE cc PE	cc PS.	EFFECT # UNSTAINED	# STAINED	% UNSTAINED	REMARKS
1	E	0.02	0.08	45	55	45	Storing the exudates for 6 hrs. in the refrigerator markedly increases the number of bac who do not take dye in the presence of PS. Serum. Will repeat ± one + 2 hr storage periods.
2	6	0.02	0.08	56	44	56	
3	H	0.02	0.08	66	34	66	
4	MIXT.	0.02 cc E, 6, H.	0.24	50	50	50	

15 April 1948

Additional In Vitro Toxoplasma Tests With Normal Human Sera.

Toxoplasma: PE's were harvested from several mice, four days after inoculation (on 11 April c̄ 10% MB) for exudate. One of these exudates contained a moderate number of extracellular parasites & so was selected for further study.

Tests: The selected PE was made to 1/5 in NaCl & various sera, as listed below, incubated for one hour @ 37° & then examined c̄ the "old" & "new" (3 April) Meth. Blue.

Tube	# unstained / # stained	% unstained	
PE 1/5 in NaCl	0/50	0 %	using 3 ~~drops~~ loops of "old" meth. bl.
	0/50	0%	using one loop of "new" meth. bl.
PE 1/5 in Schloss	20/50	40 %	3 loops of "old" meth. bl.
"	18/50	36%	1 loop "new" meth. blue.
PE 1/5 in Buescher	11/50	22%	3 loops "old" meth. blue.
"	9/50	18%	1 loop "new" " "
"	10/50	20%	2 loops " " " "
"	5/50	10%	3 loops "new" " "

In view of the above it was decided: a) To compare Schumacher serum c̄ Schloss & Buescher; b) to use the "new" meth bl. in subsequent tests.

Consequently another series of tubes were set up several hours later, using the same PE which had now been stored in the refrigerator.

~~in the refrigerator~~ for 3 1/2 hrs. All tubes incubated for one hour @ 37°C & then examined in wet-film ~ one drop of "new" methylene blue.

Tube		% contained
PE 1/5 in NaCl	3/50	6%
~~Sch~~ PE 1/5 in Schumacher	23/50	46%
PE 1/5 in Buescher	20/50	40%
PE 1/5 in Schloss	21/50	42%

APR 16 1948

In Vitro Toxoplasma Tests With "Normal" Human Sera.

TOXOPLASMA: PE pooled from 2 mice (IP Pass of 4-12-48) = 4 day exudate. Contains numerous parasites.

Tests: Immediately after obtaining PE, it was diluted 1:5 in both Peter Schlorr's & E. Bueschel's serum, incubated for one hour in the 37° C H_2O bath & then examined in wet films & one drop of meth. blue pH 11.0 (made 3 April) Differential counts as follows:

[redacted] = 82/200 unstained = 41% unstained.

= 118/200 unstained = 59% unstained.

This exudate was then stored in the refrigerator for 5 hrs. at which time another series of tests were set up. The PE was diluted 1/5 in each of the following, & then incubated @ 37° C for one hour. Wet films using one drop of the new (3 April) methylene blue were then examined.

Tube	# unstained / # stained	% unstained
[redacted]	13/100	13
	40/100	40
	9/100	9
	53/100	53
	12/100	12
	58/100	58

not examined.

APR 21 1948

In Vitro Tests With Several RH & P-17 Exudates.

Peritoneal Exudates: a) 4 mice of the RH toxo IP passage of 17 April harvested. Of these 2 had good extracellular exudates & were selected for trial. Numbered 1 & 2.

b) 4 mice of P-17 toxo IP passage of 17 April harvested & of these 2 had good exudates & were selected for trial. #1 & 2.

Test: PE's made to 1/5 in NaCl or Buescher serum, as indicated below, incubated for 1 hr @ 37° C, then examined in wet-film c̄ one loop of new meth blue.

Tube #	PExudate	1/5 in	EFFECT # UNSTAINED	# STAINED	% UNSTAINED	
1	RH #1	NaCl	3	47	6	RH #1 used for human sera test of to-day.
2	RH #1	EB.	3	47	6	
3	RH #2	NaCl	2	48	4	
4	RH #2	E.B	12	38	24	
5	P-17 #1	NaCl	2	48	4	
6	P-17 #1	EB	3	47	6	
7	P-17 #2	NaCl	1	49	2	
8	P-17 #2	EB.	10	90	10	

APR 21 1948

In Vitro Toxoplasma Tests With Human Sera.

Toxoplasma: PE #1 which in a preliminary test with Buescher serum (see other sheet for to-day) had yielded 3/50 unstained parasites after one hour at ~~—~~ 37° C was used for this test. In the interim it had stood for three hours in the refrigerator.

Test: 0.1cc serum + 0.1cc PE 1/5 in NaCl a Buescher serum. 1 hr at 37° C & then 0.02 ml + ~~1~~ 1 loop of the new Meth Bl. (excellent staining). "F" = fresh & "H" = heated at 56° for 30' just prior to the test. Test out of H_2O bath at 3[35] pm. Tubes in refrigerator until read.

Tube #	Serum	PE 1/5 in	Time	Results			Cytotoxic effect	
				# unstained	# stained	% unstained		
1	— cont.	NaCl	4[40]	3	47	6		
2	— cont.	E.B.	"	64	36	64		←
3	[redacted]	E.B.	"	46	4	92		
4		NaCl	"	50	0	100		
5		E.B.	4[55]	47	3	94		
6		NaCl	"	50	0	100		
7		E.B.	"	49	1	98		
8		NaCl	"	49	1	98		
9		E.B.	"	48	2	96		

[redacted] – 19 yr. old girl with chorioretinitis – pos. skin test + pos. rabbit test
– patient's mother – " " " + " " "

APR 24 1948

In Vitro Test With Calf Serum.

Toxoplasma: PE harvested from each of 4 mice who had been inoc. IP on 20 April c̄ 10% MB. Of these, 2 were quite cellular & 2 had good numbers of extracellular parasites & so were used.

Test: Immediately after selection the two exudates (A & B) were ~~m~~ each made up 1/5 in saline, Buescher serum, & the ~~fragm~~ calf serum rec'd from Lilly on 21 April. Tubes then incubated for one hour @ 37° & examined in wet films c̄ one loop of meth. blue.

Exudate "A"

1/5 in NaCl = ~~[illegible]~~ 1/50 unstained.

1/5 in Buescher = 1/50 unstained

1/5 in calf serum = see discussion below.

Exudate "B"

1/5 in NaCl = 0/50 unstained

1/5 in [redacted] /50 unstained

1/5 in [redacted] = see discussion below.

Calf serum preparations:

Three different preparations were made from each of the calf serum tubes since it was thought that there might be some error. In each instance, however, the slides appeared just as in the others. Parasites were found with such rarity that they were not counted. In contrast c̄ this, the parasites were numerous in both [redacted] tubes. What few parasites were found in the calf serum were unstained entirely or, as

in the case of a few, had a single, small, round, red chromatin dot in the middle a toward one end of the organism. In the main, the parasites appeared abnormal; i.e., their margins appeared to be rough, as tho crenated. In addition, the leucocytes did not stain well & only a comparatively few stained intra-cellular toxoplasma were found. There seemed to be no clumping of toxoplasma at any point. One of the three preparations from each tube was made from 2 loops of melth blue (one loop was perfectly fine in the NaCl [redacted] tubes.) but this did not alter the picture significantly.

N.B.: a) Repeat E & without heating the calf serum. Examine immediately, @ 35° & at once.

b) When repeating, do a parasite count at start & at end of incubation period. This should help determine whether there has been lysis

APR 27 1948

In Vitro Toxoplasma Tests Including Kubin's Samples.

Toxoplasma: PE obtained from each of 4, 4 dy mice (inoc 23 April). However, three were fairly cellular while one had many extracellular toxo & therefore was selected for the tests.

Procedure: The PE was diluted to 1/5 in the following: NaCl, Buescher serum, and Schloss serum. Immediately following this as series of 8 tubes was set up as indicated below & incubated for 1° @ 37° C. This was done within 15' of obtaining the original PE. The 1/5 tubes & undil PE were placed in the refrigerator where, following storage for 2 hrs, the second set of 8 tubes (Series Two) were set up, inc. as before & again examined c̄ 1 loop of MB. (See over for Second Series)

15' correct. HHF

First Series:

Tube #	Serum	PE 1/5 in	# unstained / # counted / # ~~unstained~~ / # ~~stained~~	% unst.	
1	None - control	NaCl	3/100	3	−
2	[redacted]	NaCl	10/100	10	−
3	Schloss	NaCl	8/100	8	−
4	[redacted]	NaCl	90/100	90	+
5	[redacted]	NaCl	93/100	93	+
6	[redacted]	NaCl	5/100	5	−
7	None	[redacted]	11/100	11	−
8	None	[redacted]	11/100	11	−

N.B. [redacted] 1 & #2 = two tubes of serum were rec'd from this patient with no indication that they were from either the same or different bleedings. Therefore they were numbered 1 & 2.

Second Series:

Tube #	SERUM	PE 1/5 in	# unstained / # counted	% unstained	
1	stored in refrig. for 2 hrs. used in Series #1	Buescher	19/100	19	—
2	" " " "	Schloss	18/100	18	—
3	Baby [redacted] +	Tube #1 Buescher	18/100	18	—
4	Baby [redacted] +	Tube #2 Schloss.	30/100	30	—
5	Fresh from PE stored in refrig. for 2 hrs.	Buescher	25/100	25	—
6	" " " "	Schloss.	38/100	38	—
7	stored in refrig for 2° - used in Series #1	NaCl	8/100	8	—
8	Fresh from PE stored in refrig for 2 hrs.	NaCl.	?/100	7	—

Meaning?

Please rewrite to make meaning clear

Notes for above: (Second Series)

Tube #1 = a portion of Tube #7 of the first series incubated for 1 hr after 2 hrs. in the refrigerator to note effect of such storage.

Tube #2 = the same except that this is equivalent to tube 8 of the first series.

Tubes #3 & 4 = are made from aliquots of 1 & 2 but Baby Price serum was added.

Tube #5 & 6 = Repeats of tubes 7 & 8 of the first series but with PE stored for 2 hrs. in the refrigerator before diluting the toxo in the serum & incubating.

Tube #7 = an aliquot of tube #1 of the first series that had stood for 2 hrs in the refrig. & was then incubated @ 37°C.

Tube #8 = PE stored undiluted in the refrigerator for 2 hrs, then diluted 1/5 in NaCl & incubated for 1 hr.

What this demonstrates, essentially, is that parasites kept in the refrig. for 2 hrs do not show much deterioration when incubated in saline whereas when added to normal serum, many such parasites will not take dye. ∴ Use fresh PE.

APR. 28 1948

Search for Optimum Conditions to Carry Out In Vitro Test with Toxoplasma in Peritoneal Exudate

Note – H.F.'s inability to get reproducible results with the same "normal human activator serum" using the same peritoneal exudate on the same day – paralyzed the progress of this work during the past 6 weeks.

Examination of the data suggests the desirability of testing whether toxoplasma in peritoneal exudate react differently at different times after withdrawal of the exudate and storage in the refrigerator

Proposed Procedure: Per. exudate 1:5 in Buescher serum

1. Preliminary test with exudate within few minutes

Examine after 20 minutes at 37°C –

Same tube " 60 " at 37°C –

" " " additional hours in refrigerator –

2. Use of exudate within 30–60 minutes

Examine after 60 min. at 37°C –

Same tube " additional hours in refrigerator –

3. Use of exudate within 2 hours

Examine after 60 min. at 37°C –

Same tube after additional hours in refrigerator –

4. Use of exudate within 3 hours

Examine after 60 min. at 37°C –

Same tube after additional hours in refrigerator

Preliminary Examination of Four Exudates from 4-day mice.

Mixtures — 0.01cc of 1:200 heparin
0.05 cc of exudate
0.19-0.2cc of Buescher serum

Exudates removed 9:45 AM – 10⁰⁰ AM.

~~First mixtures prep~~ Exud. examined for numbers of Toxo: 10⁰⁰–10³⁹ AM

First mixtures prepared – 10:35 – 10:38 AM – Put in water bath

20 minute reading		1 hour	After storage in refrigerator 4²⁰
Exudate 1 –	50/50 (99/100)	99/100	81/100 stained
" 2 –	96/100	96/100	76/100 4³⁰
" 3 –	49/50	97/100	82/100 5²⁵
" 4 –	All stained ≥ 100 toxo in each field	99/100	136/200 = 68% well stained

Note: 2 loops of dye were needed for staining No. 3 & 4 for satisf.

The four exudates were pooled. At 11:30 A.M. – i.e. about 1½ hours after removal of exudate, another [redacted] marked 11:30 was prepared – as above with [redacted] serum (0.19cc), heparin 1:200 (0.01cc), and pooled exudate (0.05cc). In water bath at 11:30 A.M.

20 min. at 37°C — 86/100 well stained
60 min. at 37°C — 80/100 " "
+ 5½ hrs. in refrigerator — 54/100 " "

General Summary –

Dil 1:5 in Activ. Serum –1hr. at 37°C
Exud. at 30–45 min. — 96 to 99% well stained
" " 1½ hours — 81%
" " 3 hours — 78% " " (See other sheet)
" " 6 hours — 68% " " " " "

Toxo in exud. without activator serum after 6½ hours in refrig. (" ")
— 94% well stained

April 28, 1948

Effect of Centrifuging Toxoplasma from Peritoneal Exudate and Resuspension in Saline or Fresh Serum on Subsequent Stability as Regards "Combining Power" with Methylene Blue Complex.

Exudate Used – See previous sheet for Apr. 28.

Four exudates obtained 9:45–10:00 A.M. – all yielding 96–99% staining after one hour at 37°C – pooled at 11:30 A.M.

Centrifugation – 0.5cc undiluted exudate + 4.5cc of saline (stirred to "wash" Toxoplasma")

Two such tubes prepared and centrifuged for 10 minutes at @ 2,000 r.p.m.

Supernatant liquid removed –
one reconstituted in 0.5cc saline
other " in 0.5cc fresh Buescher serum.

Resuspension completed at 12:00 noon

Immediate examination with methylene blue:

NaCl – all very deeply stained – more than usual

E.B. serum – well stained but not as deep as in NaCl

Put in refrigerator

1:00 P.M. – 3 hours after the exudates were obtained the following mixtures were set up and incubated for 1 hour at 37°

37°C
1. Untreated exud. (0.05cc) + E.B. ser. (0.49cc) + hep. 1:200 (0.01cc) — 78/100 well stained
2. NaCl – centrif. prep. " + " " " " " " — 70/100, 60/100 } " "
3. E.B. ser. – centrif. prep. " + " " + " " " — 95/100, 95/100 } " "
4. Exud. dil. 1:5 in hep. sal. at 12 noon (0.1cc) + E.B. serum (0.1cc) — 66/100 } – well st.
5. Exud. dil 1:5 in hep. saline. — per se – control — 95/100 — " "

4:30 P.M. – Undiluted exud. in refrigerator 6½ hours.
examined ± M.B. — 94/100 well stained

(over)

4:10 PM. Same mixtures that were made at 1:00 P.M. were again made up now – to determine effect of further storage in refrigerator on stability after incubation at 37°C. for 1 hour

Untreat. exud. – 6 hrs. in refriger diluted 1:5 in E.B. serum + hep. — 68/100 well stained

Centrif. toxo in saline – dil. 1:5 in EB-ser. + hep. — 58/100 " "

" " in EB ser. – dil. 1:5 in " + " — 90/100 " "

4:45 PM. – 1cc. amounts of remaining undil. peritoneal exudate centrifuged as before after addition of 9cc of saline.

1) Resuspended in 1cc saline
2) " in 1cc E. B. serum.

} stored in refrigerator

Conclusions – Washing of toxoplasma and resuspension in fresh "normal human activator serum (Buescher) seemed to have stabilized their "combining power for M.B." when tested after incub. at 37°C for 1 hour.

N.B. – Try effect of heated normal human and rabbit serum

For elucidation of basic phenomenon – also try following:

1) (centrifuged) Exudate + heated immune ser. – 1 hr. at 37°C – centrif. – resusp sal. examine c̄ M.B – Then add activator – put at 37°C examine at intervals c̄ MB

2) (centrif) Exudate + fresh activator serum (1 hr at 37°C) – centrif + resusp in saline – examine c̄ MB then add heated immune serum – put at 37°C examine at intervals

APR 29 1948

Further observations on the centrifuged, resuspended peritoneal exudate preparations of 28 April '48.

The two sets of resuspended toxoplasma (one made yesterday morning referred to as "a. m." while the other made at 4[45] p.m. yesterday is referred to as "p. m.") removed from the refrigerator & set up in wet preparations using 0.02 ml of the condit. toxo & 1 loop of M.B. E examined on my 'scope:

"a.m."

NaCl – 80/100 stained – 20% unstained

E.B. – Innumerable toxo, ca 15-20% unstained. these parasites look "normal" & most are fully stained whereas those in NaCl are frequently smaller & stained in granular fashion.

"P.M."

NaCl – 90/100 stained. = 10% unstained

E.B. – 92/100 stained. = 8% The differences between the two preparations noted in the "a. m." set are also apparent here.

E.B. – suspension in fresh Buescher serum

Incubation Stability Test:

Immediately following the above, each suspension was diluted as follows:

Toxo – 0.05 ml.
Hyaluronidase 1/200 – 0.01 ml.
Buescher Ser – 0.2 ml.

These were placed in the 37° C H_2O bath. at 30 min., tubes removed & set up in wet films (0.02 ml mixt. plus 1 loop of M.B.) & examined ~ my scope (HP). See over for counts. Tubes immediately returned to H_2O bath to complete one hour incubation when reexamined, as above, ~ AO 5 scope.

After 30 min @ 37°

Resuspended to 1/5 in Bucher serum

Preparation	Suspended in		
"a.m."	NaCl	34/50 contained	= 68% contained.
"	E.B.	5/50 contained	= 10% contained.
"P.M."	NaCl	34/50 contained	= 68% "
	E.B.	11/50 contained	= 22% "

after one hour @ 37° C.

Preparation	Suspended in		
"a.m."	NaCl	67/100 contained	= 67% cont.
"	E.B.	31/100 "	= 31% "
"P.M."	NaCl	70/100 "	= 70% "
"	E.B.	33/100 "	= 33% "

N.B. Despite the fact that in each preparation made from the two-resuspended in Bucher serum, the parasites were well-preserved, particularly in contrast to the NaCl mixtures, it is evident that parasites that have been centrifuged & resuspended in a normal human serum & then stored for 19-20 hrs. in the refrigerator are not suitable for the in vitro toxoplasma test. It is possible that they might still be "stable" enough to be used thruout the day on which they are prepared.

APR 30 1948

In Vitro Tests With Human & Animal Sera.

Toxoplasma: PE's obtained from each of 3, four day mice (inoc. 26 April.)
Examined in wet films & #1 & 2 selected for prelim incubation.

Procedures: All tubes for dilution, incubation, etc. were prepared before exudates were obtained & stored in the refrig. until needed. This minimized the time lag between steps.
PE's obtained @ 11 50, dil. 1:5 c̄ Buescher serum & hep 1:5000 & in 37°C H_2O bath for 20' prelim incubation at 11 59.
Examined @ 20' c̄ 1 loop of Meth. Blue = PE #1 = 4/50 unstained
PE #2 = 8/100 unstained
PE #2 selected for test. Dil. 1:5 c̄ E.B. & hep 1:5000 as well as in similar fashion c̄ NaCl for use below. Distributed in 0.1cc amts to proper tubes & into H_2O bath (37°C) for 1 hr beginning at 12 38. Out at 1 32 pm & examined c̄ 1 loop of MB.

See over

Tube #	SERUM.	PE's IN	RESULT # UNSTAINED	# STAINED	% UNSTAIN		
1							
2							
3							
4							
5							
6							
7							
8							
9							

Mixtures in water bath 37°C at 12:32 P.M. — Out 1:32 P.M.
Then in refrigerator

Tube No.	Serum	Per. Exud 1:5 in	TIME	No. UNSTAIN.	No. STAINED	% UNSTAIN	Cytoplasmic Effect
1	[redacted] N. Orleans)	Saline	1:33	85	15	85	+
2	" "	EB.	"	95	5	95	+
3	[redacted] repeat	Saline	"	7	43	14	−
4	" " — "	E.B.	"	48	2	96	+
5	[redacted] n — Utah	Saline	1:48	36	64	36	?
6	" — "	EB.	"	44	56	44	?
7	[redacted] "	Saline	1:59	44	6	88	+
8	" "	EB	"	45	5	90	+
9	LAMB 1	Saline	2:02	45	5	90	+
10	" 2	"	"	48	2	96	+
11	" 3	"	2:10	50	0	100	+
12	Chicken	"	"	Difficult to find to toxo. Floccul. present; complete lysis? ??			
13	Heated [redacted]	"	2:16	1	49	2	—
14	" "	EB	"	10	40	20	—

Note: [redacted] — serum sent by air mail, special delivery without refrigeration on 4/23 from New Orleans Arrived in 24 hours. Then frozen in CO_2

[redacted] — serum sent from Holland by air mail. Arrived in 4 to 5 days; then frozen Note that reaction is neg. in saline + positive with E.B. (Buescher-activator serum)

[redacted] sera — sent frozen from Utah but with insufficient dry ice — arrived fluid within 24 hours — then frozen.

MAY 10 1948

AC-GLOBULIN (Seeger) As An Activator In The In Vitro Toxoplasma Test.

AC-GLOBULIN : Rec'd as a dried powder from Seeger (Wayne Univ.) on 8 May 48. Stored in refrigerator. Bottle labelled: "Ac-Globulin 480503 1000 units (Serum type)". Total weight = 44.0 mgs (to-day). 10.7 mgs. weighed out & dissolved in 1.0 cc NaCl containg heparin 1/5000. This was used as the 1% tube altho not exactly so because of bubbles in the suspending tube, it was not possible to accurately measure the column. This suspending was done in a graduated centrifuge tube. Ten-fold dilutions were then made with heparinized saline to make 0.1% & 0.01% Ac-Glob. & stored in the refrigerator until used.

Toxoplasma : 5 mice were sacrificed, four days after IP inoc. c undil PE. & their PE's smeared. The yields were very poor & only four had sufficient fluid to warrant further examination. One loop of each was immediately examined c 1 loop of NB. Two were almost all cells while 2 had many extracellular parasites.

See over for time schedule

Prelim. Test : The two good exudates were immediately dil as follows:

PE = 0.05 cc
Hep 1/200 = 0.01 cc
Buffer sol = 0.20 cc

After 10' incubation @ 37°, .002 cc of each was mixed c 1 loop of NB & counted as follows : PE #4 = 0/51 contained. Many two & also many cells.

PE #5 = 7/64 contained. Many two. Stain light.

PE #4 used in test

(OVER)

Test Suspensions: 0.05 ml amounts of the selected PE were then added to 0.2 ml amts of each of the heparinized Ac-Glob solutions & to 0.2 ml of fresh Buescher serum plus 0.01 ml hep 1/200 & immediately distributed as indicated in the protocol.

Temporal Data:

3^{32} - 3^{42} — PE's harvested.

3^{55} — PE #4 & #5 dil. c̄ Buescher serum & into incubator (3^{59}↑) for 10' prelim test.

4^{09} — out of incubator & counted & PE #4 selected for test.

4^{28} — Final test into incubator for 1 hr.

5^{28} — out of incubator for final counts.

Immune Serum: R. Black, heated for 30' @ 56° @ joint prior to test.

Tube #	SERUM	PE 1/5 IN (Hep 1/5000)	RESULT # UNSTAINED	# STAINED	% UNSTAINED	
1	[redacted] (H)	[redacted]	47	3~~47~~	94	AC-GLOB. (SEEGER) IN
2	[redacted] (H)	Ac-Gl 1%	2	98	2	1%, 0.1%, & 0.01% CON-
3	[redacted] (H)	Ac-Gl 0.1%	4	96	4	CENTRATION DOES NOT
4	[redacted] (H)	Ac Gl. 0.01%	0	100	0	REACTIVATE HEATED
5	NONE - CONT.	[redacted]	18	82	18	IMMUNE SERUM NOR DOES
6	NONE - CONT.	Ac-GL 1.0%	2	98	2	IT DIRECTLY AFFECT
7	NONE - CONT.	Ac-GL. 0.1%	0	100*	0	TOXO PLASMA (SEE CONTROLS)
8	NONE - CONT.	Ac-GL 0.01%	0	100*	0	

* SLIGHT AGG.

MAY 12 1948

Titrations of Reactivating Power & Non-Specific "Active" Factor in Normal Rabbit Serum.

Obj: There are several objectives of this experiment. a) What is the titer of the non-specific factor in fresh rabbit serum; b) What is the titer of activator in normal rabbit serum.

Toxoplasma: Small amounts of PE obtained & pooled from each of 2, four day mice = innumerable extracellular parasites.

Prelim. Test: PE made to 1:5 in Buescher serum c̄ heparin 1/5000, incubated for 20' @ 37° & examined c̄ 1 loop MB. Numerous toxo seen. All stained.

Procedure: Immediately after prelim test, rabbit serum (#365, pre-inoc. obtained 4 March 1948 & frozen since. This is a non-reactivatable serum) diluted in saline, & various PE's made, until had been kept in refrig. each c̄ heparin 1/5000. 0.1cc quantities of serum + 0.1cc PE 1/5, immediately to 37° @ H_2O bath. Tests refrigerated until read after the one hours incubation.

Sera: Rabbit = #365, pre-inoc. bleeding of 4 March. Non-reactivatable.
* [redacted] clinical sample rec'd from N. Orleans on 8 May 48. ~~No letter~~
[redacted] obtained from Dr. I. Young to-day.

Time schedule:

1) PE obtained @ 2 40 p.m.
2) 20' prelim inc. began @ 2 44 p.m.
3) Final test - one hour inc. began @ 3 33 p.m.
4) Readings began @ 4 34 pm.

* Cannon - 5 yr old wh male. Normal physical & mental development except for "behavior disturbance & excessive emotional response". Onset convulsions 20 April '48. Suggestive area calcification left occipital region. Evidence of increased intracranial pressure.

(OVER)

MAY 12 1943

TUBE #	SERUM	PC 1/5 IN	RESULT # UNSTAINED	# STAINED	% UNSTAINED	EFFECT	TIME	
1	NONE	NaCl	4	46	8	—	4 34	
2	Rab #365 (F) undil	NaCl	47	3	94	+	"	Intracellular type of ppt.
3	" (F) 1:2 Sal.	NaCl	45	5	90	+	"	"
4	" (F) 1:4 Sal	NaCl	66	34	66	+?	"	" Non-specific ppt
5	" (F) 1:8 Sal	NaCl	10	40	20	—	"	goes to 1:4
6	" (F) 1:16 Sal	NaCl	4	46	8	—	"	
7	NONE	R.B.*	2	98	2	—	5 00 pm.	
8	[redacted]	RB.	97	3	97	+	"	
9	Rab. #365 (F) undil.	RB	98	2	98	+	"	
10	" (F) 1:2 Sal.	R.B.	95	5	95	+	"	Reactivity, power goes to 1:16
11	" (F) 1:4 Sal	RB	85	15	85	+	"	
12	" (F) 1:8 Sal	R.B.	79	21	79	+	"	
13	" (F) 1:16 Sal.	R.B.	52 57	48 43	54	5 +0 —	5 10	
14	NONE	E.B.	16	84	16	—	5 14	
15	[redacted] (F)	NaCl	10	90	10	—	5 22	
16	[redacted] (F)	E.B.	17	83	17	—	5 22	
17	[redacted] (F)	NaCl	55	45	55	±	5 32	
18	[redacted] (F)	E.B.	59	41	59	±	"	

* R.B. = [redacted] serum, heated for 30' @ 56° C just prior to test.

MAY 18 1948

In Vitro Titration of Serial Sera From Toxoplasma Infected (Experimental) Rhesus #152

Obj: To determine whether antibody can be titered to different levels in serial serum samples obtained from an experimentally infected animal (monkey)

Toxoplasma: yields extremely poor. Finally used pool from 1,4 dy & 1,3 dy mice. Direct film exam revealed many toxo but since only 0.3cc PE available, tested @ 1:10 c̄ 10^{55} a.m. Buescher (17 May) serum & heparin 1/5000. PE obtained @ 10^{35} a.m. Prelim incubation for 20' @ 37° exam c̄ 1 loop MB. = 1/100 unstained.

Test Sera: Pre-inoc., 7th, 14th, 21st, 26th post inoculation days from Ph #152. This animal died on the 26th day.

Test: All sera inactivated for 30' @ 56°C in the undil state just prior to test. When prelim test was ok, serial four-fold dilutions in NaCl were made beginning with 1:4 & ending c̄ 1:1024 for each sample except the pre-inoc which was tested as undiluted only. 0.1cc of each serum dilution & 0.1cc of PE 1/10 in Buescher serum c̄ heparin 1/5000 were mixed, incubated for one hour (beginning @ 12 noon) & then examined in wet-film c̄ 1 loop of Meth. blue. Tubes ~~incubated~~ refrigerated until read.

(over)

ALL DILUTIONS IN NaCl ALL SERA, EXCEPT EB, INACTIVATED

COMPARE WITH TEST OF 29 MARCH.

Tube #	SERUM	PE½ IN (HEP.)	RESULT # UNSTAINED	RESULT # STAINED	RESULT % UNSTAINED	EFFECT	TIME	
1	CONTROL	E.B.	9/8	91/42	9/16	=/=	1.05/3.15	
2	RH. #152 Pre-immun.	"	22	78	22	—	"	
3	RH. #152 7dy 1:4	"	31	19	62	+	"	
4	" " 1:16	"	27	23	54	+-	"	
5	" " 1:64	"	24	26	48	+-	1.27	END-POINT?
6	" " 1:256	"	12	38	24	—	"	
7	" " 1:1024	"	8	42	16	—	1.40	
8	" 14dy 1:4	"	29	21	58	+	1.40	
9	" " 1:86	"	37	13	74	+	1.50	
10	" " 1:64	"	42	18	84	+	"	
11	" " 1:256	"	37	13	74	+	1.58	
12	" " 1:1024	"	36	14	72	+	"	NO END-POINT
13	" 21 dy 1:4	"	32	18	64	+	2.08	
14	" " 1:86	"	37	13	74	+	"	
15	" " 1:64	"	38	12	76	+	2.20	
16	" " 1:256	"	43	7	86	+	"	
17	" " 1:1024	"	39	11	78	+	2.25	NO END-POINT
18	" 26 dy 1:4	"	31	19	62	+	"	
19	" " 1:16	"	34	16	68	+	2.40	
20	" " 1:64	"	42	8	84	+	"	
21	" " 1:256	"	35	15	70	+	"	
22	" " 1:1024	"	38	12	76	+	"	NO END POINT

MAY 21 1948

TITRATION BY IN VITRO TOXOPLASMA TEST OF SERIAL SAMPLES FROM RH. #152

Aby: To determine the pattern of antibody formation by Rh #152 by titrating all serum samples simultaneously in the in vitro test.

Procedure: All sera were inactivated for 30' @ 56°C just prior to the tests while undiluted. Dilutions then made (0.1 + 0.3 cc) in saline & ~~tubes refrigerated~~ simultaneously distributed to the final incubation tubes. These were then refrigerated until the addition of the toxo about one hour later.
0.1 cc of toxo 1:5 in fresh Buescher serum with heparin 1/5000 (stock 1/100) + 0.1 cc of the serum dilution mixed, incubated for 1 hour @ 37°C (H_2O bath) & then read as indicated in the protocols using 0.02 ml mixture plus 1 loop Meth Bl. Tubes refrigerated until read. Organisms were numerous & readings were readily accomplished.

Toxoplasma: PE pooled from 5 mice (inoc. IP on 18 May), 0.05 ml immediately made to 1/5 in Buescher serum with heparin 1/5000, incubated for 20' @ 37° & read in films = 1/100 contained. Then made to 1/5 using 1.1 cc PE & 4.4 cc Buescher plus 0.2 ml hep 1/200. & immediately distributed to the prepared serum dilution tubes, using a fresh pipette for each series, mixed, & incubated.

Time Schedule: PE obtained @ 11 45 a.m. 20' test read @ 12 10 p.m.
Final test into water bath @ 12 37 pm. Out at 1 37 pm.
& final readings begun immediately.

SERIAL RH #152 SERA

ALL SERA HEATED.

MAY 21 1948

TUBE #	SERUM SOURCE	SERUM DILUTION	PE ½ IN	TIME	EFFECT # UNSTAINED	EFFECT # STAINED	EFFECT % UNSTAINED	RESULT	
1	—	—	NaCl	1 45	2	48	4	−	
2	—	—	EB	"	9	91	9	−	
				4 55	3	47	6	−	
3	[redacted] (H)	UNDIL.	E.B.	1 45	44	6	88	+	POS. CONTROL
4	RH #152 PRE-INOC.	UNDIL.	"	"	2	48	4	−	
5	RH #152 - 3rd day	UNDIL.	"	2 18	7	43	14	−	
				2 40	4	46	8	−	
6	" "	1:4	"	2 18	15	35	30	−	NEG
7	" "	1:16	"	2 24	6	44	12	−	
8	" "	1:64	"	"	6	44	12	−	
9	" 5th dy	UNDIL.	"	2 29	36	14	72	+	
10	" "	1:4	"	"	35	15	70	+	1:4
11	" "	1:16	"	2 40	17	33	34	−	
12	" "	1:64	"	"	10	40	20	−	
13	" "	1:256	"	2 45	6	44	12	−	
14	" 7th dy	UNDIL.	"	2 45	32	18	64	+	
15	" "	1:4	"	2 50	26	24	52	+	
16	" "	1:16	"	"	30	20	60	+	1:16
				3 00	27	23	54	+	
17	" "	1:64	"	2 55	3	47	6	−	
18	" "	1:256	"	"	2	48	4	−	
19	" 10th dy	undil	"	3 00	27	23	54	+	
20	" "	1:4	"	"	40	10	80	+	
21	" "	1:16	"	3 05	45	5	90	+	
22	" "	1:64	"	"	43	7	86	+	
23	" "	1:256	"	3 15	32	18	64	+	1:256

ALL SERA HEATED

MAY 21 1948

TUBE #	SERUM SOURCE		DILUTION	PE 1/5 IN	TIME	EFFECT # UNSTAINED	EFFECT # STAINED	EFFECT % UNST.	RESULT.	
24	RH #152 -	10th dy	1:1024	EB	3 15	8	42	16	-	
25	"	"	1:4096	"	3 20	3	47	6	-	
26	"	"	1:16,384	"	2 13	5	45	10	-	
27	"	"	1:65,536	"	"	4	46	8	-	
28	"	14th dy	undil.	"	3 20	15	35	30	-	
29	"	"	1:4	"	3 28	18	32	36	- ?	
30	"	"	1:16	"	"	40	10	80	+	
31	"	"	1:64	"	3 30	40	10	80	+	
32	"	"	1:256	"	"	40	10	80	+	
33	"	"	1:1024	"	3 50	39	11	78	+	1:1024
34	"	"	1:4096	"	"	16	34	32	- ?	
35	"	"	1:16,384	"	2 05	8	42	16	-	
36	"	"	1:65,536	"	"	1	49	2	-	
37	"	21st dy	undil	"	3 50	19	31	38	- ?	
38	"	"	1:4	"	"	28	22	56	+	
39	"	"	1:16	"	4 14	43	7	86	+	
40	"	"	1:64	"	"	44	6	88	+	
41	"	"	1:256	"	"	43	7	86	+	
42	"	"	1:1024	"	"	44	6	88	+	
43	"	"	1:4096	"	4 28	57	43	57	+	1:4096
44	"	"	1:16,384	"	2 05	7	43	14	-	
45	"	"	1:65,536	"	"	2	48	4	-	

SERIAL RH #152 SERA

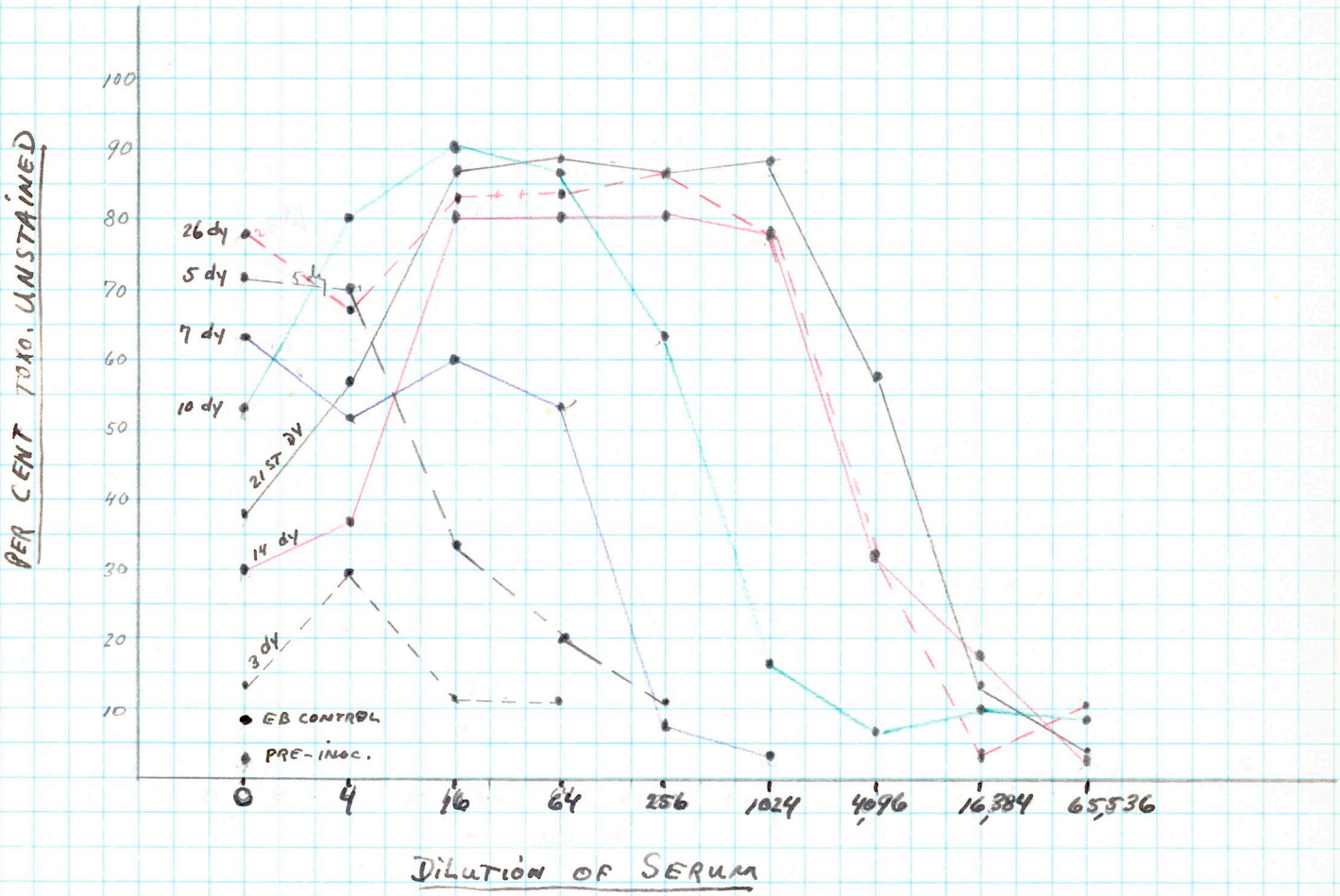
All SERA HEATED

MAY 21 1948

TUBE #	SERUM SOURCE		DILUTION	DEYS IN	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT	
46	RH #152	26 dys	UNDIL.	E.B.	4 25	39	11	78	+	
47	"	"	1:4	"	4 30	34	16	68	+	
48	"	"	1:16	"	"	51	11	82	+	
49	"	"	1:64	"	4 32	42	8	84	+	
50	"	"	1:256	"	"	43	7	86	+	
51	"	"	1:1024	"	2 22	39	11	78	+	1: 1024
52	"	"	1:4096	"	2 09	16	34	32	−	
53	"	"	1:16,384	"	2 00	2	48	4	−	
54	"	"	1:65,536	"	"	5	45	10	−	

21 MAY 48

SUMMARY OF IN VITRO TITRATIONS OF SERIAL SERA FROM RH. #152



MAY 22 1948

In Vitro Titration of Browning, Young, & Bruce Johnson Sera.

Toxoplasma: PE pooled from 2, four dry mice. Immediately made 1/5 c̄ Buescher serum & heparin 1/5000 & incubated for 20' @ 37°C. = 2/50 unstained.

Test: Sera inactivated @ 56° C just prior to test & dil in Na Cl. Immediately upon completion of prelim test, toxo made to 1/5 in Buescher c̄ heparin 1/5000 & 0.1cc amts. mixed c̄ 0.1cc serum. Incubated 1 hr @ 37° C & then read c̄ 1 loop of meth bl. Tubes refrig. until read.

Sera: Browning of 5 Feb, '48., Bruce Johnson of 15 April '48, & I. Young of 14 May '48

Time schedule: PE obtained @ 10^{50} a.m. Prelim test completed @ 11^{15} a.m. Final test in incubator @ 11^{35} am. Out at 12^{35} pm. Toxo few, counts prolonged.

Tube #	SERUM		PE 1/5 in	TIME	EFFECT			RESULT.	
	SOURCE	DILUTION			# UNSTAIN.	# STAINED	% UNSTAIN.		
1	CONTROL	—	EB	12^{40}	13	27	26	−	
2	[redacted] (H)	UNDIL.	"	"	43	7	86	+	
3	"	1:2	"	"	41	9	82	+	
4	"	1:4	"	"	31	19	62	+	1:4
5	"	1:8	"	1^{22}	16	34	32	−	
6	"	1:16	"	"	14	36	28	−	
7	[redacted]	UNDIL.	"	"	43	7	86	+	
8	"	1:2	"	"	40	10	80	+	
9	"	1:4	"	1^{50}	37	13	74	+	
10	"	1:8	"	"	28	22	56	+ ?	1:8
11	[redacted]	UNDIL.	"	"	28	32	47	+−	undil
12	"	1:2	"	"	11	39	22	−	
13	"	1:4	"	2^{35}	17	33	34	−	
14	"	1:8	"	"	17	43	28	−	

MAY 24 1948

IN VITRO TITRATION OF TWO (2) IMMUNE GUINEA PIG SERA AND TESTS OF THREE NORMAL MOUSE SERA

TOXOPLASMA: PE pooled from 2, four day mice, immediately set up @ 1/5 in EB with hep 1/5000 & incubated 20' @ 37°C. for prelim test. Rich exudate.

SERA: GP sera recd from [redacted] from hyperimmunized animals. Both have C-F titers of 1:256 & GP#974 had a positive rabbit neut. Since only 0.05 cc of GP#1037's serum remained this was started @ 1:4 to-day.

Mouse sera: obtained from hep bl from normal Ch Alb on 24 Mar & frozen since. These mice challenged IP & succumbed in 5-6 days.

Mouse #1 = pooled from 2 mice. Mouse #2 & #3 = single mouse sera.

TEST: All sera (except "F") inc. 30' @ 56°C just before test. Dilutions prepared in NaCl & distributed. Stored in refrig. until prelim test ok (6/100 unst.), quickly made to 1/5 c̄ EB & hep 1/5000 & distributed to the prepared tubes, incubated for one hour @ 37°C

(OVER)

TUBE #	SERUM SOURCE	DILUTION	PE 1/5 IN	TIME	EFFECT # UNSTAINED	# STAINED	% UNSTAINED	RESULT.	
1	—— CONT.	—	EB	1^{38}	12	88	12	−	
				3^{20}	9	41	18	−	
2	GP # 974 (7-24-49)	undil	"	1^{55}	3	47	6	−	
3	" NaCl	1:4	"	2^{00}	20	80	20	−	
4	" "	1:16	"	"	50	50	50	+	
5	" "	1:64	"	2^{05}	61	39	61	+	
6	" "	1:256	"	2^{05}	81	19	81	+	
7	" "	1:1024	"	2^{15}	90	10	90	+	
8	" "	1:4096	"	"	69	31	69	+	← TITER = 1:4096
9	" "	1:16,384	"	2^{20}	27	73	27	−	
10	" "	1:65,536	"	1^{40}	4	46	8	−	
11	" "	1:262,134	"	1^{38}	5	45	10	−	

MAY 24 1948

TITRATION IMM.G.P. (CONT.)

TUBE #	SERUM SOURCE	DILUTION	PE 1/5 IN	TIME	EFFECT # UNSTAINED	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT.	
12	GP # 1037 (10-10-47)	1:4	EB	2^{20}	35	65	35	−	
13	" " NaCl	1:16	"	2^{45}	67	33	67	+	
14	" " "	1:64	"	"	82	18	82	+	
15	" " "	1:256	"	"	93	7	93	+	
16	" " "	1:1024	"	"	80	20	80	+	
17	" " "	1:4096	"	3^{00}	47	53	47	+	← TITER = 1:4096
18	" " "	1:16,384	"	"	20	80	20	−	
19	" " "	1:65,536	"	1^{55}	4	46	8	−	
20	" " "	1:262,134	"	1^{40}	18	82	18	−	
21	MOUSE #1 (F) (pool of 2)	—	NaCl	3^{07}	2	48	4	−	FRESH MOUSE SERA DO NOT HAVE NON-SPECIFIC EFFECT BUT BEHAVE LIKE HUMAN SERA
22	" " (H)	—	EB	"	2	48	4	−	
23	MOUSE #2 (F)	—	NaCl	3^{15}	5	45	10	−	
24	" " (H)	—	EB	"	6	44	12	−	
25	MOUSE #3 (H)	—	EB	3^{20}	3	47	6	−	
26	—— CONT.		NaCl	"	0	50	0		

TEST. (CONT.) = Then 0.1cc units mixed c̄ 1 loop Meth bl. & examined in wet film. Tubes refrigerated until read.

Time schedule: PE obtained @ 11^{35} a.m., Prelim test read @ 12^{05}, final test in @ 12^{36} pm., out @ 1^{36} pm. & final readings begun.

MAY 25 1948

Titration of Several Human and One Monkey Serum In The In Vitro Test.

Toxoplasma: Pooled from 3 four day (IP) mice. Prelim test (1/5 in Bruscher serum ± hep 1/5000) set up immediately & read @ 20' = 2/100 unstained.

Sera: [redacted] 23 Dec 48, has characteristics.

[redacted] Jan 48, unspecific infection.

[redacted] Jan 48, " "

[redacted] 26 Dec 47, " "

e) Rh #142, 1 Mar 48, serum reactivatable after heating.

[redacted] 22 May 48, Recd unfrozen today. In mail since 28 May. } L.S.U. New Orleans

[redacted] 22 May 48. " " " " " " " "

Test: All sera inactivated 30' @ 56° C as undiluted serum, just prior to test. All dilutions then made in Na Cl & distributed to final tubes which were stored in refrigerator until used. Immediately after prelim test, top made to 1/5 in EBruscher serum ± hepsin 1/5000 & 0.1 cc units added to serum dilutions using separate pipette for each series. Incubated 1 hr @ 37° C & then read ± 1 loop Meth Blue - tubes refrig. until read.

Time schedule: PE obtained @ 1, 30 pm. Prelim test completed @ 2 15 pm. Final test into incubator @ 2 40 pm. Out @ 3 40 pm. when reading began.

(OVER)

N.B. These sera recd in mail this afternoon (unfrozen), included in test as unheated sera but with same PE 1/5 in Bruscher serum. Read @ 5 30 pm.

#25	[redacted]	40/50 unstained = 80% unst.	+
#26	[redacted]	6/50 " = 12% unst.	−

MAY 25 1948

TUBE #	SERUM SOURCE	DILUTION	PE'S IN	TIME	EFFECT # UNSTAINED	EFFECT # STAINED	EFFECT % UNSTAINED	RESULT.	
1	NaCl	—	EB	3^{45}	4	46	8	−	
				5^{30}	6	44	12	−	
2	[redacted]	UNDIL.	"	3^{45}	45	5	90	+	
3	"	1:4	"	"	46	4	92	+	
4	"	1:16	"	"	29	21	58	+	← TITER 1:16
5	"	1:64	"	3^{50}	6	44	12	−	
6	"	1:256	"	"	2	48	4	−	
7	"	1:1024	"	4^{30}	2	48	4	−	
8	[redacted]	UNDIL.	"	"	35	15	70	+	
9	"	1:4	"	"	43	7	86	+	
10	"	1:16	"	"	44	6	88	+	
11	"	1:64	"	4^{33}	37	13	74	+	← 1:64
12	"	1:256	"	"	11	39	22	−	
13	[redacted]	UNDIL.	"	4^{42}	46	4	92	+	
14	"	1:4	"	"	47	3	94	+	
15	"	1:16	"	4^{50}	38	12	76	+	← 1:16
16	"	1:64	"	"	15	35	30	− ?	
17	"	1:256	"	5^{05}	4	46	8	−	
18	[redacted]	UNDIL	"	"	35	15	70	+	
19	"	1:4	"	5^{09}	40	10	80	+	
20	"	1:16	"	"	45	5	90	+	
21	"	1:64	"	5^{15}	23	27	46	+	← 1:64
22	RH #142 (H)	undil	"	"	38	12	76	+	
23		1:4	"	"	39	13	74	+	
24		1:16	"	5^{25}	29	21	58	+	← 1:16

MAY 26 1948

In Vitro Titration of Ruckman's Monkey Serum And Effect of Ascitic Fluid As An Activation.

Toxoplasma: PE pooled from 3 four day mice. Prelim test in E Buescher (hep/500) serum set up immediately = 3/100 unstained (20' @ 37°C).

Sera: Rh #2 (Ruck.) 3rd week after inoc. Collected & frozen on 25 April '47. (Same sample previously tested to 1/256)

[redacted]: fresh. Used for inactivation test.

Ascitic Fluid: Collected this a.m. from nephrotic patient Roger Dunn, age 3 yrs. at Children's. Refrigerated immediately thereafter. On Duracillin. Readm. 2/18/48. Tapped q̄ 1-2 weeks.

Test: Rh #2 & [redacted] sera inac. @ 56° for 30' just before test. Rh serum diluted in NaCl & distributed. Tubes stored in refrig. until used. 0.1cc serum + 0.1cc PE 1/5 in [redacted] c̄ hep 1/5000 mixed, incubated for 1 hr @ 37°C & then read c̄ 0.02 mixt plus 1 loop Meth Bl. Ascitic fluid c̄ PE 1/5 prepared without heparin.

Time schedule: PE collected @ 1 25 pm. Prelim test begun @ 1 30 pm. Final test in @ 2 14 pm. Out @ 3 14 pm.

MAY 26 1948

Tube #	SERUM SOURCE	Dilution	PE in	Time	EFFECT # UNSTAINED	# STAINED	% UNST.	RESULT	
1	NaCl	—	EB	3 15	3	47	6	−	
				3 58	1	49	2	−	
2	Rh #2 (N) {3 mths. Ruch. 4-26-47}	Undil	EB	3 15	34	66	34	− ?	
3	"	1:4	"	3 29	26	24	52	+	See expt of 18 Feb (bad slide)
4	"	1:16	"	"	45	5	90	+	
5	"	1:64	"	3 22	44	6	88	+	
6	"	1:256	"	"	43	7	86	+	
7	"	1:1024	"	3 25	33	17	66	+	← TITER = 1:1024
8	"	1:4096	"	"	6	44	12	−	
9	"	1:16,384	"	3 42	3	47	6	−	
10	"	1:65,536	"	"	3	47	6	−	
11	"	1:262,144	"	3 45	3	47	6	−	
12	NaCl	Undil	Ascitic Fluid (F)	"	0	50	0	−	Ascitic fluid does not reactivate
13	[redacted]	"	"	3 55	1	49	2	−	
14		"	EB	"	44	6	88	+	
15		"	Ascitic Fluid Heated	3 58	1	49	2	−	

MAY 27 1948

Titration of Rat "Y" Serum & Effect of Heat Upon Diluted Monkey Toxoplasma Antibody.

Toxoplasma: Pooled from 2 four day mice. Preli test set up immediately 1/5 in Bruschen serum ± hep 1/5000. Read @ 20' = 1/50 untained.

Sera; Rat "Y". No pre-inoc. serum remained so only the 5th & 7th post-inoc. day sera tested to-day. These consumed in their entirety. Both inactivated for 30' @ 56° C & undiluted sera just before test.

Monkey #2 : Same [redacted] serum obtained 21 days after inoc. 4-25-47. Inactivated as follows: One specimen inactivated while undiluted whereas an aliquot was diluted in Na Cl as indicated in the protocol & then inactivated.

Test: All tubes prepared in advance & stored in refrigerator. Immediately upon completion of the preli test, PE made to 1/5 in [redacted] serum ± hep 1/5000 distributed in 0.1cc amts, using a separate pipette for each series, to the 0.1cc of serum dilution. Then into 37° C H_2O bath for 7 hours. Then read ± 1 loop of meth. blue.

Time schedule: PE collected @ 1 32 pm. Preli test @ 1 35. Final test in to water bath @ 2 08 pm. & out at 3 08 pm.

MAY 27 1948

TUBE #	SERUM SOURCE	DILUTION	PE'/5 IN	TIME	EFFECT # UNST.	EFFECT # STAINED	EFFECT % UNST.	RESULT	
1	CONT. NaCl.	—	EB	3[14]	4	46	8	—	
2	RAT "Y(H)" 5[dy]	UNDIL	"	"	43	7	86	+	
3	" " "	1:4	"	3[25]	46	4	92	+	
4	" "	1:16	"	"	42	8	84	+	← TITER 1:16
5	" "	1:64	"	3[39] ←	19 (21)	31 (29)	38 (42)	-?	
6	" "	1:256	"	"	16	34	32	-	
7	" "	1:1024	"	3[40]	2	48	4	—	
8	RAT "Y(H)" 7[dy]	UNDIL	"	"	18	32	36	-?	
9	" "	1:4	"	3[50]	34	16	68	+	
10	" "	1:16	"	"	43	7	86	+	
11	" "	1:64	"	4[00]	46	4	92	+	
12	" "	1:256	"	"	34	16	68	+	
13	" "	1:1024	"	4[15]	2[7] (29)	23	54	+	← TITER 1:1024
14	" "	1:4096	"	4[05]	2	48	4	—	
15	RH#2 [redacted] wks. (HEATED UNDIL.)	1:4	"	4[20]	35	15	70	+	
16	"	1:64	"	"	39	11	78	+	
17	"	1:256	"	4[25]	41	9	82	+	
18	"	1:1024	"	"	40	10	80	+	← TITER 1:1024
19	"	1:4096	"	4[33]	16	34	32	—	
20	RH.#2 [redacted] wks. (HEATED. DILUTED)	1:4	"	"	30	20	60	+	
21	" "	1:64	"	4[43]	43	7	86	+	
22	" "	1:256	"	"	40	10	80	+	
23	" "	1:1024	"	4[50]	35	15	70	+	← TITER 1:1024
24	" "	1:4096	"	"	0	50	0	—	

MAY 28 1948

IN VITRO TITRATION OF SERIAL SERA OF RAT "R"

Toxoplasma: PE pooled from 4 four day mice. Prelim test set up immediately. Read at 20' = 0/50 unstained. Final test set up immediately.

Sera: Serial samples derived from Rat "R" which recd toxo IP on 25 Feb '48

1) Pre-inoc : hep. plasma 24 Feb 48
2) 5th post-inoc. day : hep. plasma 1 March 48
3) 7th " " " : " " 3 March 48
4) 14th " " " : " " 10 March 48
5) 21st " " " : " " 17 March 48 – (no more than 0.05 ml. probably less)
6) 28th " " " : " " 24 March 48

All sera unhibited inactivated for 30' @ 56°C just before test. Dilutions made in NaCl immediately thereafter & stored in refrig until toxo added. Simultaneously distributed to final tubes.

Test: Upon completion of prelim test, toxo made to 1/5 in Bruescher serum – heparin 1/5000, distributed in 0.1cc amts to the 0.1cc of serum dilutions previously distributed. After incubation for 1 hr @ 37°C H2O bath, tubes stored in refrigerator until read using 1 loop of Meth Bl to 0.02 ml toxo-serum mixt.

Time schedule: PE obtained @ 11^09 a.m. Prelim test begun @ 11^13 a.m. Final test into H2O bath @ 11^50 a.m., out @ 12^50 pm.

(over)

SERIAL SERA RAT "R"

MAY 28 1948

TUBE #	SERUM SOURCE	DIL.	PE % IN (Hep.)	TIME	EFFECT # UNST.	# STAINED	% UNST.	RESULT.	
1	CONT. Na Cl	—	EB	12^{54}	4	46	8	—	
2	RAT "R" PRE-INOC 24 FEB	UNDIL.	"	"	4	46	8	—	
3	RAT "R" -5d DAY 1 MAR	UNDIL.	"	12^{56}	35	15	70	+	
4		1:4	"	"	32	18	64	+	
5		1:16	"	1^{20}	27	23	54	+	
6		1:64	"	"	27	23	54	+	← TITER 1:64
				4^{15}	50	50	50		
7		1:256	"	1^{22}	18	32	36	—	
8	RAT "R" - 7d DAY 3 MAR.	UNDIL.	"	"	23	27	46	+	
9		1:4	"	1^{30}	43	7	86	+	
10		1:16	"	"	33	17	66	+	
11		1:64	"	1^{45}	37	13	74	+	
12		1:256	"	"	38	12	76	+	
13		1:1024	"	1^{49}	31	19	62	+	← TITER 1:1024
14		1:4096	"	"	11	39	22	—	
15	RAT "R" 14d DAY 10 MAR	UNDIL.	"	2^{00}	7	43	14	—	
16		1:4	"	"	24	26	48	+	
17		1:16	"	2^{03}	31	19	62	+	
18		1:64	"	"	46	4	92	+	
19		1:256	"	2^{12}	42	8	84	+	
20		1:1024	"	"	24	26	48	+	← TITER 1:1024
				3^{47}	54	46	54		
21		1:4096	"	2^{17}	13	37	26	—	
22		1:16384	"	"	9	41	18	—	

SERIAL SERA RAT "R" (CONT.)

MAY 28 1948

Tube #	SERUM SOURCE		DILUTION	PE'S IN (Hlp)	TIME	EFFECT # UNST.	EFFECT # STAINED	EFFECT % UNST.	RESULT	
23	RAT "R"	21 dys	undil	EB		gms	—	—		Heated undil. & then dil'd 0.15 cc NaCl to make "1:4" but there was probably no more than 0.04 cc serum. This may account for drop in titer. ↓
24			1:4	"	2^{20}	22	28	44	+	
25			1:16	"	"	38	12	76	+	
26			1:64	"	2^{30}	45	5	90	+	
27			1:256	"	"	40	10	80	+	← TITER 1:256
28			1:1024	"	2^{45}	6	44	12	−	
					3^{49}	24	76	24		
29			1:4096	"	"	10	40	20	−	
30			1:16,384	"	2^{41}	8	42	16	−	
31	RAT "R"	28 dys	undil	"	"	14	36	28	−	
32			1:4	"	2^{50}	30	20	60	+	
33			1:16	"	"	40	10	80	+	
34			1:64	"	2^{55}	45	5	90	+	
35			1:256	"	"	46	4	92	+	
36			1:1024	"	3^{00}	21	29	42	+	← TITER 1:1024
					4^{15}	49	51	49		
37			1:4096	"	"	4	46	8	−	
38			1:16,384	"	3^{05}	5	45	10	−	

SUMMARY:

RAT "R" SERUM	TITER
PRE-INOC.	NEG.
5th DAY	1:64
7th DAY	1:1024
14th DAY	1:1024
21st DAY	1:256
28th DAY	1:1024

MAY 29 1948

In Vitro Tests Of Kope (Cleveland Clinic) Sera.

Toxoplasma: PE pooled from 2 four day mice. This was immediately distributed for the final test without a 20' prelim reading.

Sera: Rec'd this a.m. unfrozen via air-mail special delivery mail as ampouled sera labelled as follows: a) "Sterile serum of Mrs. D.E. Kope, mother of Danny Arnold. Blood drawn 5-28-48, 11^{20} a.m."
b) "Sterile serum from Danny Arnold. Blood drawn 5-28-48, 11^{15} a.m."
These were sent by Dr. A.T. Bunts, Cleveland Clinic, 2020 E. 93rd St., Cleveland, Ohio.

Test: Sera diluted in saline & used without inactivation. Pooled PE immediately dil to 1/5 in Buescher serum c̄ heparin 1/5000. & 0.1cc amts added to 0.1cc of serum dilution; incubated for 1 hr @ 37°C & then read.

Time schedule: PE obtained @ 11^{20} a.m. Final test in @ 11^{25} a.m. Out of H_2O bath @ 12^{25} p.m.

TUBE #	SERUM SOURCE	DILUTION	PE 1/5 IN	TIME	EFFECT # UNSTAINED	# STAINED	% UNST.	RESULT.	
1	NaCl - CONTROL	—	EB	12^{33}	4	46	8	—	
2	[redacted] (F)	UNDIL.	"	"	47	3	94	+	
3	" " "	1:4	"	"	47	3	94	+	
4	" "	1:16	"	"	50	0	100	+	
5	" "	1:64	"	12^{47}	75	25	75	+	NO END-POINT @ 1:64
6	[redacted]	UNDIL.	"	"	49	1	98	+	
7	" "	1:4	"	"	45	5	90	+	
8	" "	1:16	"	12^{52}	45	5	90	+	
9	" "	1:64	"	"	86	14	86	+	NO END-POINT @ 1:64

JUN 1 1948

Titration of P-17, Rab. #349 & #398 [redacted]
With the [redacted] Test

Toxoplasma: PE pooled from 2 four day mice & immediately set up in 1/5 ± [redacted] serum & kept 1/5000 for 20' prelim test. 9/60 contained so immediately made up for final test.

Sera: a) P-17 – obtained 7 Aug 47 from Eden Park physician which had toxo infection

b) Rab. #349 – rabbit immune to toxo. Bled 23 Dec. '47.

b) Rab #398 – " " " " " 24 Dec '47

Test: All sera inactivated as undiluted for 30' @ 56°C just prior to test. Then dil in Na Cl & distributed to final tubes. 0.1cc of serum mixed ± 0.1cc of toxo 1/5 in EB ± hepar 1/5000 & incubated for one hour @ 37° C. Then read ± 1 loop of mixture for 0.02 ml toxo-serum mixt. Tubes refrigerated until read.

Time schedule: PE obtained @ 1 0⁹ pm. Prelim test in @ 1 0⁹ pm. Final test in @ 1 4⁵, out @ 2 4⁵ pm.

(OVER)

JUN 1 1948

TUBE #	SERUM SOURCE	DILUTION	PE'S IN (HGR)	TIME	EFFECT # UNST.	EFFECT # STAINED	EFFECT % UNST.	RESULT	
1	CONTROL - Nell	—	EB	2 45 / 4 60	5 / 2	45 / 48	10 / 4	− / −	
2	P-17 (H) (7 Aug 47)	undil	"	"	7	43	14	−	
3	"	1:4	"	2 50	33	67	33 33	− ?	
4	"	1:16	"	"	35	15	70	+	
5	"	1:64	"	2 54	38	12	76	+	
6	"	1:256	"	"	51	49	51	+	← TITER
7	"	1:1024	"	3 02	10	40	20	−	1:256
8	"	1:4096	"	"	2	48	4	−	
9	RAB. #398 (H) 24 Dec 47	undil.	"	3 05	8	42	16	−	
10	"	1:4	"	"	19 / 23	31 / 27	38 / 46	− ?	
11	"	1:16	"	3 25	35	15	70	+	
12	"	1:64	"	"	46	4	92 2	+	
13	"	1:256	"	3 25	42	8	84	+	
14	"	1:1024	"	"	35	15	70	+	
15	"	1:4096	"	3 50	42	58	42	+ ?	TITER 1:4096 (?)
16	RAB. #349 (H) 23 Dec.	undil	"	"	8	42	16	−	
17	"	1:4	"	3 55	7	43	14	−	
18	"	1:16	"	"	15 / 14	35 / 36	30	−	
19	"	1:64	"	4 10	38	12	76	+	
20	"	1:256	"	"	44	6	88	+	
21	"	1:1024	"	"	43	7	86	+	TITER 1:4096 (?)
22	"	1:4096	"	"	57	43	57	+	

JUN 2 1948

Titration Of Paired Mother & Child Sera

Obj: When undiluted serum, only, was tested previously, [redacted] was positive & the child neg. whereas Bruce Johnson was positive & his mother negative. The current test is to determine whether zones are present in either instance.

Toxoplasma: PE pooled from 2 four day mice & immediately set up as 1/5 in Buescher with hep 1/5000 for the prelim test. Examined @ 10' = 1/50 constant. Immediately dil 1/5 in Buescher c̄ hep 1/5000 for final test.

Sera: [redacted]

[redacted] chorioretinitis, no calci.

[redacted]

[redacted]

Test: All sera inactivated for 30' @ 56° as undiluted just prior to test. Diluted in NaCl & distributed to "final" tubes in 0.1cc amts. 0.1cc PE 1/5 in EB c̄ hep 1/5000 added to each, incubated for 1 hr @ 37° C & then read c̄ loop of mech bl per 0.02 ml toxo-serum dil. Tubes refrig. until read.

Time schedule: PE obtained @ 10^{06} a.m. Prelim test in @ 10^{08} - out @ 10^{18} a.m. Final test in @ 10^{35} a.m. Out @ 11^{35} a.m.

JUN 2 1948

TUBE #	SERUM SOURCE	DILUTION	BE's IN (HCP)	TIME	EFFECT # UNST.	# STAINED	% UNSTAIN.	RESULT	
1	NaCl - CONT.	—	EB	11 35	3	47	6	−	
				1 55	5	45	10	−	
2	[redacted]	UNDIL.	"	"	39	11	78	+	
3	"	1:4	"	"	31	19	62	+	← 1:4
4	"	1:16	"		15	35	30	−	
5	"	1:64	"	11 55	0	50	0	−	
6	"	1:256	"	"	1	49	2	−	
7	[redacted]	UNDIL.	"	12 15	2	48	4	−	
8	"	1:4	"	"	3	47	6	−	
9	"	1:16	"	"	5	45	10	−	
10	"	1:64	"	"	2	48	4	−	
11	"	1:256	"	12 25	2	48	4	−	NEG.
12	"	1:1029	"	"	4	46	8	−	
13	"	1:4096	"	12 30	6	44	12	−	
14	[redacted]	UNDIL.	"	"	1	49	2	−	
15	"	1:4	"	12 33	2	48	4	−	
16	"	1:16	"		2	48	4	−	
17	"	1:64	"	12 40	3	47	6	−	
18	"	1:256	"		2	48	4	−	NEG.
19	"	1:1629	"	"	4	46	8	−	
20	[redacted]	UNDIL.	"	"	41	9	82	+	
21	"	1:4	"	1 55	40	10	80	+	
22	"	1:16	"	"	43	57	43	±	← 1:8? ← ~~1:16~~
23	"	1:64	"	"	3	47	6	−	

TITRATION OF SEVERAL HUMAN SERA & THE ~~DETERMINATION~~ JUN 8 1948

EVALUATION OF THE REACTIVATING POWER OF THE LARGE LOT OF BUESCHER SERUM.

TOXOPLASMA: Pool of 2 four day mice. Prelim test of 1:5 in EB = hep 1/5000 = 0/50 unstained @ 20'. Used immediately for final test.

SERA: [redacted] 3-18-46 } obt. from AMS via Joel Warren to determine whether antibodies present @ that time.
[redacted] -15-46 }

[redacted] -18-48 – standard positive serum.

[redacted] 6-2-48 (= "New") – sample of large pool. Stored for future activation use.

[redacted] } From Cleveland Clinic. Dated 5-28-48

[redacted] -24-47 – obt from AMS (J. Warren) as "acquired toxo inf."

[redacted] – 4 June 48 – From OPD. child has "not developed normally". No stigmata of toxo infection

Test: All sera inactivated just before test & then diluted in NaCl, dispensed to final tubes & stored in refrigerator until toxo-activation added; at that time incubated one hour @ 37°C & then examined in wet film, 0.02 ml + 1 loop meth. blue. Tubes refrig. until read.

Time schedule: PE obtained @ 12^{28} pm. Prelim began @ 12^{30} pm. Final test in @ 1^{15} pm. Out @ 2^{15}.

JUN 8 1948

Tube #	SERUM SOURCE	DIL.	PE 1/5 IN	TIME	EFFECT # UNST.	# STAINED	% UNST.	RESULT	
1	NaCl - CONT.	—	EB	2^{22}	3	47	6	−	
2	[redacted]	UNDIL	"	3^{27}	40	10	80	+	} POSITIVE
3		"	"	"	44	6	88	+	
4		"	NEW E.B.	3^{50}	40	10	80	+	REACTIVATES
5		1:4	0.2 NaCl	"	41	9	82	+	(Activator 1:4) - Pos
6		1:4	0.2 NaCl	"	52	48	52	+	EB reactivates 50% in concentration of 1:8
7		UNDIL.	NEW E.B.(H)	"	6	44	12	−	
8		UNDIL.	E.B.	"	4	46	8	−	NEG.
9	[redacted]	1:16	"	2^{22}	40	10	80	+	
10	"	1:64	"	"	30	20	60	+	TITER 1:64
11	"	1:256	"	"	14	86	14	−	
12	"	1:1024	"	2^{50}	6	44	12	−	
13	[redacted]	1:16	"	"	48	2	96	+	
14	"	1:64	"	"	37	13	74	+	
15	"	1:256	"	"	42	58	42	+	TITER 1:256
16	"	1:1024	"	3^{10}	7	43	14	−	
17	[redacted]	UNDIL	"	"	19	81	19	−	
18	"	1:4	"	"	41	59	41	+	
19	"	1:16	"	"	46	4	92	+	
20	"	1:64	"	3^{15}	35	15	70	+	TITER 1:64
21	"	1:256	"	"	20	80	20	−	
22	"	1:1024	"	3^{20}	3	47	6	−	
23	"	1:4096	"	"	5	45	10	−	

JUN 9 1948

Toxoplasma Antibody Studies With 7 Pigeon Sera.

Toxoplasma: PE pooled from 2-four day mice. Prelim test set up immediately = 0/50 unst. @ 20'

SERA: Obtained from Eden Park pigeons in Aug. '47 & stored as frozen to date. These seven tested to-day, were selected at random.

Test: All sera inactivated just before test -30' @ 56°C. Then diluted in NaCl & distributed to final tubes. Immediately after prelim test finished, PE made to 1/5 in Buercher serum ≈ heparin 1/5000. This distributed as 0.1cc to 0.1cc of the serum dilution. Then incubated for 1 hr @ 37°C & read ≈ 1 loop meth blue to 0.02 ml mixt. Tubes refrigerated until read.

Time Schedule: PE obtained @ 11^15 a.m., Prelim in @ 11^20. Final test in @ 11^55, out @ 12^55 pm.

TUBE #	SERUM SOURCE	SERUM DILUTION	PE 1/5 IN	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN	RESULT	
1	NaCl - CONT.	—	EB	1^07	6	44	12	—	
2	P-2 5 AUG.	UNDIL.	"	"	13 6	37 44	~~19~~	—	
3	"	1:4	"		2	48	4	—	NEG.
4	"	1:16	"		3	47	6	—	
5	"	1:64	"	1^15	3	47	6	—	
6	P-6 5 AUG.	UNDIL.	"	"	4	46	8	—	
7	"	1:4	"	1^17	5	45	10	—	NEG.
8	"	1:16	"	"	2	48	4	—	
9	"	1:64	"	1^25	3	47	6	—	

JUN 9 1948

TUBE #	SERUM SOURCE		DILUTION	PE 1/5 IN Hep	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
10	P-9	5 AUG	UNDIL.	EB	1^{25}	1	49	2	—	
11	"		1:4	"	"	5	45	10	—	NEG
12	"		1:16	"	"	8	42	16	—	
13	"		1:64	"	1^{30}	9	41	18	—	
14	P-10	5 AUG.	UNDIL	"	"	3	47	6	—	
15	"		1:4	"	"	66	34	66	+	Repeat this
16	"		1:16	"	"	42	58	42	+ ?	slim
17	"		1:64	"	1^{35}	14	36	28	—	
18	P-16	7 AUG.	UNDIL.	"	"	6	44	12	—	
19	"		1:4	"	"	3	47	6	—	Neg
20	"		1:16	"	"	1	49	2	—	
21	"		1:64	"	1^{45}	3	47	6	—	
22	P-20	7 AUG.	UNDIL	"	"	4	46	8	—	
23	"		1:4	"	"	3	47	6	—	Neg
24	"		1:16	"	"	3	47	6	—	
25	"		1:64	"	1^{55}	3	47	6	—	
26	P-21	7 AUG.	UNDIL.	"	"	3	47	6	—	
27	"		1:4	"	2^{00}	4	46	8	—	Neg
28	"		1:16	"	"	8	42	16	—	
29	"		1:64	"	"	2	48	4	—	

JUN 10 1948

TITRATION OF HUMAN TOXOPLASMA-IMMUNE SERA.

Sera: [redacted] A45 & A47 = mothers of proved toxoplasma infected babies [redacted] born in May 1947. This bleeding = 9 months later.

[redacted]: Pos. skin test in survey at Children's Hosp., Cinci. = 8 1/2 yr. old.

[redacted]

Toxoplasma: PE pooled from 2, three day mice. Prelim: immediately set up = 2/100 cmct.

Test: All sera inactivated for 30' @ 56°C just before test. Dilutions then made in NaCl & distributed for final test. PE made to 1/5 ± Bruchus serum & kept 1/5000, 0.1cc of each mixed & incubated 1 hr @ 37°C. Then refrigerated until read ± 1 loop of meth blue.

Time schedule: PE obt. @ 11^{08} a.m. Prelim in @ 11^{10} a.m. Final testing @ 11^{45} a.m. & out @ 12^{45} p.m.

TUBE #	SERUM SOURCE	DILUTION	PE 1/5 in (HEP)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT.	
1	NaCl - CONT.	—	EB	12^{50}	0	50	0	—	
				3^{42}	2	48	4	—	
2	[redacted]	UNDIL	"	"	45	55	45	+	
3	"	1:4	"	12^{53}	40	10	80	+	
4	"	1:16	"	"	44	6	88	+	
5	"	1:64	"	1^{05}	36	14	72	+	
6	"	1:256	"	"	55	45	55	+	← 256 ?
7	[redacted]	UNDIL	"	1^{09}	52	48	52	+	
8	"	1:4	"	"	32	18	64	+	
9	"	1:16	"		44	6	88	+	
10	"	1:64	"	1^{10}	43	7	86	+	
11	"	1:256	"		53	47	53	+	← 256
12	"	1:1024	"		6	44	12	—	

JUN 10 1948

TUBE #	SERUM SOURCE	DILUTION	PE 1/5 IN (HEP.)	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
13	[redacted]	UNDIL	EB	1^{45}	8	42	16	−	
14		1:4	"	"	54	46	54	+	
15		1:16	"	1^{30}	59	41	59	+	
16		1:64	"	"	44	6	88	+	REPEAT
17		1:256	"	1^{52}	44	6	88	+	
18		1:1024	"	"	54	46	54	+	← 1024 ?
19	[redacted]	UNDIL	"	2^{05}	32	18	64	+	
20		1:4	"	"	44	6	88	+	
21		1:16	"	2^{30}	59	41	59	+	← 16
22		1:64	"	"	22	78	22	−	
23		1:256	"	3^{03}	3	47	6	−	
24	[redacted]	UNDIL.	"	"	50	50	50	+	
25		1:4	"	3^{05}	44	6	88	+	
26		1:16	"	3^{05}	45	5	90	+	
27		1:64	"	3^{30}	43	7	86	+	← 64
28		1:256	"	3^{30}	28	72	28	−	
29	[redacted]	UNDIL.	"	3^{40}	47	3	94	+	
30		1:4	"	"	48	2	96	+	
31		1:16	"	3^{45}	37	13	74	+	← 16
32		1:64	"	"	10	40	20	−	
33		1:256	"	"	5	45	10	−	

JUN 11 1948

IN VITRO TITRATION SERIAL SERA FROM RAT "BLUE"

SERA: the following sera were available from Rat "Blue" (see protocol for dilution scheme) & were heated for 30' @ 56°C just prior to the test. Serial dilutions c̄ NaCl were made from the heated, undiluted sera. Certain adjustments were made because of small quantities. All specimens were exhausted except for the 35 day serum.

Date	Stage	Quant. For Dilution
24 Feb 48	Pre-inoc.	0.05 cc
1 March 48	5th dy.	0.2 cc
3 Mar 48	7th dy	0.1 cc
10 Mar 48	14th dy	0.2 cc
17 Mar 48	21st dy	0.1 cc
24th Mar 48	28th dy	0.15 cc
31 Mar 48	35th dy	0.2 cc

Toxoplasma: PE pooled from 3, four day mice. 20' prelim in EB = 2/100 unit.

Test: Heated sera dil. in NaCl & simultaneously distributed in 0.1 cc amts to final tubes. Following prelim test, PE made to 1/5 in Bruescher ser c̄ hep 1/5000 & 0.1 cc amts (separate pipettes for each series) added to sera. Incubated 1 hr @ 37°C & then read c̄ 1 loop of MBlue. Tubes refrig. until read.

Time schedule: PE obt. @ 10^{38} a.m. Prelim in @ 10^{40} a.m. Final test in @ 11^{15} am & out @ 12^{15} p.m.

RAT "BLUE"

JUN 11 1948

ALL SERA INACTIVATED

TUBE #	SERUM SOURCE	DIL.	PE 1/5 IN (HEP)	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
1	NaCl - CONT.	—	EB	12^{20}	5	45	10	—	
				2^{49}	7	43	14	—	
2	PRE-INOC. 2-24-48	1:4	"	"	3	47	6	—	
3	"	1:64	"	"	5	45	10	—	
4	5th POST-INOC. DAY 3-1-48	UNDIL.	"	"	25	25	50	+	dirty serum. Repeated c̄ thionin = 54% unst.
5		1:4	"	12^{40}	42	8	84	+	
6		1:16	"	"	68	32	68	+	
7		1:64	"	12^{42}	52	48	52	+	← 1:64
8		1:256	"	"	17	33	34	—	
9	7th POST-INOC. dy 3-3-48	1:4	"	12^{44}	35	15	70	+	
10		1:16	"	"	45	5	90	+	
11		1:64	"	1^{05}	44	6	88	+	
12		1:256	"	"	37	13	74	+	← 1:256
13		1:1024	"	"	40	60	40	+	44% unst. c̄ thionin
					44	56	44 — thionin		
14		1:4096	"	"	10	40	20	—	
15		1:16384	"	"	8	42	16	—	
16	14th POST-INOC. dy. 3-10-48	UNDIL.	"	1^{25}	14	36	28	—	
17		1:4	"	1^{12}	54	46	54	+	
18		1:16	"	"	43	7	86	+	
19		1:64	"	1^{20}	40	10	80	+	
20		1:256	"	"	38	12	76	+	← 1:256
21		1:1024	"	1^{22}	33	67	33	—	
22		1:4096	"	"	8	42	16	—	
23		1:16384	"	"	6	44	12	—	

"RAT BLUE"

JUN 11 1948

ALL SERA INACTIVATED

TUBE #	SERUM SOURCE	DIL.	DE/S IN (Hep)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT	
24	21st Post-Inoc. dy 17 Mar.	1:4	EB	1^{30}_{-}	37	13	74	+	
25		1:16	"	"	40	10	80	+	
26		1:64	"	2^{05}_{-}	38	12	76	+	
29		1:256	"	"	60	40	60	+	← 1:256
28		1:1024	"	"	10	40	20	−	
29		1:4096	"	"	9	41	18	−	
30		1:16384	"	2^{12}_{-}	5	45	10	−	
31	28th Post-Inoc. dy, 3-24-48	1:2	"	"	37	13	74	+	
32		1:4	"	2^{15}_{-}	41	9	82	+	
33		1:16	"	"	46	4	92	+	
34		1:64	"	2^{22}_{-}	37	13	74	+	
35		1:256	"	"	36	14	72	+	← 1:256
36		1:1024	"	2^{25}_{-}	10	40	20	−	
37		1:4096	"	"	7	43	14	−	
38		1:16384	"	2^{32}_{-}	5	45	10	−	
39	35th Post-Inoc. dy. 3-31-48	undil.	"	"	34	16	68	+	
40		1:4	"	2^{35}_{-}	40	10	80	+	
41		1:16	"	"	46	4	92	+	
42		1:64	"	2^{39}_{-}	44	6	88	+	
43		1:256	"	2^{39}_{-}	65	35	65	+	← 1:256
44		1:1024	"	2^{45}_{-}	12	38	24	−	
45		1:4096	"	"	5	45	10	−	
46		1:16384	"	$2^{?}_{-}$	5	45	10	−	

JUN 12 1948

The Effectiveness of Various Dyes In the In Vitro Toxoplasma Test.

DYES:

a) METHYLENE BLUE – REAGENT GRADE, G. FREDERICK SMITH CHEMICAL CO., COLUMBUS, OHIO. $C_{16}H_{18}N_3ClS \cdot 3H_2O$ (Abt. from S. Report.)

b) THIONIN (LAUTH'S VIOLET) – COLEMAN & BELL. CERT. #-CT-1 87% dye.

c) THIONIN BLUE GO – COLEMAN & BELL. #380802.

d) SODIUM 2,6-DICHLOROBENZENONE-INDOPHENOL – #3463, EASTMAN KODAK CO.

PREPARATION:

Each of the above dyes was weighed out this morning & dissolved in distilled H_2O shortly before use to make 0.5% solutions. The actual amounts were as follows:

a) Meth. Blue = 0.1006 gms.
b) Thionin = 0.1012 gms
c) Thionin Blue GO = 0.1043 gms.
d) Na – indophenol = 0.1 gms

} 20 cc dist. H_2O added to each.

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Observations On Centrifuged Toxoplasma ~~Suspended In~~ OBTAINED FROM 1)

Normal & Immune Serum. MIXTURES

Sera: [redacted] c̄ PE 1/5 & hep 1/5000 (Normal)

Kope dil 1:1 with PE 1/5 in Buescher (of above)

Suspensions: Incubated for 1 hr @ 37° C & then stored in refrig. for

Centrifugation: 1.0cc of each of above spun for 15' at ca 2000 rpm. Super discarded, sides of tubes washed c̄ 1.5cc NaCl, this removed, and the buttons washed into 0.5cc NaCl. This was done c̄ difficulty leaving a granular suspension.

Slide examinations:

a) Meth. blue ph 11.0. 0.02 cc susp. 1 loop meth bl. 2^{22} pm.
Description holds for 3^{40} pm.

(1) Control: Toxo more deeply stained than in presence of serum. Chromatin not visualized.

2) Immune: No staining of toxoplasma c̄ the few expected exceptions. Toxo grey under high dry but these are found to be free of dye when examined under oil. Chromatin not visualized under oil.

b) Thionin (Lauth's Violet) 1:5 of 0.5% Prep. 2^{40} pm. Final exam @ 3^{45} pm.

(1) Control: High dry - normal deep purple marginal staining of cytoplasm. Chromatin seen as separate structure. Toxo appear mostly rounded. Some leucocyte nuclei are stained red-purple, others not stained. Those leucocytes that have stained nuclei appear to be lymphocytes.

(2) Immune: Toxo are lanceolated c̄ red chromatin & grey cytoplasm under high dry while under oil, chromatin is red but the cytoplasm is clear so that the greyish appearance of the

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cytoplasm is not due to blue dye but rather results from diffraction. Those few tryp which have resisted the antibody also are recorded in this preparation as in the control.

c) Nile Blue Sulfate – (Sat. aqueous sol. old – 0.7-3.4% ?) 0.02 cc plus 2 loops of dye. Prep. @ 3^{24} Recorded @ 4^{24}.

1) Control: Under high dry, intense blue staining of tryp as well as of leucocytes. Bright-red staining droplets noted in tryp but especially prominent in leucos. Under oil, tryp appear lanceolated, diffusely stained blue with, as a rule, not more than one red droplet in each. Intensity of staining makes it difficult to find the red droplet in the tryp but some have as many as 3-4 of these minute droplets.

2) Immune: Under high dry, tryp also stained an intense blue, cannot be differentiated from the control. Under oil, the tryp are seen to be deeply stained, but the outline of the parasites is not quite normal. The cell margin suggests crenation. The red-staining globules appear about as often & are no larger or smaller than in the control.

d) Nile Blue Sulfate (Prep. in Felix Hoff. = 0.5% dye, 20% alcohol) Slide prep. @ 3^{15} μm. Recorded @ 4^{15}

1) Control: Tryp have faint shade of blue under high dry but color is paler under oil (stronger light ?) and minute red globules are seen with difficulty.

2) Immune: High dry – tryp appear almost without blue coloration altho a faint blue reflex is obtained on focusing.

d) Cont. :

2) Immune (cont). : Oil : toxo appear without any blue coloration although tiny red droplets may be seen.

e) Sudan IV (Sat. alc. sol., old bottle) - 0.02 cc + 2 loops dye @ 3^{05} pm. Recorded @ 4^{30} pm.

1) Control : High dry - much ppt. Oil - each toxo has a varying number (but not exceeding 6-8) of minute red-staining globules. No other staining except for the red globules.

2) Immune : High dry : immediately after preparation, toxoplasm showed what appeared to be somewhat larger (both number & size) red globules.

f) Na - Indophenol (0.5% aqueous). Prep @ 4^{55} - Exam. 5^{05} - 5^{15} pm.

1) Control : High dry - degs blue background, toxo appear darker than background but difficult to tell color.

Cont. toxo stained because stain mixtures did not appear to take up indophenol.

Oil : No staining what-soever of toxo except for tiny dark globules similar in size, shape, & position to those staining red = Nile Blue SO_4 & Sudan IV.

2) Immune : Exactly like the control under high dry & oil, including tiny globules.

3) PE undiluted - used to-day - also examined at same time (5^{15} pm.) - 0.02 cc + 1 loop dye. Some leucos have granules which take deep blue stain but intracellular & extracellular toxo are contained except for the granules described above.

∴ Toxoplasma, intra- & extra cellular, do not take up any indophenol - even those that have stood for 3 hrs. = the indicator.

(see p. 4)

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Uncentrifuged Serum - Toxo Preparations

1. Na – indophenol: Prepared @ 1:15 pm. Recorded @ 4:40.

a) Control - high dry. At this time toxo appear to be barely stained light blue (when freshly prepared, appeared definite blue).

(See Cont.) No dye taken up by toxo.

2. Meth. Blue 0.5% aqueous. Prep. @ 1:57 pm. Exam @ 5:30.

a) Control:
Oil & high dry – both immediate & now, toxo deeply stained blue all over. Some are rounded up & concentration of dye on periphery as with thionin. Chromatin when seen is stained blue.

b) Immune: both immediately & now, cytoplasm unstained but the toxo chromatin is a deep blue.

3. Thionin 0.5% aqueous Prep. @ 1:37 Exam. @ 5:35 pm.

a) Control - marked precipitation of dye. Typical hepatic inclusion appearance & magnification.

b) Immune: chromatin red, distinctly outlined & cytoplasm is completely unstained. No muddy appearance as with methylene blue. Background clear.

4. Thionin 0.5% aqu 1:5 & dist H_2O. Prep 2:52 Exam 5:40 pm.

a) Control – immediately and after 3 hours toxoplasma show only a faint pink hue – high dry and oil

b) Immune – cytoplasm unstained; chromatin unstained in most – faint pink in few.

Too dilute for use

JUN 12 1948 (5)

5. Thionin 0.5% aqueous 1:2 ± dist. H_2O – prep. 5:57

Control – Immed. & 30 min. later – toxo appear only pink throughout without deep st. of chromatin

Immune – as with 1:5

Note: Per. exudate undil. + thionin 0.5%-undil. – some herpetic like but many not
6:15 PM " " + thionin (0.5%) 1:2 – most poorly stained

6. Repeat – Thionion – 0.5% aqueous – undil.
6:25 P.M.

Control – no ppt. this time – some toxo typical but very many not – only bluish staining – not too good

Immune – some slight ppt. – toxo gray cytoplasm red chromatin

Note: apparently this concentration is on borderline of giving regular differential staining – and slight differences in quantities can influence results.

7. Comparison with old (Feb. 4) Thionin blue GO 0.5% in alcohol & water. 6:45 PM

Control – some typical; others not (sl. ppt).

Immune – Almost all very clear, unstained cytoplasm – very red chromatin

8. Thionin blue GO of 6-12-48

Prep. 1:50 P.M. – reexamined 6 P.M.

Control & Immune – just like the methylene blue – i.e. deep staining in control & in immune – no staining of cytoplasm chromatin ~~red~~ blue.

Centrifuged preparations (continued)

JUN 12 1948

Methyl Violet (prep. of Feb. 4) – 0.5% in alcohol + water used undiluted

1:40 P.M. Control + Immune – very deeply stained – cannot tell any difference.

Methyl Violet – above preparation 1:5 in dist. H_2O

Control – very deep violet staining and irregular granulation in cytoplasm. Chromatin also stained violet hue.

Immune – also stained throughout except that cytoplasm doesn't show as much granulation as in control while chromatin is deeply stained

Repeat with Thionin – undiluted (same prep used earlier 1:5)

Too much ppt. on dirty slide to be able to tell — should be repeated.

JUN 12 1948

In Vitro Titration Of Olsen (Detroit) Sera

Sera: Rec'd from Dr. Montgomery, Detroit, Mich. on 10 June 48. Had been mailed 9 June 48 & were partially frozen (c̄ free rbc) on arrival here. Blood specimens had been obtained on 1 June 48 & kept frozen in a deep freeze until shipment.

a) [redacted] born 22 Feb '47. Convulsive movements face began @ 2 mos c̄ onset of periodic epidodes of fever, convulsions, & diminution of fever @ 6 mos. of age. Has bilateral chorioretinitis but no cerebral calcifications.

b) [redacted] Normal & well. Had previous child in 1943 who is also well.

Test: Sera are hemolyzed. Diluted to 1/64 c̄ NaCl but not inactivated. 0.1cc amts of serum mixed c̄ PE dil 1/5 in Buescher serum c̄ hep 1/5000. Incubated 1 hr @ 37°C & then read c̄ 1 loop of meth blue.

Toxoplasma: Pooled from 3 four day mice. Set up in test without prelim test. Parasites nice looking but less numerous than usual.

Time schedule: PE obt. @ 10^{30}. Test in @ 10^{45} a.m. Out @ 11^{45} a.m.

Tube #	SERUM SOURCE	SERUM DILU.	PE 1/5 in (HEP.)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT.	
1	NaCl –	—	EB	11^{45}	3	47	6	—	
2	[redacted]	undil	"		4	46	8	—	
3	"	1/16	"		3	47	6	—	[illegible]
4	"	1/64	"		2	48	4	—	
5	[redacted]	undil	"	12^{10}	4	46	8	—	
6	"	1/16	"		5	45	10	—	[illegible]
7	"	1/64	"		3	47	6	—	

Effect of Hydrogen Peroxide

JUN 14 1948

Studies on the Mechanism Of The "Non-Staining" of Toxoplasma By ~~with~~ Meth. Blue In The Presence of Immune Serum.

Plan: To incubate PE & serum for 1 hr, then add meth blue to tube, wash free meth blue away with saline (cent.), then add H_2O_2 to determine whether non-stained parasites will become blue. If so, then they apparently take up the dye but reduce it to the leuco-base.

Procedure:

1) A large amount of "A" exudate was pooled from 3, four day mice at 2:5 p.m. & when it was found to have many toxoplasma, 2 cc were made to 1/5 with 8.0 cc Bruscher serum & hep 1/5000 (final). Five cc of this was immediately mixed ā 5.0 cc of Rh 152, 21 day serum diluted 1/64 with saline. (The latter was made in a flask by pipetting in 0.2 cc of the serum & then adding 12.6 cc Na Cl). For control, 2 cc of the 1/5 PE was mixed ā 2.0 cc Na Cl. These tubes were then incubated for 1 hr @ 37° C & examined in the wet film with ± 2 loops of meth blue. Surprisingly, the monkey serum-toxo mixture contained almost all stained parasites, just like the control. Numerous bacteria were noted in the exudate & so it was thought that perhaps they had produced an "inhibitor" to the reaction. Consequently, it was decided to investigate this point by using another exudate. (see below)

Second Test: To determine whether Rh #152 ser (21st dy) or the exudate used above was at fault in giving the negative test. Toward this end, a single three day exudate was obtained @ 4:30 p.m & labelled "B." The following test was then set up:

(over)

JUN 14 1948

Tube #	SERUM	EXUDATE 1/5 IN BUESCHER (HEP. 1/5000)	after 1h @ 37°C
1	[redacted]	B (1.0 cc)	almost all unstained.
2	* RH. 152-21st dy. 1/64 (1.0 cc)	B (1.0 cc)	almost all stained
3.	NaCl (1.0 cc)	B (1.0 cc)	" " "
4	[redacted]	A (0.1 cc)	almost all unstained
5	* 0.1 cc RH 152-21st dy 1/64	A "	almost all stained
6	0.1 cc NaCl.	A "	" " "

* This was the same 1/64 serum used in the first test described on the other side of this sheet.

Interpretation: Since the two exudates are active with Rose serum, the fault lies with the monkey serum. The possibilities rest between:

a) The serum is not active & previous tests are wrong. This is unlikely & will be retested to-morrow.

b) No serum was used to make the dilutions. But I remember distinctly placing it in the flask first before adding saline.

c) There was some toxic residue in the bottom of the flask which inactivated the 0.2 cc serum first placed there. Most likely.

d) This serum was diluted "fresh" to-day whereas in previous titrations it was inactivated, thus an extremely high zone was present to-day because of no antibody knock-down by heat. Very unlikely.

W. RONG → SALINE HYPERTONIC

JUN 15 1948

In Vitro Checks On Monkey Serum (Rh #152) @ 1:64

Obj: The 21 day serum of Rh #152 which when titrated on 21 May '48 yielded an in vitro titer of 1:4096 was used yesterday at a dilution of 1/64, unheated, but was negative in the in vitro test. This was difficult to explain except that heated (in the titration) & unheated serum might differ to that extent & thus we had run into a zone or that something was inherently wrong with the 2 exudates used yesterday – the latter is hardly likely. The toxo were shrunken in the 6/14 test

Morning test:

1. Sera: The 21st & 26th post-inoc. day sera from Rh #152 were inac. as usual. serum for 30' @ 56°C just before the test & then dil. to 1:64 c Na Cl. & 0.1cc distributed to appropriate tubes. Simultaneously similar amounts of fresh, unheated serum were diluted in the same fashion & also distributed.

2. Toxoplasma: PE "B" obtained from 1 four day mouse for the photographic studies was dil to 1/5 c Bunker serum & kep 1/500. to make kep. 1/5000. This exudate has been in the refrigerator for 1 hr & 5 min. before use in this test.

3. Test: 0.1cc toxo in EB plus 0.1cc serum-mixt. incubated 1 hr @ 37°C & then read immediately thereafter using 0.02 ml of the mixt. & 1 loop of meth blue 11.0 made yesterday & /or that made on 3 April & used for all titrations past several weeks. There was so much ppt in the meth blue made yesterday that it was impossible to any sort of counting. Consequently, the "old" meth. blue was used & yielded good differentiation with a perfectly clean background.

Test out of incubator @ 11:00 a.m.

JUN 15 1948

Tube #	SERUM SOURCE	DIL.	PE VS in (HEP.)	TIME.	EFFECT # UNSTAIN.	# STAINED	% UNST.	RESULT.	
1	NaCl - CONT.	—	EB	11 45	22	78	22	—	Figures recorded are those obtained with the old "meth blue."
2	RH. 152 - 21dy (F)	1/64	"		79	21	79	+	← ē "new" dye = 39/100 unst.; BUT MARKED PRECIPITATE INTERFERED WITH READING UNSTAINED TOXO.
3	" 21dy (H)	1/64	"		66 65	34 35	66 65	+	← ē new dye = 39/50 unst.;
4	RH. 152. 26dy. (F)	1/64	"		82	18	82	+	
5	" " (H)	1/64	"		85	15	85	+	

AFTERNOON TEST:

Because the ~~afternoon~~ morning test had been done with an exudate that was over an hour old, the entire test was repeated from scratch with a new exudate obtained from a single four day mouse. The test was set up within 15 min. after the exudate was obtained. All inactivation & dilutions & incubation performed as in the morning. All tests read by ABS using the meth bl pH 11.0 made on 14 June '48. This time there was little precipitate in some & almost none in other tubes.

1 HR incubation completed @ 3 10 pm. Tests read from 4 15 on. In refrig. until read. Remtn serum obt. yesterday prior to this test.

TUBE	SERUM SOURCE	DILUTION	PE VS in (HEP.)	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
1	NaCl. - CONT.	—	EB	4	21	8	—	V. FEW TOXO. READ WITH DIFFICULTY.
2	RH. 152 - 21dy (F)	1/64	"	—	—	88	+	
3	" " (H)	1/64	"	28	22	56	+	
4	RH. 152 - 26 dy (F)	1/64	"	45	5	90	+	
5	RH 152 - 26dy (H)	1/64	"	21	4	84	+	
6	[redacted]	UNDIL.	"	99	1	99	+	←

JUN 16 1948

The Effect of Repeated Washing upon Tube-Stained Toxoplasma

Obj: Since the toxoplasma which had been treated with normal serum, then stained & washed with saline, appeared to lose their stained characteristics, this experiment will check this finding in the absence of serum, preparatory to repeating with serum.

Exudate: Pooled from four, four day mice, rich in parasites.

Procedure: 1.0 cc units of PE distributed to each of two very narrow cent tubes. To one was added 0.5 cc of meth bl. pH 11.0 (made on 14 June from sat. alc. meth. bl.) while the other rec'd 0.5 cc of 0.5% aqueous meth. blue (made on 12 June 48). After thoro mixing, each was examined in a wet film. The preparation made from the aqueous meth blue contained many well stained parasites but also about 30% unstained and so this tube was discarded. (0.5 cc more dye was added but this did not change the picture.) On the other hand, the preparation made from the meth bl pH 11.0 contained almost all beautifully stained parasites & so was selected for further trial.

Further studies with PE + Meth Blue pH 11.0: Approx 2.5 cc of NaCl were added to the PE-dye mixture, well mixed & this again examined under the scope. Tox staining unchanged so this was cent. for 10' @ 2000. The deep blue pellet was easily seen against a bright light so the supernatant was aspirated off. This was then rinsed 3x with NaCl as until the super was clear. Then the pellet was resuspended in 0.5 cc NaCl (became light blue) & reexamined in the scope. Tox staining unchanged, background cleaner. Tube filled with saline, cent again for 10' @ 2000. Super very light

blue, sediment deep blue. Super removed, pellet deep blue. Rinsed 2x with NaCl, pellet suspended in 0.5 cc NaCl (light blue) & examined. Toxo still well stained. Again filled w NaCl & cent 15' @2000. Rinsed 2x, super clear, pellet blue so latter resuspended in 0.5 cc NaCl = well-stained toxo. Tube filled, spun 10' @2000, super removed, blue pellet rinsed 2x, then resuspended & spun for 10' @2000, super removed, pellet rinsed 2x NaCl then resuspended in 0.5 cc NaCl & examined. Toxo perfectly well stained, both intra & extracellularly.

∴ Repeated rinsing of dog plasma derived from PE mixed w a half volume of Methyl Blue pH 11.0 fails to obtain the parasites

This may be true of alcoholic

not found to be true of aqueous

pH 11 on 6/26/48

17 June 48

In Vitro Check On Toxoplasma Skin tests

Toxoplasma: Pooled from 2-four day mice. Toxo numerous.

Sera: [redacted] (works for A.J. Steigman.) Serum obt. prior to skin test on 1st Jun.

b) [redacted]: In Ch. Hosp with chorea. Skin test mild pos. Serum drawn this a.m.

c) [redacted] Has had FMO for 1 year. Skin test markedly positive yesterday. Serum obtained this a.m.

Test: All sera unheated. 0.1cc amts mixed c̄ 0.1cc PE 1/5 in Buesleher serum c̄ hep 1/5000. Inc. 1 hr @ 37° C & then read as usual.

Tube.	Serum	PE 1/5 in	Result		
1	NaCl	EB	6/50 unstain.	−	12%
2	[redacted]	"	49/50 "	+	98%
3		"	49/50 "	+	98%
4		"	50/50 "	+	100%

Note — the very high percentage of unstained toxo in mixtures with fresh unheated sera and toxo in activator. This suggests that the amount of activator ~~is~~ determines whether or not close to 100% fail to stain, and that the 50% of fresh serum usually used does not contain enough for 100% effect

See test of 18 June c̄ heated Bernstein serum

Mechanism of the Toxoplasma - Antibody-Accessory Factor Reaction — as determined by the dye affinity test

JUN 17 1948

Questions I) Does the antibody in immune serum combine with the toxoplasma in the absence of "accessory factor"?

II) Does the "accessory factor" ~~combine~~ in normal serum combine with toxoplasma in the absence of antibody.

Reagents: Toxoplasma — peritoneal exudate from two mice inoculated i.p. 4 days before; many toxoplasma present; diluted with equal volume of saline

~~Immune~~ Antibody — Serum of Bernstein (previously found to yield 99% unstained toxo with fresh serum) — heated at 56°C for ½ hour.

Accessory Factor — Serum of Buescher frozen since

Methylene Blue —

Procedure — to answer question I:

1) 0.5cc. heated antibody + 0.5cc. toxoplasma + heparin (0.01cc of 1:100)
2) Mixture incubated at 37°C (water bath) for one hour
3) 0.02cc of incubated mixture + 1 loop of methylene blue → all toxoplasma deeply stained (i.e. no antibody effect)
4) Mixture centrifuged (after addition of 1cc saline) in narrow conical tube for 10 min. at @ 2000 rpm. on horizontal centrifuge — sediment forms tiny pellet at bottom (estimated at about 0.01cc in volume or less). Sn. liquid drawn off to pellet. 4cc of saline added (dilution of serum now 1:8000) and pellet broken up with wooden applicator. Recentrifuged for 10 min. at 2,000 rpm. — sn. liquid drawn off. 0.5cc of saline added and pellet again broken up (dilution of original serum now 1:400,000)
5) 0.5cc. washed "antibody-treated" toxoplasma + ~~=~~ 0.5cc fresh Buescher serum (accessory factor). (dil. antibody now 1:800,000)

Examinations — 0.02cc. mixture + 1 loop ~~stain~~ meth. blue.

Immediate examination — all toxoplasma stained

After 30 min. at 37°C — 78% of toxoplasma unstained (chromatin and intracellular toxoplasma deeply stained)

60 min at 37°C — 92% of toxoplasma unstained, i.e. antibody combined with toxo in absence of accessory factor

(over)

Procedure: Answer to question II:

1. 0.5cc accessory factor + 0.5cc toxoplasma + heparin (0.01cc of 1:100)
2. Incubated water bath at 37°C for 1 hour
3. Mixtures examined as in I – approximately 95% of toxoplasma deeply stained
4. 1cc of saline added and centrifuged for 10 min at 2000 rpm in narrow conical tube. Sn. liquid removed; 4cc of saline added to tiny pellet – mixed with wooden applicator. Recentrifuged at 2,000 rpm for 10 min. Sn. liquid discarded. Resuspended in 0.5cc saline.
5) 0.5cc of washed, "accessory-factor-treated" toxoplasma + 0.5cc of heated antibody

Immediate examination – toxoplasma almost all stained (not counted but estimate of 90-95% stained)

30 min. at 37°C – 90-95% deeply stained

60 min at 37°C – 78% deeply stained

∴ accessory factor, unlike antibody, did not combine with toxoplasma in absence of antibody.

Preliminary

Check on heated antibody and accessory factor used in test
4 Mixtures 1 hour at 37°C

Saline + toxoplasma 1:5 in Buescher serum – 6/50 unstained = 12% unstained

Heated antibody (Bernstein) + " " " " – 49/50 " = 98% "

MAIN CONCLUSIONS

1) Specific antibody combines with toxoplasma in absence of "accessory factor" but this does not interfere with ~~staining~~ staining of toxo cytoplasm by dye

2) "Accessory factor" does not combine with toxoplasma but it acts after toxoplasma have combined with antibody, and this action results in a [illegible] staining of the toxo cytoplasm

JUN 17 1948

Significance of Lack of Cytoplasm Staining in Antibody-Treated Toxoplasma.

Questions: I Does it mean that the dye combines with the cytoplasm but is reduced to its "leuko" base?

or

II Does it mean that the combining groups which have an affinity for the dye have combined with the antibody or been changed as a result of antibody-accessory factor action?

Plan of Experiment:

Procedure: Toxoplasma + "Antibody-Accessory Factor" — 37°C for 1 hour
Add Methylene Blue
Centrifuge and wash toxoplasma
Add ~~2.5%~~ fresh H_2O_2 to final concentration of 0.5% — mix and examine.

If cytoplasm of Toxoplasma remain unstained — combining groups are locked up, altered or destroyed

If cytoplasm of toxoplasma stains blue on addition of hydrogen peroxide — we must interpret reaction as indicating that the dye combines with the cytoplasm but is reduced to its "leuko" base in the antibody treated parasites.

Procedure: Reagents — Toxoplasma — undilut. perit. exudate from two mice - 4 days after inoculation

Antibody — Bernstein serum — frozen

Normal Serum — Buescher frozen

A — 1cc. toxoplasma + 1cc antibody serum
B — 1cc " + 1cc normal serum
} Incubate at 37°C for 1 hour

Examined with methylene blue and almost all stained in B and unstained in A.

1cc of methylene blue (sat. alcoholic + borate buffer pH 11) added to each tube.

Both tubes centrifuged at 2,000 rpm for 10 min. Sn. blue liquid removed leaving pellet at bottom. Sides of tubes washed without disturbing pellet. 5cc of saline added to each and pellet resuspended. Centrifuged again at 2,000 rpm. for 10 min.

(over)

Sn. liquid removed from each tube and pellet resuspended in 0.5cc saline.

0.02cc of each examined in cover-slip preparation:

Normal — almost all deeply stained

Antibody — great majority unstained but quite a few stained — it appeared as if many of the intracellular masses had ruptured.

Hydrogen peroxide — fresh bottle of superoxol — 1% H_2O_2 in saline prepared.

0.5cc of 1% H_2O_2 in saline + 0.5cc resuspended toxoplasma (A) - also (B)
Stirred thoroughly by repeated pipetting.

Allowed to stand for 15 to 20 min. and

0.02cc from each tube examined microscopically.

Antibody-treated toxo — no change — cytoplasm remained unstained; chromatin deeply stained as before.

"Normal" toxo — no change — all deeply stained

(Much free oxygen bubbled up underneath cover slips)

Conclusion: Hydrogen peroxide did not bring about staining of cytoplasm in antibody-treated toxoplasma which had been mixed with methylene blue.

Therefore:

Methylene blue did not combine with antibody-treated toxoplasma cytoplasm

JUN 18 1948

Re-check on Zone Phenomenon Observed in Previous Tests.

REAGENTS – TOXOPLASMA – peritoneal exud. from 2 mice 4 days after i.p. – obtained 10:23 AM – abundant toxo seen in cover slip preparation

Preliminary test with exud. 1:5 in Buescher serum + heparin (ad final conc. 1:5000) – 10:40 AM 20 min at 37°C = 2/100 unstained; all others deeply stained & in good condition

SERA – Farber A-47, Rabbit 349, & Rudnick Rhesus No. 2 – (14 weeks after inocul.)

Sera heated at 56°C for 30 min. before obtaining exudate and dilutions prepared in saline (fresh sterile bottle from media Kitchen)

Peritoneal exudate 1:5 in Buescher serum added to all tubes and put in at 37° at 11:15 AM Out at 12:15 P.M.

TUBE	Serum		No. UNSTAIN	No. STAIN	% UNSTAIN	Remarks
1	None - saline control		0	50	0	All crenated
2	[redacted] – A47 –	undilut.	32	68	32	No crenation seen
3	"	1:4	1	49	2	All crenated
4	"	1:16	0	50	0	" "
5	"	1:64	1	49	2	" "
6	"	1:256	0	50	0	" "
7	"	1:1024	0	50	0	" "
8	"	1:4096	0	50	0	" "
9	Rabbit 349 –	undilut.	7	93	7	No crenation seen
10	" –	1:4	0	50	0	All crenated
11	"	1:16	0	50	0	" "
12	"	1:64	0	50	0	" "
13	"	1:256	0	50	0	" "
14	"	1:1024	} not examined			
15	"	1:4096				
16	"	1:16384				
17	Rhesus 2 (14wks)	undil.	40	10	80	No crenation
18		1:4	0	50	0	all crenated
19		1:16	0	50	0	" "
20		1:64	} not examined			
21		1:256				
22		1:1024				
23		1:4096				
24		1:16,384				

(over)

NOTE: It became apparent that the toxoplasma were markedly shrunken and crenated (although deeply stained) in all tubes to which saline had been added, but not in tubes with only serum present or in exudate per se.

It was also recalled that on the two previous occasions when "things went bad", the same crenations was noted.

The following tests were therefore carried out:

Toxoplasma 1:5 in [redacted] serum by itself — no crenation
" " " " " + equal part of saline used in test — all crenated
" " " " " + " " of Saline in capped bottle (Abbott's commercial) — no crenation.

It was apparent, therefore, that the particular batch of saline used in the test was responsible for the crenation — and the following tests were then carried out on various batches of saline:

2 loops of saline + 1 loop fresh peritoneal exudate + 1 loop methylene blue.

SALINE from big bottle prior to sterilization — no crenation

Other bottles from same shelf — Bottle No. 1 — many good; some slightly shrunk about 10-20% crenated
" " 2 — O.K. (toxo mostly all rounded)
" " 3 — OK.
" " 4 — only few slightly shrunk?
" " 5 — all good with few questionable exceptions
" " 6 — OK
" " 7 — OK
" " 8 — OK
" " 9 — all markedly crenated
" " 10 — OK
" " 11 — OK
(Bottle originally used in test) " " 12 — all crenated.

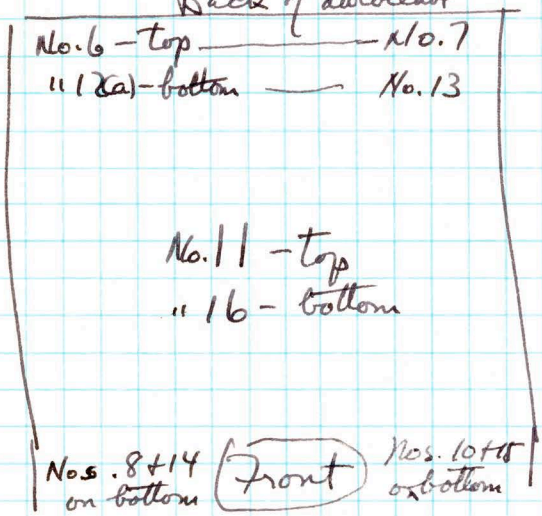
JUN 18 1948

Investigations of Factors Responsible for "Bad" Creating Quality of Saline in Some Sterilized Bottles:

I Test of Saline in Bottles Prior to Autoclaving

Bottle No. 12a – O.K. (no creation
" " 13 – "
" " 14 – "
" " 15 – "
" " 16 – "

II Check on possibility that "some impurity" comes in with the steam during autoclaving – going through the paper and cotton plugs. The steam comes in from a feed pipe in front of the autoclave. Therefore, 10 bottles, tested earlier and found O.K. were put in various locations in the autoclave to determine whether those directly under the steam inlet will develop the impurity.



Bottles 6, 7, 8, 10, 11, 12a, 13, 14, 15, and 16 autoclaved 15 lbs. for 20 minutes

June 19 – All above tested with fresh peritoneal exudate and none produced creation of toxoplasma.

Note !!! – Chemical analysis of bad saline revealed that it was 8.5% instead of 0.85%

18 June 48

Titration of Bernstein Serum

Toxoplasma: pooled from 4-4 dy mice.

Serum: [redacted] (lab. asst.) of 14 June. 48

Test: Serum inac for 30' @ 56° C just prior to test & then diluted in saline. 0.1cc amts of serum & toxo 1/5 in Bruescher serum c̄ hep 1/5000 incubated 1 h @ 37° C & then read c̄ 1 loop of meth bl for 0.02 ml of mixture. In addition, fresh Bernstein & Bruescher sera (0.5cc) were set up with PE 1/5 in NaCl 0.5cc for ABS.

Time: PE obt @ 2^05 pm. & final test in @ 2^30 pm.

Tube	Serum		PE 1/5	Result	
1	NaCl		EB	4/50 unst. = 8%	—
2	[redacted]	undil.	"	48/50 " = 96%	+
3	"	1:4	"	50/50 " = 100%	+
4	"	1:16	"	32/50 " = 64%	+
5	"	1:64	"	9/50 " = 18%	—
6	"	1:256	"	4/50 " = 8%	—
7	"	1:1024	"	2/50 " = 4%	—

TITER = 1:16

Check on Zone Phenomenon

JUN 19 1948

Repeat test on serum of Rabbit 349 which did not work yesterday – "because of bad saline".

Toxoplasma – Peritoneal exudate of two 4-day mice

Preliminary test – Per. exud. – 0.05 cc
Heparin 1:500 – 0.025 cc
Buescher serum – 0.20 cc
Saline – 0.25 cc
} after 20 min. at 37°C
1/50 – unstained; all others in good condition. No shrinkage

– Saline test – exudate 1:20 in saline – no "

For final test – Per. exud. – 0.2 cc
Hep. 1:100 – 0.02 cc
Buesch. ser – 0.8 cc

Serum – heated at 56°C and dilutions prepared prior to testing of toxoplasma. Mixtures – 1 hour at 37°C

Tube	Serum	No. unstain.	No. stain	% unstained
1	Saline	0	50	0
2	Rabb. 349 – heat. – undil.	2	48	4
3	" " – " – 1:4	28	72	28
4	" " " 1:16	25	75	25
5	" " " 1:64	38	12	76
6	" " " 1:256	41	9	82
7	" " " 1:1024	38	12	76
8	" " " 1:4096	38	12	76
9	" " " 1:16,384	9	41	18

(over)

Test for "Spontaneous" Activity of Pool of Guinea Pig Serum Used for Complement

JUN 19 1949

G.Pig Pool – Lot of 4-14-48 frozen in dry ice

Repeated titrations have shown that it contains 200 units of complement per cc. (i.e. 0.25cc of 1:50 is one unit in presence of antigens used – without antigen it titers 0.2cc of 1:30 or 150 u/cc)

Per. expd. (same as for other test) diluted 1:5 in saline

Mixtures incubated 1 hour at 37°C

	No. Unstain	No. Stain	% Unstain
G. pig pool – undil	45	5	90
" 1:2	37	13	74
" 1:4	7	43	14
" 1:8	5	45	10
Saline Control	0	50	0

JUN 19 1948

Immune Phenomenon with Toxoplasma Previously Stained with Methylene Blue

Note: It had been observed previously that when mixtures of toxo, immune serum (active) and methylen blue were made directly on the slide — and observed for several hours at room temperature — no decolorization of the cytoplasm appeared

Purpose: to determine whether in the spots when methylene blue combines first with the toxoplasma — the immune phenomenon can no longer be demonstrated

Reagents — 1cc of perit. exudate + 0.02cc of heparin 1:100

Immune Serum — Bernstein
Normal " — Buescher

Methylene blue — pH 11, borate buffer preparation using sat. alcoholic M. Blue. (i.e. finals 0.1% in 4% alcohol)
— 0.5% solution freshly prepared in dist. H_2O just before use.

Procedure: Controls for Effectiveness of Sera:

Hep. Exudate (0.1cc) + Immune Serum (0.1cc) — 85/100 unstained
" " + Normal serum — 2/100 "

These mixtures were incubated for 1 hour at 37°C

Then to 0.02cc of mixture was added 1 loop (0.01cc) of methylene blue + examined

Note — the freshly prepared 0.5% methylene blue in water needed time for staining of normal toxo to occur — at least 30+ minutes for optimum effect.

Preliminary Addition of Methylene Blue —

Hep. exudate — (0.2cc) + 0.1cc of Meth. Blue (pH 11)

0.02cc immediately examined — all deeply stained

Then added 0.3cc of immune serum and incubated in

water bath at 37°C for 1 hour. An identical mixture was made with normal serum.

After incubation — 0.02cc was examined under cover slip microscopically and all toxoplasma appeared deeply stained — same in immune as in normal mixture.

Conclusion: If methylene blue is added to exudate first immune phenomen does not occur, but from this exp't it is not clear whether methylene blue interferes with action of activator or antibody.

Since previous tests have shown that when antibody combines with toxoplasma, the cytoplasm still takes up the dye, it cannot be said that the antibody has an affinity for the same groups ~~which~~ in the toxo cytoplasm which combine with the dye. However after the "activator" has acted on the sensitized toxoplasma — the affinity for the dye is gone. (in absence of activator)

It should be determined whether "dyed" toxoplasma washed in saline still have an affinity for antibody (by absorption)

Same Result was Obtained When Aqueous Methylene Blue — 0.5% was used — but 40 minutes were allowed for the methylene blue to stain the toxo before the immune serum was added.

JUN 21 1948

Effect of Aqueous Solutions of Certain Dyes on Toxoplasma in Normal and Immune Serum Mixtures

The following dyes were freshly prepared this morning from powder dissolved in distilled water:

Oxazin Group – Brilliant Cresyl Blue – (Coleman & Bell, 60% dye content)
(0.5%) – 0.1123 Gm. in 22cc dist H_2O

Cresyl violet (Natl. Aniline NW14) – 0.0357 Gm in 16cc
or 0.22%

Amino-azin Group – Neutral Red – (Coleman & Bell) – 0.0951 Gm in 19cc
0.5%

Safranin Y – (Coleman & Bell) – 0.1106 Gm in 22cc
0.5%

Tri-amino tri-phenyl methane group

Crystal Violet – (Coleman & Bell – 88% dye)
0.1000 in 20cc
0.5%

Nile Blue Sulphate – 0.5% freshly prepared
Incompletely dissolved – Used as such

The following dyes were taken from stock:

Basic fuchsin – 1% aqueous prepared 4-21-48
(some ppt. dye on bottom and sides)

2½ Phloxine – aqueous –

Congo Red – old bottle – conc. unknown – used for exploration only

TOXOPLASMA – Exudate from "4-day mice"
Very rich in toxoplasma – diluted
1:3 in saline and heparin added to 1:500
before mixture with sera

Sera – Normal – frozen Buescher
Immune – frozen Bernstein

Mixtures – 0.5cc of diluted exudate
+ 0.5 of serum
Mixtures incubated 1 hour at 37°C

Examinations – 0.02cc mixture + 1 loop dye (0.01cc)
Examined immediately and
at varying intervals thereafter.

Notes on Observations in General + Dyes not Tabulated

1. In each instance the intracellular toxoplasma in the immune mixtures behaved like those in the normal mixtures – i.e. stained or not depending on the dye.
2. In all preparations (normal + immune) there was a certain small proportion of toxo, which as usual, either stained or not depending on the dye.

NILE BLUE SULPHATE – the dye was incompletely dissolved.
Immediate – no staining in either normal or immune mixture
60 min – Normal – Toxo – faint blue cytoplasm
Immune – Mostly negative except for red globules, and intracellular which stain bluer than extracell. in normal.

Congo Red – Immediate and 40 min. – no staining at all in normal or immune mixtures.

JUN 22 1948

Titration of Clinical Toxoplasma Sera

Ser[redacted]16-44 delivered power toxo infant 40 days earlier. (Ams.)

[redacted]-5-22-48 } rec'd from Wegman @ LSU.

[redacted] 5-22-48

[redacted] April 48

[redacted] April 48

6) Eliot Ray -1-15-47- [redacted] has had repeatedly negative rabbit nerves.

Toxoplasma: PE pooled from 5, four day mice. Very rich in toxo. Prelim = 1/5 in EB c̄ hep 1/5000.

Test: All sera inac. for 30' @ 56° just before test, then diluted in good saline & distr. lited to tubes. 0.1cc of toxo in Buescher serum c̄ hep 1/5000 added to 0.1cc of serum dil. Incubated for 1 hr @ 37°C & then exam. c̄ 1 loop of meth bl to 0.02 ml of mixt. Tubes refrig until read.

Time schedule: PE obtained @ 1:00 pm. Prelim began @ 1:10 pm. Final test in @ 1:46 pm; out @ 2:46 pm.

All sera inactivated

TUBE #	SERUM SOURCE	DILUTION	PE 1/5 IN (HEP)	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
1	Na Cl. — CONT.	—	EB	1:49	1	49	2	—	
2	[redacted] 1-16-44	UNDIL.	"	"	61	39	61	+	
3	"	1:4	"	"	55	45	55	+	
4	"	1:16	"	"	77	23	77	+	
5	"	1:64	"	3:14	42	8	84	+	
6	"	1:256	"	"	41	9	82	+	
7	"	1:1024	"	"	62	38	62	+	← 1:1024
8	"	1:4096	"	"	27	73	27	—	

all sera inactivated

JUN 22 1948

Tube #	SERUM SOURCE	Dilution	PE 1/5 IN	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT	
9	[redacted]	undil	EB	3 35	8	42	16	—	
10	"	1:4	"	"	11	39	22	—	
11	"	1:16	"	"	12 21	38 29	24 42	±	
12	"	1:64	"	"	37 79	13 21	74 79	+	No end point (1:16,384 on 23 June)
13	[redacted]	undil	"	3 58	42	8	84	+	
14	"	1:4	"		40	10	80	+	
15	"	1:16	"		39	11	78	+	
16	"	1:64	"		47	3	94	+	
17	"	1:256	"	4 15	37	13	74	+	No end point (1:1024 on 23 June
18	[redacted]	undil	"		41	9	82	+	
19	"	1:4	"		44	6	88	+	
20	"	1:16	"		35	15	70	+	
21	"	1:64	"	4 31	27	23	54	+	1:64
22	"	1:256	"	"	11	39	22	—	
23	[redacted]	undil	"	"	38	12	76	+	
24	"	1:4	"		42	8	84	+	
25	"	1:16	"	4 45	32	18	64	+	1:16
26	"	1:64	"		10	40	20	—	
27	"	1:256	"		5	45	10	—	
28	[redacted]	undil	"	"	31	19	62	+	undil
29	"	1:4	"	5 00	7	43	14	—	
30	"	1:16	"		3	47	6	—	
31	"	1:64	"		1	49	2	—	

JUN 23 1948

In Vitro Titration of AMS & Paired Mother-Child Sera

Toxoplasma: Pool of 5 four day mice.

Sera: a) D. Lehman, Mrs. Lehman, Torres, Mrs. Wright were sent by Joel Warren from AMS.

b) Baby Price & Mrs. Price (Spec. #2) from Kulens @ Sam Houston. This is a check to see whether baby has a zone & what mother's titer is.

c) Rudolf Schneider & Mrs. Schneider - from Chzech. - same purpose as Price.

d) Gladys Johnson & Jenny Johnson from L.S.U. - to determine end-points.

Test: All sera inac. for 30' @ 56° C just prior to test, then diluted with NaCl. Distributed in 0.1cc units & 0.1cc of PE 1/5 in Bruenhen serum c̄ hep 1/5000 added, incubated for 1 hr @ 37° C & then read c̄ 1 loop of meth bl for 0.02 ml of mixture. Tubes incubated until read.

Time schedule: PE obt. @ 11 58 a.m. Prelim (c̄ NaCl + EB) in @ 12 03 (2/50 unit aim) & final test in @ 12 40, out @ 1 40 pm.

JUN 23 1948

Tube #	SERUM SOURCE	DILUTION	PE 1/5 in (Hop.)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT.	
1	NaCl - CONT.	—	EB	$2^{0\underline{9}}$	1	49	2	—	
2	[redacted]	undil	"	"	41	9	82	+	
3	"	1:4	"	"	40	10	80	+	
4	"	1:16	"	$2^{2\underline{9}}$	40	10	80	+	
5	"	1:64	"	"	34	16	68	+	
6	"	1:256	"	"	39	11	78	+	
7	"	1:1024	"	$2^{4\underline{5}}$	32	18	64	+	← 1024
8	"	1:4096	"	"	16 36	34 70	32 34	− −	
9	[redacted]	undil.	"	$3^{0\underline{5}}$	42	8	84	+	
10	"	1:4	"	"	38	12	76	+	
11	"	1:16	"	$3^{0\underline{9}}$	43	7	86	+	
12	"	1:64	"	"	40	10	80	+	
13	"	1:256	"	$3^{4\underline{5}}$	32	18	64	+	← 256
14	"	1:1024	"	"	11	39	22	−	
15	"	1:4096	"	$4^{1\underline{9}}$	5	45	10	−	
16	[redacted]	undil.	"	$3^{2\underline{5}}$	43	7	86	+	
17	"	1:4	"	$4^{1\underline{9}}$	50	0	100	+	
18	"	1:16	"	[illegible]	44	6	88	+	
19	"	1:64	"	"	43	7	86	+	
20	"	1:256	"	"	38	12	76	+	← 256
21	"	1:1024	"	"	14	36	28	−	
22	"	1:4096	"	"	8	42	16	−	

JUN 23 1948

TUBE #	SERUM SOURCE	DILUTION	PE VS IN (HOP)	TIME	EFFECT % UNSTAIN.	% STAINED	% UNSTAIN.	RESULT.	
23	[redacted]	undil	EB		35	15	70	+	
24	"	1:4	"	4[10]	33	17	66	+	
25	"	1:16	"	4[36]	40	10	80	+	
26	"	1:64	"	4[26]	38	12	76	+	
27	"	1:256	"		30	20	60	+	← 256
28	"	1:1024	"	4[32]	14	36	28	−	
29	"	1:4096	"		3	47	6	−	
30	[redacted]	undil.	"		9	41	18	−	
31	"	1:64	"	4[57]	1	49	2	−	NEG
32	[redacted]	undil	"	"	49	1	98	+	
33	"	1:4	"	4[59]	43	7	86	+	
34	"	1:16	"	"	28	21	56	+	← 16
35	"	1:64	"	"	5	45	10	−	
36	"	1:256	"	"	1	49	2	−	
37	[redacted]	undil	"	5[15]	1	49	2	−	
38	"	1:64	"	"	0	50	0	−	Neg
39	[redacted]	undil	"	5[37]	46	4	92	+	
40	"	1:4	"	"	45	5	90	+	
41	"	1:16	"	5[50]	25	25	50	+	← 16
42	"	1:64	"		5	45	10	−	
43	"	1:256	"	6[00]	2	48	4	−	

JUN 23 1948

Tube #	SERUM SOURCE	Dil.	PEY/S (HEP.)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN	RESULT.	
44	[redacted]	1:16	"	6^{30}	22	28	44	±	
45		1:64	"	6^{10}	33	17	66	+	
46		1:256	"	"	43	7	86	+	
47		1:1024	"	6^{15}	45	5	90	+	
48		1:2048	"	"	44	6	88	+	
49		1:4096	"	6^{20}	40	10	80	+	
50		1:8192	"	6^{28}	38	12	76	+	
51		1:16384	"	6^{25}	24	26	48	+	← 16,384 !
52	[redacted]	:64	"	"	41	9	82	+	
53		1:256	"	6^{50}	44	6	88	+	
54		1:1024	"	"	32	18	64	+	← 1024
55		1:4096	"	"	3	47	6	−	

24 June 48

The Reactivating Power of GP ~~&~~ Mouse Sera And Tests On Several Clinicals.

Quest: a) To determine whether gp serum of high complement value will reactivate any of several immune sera from different species.

b) Will normal mouse serum ~~also~~ reactivate heated immune serum in view of its lack of "non-specific" activity.

Toxoplasma: pooled from 4-4 dy mice - poor in toxoplasma so used as 1/3 PE.

Sera: a) GP #974 - immune. rec'd from AMS. Serum date = 7-24-47

b) D.E. Kope: 5-28-48. Immune from Cleveland.

c) Rh #152 - 26th dy after inoc. Immune monkey.

d) GP pool - normal. Obtained on 4-4-48 & used for toxo test on 6-19-48

e) Ineke Bonsink - rec'd to-day unfrozen from Van Kreveldt.

f) J. Weigand (4-27-48) & S. Harrison (8-26-48) rec'd from AMS.

g) Mouse serum.: Pool of "yellow heads" - 2 - normal mouse serum.

Test: All test sera inac. for 30' @ 56° C just prior to test & then diluted c̄ NaCl. 0.1cc amts dispensed & then toxo added as 0.1cc of ~~1/5~~ 1/3 dil in EB, GP pool 1/4 or mouse serum (1/4 in mouse) Incubated 1 hr @ 37° C & then read. c̄ 1 loop of meth blue to 0.02 cc mixt. Toxo content so low that not all tests read.

Note 1:3 ↓

24 June 48

Tube #	SERUM SOURCE	Dil.	PE 1/3 in (Hya)	Time	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
1	NaCl	—	GP 1/4	1 45	26	20	50 HF		
				2 45	39	11	78 ABS		
2	NaCl	—	EB	1 45	9	43	14 HF		
				2 45	6	23	20 ABS		
3	NaCl	—	MOUSE	3 15	0	35	0		PE 1/4 in mouse serum.
4	GP #974 (H)	1:256	EB	NOT READ					
5	"	1:256	GP 1/4	"	"				
6	DE. KOPE (H)	1:16	EB	—	42	8	84 F	+	Normal mouse serum dilutions not recorded
7	"	"	GP 1/4	-	NOT READ.				
8	"	"	MOUSE	—	1	24	4	—	PE 1/4 in MOUSE SERUM.
9	Rh #152 (H) -26 2 dy	1/64	EB	NOT READ.					
10		1/64	GP 1/4	"	"				
11	[redacted] -2748	undil	EB	"	"				
12	"	1/4	"	"	"				
13	"	1/16	"	"	"				
14	"	1/64	"	"	"				
15	[redacted] -26-48	undil	"	"	"				
16	"	1:4	"	"	"				
17	"	1:16	"	"	"				
18	"	1:64	"	"	"				
19	[redacted] (H)	undil	"		27	3	90	+	Titer lower than 1:64
20	" " (H)	1:64	"		6	24	20	—	

Repeat Test with GP serum

25 June 1948

The Reactivating Power Of Guinea Pig Serum

Quest: Will guinea pig serum of high complement activity reactivate either (or both) heated immune gp or human ~~to~~ serum?

Toxoplasma: Pooled from 3 four day mice, poor in toxoplasma but used at 1/5.

Sera: a) Buescher - normal human control.

b) Daniel Kope - immune human. (5-28-48)

c) GP #974 - obt. from J. Warren. Titers to 1:4096 (7-24-47)

Test: All test sera inac. for 30' @ 56° @ just before test & then diluted c̄ saline & distributed to proper tubes. GP serum (normal complement) obtained on 4-14-48 & used for toxo titration on 6-19-48, thawed, dil. c̄ saline & also distributed as indicated. PE 1/5 in NaCl c̄ hep. 1:5000 then added, tubes incubated for 1 hr @ 37° & then read c̄ 1 loop of meth bl for 0.02 cc of toxo-serum mixture. Tubes refrigerated until read.

Quantities: 0.1 cc of test serum + 0.1 cc of gp dil. + 0.2 cc of PE 1/5 in NaCl.

Time schedule: PE obtained @ 12^{00} noon. Prelim test in @ 12^{05} (2/50 unit.).

Final test in @ 12^{43} pm. & out @ 1^{43} pm.

(OVER)

Tube #	SERUM		PE 1/5 in (Hep.)	Time	EFFECT			RESULT.	
	SOURCE +	GP SER IN NaCl			# UNSTAIN.	# STAINED	% UNSTAIN		
1	NaCl - 0.2 cc	—	NaCl	1^{43}	3	47	6		Counting very diff because of few texts
2	[redacted]	1:2	"	"	25	25	50		
3	" "	1:4	"	"	6	44	12		
4	" "	1:8	"	"	1	49	2		
5	GP#974 (H) 1/64	1:2	"	2^{09}	12	38	24		
6	" "	1:4	"	"	5	45	10		
7	" "	1:8	"	"	3	47	6		
8	[redacted]	1:2	"	2^{40}	33	17	66		
9	" "	1:4	"	"	23	27	46		
10	" "	1:8	"	"	1	49	2		

Repeat c̄ controls using fresh Bruce ch serum to check Ab content of immune sera

25 June 48

In vitro Titration of Bonsink Family Sera

(+ Olive Cannon)

Toxoplasma: Same PE used for gp tests to-day. PE pooled from 3 fresh day mice; toxoplasma content low but used 1:5 for test.

Sera: [redacted] – child. (spec. of Aug. 1947)

[redacted] – spec. recd ~~May~~ April 1948 d) Olive Cannon

[redacted] recd 24 June 1948

Test: Bonsink sera used unheated all dilutions in Nacl. Cannon inactivated just prior to test & then diluted. PE dil 1:5 c Ringer serum & hep 1/5000 & used in 0.1cc amts c 0.1cc serum dilution. Incu. 1 hr @ 37°C & then read c 1 loop of methy. blue.

Time schedule: Same as for gp serum tests to-day.

Tube #	SERUM SOURCE	Dil.	PE & in (Hep)	Time	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN	RESULT.	
11	Nacl	—	EB.	3 05	8	42	16	—	
12	[redacted]	undil	"	"	23	27	46	+-	NO ZONE
13	" "	1:64	"	"	13	37	26	—	
14	[redacted]	undil	"	3 45	46	4	92	+	
15	"	1:4	"	"	45	5	90	+	
16	"	1:16	"	3 58	46	4	92	+	
17	"	1:64	"	"	42	8	84	+	
18	"	1:256	"	4 30	36	14	72	+	
19	"	1:1024	"	"	35	15	70	+	← 1:1024
20	"	1:4096	"	4 40	19	31	38	—	
21	"	1:16384	"	"	10	40	20	—	

(OVER)

Tube #	SERUM SOURCE		Dil.	PE's in (Hep.)	Time	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN	RESULT.	
22	[redacted]		undil	EB	4 59	43	7	86	+	
23	"	"	1:4	"	"	43	7	86	+	
24	"	"	1:16	"	5 10	42	8	84	+	
25	"	"	1:64	"	"	37	13	74	+	
26	"	"	1:256	"	5 30	28	22	56	+	← : 1:256
27	"	"	1:1024	"	"	39	61	39	−	
28	[redacted]		undil	~~5 45~~ "	5 45	40	10	80	+	
29	"	"	1:4	"	"	23	27	46	±	1:4 ?
30	"	"	1:16	"	6 15	12	38	24	−	
31	"	"	1:64	"	"	12	38	24	−	

26 June 48

The Reactivating Power of Guinea Pig Serum (Repeat)

Toxoplasma: Pooled from 3 four day mice. Toxoplasma quite low, so PE used 1/2.

Sera: Same as yesterday's. (25 June 48) All inactivated as undil. before test.

Test: 0.1cc of serum dil. + 0.1cc NaCl or gp dil. + 0.2cc PE 1/2 in NaCl or Buescher serum c̄ heparin 1:5000. Inc. 1 hr @ 37°C & then read c̄ 1 loop of meth bl for 0.02 ml of mixture.

Time schedule: PE obt. @ 10^{50} a.m., Final test & preh in @ 11^{15} a.m.

1/2

Tube #	SERUM SOURCE +	GP in NaCl	PE 1/5 in (HEP.)	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN		
1	NaCl - 0.2cc	—	NaCl	12^{23}	0	50	0	—	
2	NaCl - 0.2cc	—	EB.	"	2	48	4	—	
3	[redacted]	—	"	"	3	47	6	—	
4	[redacted]	1:2	NaCl	"	30	20	60	+	Insufficient
5	" "	1:4	"	12^{44}	13	37	26	—	
6	" "	1:8	"	"	3	47	6	—	
7	GP 974 (H) 1/256 + NaCl	—	EB	"	60	40	60	+	Insufficient activator in human serum
8	GP 974 (H) 1/256	1:2	NaCl	1^{10}	52	48	52	+	
9	"	1:4	"	1^{51}	11	39	22	—	
10	"	1:8	"	1^{10}	6	44	12	—	
11	[redacted]	—	EB	"	33	17	66	+	
12	[redacted]	1:2	NaCl	1^{57}	41	9	82	+	
13	"	1:4	"	"	30	20	60	+	
14	"	1:8	"	"	13	37	26	—	

Repeat, using more human activator.

JUN 26 1948

Repetition of Experiment with ~~Previously~~ Toxoplasma Previously Stained with Methylene Blue

~~In~~ In the experiment of June 19, 1948 it was found that when the toxoplasma were first mixed with methylene blue and the immune serum was added next — no decolorization of the cytoplasm occurred.

Since it was thought that the excess of methylene blue in the fluid around the toxoplasma may perhaps have interfered indirectly with the actions of the "accessory factor" or the specific antibody, it was desirable to repeat the experiment under circumstances which would diminish the extra-toxoplasmic methylene blue to a minimum.

The experiment was designed as follows:

Toxoplasma in exudate + methylene blue ⇒ when well stained, centrifuge out toxoplasma, wash and then add normal and immune sera.

June 25 — this was started yesterday, but difficulty was encountered because the 0.5% aqueous methylene blue of June 12, 1948 left a ~~large~~ number of the toxoplasma very poorly stained even after one hour's contact.

~~Later~~ It was then found that when the 0.5% aqueous methylene blue was mixed with an equal amount of borate buffer pH 11.0, the staining was uniform deep and immediate.

June 26 — Experiment repeated using aqueous methylene 0.5% + equal volume of borate buffer pH 11.

Procedure — 1) 1cc of exudate rich in toxoplasma, diluted 1:~~8~~ with saline (without heparin) and centrifuged at 2,000 rpm for 10 min. Sn. liquid discarded. Sediment reconstituted in 2cc of saline.

Check with meth. blue pH 11 — + 0.02cc 1 evap — immediate good staining of toxo

2) 1cc of meth. blue added to 2cc of toxo Some ppt. occurred. — microscop. exam. showed that toxo were stained much deeper than in 1). 5cc of saline

immediately added – divided in 2 narrow centrif. tubes & centrif. at 2000 r.p.m. for 10 min.

Sn. liquid removed – sides of tubes rinsed with saline, and each pellet reconstituted with 1cc. saline. Microscopic examination showed toxo well stained – no heavy ppt.

Because the fluid was still quite blue – 6cc more of saline was added, mixed and recentrifuged. Sn. removed, sides of tubes rinsed and pellets reconstituted in 1cc of saline. Microscopic examination now showed that toxo were still blue but not as deep as before.

3) The following mixtures were now prepared:

a) 0.2cc of washed stained toxo + 0.2cc saline

b) " " " + 0.2cc normal fresh serum (Buescher)

c) " " " + 0.2cc immune fresh serum (Kope)

a) was grossly light blue while tubes b) + c) containing the serum immediately turned a light green.

All tubes incubated for one hour at 37°C

Microscopic examination:

a) Toxo – very faint blue – dye obviously leached out of cytoplasm

b + c) look alike – toxo almost entirely without evidence of staining in cytoplasm as seen under oil immersion.

Conclusions – The staining of toxo cytoplasm by aqueous methylene blue (pH 11) is reversible by washing, and even more so in final stage by serum which itself has an affinity for the dye.

Repetition of Experiment with Stained Toxoplasma

JUN 28 1948

Another attempt was made to see whether toxoplasma which had been washed only once would retain the dye upon addition of serum.

Procedure –1) 3cc of exudate (4-day) 1:5 in saline (without heparin) +1cc of aqueous methylene blue-borate buffer pH 11 (prepared 6-25-48 – final MB -0.25%)

Microscopic – toxo immediately deeply stained – no ppt – no distortion. Large numbers of toxo present.

2) 4cc of saline added immediately – distributed in 2 narrow tubes & centrif. 2000 rpm 10 min.

3) Sn. drawn off – sides rinsed with saline and pellets rubbed & dispersed in 1.6cc saline

4) Microscopic examination revealed toxoplasma well stained

5) a) 0.2cc of washed stained toxo +0.2cc normal serum (Brandes) (turned green

b) 0.2cc of " " " +0.2cc immune serum (Frankel) remained blue

Added controls:

c) 0.1cc of toxo 1:5 in sal.(hep.) +0.1cc normal serum
d) " " " " +0.1cc immune "

All tubes in water bath for 1 hour at 37°C

Microscopic examination of:

Tubes a + b – all extracellular toxoplasma appear unstained – neither chromatin nor cytoplasm showed anything under oil

Tubes c and d – 0.02cc +1 loop of Meth. Bl. pH 11

c – 2/100 unstained
d – 100/100 "

6) To see whether toxo in tubes a) and b) could be restained, 1 loop of dye was added to 0.02cc on slide

Tube a) – normal – 35 well stained
65 unstained cytoplasm, but chromat. stained

" b) – immune – 100/100 unstained cytoplasm with stained chromatin (over)

Conclusion – 1) Cytoplasm-dye combination is readily reversible especially upon addition of serum to washed, stained toxo

2) During the process of "destaining" about 65% of the toxoplasma become "denatured," the ~~cyst~~ cytoplasm losing the capacity to stain again on the addition of fresh dye

3) The ~~precise~~ toxoplasma which are not "denatured" by destaining are still susceptible to the "immune phenomenon"

Other Experiments with Washed Stained Toxoplasma

Purpose – to determine whether freezing and thawing or heating at 56°C for 1/2 hour will "destain" the toxoplasma.

I Freezing and Thawing – The same preparation of once-washed toxoplasma in previous experiment ③ was used. 0.3cc was quickly frozen in dry-ice-alcohol and quickly thawed at 37° water bath.

Frozen and thawed once – all toxoplasma appear well stained
" " " twice – " " " " "

II 56°C for 30 min – 0.3cc of ~~above~~ same mixture used. All toxoplasma well stained.

Conclusion – Although toxoplasma which are frozen and thawed or heated at 56°C lose their affinity for dye (except the chromatin), toxo which are stained first and submitted to these procedures subsequently do not become "destained". No evidence, therefore, for appearance of reducing groups during process.

JUN 28 1948

Pathogenicity of Stained Washed Toxoplasma Before and After Freezing and Thawing ~~& Heating~~

Purpose — 1) Are stained toxoplasma infections?

2) If they are, does the presence of the dye in them during freezing and thawing or heating at 56°C protect them from the lethal effects of these procedures?

Material — See previous experiment of JUN 28 1948 for description of material used.

GROUP I — Peritoneal Exudate 1:5 in saline; 0.5cc i.p. in 6 mice

	1	2	3	4	5	6	7	8	9	10	
1	–	–	–	Ac←	Many toxo						} Exudate used
2	–	–	–	Ac←	"	"					
3	–	–	–	–	D						
4	–	–	–	–	D						
5	–	–	–	–	D						
6	–	–	–	–	Sick D						

GROUP II — Washed stained toxoplasma – 0.25cc i.p. – equivalent to 0.5cc of Exud 1:5

	21	22	23	24	25	26	27	28
1	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–

GROUP III — Washed stained toxoplasma frozen and thawed x 2 – dose as above

1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

GROUP IV — Washed stained toxoplasma heated at 56°C for ½ hour – dose as above

1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

JUN 28 1948

Further Observations on Aqueous Methylene Blue and Aqueous Safranin Y – in 0.5% Concentration.

Contrary to observation recorded in tests of June 12 further use of ~~x~~ 0.5% aqueous methylene blue (without buffer) has indicated that normal toxoplasma do not stain immediately and require a variable time for ultimate staining. In view of this it was deemed desirable to reconsider whether or not Safranin was really different from methylene blue

Material – Tubes c + d from experiment of same day.

Dyes – 0.5% aqueous methylene blue prepared 6/25/48
0.5% " Safranin Y " 6/21/48

0.5% Aqueous Meth. Blue

Normal – Immediate – only rare toxo shows stained cytoplasm; chromatin not seen
60 min. – Many stained but many still poorly stained or unstained
120 min. – Almost all well stained now

Immune – Immediate up to 2 hours – Cytoplasm unstained
Chromatin stained deep blue

SAFRANIN

Normal – Immediate – only very few toxo stained – both cytoplasm & chromatin seen
60 min – more stained now but majority still unstained
120 min – same as 60 min.; majority unstained

Immune – Immediate + 2 hours – chromatin deep red; cytoplasm appears yellowish but cannot be said to be stained

(over)

Note: important to determine effect of Safranin as well as basic fuchsin in borate buffer at pH 11

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Safranin Y – freshly prepared 0.5% in soda-borax buffer pH 11

Normal – immediate to 60 minutes – 95% of toxoplasma show no staining of either cytoplasm or chromatin.

Few ; some chromatin only as in immune, i.e. the degenerated ones.

Immune – immediate to 60 min – cytoplasm not stained chromatin – orange red

Basic Fuchsin – 0.5% in buffer pH 11 – insoluble.
– 0.5% in distilled H_2O

Normal – some stained after 30-60 min. but 60% still unstained

Immune – Immediately – cytoplasm red granular chromatin – negative or too faint to tell

Phloxine – freshly prepared 2.5% in buffer pH 11

Normal – Immed. to 60 min. – cytoplasm and chromatin unstained (except few degenerate ones which appear red like in immune)

Immune – " – Cytoplasm red granular Chromatin negative

JUN 28 1948

Repetition of Test on Effect of 0.5% Formalin

Previous tests have shown that toxoplasma are quickly killed by this concentration of formalin, retain the cytoplasmic affinity for methylene blue, but are not "destained" by ~~the~~ fresh immune serum.

Purpose of this test: to determine whether removal of formalin will yield toxoplasma capable of reacting with immune serum.

Toxoplasma – Peritoneal Exudate from 6 four-day mice rich in toxoplasma – diluted 1:5 in saline without heparin

12 cc of diluted exudate + 12 cc of 1% Formalin (not formaldehyde – 40%) in saline. (at 2:30 P M)

Immediate Microscopic Examination

0.02 cc + 1 loop methylene blue (pH 11 – alcoholic)

Toxo deeply stained – not distorted

After 4 hours at Room Temperature –

microscopic examination same

Flask Transferred to Refrigerator after 4 hours at Room Temperature.

JUN 29 1948

8 cc of formalinized exudate centrifuged in two narrow tubes – 2000 for 10 min. – Sn. discarded – sides rinsed with saline – reconstituted in 8 cc of saline; Centrifuged again; Sn. discarded; sediment taken up in 1 cc saline.

Microscopic Examination – large numbers of toxoplasma staining deeply with methylene blue (pH 11) – not shrunken or deformed

Unwashed Formalinized Toxo – stain well same as yesterday.

(See other side for test with sera)

JUN 29 1948

Sera Used: Normal — Baescher frozen since 6/2/48
Immune — Frankel of 6/17/48

Control Toxoplasma — freshly obtained exudate from 4 day mouse
Diluted 1:5 in hep. saline — many toxoplasma

Mixtures — 0.1cc toxo + 0.1cc fresh serum
Incubated for 1 hour at 37°C in water bath
Micro-Exam — 0.02cc + 1 loop Meth. Blue — pH 11

Control for sera { Fresh toxo + normal serum — 0/100 unstained
" " + immune " — 90/100 " (except for chromatin)

Washed form. toxo + normal serum — cytoplasm unstained; chromatin stained
Intracellular — some stained
most unstained

" " " + immune " — same as with normal serum

0.5% form. toxo + normal " — all stained

" " " + immune " — " "

Conclusion: 1) 0.5% formalin interferes with immune phenomenon

2) Both normal & immune fresh sera produce a a change in washed formalinized toxo, depriving the cytoplasm of its affinity for dye.

3) The above effect on the intracellular toxo may be due either to change in permeability caused by formalin or due to capacity of "complement factor" to diffuse into cell.

Note — since this result was totally unexpected it was decided to determine whether the "complement" factor in normal serum acting on the formalinized toxoplasma was heat-labile or heat-stable

After incubation for 1 hour at 37°C — following mixtures-exam.

Saline (0.1cc) + Washed form. toxo (0.1cc) — all toxo deeply stained

Normal serum (fresh) 0.1cc + " " — all toxo (except few intracellular) unstained cytoplasm; stained chromatin

" " (heated 56°C) " + " " — as above

Conclusion — Factor is heat-stable — Therefore not ordinary complement

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Note – The mixture of washed formalinized toxoplasma with fresh normal serum was also examined immediately after mixing & just before incubating in water bath ⟶

Toxoplasma were found well stained.

One may conclude, therefore, that the peculiar "complementary activity" required time & incubation.

Summary – Toxoplasma treated with 0.5% formalin are "sensitized" in such a manner that upon the addition of normal serum (fresh or heated at 56°C) & and subsequent incubation of the mixture, something happens to the cytoplasm to deprive it of its affinity for methylene blue while the chromatin retains it — in other words a phenomenon analogous to that obtained with specific antibody except that the accessory factor here is <u>not</u> heat-labile.

JUN 29 1948

Further Repetition of Experiment with Stained Toxoplasma

Previous tests have shown that ~~even a single~~ when stained toxo are resuspended in saline + then serum added — the toxo lose their methylene blue.

Purpose of this test — to obtain condition at which toxo retain meth. blue upon addition of serum and study effect of fresh immune serum under these conditions — will they lose the dye ~~differentially~~ from the cytoplasm in the immune and not in the normal?

Sera — same as before; test for effectiveness on to-day's exudate given in previous Expt. — normal — 0/100 unstained
immune — 90/100 "

Procedure

3cc. of exudate 1:5 in saline (no heparin) — many toxo
+ 1cc of 0.25% aqueous methylene blue (pH 11)

Microscopic — immediate deep staining of toxo

Centrifuge — 2cc in each of two narrow tubes (note that no additional saline was added)
2000 rpm for 10 min.
Sn. liquid removed — sides of tubes not rinsed.

Sediment — in each tube broken up ē wooden applicator
To tube 1) added 0.2cc of fresh normal serum
" " 2) " 0.2cc " " immune serum

Incubation — 1 hour at 37°C water bath

Result

Normal — all deeply stained

Immune — all deeply stained

(over)

Conclusions: a) Immune Serum added to Stained Toxoplasma fails to bring about "destaining" of cytoplasm.

b) Methylene blue ~~xxxxxx~~ is not ~~xxxxx~~ "an innocent bystander" which becomes reduced to its <u>leuko</u> base as a result of the immune phenomenon, but probably is in combination with the groups finally affected in the terminal phase of the immune reaction.

JUN 30 1948

In Vitro Titrations of Additional Army Med. School Sera.

Toxoplasma: PE pooled from 2 foundry mice. Toxo content moderately high.
Prelim test = 1/50 unst.

Sera: [redacted] W.R.G.H. 8-26-46
[redacted], AAF R.&C. Hosp. 5-8-45
[redacted] U. Wisc. 4-27-48.
[redacted] Hopkins, 11-26-47
[redacted] Gables, 7-24-44.

all rec'd from Warren at the AMS.
All have CF titer of 1:8

N.B. [redacted] SERUM CENTRIFUGED TO CLEAR BECAUSE OF "MUCK"

Test: All sera inactivated for 30' @ 56° C just prior to testing. Then diluted in NaCl & 0.1cc amts distributed to tubes. Immediately after preliminary test, PE dil 1:5 in EB (hep 1/5000) & 0.1cc amts added to serum dilutions. Tests then incubated for one hour @ 37° C & read ≈ 1 loop Mech bl for 0.02 cc toxo-serum mixture. Tubes refrigerated until read.

Time schedule: PE collected @ 11 50 a.m., Prelim (PE 1/5 in EB ≈ hep 1/5000 + ≈ amt NaCl) in at 12 00 noon, Final test @ 12 38 p.m.

COMMENT – The following may be the reasons for the discrepant readings between HF & me

1) The activator serum tube used had been frozen & thawed at least 5 x and therefore a) the activator may have been diminished in amount & required more time for effect
b) the muck interfered with seeing the ~~toxop~~ unstained toxo

2) HF used another scope and the "muck" in the original sera obscured the "unstained toxo" OVER

Titer to 1024

On repetition use fresh tube of "clear" or clarified Bruce's serum

JUN 30 1948

All Sera Inactivated/Undiluted

Tube #	SERUM SOURCE	Dilution	PE/S in (HEP)	Time	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT
1	NaCl - CONT.	—	EB	1 42 / 5:30	0 / 2	50 / 48	0	
2	[redacted] 6-26-46	undil	EB	1 42	55 / 44	45 / 6	55 / 88	+ → P.S
3	"	1:4	"	1 56	36	14	72	
4	"	1:16	"	"	37 / 44	13 / 6	74 / 88	+ → P.S
5	"	1:64	"	1 58	54 / 48	46 / 2	54 / 96	+
6	[redacted] 8-45	undil	"	"	34	16	68	
7	" Cent. to clear prior to heating	1:4	"	2 30	41	9	82	
8	"	1:16	"	2 42	45	5	90	
9	"	1:64	"	"	27	23	54	
10	[redacted] 4-27-48	undil	"	2 55	32 / 40	18 / 10	64 / 80	+ → P.S
11	"	1:4	"	"	40	10	80	
12	"	1:16	"	3 05	25	25	50	
13	"	1:64	"	" / 6:30	7 / 11	43 / 39	14 / 22	−
14	[redacted] 11-26-47	undil	"	3 16	5 / 15	45 / 35	10 / 30	−? very dusty
15	"	1:4	"	"	25	25	50	
16	"	1:16	"	3 24	37	13	74	
17	"	1:64	"	"	39 / 32	61 / 18	39 / 64	+ → P.S
18	[redacted] 7-24-44	undil	"	3 35	25	25	50	→ too dirty to count
19	" Cent. to clear prior to heating.	1:4	"	"	34	17	68	
20	"	1:16	"	3 44	28	22	56	
21	"	1:64	"	"	15 / 31	35 / 19	30 / 62	+ → P.S

Data in this test unreliable
Preparations dirty even in 1:64 dilution
(See other side)

JUL 10 1948

INVITRO TOXOPLASMA TESTS — J. Warren SERA.

TOXOPLASMA: PE pooled from 2, four day mice. Very rich in toxoplasma.

SERA: 1) [redacted] July 48 mother of Cecilia Edwards, patient @ Children's

2) [redacted] 8 July 48. Died 9 July 48. No clinical stigmata of toxo.

Onset of convulsions @ 3-4 months. Clinical Diag: Krabbe's ?

3) Sera from [redacted]

a) Augustino 6-18-48 — 11 yrs. chorioretinitis. Noted @ 7 yrs. Mother & child pos. neut. in Boston in 1944

b) GP.'s #2464 & 2463 — inoc. ID with toxo. Neg. CF pre-inoc, & @ 2 wks., #2463 CF 1:128+ @ 34 days.

Test: All sera inoc. as undil. just before test. All sera diluted @ in NaCl. 0.1cc amts of PE 1/5 in Buescher serum c̄ heps 1/5000 mixed with 0.1cc dil. serum & incubated for one hour @ 37°. Then stored in refrigeration until read c̄ 1 loop of meth bl. to 0.02 ml serum-toxo. Numerous toxo so counting proceeded rapidly.

Time Schedule: Exudate obtained @ 11^{05} a.m. Final test in @ 11^{35} a.m. & out @ 12^{35} pm.

JUL 10 1948

ALL TEST SERA INACTIVATED

TUBE #	SERUM SOURCE	DIL.	PE ½-HR	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT	
1	NaCl - CONT.	—	EB	12^{43}	1	49	2	—	
2	[redacted]	NDil.	"	"	3	47	6	—	
3	"	1:64	"	"	2	48	4	—	NEG.
4	[redacted]	UNDil.	"	"	1	49	2	—	
5	"	1:64	"	12^{55}	1	49	2	—	NEG.
6	[redacted]	Dil	"	"	49	1	98	+	
7	[redacted]	1:4	"	"	45	5	90	+	
8	"	1:16	"	"	45	5	90	+	
9	"	1:64	"	"	33	17	66	+	← 1:64
10	"	1:256	"	"	6	44	12	—	
11	"	1:1024	"	1^{14}	1	49	2	—	
12	GP #2464 - PRE.	Undil	"	"	40	10	80	+	end point 1:16 (12 guinea pigs)
13	"	1:64	"	"	50	65	43	+	← 1:64 ?
14	GP #2464 - 2 wks.	UNDil.	"	"	47	3	94	+	
15 *	"	1:4	"	"	45	5	90	+	
16	"	1:16	"	"	44	6	88	+	
17	"	1:64	"	1^{26}	30	20	60	+	← 1:64
18	"	1:256	"	"	2	48	4	—	
19	"	1:1024	"	"	2	48	4	—	
20	"	1:4096	"	"	2	48	4	—	

* This result may indicate a stage of neg. "antibody balance" in a previously immune pig receiving a fresh dose of antigen or else this pig did not receive an challenge infection.

JUL 10 1948

ALL TEST SERA INACTIVATED

Tube #	Serum Source	Dil.	PE 1/5 in (Hep.)	Time	Effect # unstain.	# stained	% unstain.	Result	
	AMS.								
21	GP-2468 - Pre.	undil	EB	2^{01}	9	41	18	—	NEG.
22	"	1/64	"	"	0	50	0	—	
23	GP. 2463 -2 wks	undil	"	"	49	1	98	+	
24	"	1:4	"	"	45	5	90	+	
25	"	1:16	"	2^{14}	48	2	96	+	
26	"	1:64	"	"	48	2	96	+	
27	"	1:256	"	"	42	8	84	+	← 1:256
28	"	1:1024	"	"	14	36	28	—	
29	"	1:4096	"	"	1	49	2	—	
30	GP 2463 - 34 dys.	undil	"	"	3	47	6	—	"ZONE"
31	"	1:4	"	2^{30}	6	44	12	—	
32	"	1:16	"	"	18	32	36	±?	
33	"	1:64	"	"	37	13	74	+	Final end-point =
34	"	1:256	"	"	39	11	78	+	1:16, 384 (12 July 48)
35	"	1:1024	"	"	44	6	88	+	
36	"	1:4096	"	"	42	8	84	+	NO END-POINT

JUL 12 1948

IN VITRO TESTS WITH WARREN GP SERA, RH #2 (RUCH) & TWO CLINICAL SPECIMENS.

TOXOPLASMA: PE pooled from three, three day mice, rich in toxoplasma.

Sera: a) Warren GP:

(1) #2463, 34 dys – to determine end-point.

(2) #2464, pre-inoc. – " " " "

(3) #2470, pre-inoc. } to determine titers.

(4) #2470, 2 wks. }

b) RH #2, 14 weeks. Obt. from Ruckman, this is the same monkey whose 3rd week serum was titered previously. This test is to determine the persistence of titer.

c) [redacted] chorea patient at Ch. Hosp. Pos. skin test.

d) [redacted] FUO @ Ch. Hosp. Pos. skin test.

Test: All sera inac. for 30' @ 56° just before test; then diluted c̄ NaCl. 0.1cc amts mixed c̄ 0.1cc PE 1/5 in Buescher serum c̄ hep 1/5000. Incubated for one hour @ 37°C & then refrigerated until read. Read c̄ 1 loop fresh MB pH 11.0 to 0.02 ml serum-toxo mixt. Numerous toxo. Test read easily.

Time schedule: PE obt. @ 1:20 pm. Final test in @ 2:00 pm., out @ 3:00 pm.

(over)

ALL TEST SERA INACTIVATED

JUL 12 1948

TUBE #	SERUM SOURCE	DIL.	PE/5 ml (HEP)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT	
1	NaCl - CONT.	—	EB	3 41	1	49	2	—	
2	GP. 2463 - 34 dys.	1,024	"	"	44	6	88	+	
3	"	4,096	"	"	41	9	82	+	
4	"	16,384	"	3 35	50	50	50	+	16,384
5	"	65,536	"	4 00	5	45	10	−	
6	"	262,144	"	"	4	46	8	−	
7	GP 2464 - PRE	undil	"	4 06	43	7	86	+	
8	"	1:4	"	"	43	7	86	+	
9	"	16	"	"	42	8	84	+	1:16
10	"	64	"		41	59	41	±	
11	"	256	"	4 22	4	46	8	−	
12	GP-2470 - Pre.	undil	"	"	9	41	18	−	
13	"	4	"	"	5	45	10	−	
14	"	16	"	"	3	47	6	−	
15	"	64	"	"	3	47	6	−	NEG PRE.
16	"	256	"	"	4	46	8	−	
17	GP 2470 - 2 wks.	undil	"	4 39	40	10	80	+	
18	"	4	"	"	44	6	88	+	
19	"	16	"	"	42	8	84	+	
20	"	64	"	"	43	7	86	+	
21	"	256	"	"	40	10	80	+	
22	"	1024	"	"	29	21	58	+	1024
23	"	4096	"	4 58	7	43	14	−	

ALL TEST SERA INACTIVATED

JUL 12 1948

TUBE #	SERUM SOURCE	SERUM DIL.	PE 1/5 IN (HEP)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT	
24	RH. #2 - (RUCH.) 14 wks.	undil	EB	4^{58}	44	6	88	+	
25	"	4	"	"	43	7	86	+	
26	"	16	"	"	44	6	88	+	
27	"	64	"	"	45	5	90	+	
28	"	256	"	"	40	10	80	+	
29	"	1024	"	5^{35}	30	20	60	+	← 1:1024
30	"	4096	"	"	7	43	14	−	
31	[redacted]	undil	"	"	42	8	84	+	
32	"	4	"	"	43	7	86	+	
33	"	16	"	"	41	9	82	+	1:16
34	"	64	"	"	40	60	40	±	
35	"	256	"	5^{58}	3	47	6	−	
36	[redacted]	undil	"	"	46	4	92	+	
37	"	4	"	"	45	5	90	+	
38	"	16	"	"	58	42	58	+	1:16
39	"	64	"	"	10	40	20	−	
40	"	256	"	"	2	48	4	−	

JUL 15 1948

INVITRO TOXO. TESTS WITH WARREN GP's, CONT.

TOXOPLASMA: PE pooled from 3, three day mice, moderately rich in toxo.

Sera: GP sera rec'd from Warren. #'s 2467, 2468, 2472, there being a pre-inoc, 14th and 34th post-inoc. sera from each pig.

Test: all sera inoc. for 30' @ 56°C just prior to test, as undiluted serum. All dilutions then made in Na Cl. 0.1cc amts of dil mixed with 0.1cc PE 1/5 in Buescher serum c̄ hep 1/5000 & incubated for one hour @ 37°C. Tests then refrig until read with 1 loop of meth blue for 0.02 ml toxo-ser. mixt.

Time schedule: PE obtained @ 10^{30} a.m. Final test in @ 11^{15} a.m. & out @ 12^{15} p.m.

(over)

JUL 15 1948

TEST
ALL SERA INACTIVATED

Tube #	SERUM SOURCE	Dil.	PE's in (HEP.)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT.	
1	NaCl - CONT.	—	EB.	12 44	1	49	2	—	
2	GP #2467 - PRE-inc AMS	undil	"	"	4	46	8	—	
3	"	4	"		—	—	—		NEG
4	"	16	"		—	—	—		
5	"	64	"	12 44	1	49	2	—	
6	GP #2467 - 2 wks	undil	"	"	35	21	63	+	
7	"	4	"	1 05	37	13	74	+	
8	"	16	"	"	47	3	94	+	
9	"	64	"	"	44	6	88	+	
10	"	256	"	"	33	17	66	+	≤ 1:256
11	"	1024	"	1 23	15	35	30	—	
12	"	4096	"	"	6	44	12	—	
13	GP #2467 - 34 dys	undil	"	"	1	49	2	—	
14	"	4	"	"	2	48	4	—	
15	"	16	"	1 40	6	44	12	—	
16	"	64	"	"	18 38	32 62	36 38	±	
17	"	256	"	"	35	15	70	+	
18	"	1024	"	"	36	14	72	+	
19	"	4096	"	1 45	39	11	78	+	
20	"	16,384	"	"	42	8	84	+	≤ 1:16,384
21	"	65,536	"	2 00	11	39	22	—	

JUL 15 1948

ALL TEST SERA INACTIVATED

Tube #	Serum Source	Dil.	PE ½ in (Hep)	Time	Effect # unstain.	Effect # stained	Effect % unstain.	Result	
22	GP. # 2468 - Pre.	undil	EB	2^{00}	2	48	4	−	
23	" AMS	4	"	−	~~9~~	±	−		NEG
24	"	16	"	−	−	−	−		
25	"	64	"	2^{40}	3	47	6	−	
26	GP # 2468 - 2 wks	undil	"	"	37	13	74	+	
27	"	4	"	2^{16}	40	10	80	+	
28	"	16	"	"	40	10	80	+	
29	"	64	"	2^{20}	34	16	68	+	
30	"	256	"	"	27	23	54	+	← 1:256
31	"	1024	"	2^{30}	7	43	14	−	
32	"	4096	"	"	4	46	8	−	
33	GP # 2468 - 34 dys	undil	"	2^{35}	3	47	6	−	
34	"	4	"	"	4	46	8	−	
35	"	16	"	2^{59}	11	39	22	−	
36	"	64	"	"	16	34	32	±	
37	"	256	"	3^{02}	27	23	54	+	
38	"	1024	"	"	39	11	78	+	
39	"	4096	"	3^{07}	37	13	74	+	
40	"	16384	"	"	35	15	70	+	← 1:16,384
41	"	65536	"	3^{12}	7	43	14	−	

JUL 15 1948

ALL TEST SERA INACTIVATED

TUBE #	SERUM SOURCE		Dil.	PE 1/5 IN (HEP)	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN	RESULT	
42	GP#2472 - Pre-		undil	EB	3^{12}	3	47	6	—	
43	"	AMS	4	"		—	—	—		
44	"		16	"		—	—	—		NEG
45	"		64	"	3^{47}	3	47	6	—	
46	GP#2472 - 2 wks.		undil	"	"	33	17	66	+	
47	"		4	"	3^{32}	38	12	76	+	
48	"		16	"	"	43	7	86	+	
49	"		64	"	3^{32}	36	14	72	+	
50	"		256	"	"	33	17	66	+	← 1:256
51	"		1024	"	4^{02}	15	35	30	—	
52	"		4096	"	"	5	45	10	—	
53	GP#2472 - 34 dys.		undil	"	4^{07}	2	48	4	—	
54	"		4	"	"	6	44	12	—	
55	"		16	"	4^{12}	12	38	24	—	
56	"		64	"	"	49	51	49	+	
57	"		256	"	4^{20}	40	10	80	+	
58	"		1024	"	"	39	11	78	+	
59	"		4096	"	4^{32}	34	16	68	+	
60	"		16,384	"	"	27	23	54	+	← 16,384
61	"		65,536	"	4^{35}	9	41	18	—	

JUL 17 1948

IN VITRO TESTS WITH WARREN GP SERA (CONCLUDED)

Toxoplasma: PE pooled from 2 four day mice & very rich in toxo.

Serum: GP # 2473 - rec'd from Warren; three samples representing pre-inoc, & 14th & 34th day post-inoc. specimens.

Test: All sera inactivated undiluted just prior to test, then diluted in NaCl. PE made to 1/5 in Buescher serum with hep. 1/5000. 0.1cc serum dil. & 0.1cc PE Susp. mixed, incubated for one hour @ 37° & then read c̄ 1 loop meth blue + 0.02 ml serum-toxo mixt. Tubes refrigerated until read.

over

JUL 17 1948

Test sera inactivated.

TUBE #	SERUM SOURCE	DIL.	PE's IN (HEP.)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT	
1	NaCl - CONT.	—	EB	12^{40}	2	48	4	—	
2	GP #2473 - PRE	undil	"	"	10	40	20	—	
3	"	1:4	"	—	—	—	—		NEG
4	"	16	"	—	—	—	—		
5	"	64	"	"	4	46	8	—	
6	GP #2473 - 2 wks	undil	"	"	38	12	76	+	
7	"	4	"	12^{55}	36	14	72	+	
8	"	16	"	"	35	15	70	+	
9	"	64	"	"	37	13	74	+	
10	"	256	"	"	40	10	80	+	
11	"	1024	"	"	41	9	82	+	
12	"	4096	"	"	31	19	62	+	← 4096 ?
13	GP #2473 - 34 dys	undil	"	1^{20}	7	43	14	—	
14	"	4	"	"	13	37	26	—	"ZONE"
15	"	16	"	"	16	34	32	—	
16	"	64	"	"	33	17	66	+	
17	"	256	"	"	36	14	72	+	
18	"	1024	"	1^{36}	41	9	82	+	
19	"	4096	"	"	43	7	86	+	
20	"	16384	"	"	39	11	78	+	← 16,384
21	"	65536	"	"	13	37	26	—	

JUL 21 1948

In Vitro Tests - Clinical

3[10]

Toxoplasma: PE from one four day mouse - rich in toxo.

Sera: [redacted] - obt. 13 July 48 & forwarded by Zuelzer. Delivered a proven toxo-infected infant in Sept 1944.

[redacted] - pt of Dr. Ghory - quest. calcification (cerebral), quest. chorioretinitis, microcephaly, poor development. Skin test (toxo) - ?

[redacted] Delivered proven toxo infant in May 1947. Sibling delivered 18 July 48 - current samples of cord & venous blood obtained at this delivery.

Test: All sera inactivated just before test, then diluted in NaCl. 0.1cc serum dilution & 0.1cc ~~toxo~~ PE 1/5 in Bierscher serum c̄ hep 1/5000 mixed, incubated for one hour @ 37°C & then examined c̄ 1 loop of meth blue.

All test sera inactivated

Tube #	SERUM SOURCE	DIL.	PE 1/5 in (Hep.)	TIME	EFFECT #UNSTAIN.	#STAINED	% UNSTAIN.	RESULT.	
1	NaCl —	—	EB	4[15]	1	49	2	−	
2	[redacted]	undil	"	"	32	18	64	+	
3		4	"	"	70	30	70	+	
4		16	"	"	41	9	82	+	
5		64	"		42	8	84	+	
6		256	"		26	24	52	+	256
7		1024	"		3	47	6	−	

JUL 21 1948

Tube #	SERUM SOURCE	DIL.	PE 1/5 IN (Hosp.)	TIME	EFFECT % UNSTAIN.	% STAINED	% UNSTAIN	RESULT	
8	[redacted]	undil	"		38	12	76	+	
9		1:4	"		38	12	76	+	
10		16	"		38	12	76	+	
11		64	"		34	16	68	+	← 64
12		256	"		16	34	32	-	
13		1024	"		5	45	10	-	
14	[redacted]	64	"		42	8	84	+	
15		256	"		38	12	76	+	
16		1024	"		35	15	70	+	end-point ?
17	[redacted]	undil	"		35	15	70	+	
18		4	"		33	17	66	+	
19		16	"		41	9	82	+	
20		64	"		38	12	76	+	
21		256	"		40	10	80	+	
22		1024	"		30	20	60	+	end-point ?
23	[redacted]	undil	"		40	10	80	+	
24		4	"		36	14	72	+	
25		16	"		37	13	74	+	
26		64	"		42	8	84	+	
27		256	"		41	9	82	+	end-point ?
28		1024	"		34	16	68	+	

JUL 23 1948

In Vitro Tests – Zuelzer's, Adams', & Pigeon Sera.

Toxoplasma: PE obt. from one four day mouse – very rich in toxoplasma.

Sera: 1) Hemphill family: Sera rec'd from Zuelzer on 17 July via air mail. Had been drawn on 16 July.

a) Mrs. Hemphill: mother of twins – one proven toxo died, other has stigmata.

b) Woody Hemphill – older sibling; normal.

c) Luther – surviving twin – has calci, chorioretinitis, etc.

2) [redacted] tryblicky, Krogstad, rec'd from Forrest Adams.

3) Pigeon sera = part of Eden Park group.

Test: Usual. Sera inac. just before test & then dil in NaCl. 0.1cc mixed c̄ 0.1cc PE 1/5 in Buescher serum c̄ hep 1/5000. Incubated 1 hr @ 37° & read.

TUBE #	SERUM PATIENT	DIL.	PE 1/5 in (HEP).	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
1	NaCl – CONT.	–	EB		12 18	38 36	24		Because of poor control,
1 2	[redacted]	undil	"		45	5	90	+	tubes not read
3	"	4	"		44	6	88	+	completely. Test to
4	"	16	"		45	5	90	+	be redone
5	"	64	"						
6	"	256	"		41	9	82	+	
7 8	"	1024	"		42	8	84	+	
2 8	[redacted]	undil	"		18	32	36	–	
9	"	4	"						
10	"	16	"						
10 11	"	64	"		19	31	38	–	
12	"	256	"						

JUL 23 1948

	TUBE #	SERUM PATIENT	DIL.	PE ½ IN (HEP.)	TIME	EFFECT A UNSTAIN.	EFFECT A STAINED	EFFECT B UNSTAIN.	RESULT.
	13	[redacted]	1024	EB					
3	14	[redacted]	undil	"					
	15		4	"					
	16	"	16	"					
	17	"	64	"					
	18	"	256	"					
	19	"	1024	"					
4	20	[redacted]	undil	"					
	21	"	4	"					
	22	"	16	"					
	23	"	64	"					
	24	"	256	"					
	25	"	1024	"					
	26	"	4096	"					
	27	"	16384	"					
5	28	[redacted]	undil	"		41	9	82	+
	29		256	"		28	22	56	+
6	30	[redacted]	undil	"		32	18	64	+
	31	"	256	"		40	10	80	+
7	32	P-9 5 Aug 47	256	"					
8	33	P-4 5 Aug 47	undil			16	34	32	−
	34	"	256			15	35	30	−
9	35	P-7 5 Aug 47	undil			16	34	32	−
	36	P "	256			16	34	32	−

JUL 24 1948

In Vitro Tests - Zuelzer & Adams Sera

Toxoplasma: PE obtained from one four day mouse. Rich in toxo. Prelim test = 0/50 unstained. (Test = PE 1/5 in EB 1st + hep 1/5000 + NaCl 1st, 20' @ 37°)

Sera: Same sera used 23 July. This is a repeat using the same diluted, heated sera after overnight refrigeration.

Test: 0.1cc serum dilution + 0.1cc toxo PE 1/5 in Buescher serum c̄ hep 1/5000. Incubated 1hr @ 37° C & then refrig. until read using 1 loop of meth blue & 0.02 ml toxo-serum mixt.

Tube #	SERUM PATIENT	DIL.	PE 1/5 in (hep)	Time	EFFECT # UNSTAIN.	# STAIN.	% UNSTAIN.	RESULT.	
1	NaCl - cont	—	EB	11^{50}	1	49	2	−	
				2^{25}	1	49	2	−	
3	[redacted]	4	"	"	46	4	92	+	
4	"	16	"	"	46	4	92	+	
5	"	64	"	"	44	6	88	+	
6	"	256	"	12^{00}	43	7	86	+	
7	"	1024	"	"	36	14	72	+	← 1024
8	"	4096	"	"	6	44	12	−	
9	[redacted]	4	"	"	1	49	2	−	NEG.
10	"	64	"	12^{14}	2	48	4	−	
12	[redacted]	4	"	"	39	11	78	+	
13	"	16	"	"	45	5	90	+	
14	"	64	"	"	47	3	94	+	
15	"	256	"	12^{29}	42	8	84	+	
16	"	1024	"	"	38	25	60	+	← 1024
17	"	4096	"	"	3	47	6	−	

JUL 24 1948

Tube #	SERUM PATIENT	Dil	BE 1/5 in (Hcp)	Timing	EFFECT # UNSTAIN	# STAINED	% UNSTAIN	RESULT	
~~18~~	[redacted]								
19	"	4	EB	$12\frac{1}{4}$	43	7	86	+	Numerous bact
20	"	16	"	$1\frac{45}{}$	45	5	90	+	fewer bact
21	"	64	"	"	42	18	66	+	V few bact 1/64
22	"	256	"	"	7	43	14	−	no bact
23	"	1024	"	"	4	46	8	−	no bact
24	"	4096	"	$2\frac{14}{}$	4	46	8	−	"
25	"	16384	"	"	1	49	2	−	" Serum contaminated
26	[redacted]	4	"	"	47	3	94	+	
27	"	256	"	"	26	24	52	+	end point ?
28	[redacted]	4	"	$2\frac{25}{}$	40	10	80	+	
29	"	256	"		40	10	80	+	end point ?
30	P-9 -5 day 47	256	"		6	44	12	—	

JUL 26 1948

In Vitro Tests – Clinical Samples

Toxoplasma: Pool of two 3 day mice (0.15cc of one & 0.5cc of another). Very rich in toxoplasma. Prelim test = 0/50 unit.

Sera: a) [redacted] Univ. of Utah. Repeat samples drawn 6 July 48.
b) [redacted]
c) [redacted] Both rec'd from J. Warren. Both ~~have~~ are adults c̄ chorioretinitis
d) [redacted]
e) [redacted] Zuelzer's samples. Run for demonstration.

Test: All sera inactivated for 30' @ 56°C just before test & then diluted in NaCl. 0.1cc mixed c̄ 0.1cc PE 1/5 in Buescher c̄ hep 1/5000 & incubated 1hr @ 37°. Then read c̄ meth blue.

(only)

JUL 6 1948

Tube #	SERUM PATIENT	DIL.	PE/S IN (NED)	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
1	NaCl - CONT.	—	E.B.	2 20	1	49	2	—	
2	[redacted]	undil	"	"	18	82	18	—	
3	"	4	"	"	2	48	4	—	
4	"	16	"	"	4	46	8	—	
5	"	64	"	3 40	0	50	0	—	Neg
6	"	256	"	"	2	48	4	—	
7	"	1024	"	"	7	93	7	—	
8	[redacted]	undil	"	"	1	49	2	—	
9	"	256	"	3 50	2	48	4	—	Neg.
10	[redacted]	undil	"	"	44	6	88	+	
11		4	"	"	49	1	98	+	
12	"	16	"	"	41	9	82	+	← 1:16
13	"	64	"	4 17	35	65	35	— ?	
14	"	256	"	"	4	46	8	—	
15	"	1024	"	"	2	48	4	—	
16	[redacted]	undil	"	"	38	12	76	+	
17		4	"	4 45	46	4	92	+	
18	"	16	"	"	43	7	86	+	← 1:16
19	"	64	"	"	37	65	33	— ?	
20	"	256	"	"	3	47	6	—	
21	"	1024	"	"	2	48	4	—	
22	[redacted]	undil	"	"	45	5	90	+	Pos
23		~~256~~ undil	"	2 45	42	8	84	+	Pos.
24		undil	"	"	2	48	4	—	Neg.

JUL 27 1948

In Vitro Tests - Clinical.

Toxoplasma: PE from one four day mouse. Quite rich in toxo.
Prelim test = 1/50 unstained.

Sera: a) [redacted] drawn this a.m. Skin test looks pos. @ 24 hrs.
b) [redacted] - serum drawn yesterday just before skin test which looks positive to-day. Is "Jackie" Woerner's mother.
c) [redacted] Infant Cheryl has been convulsing since birth ca 5 mos. ago. Has hydrocephalus but no other stigmata of toxo. infection.
d) [redacted] for titration. Is a 19 month baby on ward c̄ possible of brain tumor. No signs of toxo.

Test: All sera inac. just before test, then diluted in NaCl. 0.1cc mixed c̄ 0.1cc PE 1/5 in Buescher c̄ hep 1/5000. Incubated one hour @ 37°C & then read c̄ meth. blue.

JUL 27 1948

Tube #	SERUM SUBJECT	Dil.	PE's in (Rep)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT.	
1	[redacted]	0	EB	3 50	3	47	6	−	
1 2	[redacted]	undil	"		41	9	82	+	
3	"	4	"		46	4	92	+	
4	"	16	"		27	23	54	+	← 16
5	"	64	"		10	40	20	−	
6	"	256	"		1	49	2	−	
2 7	[redacted]	undil	"	4 17	38	12	76	+	
8	"	4	"		44	6	88	+	
9	"	16	"		42	8	84	+	
10	"	64	"		38	12	76	+	← 64
11	"	256	"		17	33	34	−	
12	"	1024	"		7	43	14	−	
3 13	[redacted]	undil	"	4 35	38	12	76	+	end point ?
14	"	64	"		42	58	42	+?	
4 15	[redacted]	undil	"		46	4	92	+	end-point ?
16	"	64	"		7	43	14	−	
5 17	[redacted]	undil	"	4 58	0	50	0	−	
18	"	256	"		3	47	6	−	Neg
6 19	[redacted]	undil	"	"	47	3	94	+	end point ?
20	"	256	"		1	49	2	−	

JUL 29 1948

In Vitro Tests - Clinical.

Toxoplasma: PE from 1 four day mouse. Quite rich in toxo. Prelim ok.

Sera: 1 [redacted] 7 - repeat to test zone & end-point.

2 [redacted] - reid from J. Warren. Mother age 30 yr. of child c̄ cong. cataracts, only.

3 [redacted] id from Warren. Died of pneumonia. age 55. No toxo reported.

4 [redacted] reid from Warren. 30 yr. old. Has old chorioretinitis

[redacted] - Dilutions stored in refrigerator since 29 July. 48

[redacted] ilutions made on 27 July & stored in refrig. since.

Test: All sera except Reynolds & Craig inactivated just before test & then diluted in NaCl. 0.1cc of serum + 0.1cc PE 1/5 in Buescher serum c̄ hep 1/5000 mixed & incubated for 1 hr @ 37°C. Then read c̄ meth blue. in rapid order.

JUL 29 1948

Tube #	SERUM SOURCE	Dil.	PE's in (Hep.)	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT:	
1	Na Cl – cont.	—	EB.	1^{05}	1	49	2	−	
2	[redacted]	undil	"	"	11	39	22	−	
3	"	4	"	"	28	22	56	+	
4	"	16	"	"	35	15	70	+	
5	"	64	"	1^{24}	43	7	86	+	
6	"	256	"	"	46	4	92	+	
7	"	1024	"	1^{40}	47	3	94	+	
8	"	4096	"	"	31	19	62	+	4096
9	"	16384	"	1^{54}	7	43	14	−	
10	[redacted]	undil	"	"	42	8	84	+	
11	"	4	"	"	48	2	96	+	
12	"	16	"	"	46	4	92	+	
13	"	64	"	2^{10}	38	12	76	+	
14	"	256	"	"	50	50	50	+	256
15	"	1024	"	2^{35}	3	47	6	−	
16	[redacted]	undil	"	2^{10}	41	9	82	+	v dirty serum.
17	"	4	"	"	45	5	90	+	
18	"	16	"	2^{35}	49	1	98	+	
19	"	64	"	"	42	8	84	+	
20	"	256	"	"	33	17	66	+	256
21	"	1024	"	2^{58}	7	43	14	−	
22	"	4096	"	"	–	–	–		
23	"	16384	"	"	–	–	–		

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Tube #	SERUM SOURCE	Dil.	PE 1/5 in (Kap.)	Time	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
24	[redacted]	undil	EB	2 58	20	30	40	±	very dirty serum
25	"	4	"	"	44	6	88	+	
26	"	16	"	"	46	4	92	+	
27	"	64	"	3 ??	34	16	68	+	64
28	"	256	"	"	17	33	34	−	
29	"	1024	"	"	8	42	16	−	
30	"	4096	"	"	6	44	12	−	
31	[redacted]	4	"		43	7	86	+	
32		16	"		46	4	92	+	
33	"	64	"		40	10	80	+	1:64
34	"	256	"		13	37	26	−	
35	[redacted]	4	"		46	4	92	+	
36		16	"		25	25	50	+	1:16
37	[redacted]	4	"		31	19	62	+	1:4
38		16	"		10	40	20	−	
39	"	64	"		4	46	8	−	

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In Vitro Toxoplasma Tests

Sera: a) GP #2473 - 2 wks bleeding, rec'd from Warren.
[redacted] 29 Dec 47 - one of Eden Park Groups. Neg. skin test. CF = Pos 1/8
[redacted] rec'd from Forrest Adams - has Still's disease.
[redacted] " " " " " "
[redacted] 4 mos. Has convulsions, blindness, chorio retinitis ?. Rec'd from Riley @ U. of Ark.
[redacted] - bled 6-30-48. Rec'd as serum.
[redacted] Koetters, Harrison - first two had neg skin tests
[redacted] positive in recent survey on Psychiatric Survey @ General Hosp.

Test: Sera inactivated as undil on 28 July 48 & stored in refrigerator since. Dilutions made in NaCl just before test this a.m. 0.1cc amts mixed c̄ 0.1cc of PE 1/5 in Buescher serum c̄ hep 1/5000 & then read after one hour @ 37°C using 1 loop of meth bl to 0.02cc serum two mixt. Test out @ 1^25 pm.

Toxoplasma: PE from single 4 dy mouse, quite rich in toxo. Prelim test of PE 1/5 in Buescher + hep 1/5000 & equal quant of NaCl = 1/50 unst. after 20' @ 37°C.

Tube #	SERUM SOURCE	DIL.	PE 1/5 in (HEP.)	TIME	EFFECT # UNSTAIN	# STAINED	% UNSTAIN.	RESULT	
1	NaCl - cont.	—	EB	1^42	2	48	4	—	
1 2	GP. #2473 - 2 wks	1024	"	"	41	9	82	+	
3	"	4096	"	"	42	8	84	+	1:4096
4	"	16384	"	"	4	46	8	—	
2 5	[redacted]	undil	"	2^05	47	3	94	+	dirty ser
6	"	256	"	"	31	19	62	+	run to 512 1:256 ?
3 7	[redacted]	64	"	"	34	16	68	+	1:64
8	"	256	"		11	39	22	—	
9	"	1024	"	2^30	2	48	4	—	
10	"	4096	"	"	0	50	0	—	

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	Tube #	SERUM SOURCE	Dil.	PE's in (HEP.)	Time	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
4	11	[redacted]	64	EB	2^{30}	26	24	52	+	1:64
	12	"	256	"	"	9	41	18	−	
	13	"	1024	"	"	—	—	—		
	14		4096	"	"	—	—	—		
5	15	[redacted]	undil	"	2^{45}	3	47	6	−	
	16	"	64	"	"	2	48	4	−	Neg
6	17	[redacted]	undil	"	2^{50}	41	9	82	+	
	18	"	4	"	"	36	14	72	+	1:4
	19	"	16	"	2^{57}	10	40	20	−	
	20	"	64	"	"	1	49	2	−	
	21	"	256	"	3^{02}	2	48	4	−	
7	22	[redacted]	undil	"	"	1	49	2	−	
	23	"	64	"	3^{06}	4	46	8	−	Neg
8	24	[redacted]	undil	"	"	47	3	94	+	
	25	"	4	"	3^{11}	39	11	78	+	
	26	"	16	"	"	40	10	80	+	1:16
	27	"	64	"	"	5	45	10	−	
	28	"	256	"	"	3	47	6	−	
9	29	[redacted]	undil	"	3^{22}	5	45	10	−	
	30	Rabbit " 4 mos. 6-30-48 - Anti	256	"	"	5	45	10	−	Neg

AUG 3 1948

In Vitro Tests, Clinical & Pyrian Sera

Toxoplasma: One 4 dy PE - terrifically rich in toxo. so counts not done. Prelim = 1/100 unt.

Sera: a) [redacted] - Akron. Tom had fever, convulsions only & died @ 5 mos. No path.
b) [redacted] part of Eden Park group.
c) [redacted] repeats for titers.
d) [redacted] obtained in Edenpark study.
e) [redacted]: rec'd to-day from Dr. Debré. Unfrozen. No data but apparently mother & child.

Test: All sera except the Couriacs' inactivated at 56° C for 30', undiluted just before test. All dilutions in NaCl. 0.1cc serum dilution & 0.1cc of toxo 1:5 in Buescher serum c̄ hep 1/5000 incubated one hour @ 37° C & then read c̄ 1 loop meth bl & 0.02 cc serum-toxo. Toxo so numerous that all readings completed within 1 1/2 hrs.

	TUBE #	SERUM PATIENT	DIL.	PE 1/5 IN (HEP.)	TIME	EFFECT # UNSTAIN	# STAINED	% UNSTAIN.	RESULT	
	1	NaCl - CONT.	—	EB	2:15	0	50	0	—	
1	2	[redacted]	undil	"		al 100%			+	
	3	"	4	"		"			+	1:8 or 16 ?
	4	"	16	"		28	33	46	+?	
	5	"	64	"			al 100		—	
	6	"	256	"			al 100		—	
	7	"	1024	"			"		—	
2	8	[redacted]	undil.	"			"	—	—	
	9	"	4	"			"		—	
	10	"	16	"			"		—	Neg
	11	"	64	"			"		—	

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	Tube #	Serum Patient	Dil.	Pe 1/5 in (Hep.)	Time	Effect # Unstain.	Effect # Stained	Effect % Unstain.	Result.	
3	12	[redacted]	256	EB			al 100%		—	
	13	"	1024	"			"		—	
	14	"	4096	"			"		—	
3	15	[redacted]	64	"		Pos 85?			+	
	16	"	256	"		80?			+	1:256
	17	"	1024	"			Ca 85%		—	
	18	"	4096	"			al 100%		—	
4	19	[redacted]	64	"		Pos 80?			+	
	20	"	256	"		44	56	44	+?	256 ?
	21	"	1024	"			neg al 100			
	22	"	4096	"			"			
5	23	[redacted]	64	"		Pos. 80%			+	
	24	"	256	"		43	57	43	+?	256 ?
	25	"	1024	"			al 100			
	26	"	4096	"			"			
6	27	[redacted]	undil	"			al 100	—	—	
	28	"	64	"			"		—	neg.
7	29	~~[illegible]~~ P-5 5 day 47	undil	"			"		—	
	30	"	64	"			"		—	neg.
8	31	[redacted]	undil	"		Ca 80%			+	
	32	"	4	"		"			+	
	33	"	16	"		"			+	
	34	"	64	"		44	15	74	+	
	35	"	256	"		65	35	65	+	256 ?

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	Tube #	SERUM SOURCE		Dil.	PE 1/5 in (Hyp.)	Time	EFFECT A UNSTAIN.	EFFECT A STAINED	EFFECT B UNSTAIN.	RESULT	
9	36	P-11	5 Aug	undil						−	
	37	"	"	64				"		−	Neg
10	38	P-12	5 Aug	undil				"		−	
	39	"	"	64				"		−	Neg
11	40	P?	5 Aug	undil				"		−	
	41	"	"	64				"		−	Neg
12	42	P-19	7 Aug	undil			15	35 al 100% all sat.	30	−	
	43	"	"	64						−	Neg
13	44	P-18	7 Aug	undil				"	"	−	
	45	"	"	64				"		−	neg
14	46	P-22	7 Aug	undil				"		−	
	47	"	"	64				"		−	Neg.
15	48	P-23	7 Aug	undil				"		−	
	49	"	"	64				"		−	Neg.
16	50	[redacted]		undil		2 15	al 100%		ca 100	+	
	51			256		"	al 0		neg.	−	Pos. Titer?
17	52	[redacted]		undil		2 15	"		"	+	
	53			256		"	"		"	−	"

AUG 7 1948

In Vitro Tests Of Pooled Mouse (Infected) Sera And Sera From Skin-Test Positive & Negative Humans.

Toxoplasma: PE from single four day mouse - extremely rich in toxo. Saline test = 0/50 cmt.

Sera: a) [redacted] 20 dy infant @ Cinc. Ch. Hosp with acute purulent asymptomatic purulent meningitis.

b) 5 dy Mouse Pool: Serum pooled from 3 Ch alb mice, 5 dy post-inoc. day (IP), well-infected. Obtained & frozen since 27 May 48.

c) 6 dy Mouse Pool: Serum pooled from 2 Ch alb mice, 6 dy post-inoc. day (IP), well-infected. Obtained & frozen since 27 May 48.

d) Skin test Groups (16 July 48) Psycho Service of CMH:

(1) [redacted]: WM 68 yrs. Pos. skin test.

(2) [redacted] WM 81 yrs. " " "

(3) [redacted] WM 59 yrs. " " " (but pos. reac. @ 24 hrs.).

Test: All sera inactivated for 30' @ 56° C just prior to test & then diluted in saline. 0.1 cc cmts mixed = 0.1 cc PE 1/5 in Baeschen serum = hep. 1/5000 & then incubated for 1 hr @ 37° C. Then read = 1 loop meth bl. to 0.02 cc serum-tox mixt.

(avg)

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	Tube #	SERUM SOURCE	DIL.	PE 1/5 IM (HEP.)	TIME	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT.	
	1	NaCl - cont.	—	EB	2^{40}	2	48	4	−	
1	2	[redacted]	undil	"	"	6	44	12	−	Neg
	3		64	"		1	49	2	−	
2	4	5 dy Mouse Pool 27 May 48	undil	"	10^{3}	2	48	4	−	dirty
	5	"	4	"	1^{50}	1	49	2	−	"
	6	"	16	"		2	48	4	−	Neg
	7	"	64	"	1^{03}	7	43	14	−	
	8	"	256	"		3	47	6	−	
	9	"	1024	"						
	10	"	4096	"						
3	11	6 dy Mouse Pool 27 May 48	undil	"	1^{50}	1	49	2	−	
	12	"	4	"						
	13	"	16	"						Neg
	14	"	64	"	1^{50}	2	48	4	−	
	15	"	256	"	2^{10}	4	46	8	−	
	16	"	1024	"	2^{25}	2	48	4	−	
	17	"	4096	"						
4	18	[redacted]	undil	"	2^{10}	5	45	10	−	
	19		64	"	"	2	48	4	−	Neg (Neg when tested)
5	20	[redacted]	undil	"	"	46	4	92	+	Dose Titer 1:16 (Neg when tested)
	21		64	"	"	2	48	4	−	
6	22	[redacted]	undil	"	"	47	3	94	+	
	23		4	"	2^{25}	45	5	90	+	
	24	"	16	"	"	32	18	64	+	1:16
	25	"	64	"	"	6	44	12	−	(Neg when tested)

AUG 13 1948

In vitro Tests With English and Hopkins Sera

Toxoplasma: PE from single 4 day mouse, containing only moderate number of toxoplasma. Dilution = 1/50 control

Sera: a) [redacted] rec'd from Johns Hopkins. ~~has bi-lateral chorio-retinitis~~ Spastic. Retarded. Child ca 1 year of age, ~~has had no convulsions~~, ~~retinal atrophy in the other eye~~, no cerebral calcifications. ~~Microcephaly?~~

b) [redacted]: rec'd from Univ. of Sheffield, England. Child ca 1 yr. has retinal atrophy but no ~~cerebral~~ cerebral calcifications

c) [redacted] rec'd from Univ. of Sheffield, England. Child ca 1 yr has retinal atrophy but no ~~cerebral~~ cerebral calcifications

Test: All sera inactivated for 30' @ 56°C & then dil'd in saline & 0.1 cc units distributed to final tubes. 0.1 cc PE 1/5 in Bulocher serum with heparin 1/5000. Incubated 1 hr @ 37°C & then read = meth blue. Toxo few in numbers but counted without too much difficulty. Control quite high but results appear valid.

(Sabin)

AUG 13 1948

all test sera inactivated.

Tube #	SERUM SOURCE	Dil.	PE's in (Hosp.)	TIME	EFFECT # UNSTAIN. STAIN	# STAINED	% UNSTAIN.	RESULT	
1	NaCl - CONT.	—	EB		8	42	16	−	
2	[redacted]	undil	"		47	3	94	+	Pos.
3	[redacted]	undil	"		41	9	82	+	
4	"	4	"		41	9	82	+	
5	"	16	"		38	12	76	+	← 1:64
6	"	64	"		27	23	54	+	
7	"	256	"		15	35	30	−	
8	"	1024	"						
9	"	4096	"						
10	"	16384	"						
11	[redacted]	undil	"		34	16	68	+	undil
12	"	4	"		20	30	40	−	
13	"	16	"		16	34	32	−	
14	"	64	"		16	34	32	−	
15	"	256	"						
16	"	1024	"						
17	"	4096	"						
18	"	16384	"						
19	[redacted]	undil	"		19	31	28	−	Neg
20	[redacted]	64	"		12	38	24	−	
21	[redacted]	undil	"		8	42	16	−	Neg
22	[redacted]	64	"		11	39	22	−	
23	[redacted]	undil	"		8	42	16	−	Neg
24	[redacted]	64	"		10	40	20	−	
25	[redacted]	undil	"		7	43	14	−	Tube #26 Mrs. Fitzgerald 1/64 Neg − 6/44 ant.

In Vitro Tests Codey (England) Family & Ochsner Sera.

SEP 7 1948

Sera: 1) Codey Family - sera obtained 10 Aug 48 - reid 26 Aug 48 - not frozen.
a) Father - L & W - 23 yrs. old. In Africa & Mediterranean during war.
b) Mother - L & W - 20 yrs. Normal pregnancy
c) [redacted] born 4 Nov 48 - Premature. Left chorio-retinitis, Microphthalmus on right. Intra-cranial calcification.
d) [redacted] Two sets, each reid on different days with no identifying labels. One group from mother & child c̄ rubella syndrome, the other from a 10 year old c̄ epilepsy, Paul Galatas & mother
Rubella group = Robert Heroman, born 16 Dec 46
e) [redacted] reid in A mail from Van Creveld to-day. Contaminated & unfrozen.

Toxoplasma: Derived from a single four day mouse. Mod. # toxoplasma. Prelim OK.

Test: Usual. Sera dil in NaCl after inactivation. 0.1 cc mixed c̄ PE 1/5 in Buescher serum c̄ hep 1/5000 (heparin freshly made & unfiltered), incubated 1 hr @ 37°C & then read c̄ 1 loop of meth blue for 0.02 cc toxo-serum mixt.

All sera inactivated

	Tube #	SERUM SOURCE	DIL.	PE 1/5 in (HEP.)	EFFECT # UNSTAINED	# STAINED	% UNSTAIN.	RESULT	
	1	NaCl - CONT.	—	EB	5	45	10	−	
1	2	[redacted]	undil	"	4	46	8	−	ZONE
	3		1:64	"	33	17	66	+	1024
2	4		undil	"	16	34	32	+−	ZONE
	5		1:64	"	33	17	66	+	1024
3	6		undil	"	3	47	6	−	
	7		1:64	"	1	49	2	−	Neg
4	8		undil	"	2	48	4	−	
	9		1:64	"	4	46	8	−	Neg
5	10		undil	"	28	22	56	+?	loaded c̄ bacteria. Toxo diff to count
	11		1:64	"	14	36	28	−	Neg

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all sera except Brinkman inactivated.

	Tube #	SERUM SOURCE	Dic.	PE 1/5 in (Hep.)	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
6	12	[redacted]	undil	EB	2	48	4	–	Neg
	13		1:64	"	1	49	2	–	
7	14		undil	"	8	42	16	–	Neg
	15		1:64	"	2	48	4	–	
8	16		undil	"	7	43	14	–	loaded c̄ roots.
	17		1:64	"	1	49	2	–	Neg

SEP 8 1948

In Vitro Toxoplasma Tests — Clinical Samples & Skin Test Study.

Toxoplasma: Obtained from a single 4 day mouse. Many toxoplasma.

Sera: [redacted] sera inactivated 7 Sept & stored overnight in the refrigerator in the undiluted state. Diluted just before this test.

b) [redacted] spinal fluid — thawed & inactivated just before test.

[redacted] — born 7-18-48 — bled 8-30-48 — 43rd day serum. Following [redacted] Chard, Ciera, Dwyer, Byrne, — part of the toxoplasma skin test survey at CGH. All except Maher had positive skin tests. Latter is 81 yrs. old.

Test: Spinal fluid & all sera except those tested yesterday inac. for 30' @ 56°C before test. Dilutions in NaCl. 0.1cc amts mixed c̄ 0.1cc PE 1/5 in Buescher serum c̄ hep. 1/5000. Incubated one hour @ 37°C & then read c̄ meth blue. Tubes read very rapidly because of large number of toxoplasma.

(pub)

all sera inactivated

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	Tube #	SERUM SOURCE	Dil.	PE in (Hep)	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
	1	NaCl - cont.	—	EB	1 40	1	49	2	−	
1	2	[redacted]	undil	"	"	7	43	14	−	
	3		4	"	1 55	8	42	16	−	"ZONE"
	4	"	16	"	"	19	31	38	±	
	5	"	64	"	"	40	10	80	+	
	6	"	256	"	"	46	4	92	+	
	7	"	1024	"	2 20	46	4	92	+	1024
	8	"	4096	"	"	14	36	28	−	
	9	"	16384	"	"		almost all stained		−	
	~~10~~	"	65536	"	"				−	
2	11	[redacted]	undil	"	2 55	10	40	20	−	
	12	"	4	"	"	19	31	38	±	
	13	"	16	"	"	33	17	66	+	
	14	"	64	"	"	40	10	80	+	
	15	"	256	"	"	43	7	86	+	
	16	"	1024	"	"	30	20	60	+	1024
	17	"	4096	"	3 16	5	45	10	−	
	18	"	16384	"	"	0	50	0	−	
3	19	[redacted]	undil	"		37	13	74	+	
	20	"	4	"		44	6	88	+	
	21	"	16	"		48	2	96	+	
	22	"	64	"		47	3	94	+	
	23	"	256	"		30	20	60	+	256
	24	"	1024	"		5	45	10	−	

SEP 8 1948

All sera inactivated

Tube #	SERUM SOURCE	DIL.	PE 1/5 in (Lep)	Time	EFFECT # UNSTAIN	# STAINED	% UNSTAIN	RESULT	
25	[redacted]	undil	EB		47	3	94	+	
26	"	4	"		38	12	76	+	1:4
27	"	16	"		13	37	26	−	
28	[redacted]	undil	"		38	12	76	+	
29	"	4	"		37	13	74	+	
30	"	16	"		38	12	76	+	
31	"	64	"		39	11	78	+	endpoint ? 1:256
32	[redacted]	undil	"		39	11	78	+	
33	"	4	"		47	3	94	+	
34	"	16	"		34	16	68	+	1:16
35	"	64	"		4	46	8	−	
36	[redacted]	undil	"		34	16	68	+	
37	"	4	"		40	10	80	+	
38	"	16	"		36	14	72	+	1:16
39	"	64	"		20	45	30	−	
40	[redacted]	undil	"		40	10	80	+	
41	"	4	"		45	5	90	+	
42	"	16	"		27	23	54	+	1:16
43	"	64	"		6	44	12	−	
44	[redacted]	undil	"		41	9	82	+	
45	"	4	"		40	10	80	+	
46	"	16	"		44	6	88	+	1:64
47	"	64	"		30	20	60	+	1:64 ?
48	[redacted]	undil	"		45	5	90	+	titer ? 1:16
49		64	"		5	45	10	−	

SEP 9 1948

Completed Titrations of Clinical Samples Tested On 8 Sept 48

Toxoplasma: Pooled from 2-3 dy mice - same PE used for Serum Fraction Tests of to-day.

Sera: Joseph Codey spinal fluid, Wade, & Byrne - inactivated & diluted 8-Sept & stored in refrigerator overnight. Additional dilutions from 1/64 made just before test.

Test: 0.1cc serum dil & 0.1cc PE 1/5 in Buescher serum c̄ hep 1/5000 incubated one hour @ 37° C & then read as usual c̄ meth blue.

Tube #	Serum Source	Dil.	PE 1/5 in (hep.)	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
30	[redacted]	1:4	EB	43	7	86	+	
31		1:16	"	38	12	76	+	1:16
5 32		64	"	48	2	96	+	
33		256	"	30	25	54	+	1:256
34		1024	"	11	39	22	−	
9 35		64	"	36	14	72	+	1:64
36		256	"	10	38	20	−	
37		1024	"	3	47	6	−	

AUG 4 1948

In Vitro Tests – Harvard Plasma Fractions & Titration of Debre's Case.

Toxoplasma: PE obt from one ~~#~~ 3 dy mouse. Vrich in toxo but gns of thick so peritoneal cavity washed = small amt of NaCl & this added to exudate. This PE-saline mixt considered the undil PE.

Sera: a) [redacted] had been diluted yesterday from the "fresh" state (i.e. not inactivated) & stored in the refrigerator overnight.

[redacted] reid as dried powder in rubber capped vials, having been 3 days en route via RR Express, unrefrigerated. Kept in refrigerator here until suspended to-day.

The whole plasma was dissolved in ~~saline~~ distilled H_2O

The fractions were dissolved in saline and all were acid to phenol red. The amount of N/100 NaOH required to neutralize was determined on a small aliquot and the remainder adjusted with N/1 NaOH.

All frozen, uncentrifuged.

AUG 4 1948

Tube #	SERUM SOURCE	DIL.	PE 1/5 in (Hep)	TIME	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
1	NaCl - cont.	—	EB	5 1/2	3	47	6	—	
2	EB - cont.	—	NaCl		3	47	6	—	
3	[redacted]	4	EB		40	10	80	+	
4	"	16	"		35	15	70	+	1:16
5	"	64	"		14	36	28	—	
6	"	256	"		5	45	10	—	
7	[redacted]	4	"		38	12	76	+	1:8?
8	"	16	"		40	60	40	±	
9	"	64	"		3	47	6	—	
10	"	256	"		2	48	4	—	
11	WH. PLASMA - [redacted]	2.5%	NaCl		4	46	8	—	
12	~~FRACTION I~~ wh. Plasma	5.0%	"		1	49	2	—	
~~13~~	"								
14	FRACTION II + III	2.5%	"		0	50	0	—	Full of plate-like yellow crystals and amorphous material, holding texo
15	"	1.0%	"		0	50	0	—	Texo enmeshed in ppt. "fibrin" network
16	FRACTION IV - 1	2.5%	"		10	40	20	—	Many texo trapped in "fibrin" network stained
17	"	1.0%	"		0	50	0	—	Extensive fibrin network full of texo
18	FRACTION IV - 4	2.5%	"		2	48	4	—	Full of crystal "needle like plates (yellow) "no fibrin network"
19	"	1.0%	"		1	49	2	—	"fibrin" network trapping texo
20	FRACTION V	2.5%	"		0	50	0	—	Almost no network
21	"	1.0%	"		0	50	0	—	"fibrin" network enmeshing everything
22									

Note - it is not clear whether the "fibrin" network is due to ppt. by stain or to reaction of fraction with exudate

Presence of Activator in Various Human Plasma Fractions

Obtained from Harvard Department of Physical Chemistry.

SEP 9 1948

Fractions - see protocol of Aug. 4, 1948; Kept frozen as 7.5% solutions. Prior to use in present test the 7.5% and 1% solutions will be centrifuged. All 7.5% dilutions to 1% made ⊂ Saline

SERA — IMMUNE — MRS. D.E. ROPE, 5-19-48. CENT. 6-15-48. Heated 30' @ 56°C just before test.
Normal — Buescher - cent & heated 30' @ 56° just before test.

Toxoplasma - pooled from 2, three day IP inoc. mice. Moderate # of toxo.

Tube	Heated Immune Serum	DILUENT	Toxo in 0.2cc		No. unstained	% unstained
1	None	0.2cc saline	Saline *		1/50	2%
2	0.02 cc	0.18cc "	"		3/50	6%
3	"	" white pl. 7.5%	"		4/50	8%
4	"	" " " 1%	"		4/50	8%
5	"	" FR. II + III - 7.5%	"	very murky	1/50	2
6	"	" " " - 1%	"	almost clear	3/50	6
7	"	" FR. IV-1 - 7.5%	"	clear	0/50	0
8	"	" " " -1 - 1%	"		2/50	4
9	"	" FR IV-4 - 7.5%	"		2/50	4
10	"	" " - 1%	"		1/50	2
11	"	" FR V - 7.5%	"		1/50	2
12	"	" " - 1%	"		0/50	0

* PE 1/5 in NaCl ⊂ hepar 1/5000

Usual procedure, tubes read ⊂ methylene blue after one hour @ 37° C.

STANDARD B&P "NOTEAR"

Capacity of Various Human Plasma Fractions to Reactivate Heated Activator

SEP 9 1948

TUBE	Heated Immune Serum	DILUENT	Toxo in 0.2cc		No. unstained	% unstained	
13	0.02cc	0.18cc saline	Heated E.B. serum *		1/50	2	−
14	"	0.18cc fresh E.B. serum	"		46/50	92	+
15	"	" whole pl. − 7.5%	"		12/50	24	−
16	"	" " " − 1%	"		6/50	12	−
17	"	" Fr. II + III − 7.5%	"	vg mucky	0/50	0	−
18	"	" " " − 1%	"	sl mucky	2/50	4	−
19	"	" Fr. IV − 1 − 7.5%	"		18/50	36	?−
20	"	" " " − 1%	"		1/50	2	−
21	"	" Fr IV − 4 − 7.5%	"	some granular ppt.	6/50	12	−
22	"	" " − 1%	"		1/50	2	−
23	"	" Fr. V − 7.5%	"		2/50	4	−
24	"	" " − 1%	"		1/50	2	−
25	None	0.2cc saline	"		1/50	2	−

* PE 1/5 in Buescher serum - heated for 30' @ 56°C just before test — & containing heparin 1/5000

STANDARD B&P "NOTEAR"

SEP 10 1948

Complementary Activity of Various Human Plasma Fractions for Sensitized Sheep RBC and Capacity of Various Fractions to Reactivate Heated G. pig Complement

Plasma Fractions – Same centrifuged preparations used in Toxo test on 9/9 kept frozen in dry ice

Sensitized Sheep RBC – 3-4 units of ambocepter

Tube	Material added to 0.5cc sensitized RBC	Hemolysis ½ hr. at 37°C	Hemolysis Overnight in refrigerator
1	0.5cc saline	0	0
2	0.5cc freshly thawed frozen guinea pig serum 1:10	4	4
3	0.5cc of whole plasma 7.5%	3?	3.5
4	" " " " 1%	0	0
5	0.35 " " Fr. II+III – 7.5%	0	0
6	0.5 " " " " " — 1%	0	0
7	" " Fr. IV-1 – 7.5%	0	0
8	" " " " — 1%	0	0
9	" " Fr. IV-4 – 7.5%	0	0
10	" " " " — 1%	0	0
11	" " Fr. V – 7.5%	0	0
12	" " " — 1%	0	0
13	0.1cc each of Fr. II+III, Fr. IV-1, Fr IV-4, + Fr. V,* saline	0	0
14	0.1cc of heated g. pig ser. + 0.4cc saline	0	0
15	" " " " + 0.4cc Fr II+III – 7.5%	0	0
16	" " " " + 0.4cc. Fr IV-1 – "	0	0
17	" " " " + 0.4cc Fr IV-4 – "	0	0
18	" " " " + 0.4cc Fr V – "	0	0
19	" " " " + II+III, IV-1, IV-4, V**	0	0

* All the fractions used in tube 13 were 7.5%

** In tube 19 – 0.5cc of 1% II+III; 0.1cc of 7.5% of other fractions

STANDARD B&J "NOTEBAR"

SEP 10 1948

In Vitro Toxoplasma Tests

Toxoplasma: PE from a single 4 day mouse. Moderate number of toxo. Pullin = 0/50 unit.

Sera: a) Wilson, Watson, Bowling – all skin test positive in the survey on Psycho (CGH.)

b) P-14 – one of the Eden Park pigeons.

Test: All sera inac. for 30' @ 56°C just prior to test, then dil. in NaCl & 0.1cc amts distributed to tubes. PE 1/5 in Buescher serum c̄ heparin 1/5000 then added in 0.1cc amts & incubated for one hour @ 37°C. Read with meth. blue. PE used in final test within 35' of withdrawal from mouse.

(over)

Note: [redacted] serum used to-day had been used once before (yesterday); appears as tho activator titre had dropped, according to this test.

SEP 10 1948

all sera inactivated before test

Tube #	SERUM SOURCE		Dil.	PE 1/5 in (Dup.)	EFFECT # UNSTAIN-	# STAINED	% UNSTAIN	RESULT	
1	NaCl	—	—	EB	1	49	2	−	
2	[redacted]		undil	"	26 / 52	24 / 48	52 / 52	+	
3	"		4	"	35	15	70	+	
4	"		16	"	41	9	82	+	
5	"		64	"	34	16	68	+	← 1:64
6	"		256	"	1	49	2	−	
7	[redacted]		undil	"	27	23	54	+	
8	"		4	"	38	12	76	+	
9	"		16	"	33	17	66	+	
10	"		64	"	25	25	50	+	1:64
11	"		256	"	3	47	6	−	
12	[redacted]		undil	"	36	14	72	+	
13	"		4	"	44	6	88	+	
14	"		16	"	30	20	60	+	1:16
15	"		64	"	16	34	32	−	
16	"		256	"	3	47	6	−	
17	P-14 (9 Aug 49)		undil	"	2	48	4	−	
18	"		256	"	2	48	4	−	

SEP 13 1948

In Vitro Tests - Eden Park Group & Two Clinicals

Toxoplasma: PE pooled from 2 four day mice. Very rich in Toxo. Prelim test ok.

Sera: 1) The first 14 sera in the protocol are derived from the Eden Park pneumonitis patients.

2) [redacted] 11 month infant c̄ poor development. Convulsions. No cerebr. calci. Patient @ Tulane.

3) [redacted] mother of Willie Lee.

4) [redacted] reid to-day from Jersey City Med. Center. Is 18 mos old. Was 2 1/4 lb premature. Slow development. Has bilateral chorioretinitis, microphthalmos, convulsions @ 5 & 18 months. Has questionable multiple cerebral calcifications. Serum contained some rbc which were spun out before use.

5) [redacted] mother of Cecilia.

Test: All sera inactivated just before test for 30' @ 56°C, then diluted with NaCl & 0.1cc amts mixed c̄ 0.1cc PE 1/5 in Bruescher serum c̄ hep 1/5000. Incubated 1 hr @ 37°C & then read c̄ meth blue as usual. Toxoplasma very numerous & slides read quickly.

SEP 13 1948

all sera inactivated

	Tube #	SERUM SOURCE	DIL.	PE 1/5 in (hrs.)	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN	RESULT.	
	1	NaCl —	—	EB	1	49	2	−	
1	2	[redacted]	undil	"	36	14	72	+	Titer — undil
	3	"	64	"	4	46	8	−	
2	4	[redacted]	undil	"	48	2	96	+	titer ?
	5	"	64	"	4	46	8	−	1:16
3	6	[redacted]	undil	"	~~21~~ 21	79	21	−	neg
	7		64	"	5	45	10	−	
4	8		undil	"	42	8	84	+	Titer ?
	9		64	"	4	46	8	−	1:4
5	10		undil	"	28	72	28	−	neg
	11		64	"	2	48	4	−	
6	12		undil	"	42	8	84	+	Titer ?
	13		64	"	5	45	10	−	1:16
7	14		undil	"	25	25	50	?	v dirty - repeat.
	15		64	"	4	46	8		repeat
8	16		undil	"	18	32	36	−	neg
	17		64	"	10	40	20	−	
9	18		undil	"	44	6	88	+	v dirty but easy count
	19		64	"	36	14	72	+	Titer ? 1:64
10	20		undil	"	50	0	100	+	Titer ?
	21		64	"	5	45	10	−	1:16

all sera inactivated

SEP 13 1948

	Tube #	SERUM SOURCE	DIL.	PE's IN (Hep.)	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
11	22	[redacted]	undil	EB	50	0	100	+	
	23	"	4	"	49	1	98	+	
	24	"	16	"	44	6	88	+	1:16
	25	"	64	"	14	36	28	−	
	26	"	256	"	3	47	6	−	
	27	"	1024	"	−	−	−		
12	28	[redacted]	undil	"	44	6	88	+	
	29	"	4	"	44	6	88	+	
	30	"	16	"	45	5	90	+	
	31	"	64	"	48	2	96	+	
	32	"	256	"	48	2	~~96~~	+	1:1024
	33	"	1024	"	38	12	76	+	Titer?
13	34	[redacted]	undil	"	48	2	96	+	
	35	"	4	"	49	1	98	+	
	36	"	16	"	46	4	92	+	
	37	"	64	"	49	1	98	+	
	38	"	256	"	35	15	70	+	256
	39	"	1024	"	13	37	26	−	
14	40	[redacted]	undil	"	47	3	94	+	
	41	"	4	"	47	3	94	+	
	42	"	16	"	49	1	98	+	
	43	"	64	"	47	3	94	+	
	44	"	256	"	46	4	92	+	256
	45	"	1024	"	12	38	24	−	

SEP 13 1948

All sera inactivated

	Tube #	SERUM SOURCE	Dil.	RBC's in (kep.)	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
15	46	[redacted]	undil	EB	9	41	18	—	Neg.
	47		64	"	6	44	12	—	
16	48	[redacted]	undil	"	46	4	92	+	Titer ?
	49		64	"	46	4	92	+	1:256
17	50	[redacted]	undil	"	8	42	16	—	Bact. Neg @ 1024
	51	" Jersey City.	64	"	7	43	14	—	Zone ?
18	52	[redacted]	undil	"	4	46	8	—	Neg Zone ?
	53	"	64	"	10 22	40 78	20 22	—	Neg @ 1024

SEP 14 1948

Completion of In Vitro Tests of 13 Sept 1948

Toxoplasma: PE obtained from a single 4 day mouse, Mod. rich in toxo. Prelim test ok.

Sera: Inactivated & diluted 13 Sept & stored in refrigerator since. Additional dilutions made this a.m. where indicated.

Test: 0.1cc serum dilution plus 0.1cc PE 1/5 in Buescher serum c̄ hep. 1/5000, incubated 1 hr @ 37°C & then read as usual c̄ meth blue in wet film.

(over)

SEP 14 1948

	Tube #	SERUM SOURCE	Dil.	PE's in (Rep.)	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
	1	NaCl - cont	–	EB	2	48	4	—	
1	2	[redacted]	4	"	5	45	10	–	
	3		16	"	4	46	8	–	neg. Titer = undil
2	4	[redacted]	4	"	38	12	76	+	
	5	"	16	"	26	24	52	+	1:16
4	6	[redacted]	4	"	28	22	56	+	1:4
	7	"	16	"	2	48	4	–	
6	8	[redacted]	4	"	39	11	78	+	
	9	"	16	"	39	11	78	+	1:16
9	10	[redacted]	64	"	34	16	68	+	1:64
	11	"	256	"	8	42	16	–	
	12	"	1024	"	—	—	—		
10	13	[redacted]	4	"	42	8	84	+	
	14	"	16	"	32	18	64	+	1:16
12	15	[redacted]	1024	"	42	8	84	+	1:1024
	16	"	4096	"	8	42	16	–	
	17	"	16384	"	—	—	—		
15	18	[redacted]	256	"	6	44	12	–	neg
16	19	[redacted]	64	"	42	8	84	+	
	20	"	256	"	38	12	76	+	1:256
	21	" Tulane.	1024	"	14	36	28	–	
17	22	[redacted]	1024	"	6	44	12	–	
18	23	[redacted]	1024	"	2	48	4	–	

SEP 15 1948

Comparative In Vitro Titrations of Immune Pigeon and Human Sera Vs. "RH" & P-17 Strain Toxoplasma.

Obj: To determine whether immune pigeon serum (P-17) & immune human serum (Rainey) will yield different titers when tested against toxoplasma of human origin (RH) and toxoplasma of pigeon origin (P-17 strain).

Toxoplasma:
a) "RH" - 4 dy PE from 2 mice - mod. # tox. Prelim test OK.
b) P-17 - 4 dy PE from 1 mouse, very rich in tox. Prelim test OK.

Sera: a) P-17 & Rainey sera. The latter is from the mother of a proven congenital case of toxoplasmosis & in previous tests has had the same titer as the P-17 serum; however, the Rainey serum has not had a zone.
b) P-10 - recheck on a previous test.
c) P-8 & P-3 (label difficult to read but looks like P-3) - survey sera.

Test: Sera inactivated just before test & then diluted ē saline, distilled to both the RH & P-17 series from the same dilution tubes. Each PE diluted 1/5 with Boeschen serum & hep 1/5000 added. 0.1cc serum dilution & 0.1cc PE in EB incubated 1 hr @ 37° C & then read ē methylene blue. Tox plentiful & tests read without difficulty.

SEP 15 1948

PIGEON & HUMAN SERA VS. "RH" TOXO

all sera inactivated before test

	Tube #	SERUM SOURCE		DIL.	PE 1/5 IN (HRP)	EFFECT # UNSTAIN	# STAINED	% UNSTAIN	RESULT	
	1	NaCl - cont.		—	EB	4	46	8	—	
1	2	P-17	7 Aug 47	undil	"	9	41	18	—	
	3	"		4	"	10	40	20	—	
	4	"		16	"	28	22	56	+	ZONE
	5	"		64	"	45	5	90	+	
	6	"		256	"	36	14	72	+	
	7	"		1024	"	48	52	48	+	1024
	8	"		4096	"	6	44	12	—	
2	9	[redacted]		undil	"	37	13	74	+	
	10	"		4	"	44	6	88	+	
	11	"		16	"	45	5	90	+	
	12	"		64	"	43	7	86	+	
	13	"		256	"	34	16	68	+	
	14	"		1024	"	46	54	46	+	~~1024~~ 256-512
	15	"		4096	"	4	46	8	—	
3	16	P-10	5 Aug. 47	undil	"	10	40	20	—	
	17	"		4	"	30	20	60	+	
	18	"		16	"	30	20	60	+	? Titer
	19	"		64	"	29	21	58	+	Titrate further
4	35	P-8	5 Aug. 47	undil	"	5	45	10	—	
	36	"		256	"	5	45	10	—	
5	37	P-3 (?)	5 Aug. 47	undil	"	7	43	14	—	
	38	"		256	"	6	44	12	—	

SEP 15 1948

PIGEON & HUMAN SERA VS. P-17 TOXO.

All sera inactivated before test

TUBE #	SERUM SOURCE	DIL.	PEYS IN (Leg.)	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
20	NaCl - cont	—	EB	4	46	8	−	
21	P-17 7 Aug 47	undil	"	11	39	22	−	ZONE
22	"	4	"	30	20	60	+	
23	"	16	"	38	12	76	+	
24	"	64	"	40	10	80	+	
25	"	256	"	39	11	78	+	
26	"	1024	"	27	23	54	+	
27	"	4096	"	5	45	10	−	
28	[redacted]	undil	"	43	7	86	+	
29	"	4	"	47	3	94	+	
30	"	16	"	43	7	86	+	
31	"	64	"	44	6	88	+	
32	"	256	"	39	11	78	+	
33	"	1024	"	48	52	48	+	~~1024~~ 512
34	"	4096	"	6	44	12	−	

Conclusion: Immune sera derived from human & pigeon sources yield the same titers when tested against mouse-adapted toxoplasma of human & pigeon origin.

SEP 17 1948

In Vitro Toxoplasma Tests -

Toxoplasma: PE from single 4 day mouse. Rich in toxoplasma.

Sera: 1) [redacted] pt at Ch. Hosp. (Brisc.) - about 1 month ago. Has had grossly bloody spinal fluid since about 2 wks.

serum & spinal fluid (latter very bloody. When spun, supernate appeared like red wine. Prolonged search of sediment smears revealed no toxo but many polys, some mono & lymphs, rare basophils & ca 5% eosinophiles - gram).

2) Harvard, whole plasma.: The reconstituted 7.5% plasma recd with fractions from Harvard. This is a centrifuged supernate.

3) P-10: Eden Park pigeon serum inactivated & diluted on 15 Sept 48. & stored in refrigerator since. Todays dilutions made from the 1/64 tubes.

Test: Both Gibson serum & Harvard plasma & Gibson sp. fluid inactivated for 30' @ 56°C before test, then diluted in saline. 0.1cc mixed = 0.1cc PE 1/5 in EB = dep 1/5000 & incubated for 1 hr @ 37°C. Then read as usual = methyl.

all sera inactivated

PE 1/5 in EB Glycine 1/500

SEP 17 1948

	TUBE #	SERUM	DIL.	# UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
	1	NaCl — CONT.		1	49	2	−	
1	2	[redacted]	undil	7	43	14	−	
	3	"	4	5	45	10	−	
	4	"	16					Neg.
	5	"	64	1	49	2	−	
	6	"	256	1	49	2	−	
	7	"	1024	2	48	4	−	
	8	"	4096					
2	9	[redacted]	undil	1	49	2	−	
	10		4					Neg.
	11	"	16	0	50	0	−	
	12	"	64					
	13	"	256					
3	14	HUMAN PLASMA - HARVARD	undil	24	26	48	+	undil?
	15	"	64	10	40	20	−	
4	16	P-10 5 Neg.	64	28	22	56	+	1:64
	17	"	256	13	37	26	−	
	18	"	1024	3	47	6	−	
	19	"	4096	1	49	2	−	
	20	"	16384					

SEP 28 1948

In Vitro Toxoplasma Tests

Toxoplasma: Pooled from 2 four day mice, rich in toxoplasma. Prelim test ok.

Sera: 1) [redacted] series + spinal fluid = Eden Park Pneumo. group.

2) [redacted] Infant = ~~wh~~ col O+ born 11 Sept 48. Traumatic delivery. developed jaundice, hepatomegaly, splenomegaly 2nd day. Became jittery & began vomiting. Jaundice worsened on 23 Sept. Has xanthochromic sp. fl. Question of cong. toxo.

3) [redacted] & [redacted]: This is a chorio retinitis NO calcif repeat serum from the former. Both rec'd from Van Creveld on 24 Sept 48. Drawn 16 Sept 48.

4) [redacted] rec'd same day as Brinkman from Van Creveld. For diagnosis.

5) [redacted] 6-16-48. This sample has C-F titer of 1:16 & pos. rabbit vent. Rec'd from J. Warren. Had chorioretinitis detected at age 6 yrs. Is now about 11 yrs. Both mother & she have positive vents. Now has skin disease. This is for check on titer. Previous serum tested here (date of drawing unknown) had titer of 1:64.

6) [redacted]

7) [redacted] sera rec'd from [redacted] Tray School of Med. on 9-23-48. In mail for 24 hrs. No history.

Test: All sera inactivated & diluted in NaCl on 27 Sept 48. Overnight in refrig because no good PE yesterday. 0.1cc amts mixed c̄ 0.1cc PE 1/5 in Buescher serum c̄ hep 1/5000. Incubated for 1 hr @ 37°C & then read c̄ meth blue. Many toxo, so tests read c̄ facility.

6 Nov: Letter from Van Creveld: Trompetter born in March 1948, has microcephaly, choroiditis but no cerebral calcifications.

Van Raalte is 14 yrs old, has hydrocephalus & skin disease. No chorioretinitis or cerebral calcifi.

all sera inactivated
PE 1/5 in EB c̄ Lepain 1/5000

SEP 28 1948

	Tube #	SERUM SOURCE	DIL.	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
	1	NaCl - cont	—	2	48	4	—	
1	2	[redacted]	undil	3	47	6	—	Neg.
	3		16			Neg	—	
2	4		undil	43	7	86	+	
	5		4	40	10	80	+	
	6		16	33	17	66	+	1:16
	7		64	4	46	8	—	
	8		256	4	46	8	—	
	9		1024					
	10		4096					
3	11		undil	27	23	54	+	
	12		4	39	11	78	+	
	13		16					
	14		64					
	15		256	40	10	80	+	
	16		1024	52	48	52	+	1:1024
	17		4096	1	49	2	—	
4	18		undil	28	22	56	+	
	19		4	40	10	80	+	
	20		16					
	21		64					
	22		256	37	13	74	+	1:256
	23		1024	13	37	26	—	
	24		4096	3	47	6	—	

All sera inactivated
PE 1/5 in GB & Lyon 1/5000

SEP 28 1948

	Tube #	SERUM SOURCE		Dil.	EFFECT % UNSTAIN.	% STAINED	% UNSTAIN.	RESULT.	
5	25	[redacted]		undil	44	6	88	+	Titer? undil
	26			64	3	47	6	−	
6	27			undil	7	43	14	−	Neg
	28			64	1	49	2	−	
7	29			undil	42	8	84	+	Titer? 1:16 (32?)
	30			64	20 44	30 56	40 44	−	
8	31			undil	14	36	28	−	Neg
	32			64	2	48	4	−	
9	33			undil	2	48	4	−	Neg
	34			64	3	47	6	−	
10	35			undil	43	7	86	+	
	36			4	41	9	82	+	
	37			16	37	13	74	+	1:16
	38			64	10	40	20	−	
	39			256	3	47	6	−	
	40			1024					
11	41	P-15	7 day 49	undil	5	45	10	−	
	42	"		256	49	51	49	+?	Titer? 1:16
12	43	[redacted]		undil	45	5	90	+	
	44			64	49	1	98	+	Titer? 4096
13	45			undil	47	3	94	+	
	46			64	44	6	88	+	Titer? 1024

OCT 1 1948

In Vitro Toxo Tests – Clinical & Survey

Sera:	Name.	Birth Date	Age	Spec. Date.
	[redacted]	~~9-9-48~~ 9-13-42	5 1/2	3-4-48
		11-5-42	5 2/12	1-27-48
		11-28-42	5 4/12	3-5-48
		10-13-42	5 9/12	1-29-42
		1-29-43	5 1/2	2-19-48
		10-26-42	5 5/12	3-12-48
		2-12-43	5	3-3-48
		3-24-42	5 11/12	2-14-48
		4-12-42	6 years	4-9-48
		12-22-42	5 1/12	1-27-48

The above sera were collected & used in a Lansing-virus antibody survey among children admitted to Cin. Gen. Hosp. All are in the 5 + yr age group and are being tested here as part of a toxoplasma survey.

[redacted] ant at CGH. This is retest for titer

[redacted] Mot [redacted] titer.

[redacted] Part of Eden Park group.

[redacted] 6 1/2 yr girl from Mobile (Dr. Sullivan). Has chorioretinitis, cerebral calcifications, repeated convulsions.

[redacted]

P-10 – retest of Eden Park pigeon as check.

P-15 – Eden Park pigeon, retest for titer.

[redacted] titer. Former was pt of CGH. Has microcephaly, cerebral calcification, pos. skin test.

(over)

OCT 1 1948

Toxoplasma: Pooled from 3 four day mice. Moderately rich in T&O. Preliminary.

Test: All sera inactivated 30 Sept 48 & stored in refrigerator overnight. Diluted in Na Cl just before test. 0.1 cc serum dilution plus 0.1 cc PE 15 in Bruschen serum = beg 1/5000 incubated for one hour @ 37°. Then read & methylene as usual. T&O numerous & counts made without difficulty.

all sera inactivated

PE 1/5 in EB = hyp 1/5000

OCT 1 1948

Tube #	SERUM SOURCE	Dil.	EFFECT ## UNSTAIN.	## STAINED	% UNSTAIN.	RESULT	
1	NaCl - cont	—	0	50	0	—	
2	[redacted]	undil	4	46	8	—	
3		"	0	50	0	—	
4		"	1	49	2	—	
5		"	1	49	2	—	
6		"	16	34	32	—	
7		"	1	49	2	—	
8		"	1	49	2	—	
9		"	11	39	22	—	
10		"	43	7	86	+	← Pos
11		"	1	49	2	—	
1 12		undil	34	16	68	+	undil
13		4	35	65	35	—	
14		16	3	47	6	—	
2 15		undil	0	50	0	—	Cont.
16		4			neg	—	Neg
17		16					
3 18		64	45	5	90	+	
19		256	45	5	90	+	
20		1024	30 34	20 16	60 68	+	
21		4096	24 28	26 22	48 56	+	4096 next.
4 22		64	43	7	86	+	
23		256	41	9	82	+	
24		1024	26	24	52	+	1024
25		4096	5	45	10	—	

All sera inactivated

PE 1/5 in EB = hep 1/5000

OCT 1 1948

	Tube #	SERUM SOURCE	Dil.	EFFECT # UNSTAIN	# STAINED	% UNSTAIN.	RESULT.	
5	26	[redacted]	undil	32	18	64	+	dirty
	27		4	40	10	80	+	
	28		16	26	24	52	+	1:16
	29		64	8	42	16	−	
	30		256	1	49	2	−	
	31		1024					
6	32		undil	32	18	64	+	
	33		4	37	13	74	+	
	34		16	40	10	80	+	
	35		64	29	21	58	+	1:64
	36		256	5	45	10	−	
	37		1024	4	46	8	−	
	38		4096					
7	39		undil	34	16	68	+	
	40		4	32	18	64	+	
	41		16	37	13	74	+	
	42		64	30	20	60	+	1:64
	43		256	1	49	2	−	
	44		1024					
	45		4096					
8	46	P-10 Sling 47	undil	30	30	50	+	
	47	"	4	35	15	70	+	
	48	"	16	27	23	54	+	1:16
	49	"	64	11	39	22	−	
	50	"	256	7	43	14	−	

all sera inactivated

PE 1/5 in ETB = hep 1/5000

OCT 1 1948

	Tube #	SERUM SOURCE		EFFECT Dil	% UNSTAIN.	# STAINED	% UNSTAIN	RESULT	
9	51	P-15	7 Aug 47	undil	13	37	26	−	
	52	"		4	30	20	60	+	
	53	"		16	26	24	52	+	1:16
	54	"		64	13	37	26	−	
	55	"		256	4	46	8	−	
	56	"		1024					
	57	"		4096					
10	58	[redacted]		undil	33	17	66	+	
	59	"		4	25	25	50	+	
	60	"		16	25	25	50	+	
	61	"		64	35	15	70	+	
	62	"		128	23	27	46	+	1:128
	63	"		256	7	43	14	−	
11	64	[redacted]		undil	34	16	68	+	
	65	"		4	35	15	70	+	
	66	"		16	40	10	80	+	
	67	"		64	39	11	78	+	
	68	"		128	25	25	50	+	
	69	"		256	46	54	46	+	1:256

OCT 5 1948

Toxoplasma In Vitro Tests – Clinical

Sera: a) [redacted] 15 mos. old. 48 – rec'd from Eng. Typ. toxo. picture. microcephaly, Retarded.

b) [redacted] 48 " " " " " "

c) [redacted] Aug 48, mother of Joan.

d) [redacted] 48, father of Joan — these samples rec'd unfrozen. Approx. 1.0 cc in each.

e) [redacted] Rec'd from Vanderbilt, unfrozen. Has chorio-

[redacted] brain, 14 mos. old. Also microcephaly.

f) [redacted] 8. Rec'd from Univ. of Ark. Repeat sample. No calci.

g) [redacted] ally hemolyzed = deep purple wine. Parents of Barbara.

h) [redacted]

h) [redacted] of infant McDowell. Check on titer.

i) [redacted] Part of toxo. Survey. Case # 60953, Cin. Ch. Hosp.

j) [redacted] Renwld. Mother of Eliz. Brinkman.

k) [redacted] 8. Rec'd from Bowman-Gray. Typical cong. toxo.

l) [redacted] Dated 2 Oct 48. Rec'd from Ochsner Clinic

Baby has chorioretinitis but no cerebral calcification.

Toxoplasma: Pooled from 2 four day mice. Rich in toxoplasma. 20' prelim test. ok.

Test: All sera inactivated 30' @ 56°C just before test. Then diluted c̄ saline & 0.1cc amts distributed. 0.1cc PE 1/5 in EB c̄ hep 1/5000 added to each tube. Mixed, incubated 1 hr @ 37°C. Tests then read, as usual, with 1 loop methyl to 0.02 cc toxo-serum mixt. Toxo numerous & tests readily read.

All sera inactivated before test
PE 1/5 in EB c̄ hep 1/5000

OCT 5 1948

	Tube #	SERUM SOURCE	DIL.	EFFECT # UNSTAIN	# STAINED	% UNSTAIN.	RESULT	
	1	NaCl - cont.	—	1	49	2	−	
1	2	[redacted]	undil	16	34	32	−	← zone
	3		4	28	22	56	+	
	4		16	31	19	62	+	
	5		64	42	8	84	+	
	6		256	40	10	80	+	
	7		1024	40	10	80	+	
	8		4096	25	25	50	+	1:4096
	9		16384	2	48	4	−	
2	10		undil	40	10	80	+	
	11		4	25	25	50	+	1:4
	12		16	3	47	6	−	
3	13		undil	18	32	36	−	← zone
	14		4	25	25	50	+	
	15		16	36	14	72	+	
	16		64	39	11	78	+	
	17		256	45	5	90	+	
	18		1024	40	10	80	+	1:1024
	19		4096	11	39	22	−	
4	20		undil	5	45	10	−	← zone
	21		64	40	10	80	+	Titer ? 1024
5	22		undil	49	1	98	+	
	23		64	50	0	100	+	Titer ? 1024

Pigment Bright chorion 3 patch of cells in retain

OCT 5 1948

All sera inactivated before test
PE 1/5 in EB c̄ hep 1/5000

	Tube #	SERUM SOURCE	DIL.	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT.	
6	24	[redacted]	undil	7	43	14	−	
	25		64	2	48	4	−	neg
7	26		undil				neg −	U dirty - but all two appear
	27		64				neg −	" neg unstained
8	28		undil				neg. −	"
	29		64				neg. −	" neg
9	30		undil	0	50	0	−	
	31		4	—	—	—		neg
	32		16	1	49	2	−	
10	33		undil	46	4	92	+	
	34		4	45	5	90	+	
	35		16	46	4	92	+	1:16
	36		64	17	33	34	−	
11	37		undil	40	10	80	+	
	38		4	44	6	88	+	
	39		16	44	6	88	+	1:16
	40		64	11	39	22	−	
	41		256	0	50	0	−	
start @ 1:64 12	42		1024	47	3	94	+	
	43		4096	35	15	70	+	1:4096
	44		16384	3	47	6	−	
13	45		undil	0	50	0	−	Loaded c̄ rods (bact.)
	46		64	3	47	6	−	no bact. neg
14	47		undil	0	50	0	−	Bact.
	48		64	0	50	0	−	no bact. neg

OCT 6 1948

In Vitro Toxoplasma Tests.

Sera: [redacted] 1/4 dil of yesterday stored overnight in the refrigerator.

b) [redacted] 1/64 dil of yesterday, stored overnight in refrigerator.

c) γ [redacted] Squibb. Control # 82-3. Exp. date April 21, 1950. Fresh 2 cc vial obtained from pharmacy refrig. yesterday. This is thought to be of the same lot that Ella belle Ward rec'd 2 cc IM while a patient on [redacted] glob rec'd 6 March 1948. Bled 9 April 48. Evidence for this being the lot of γ-globulin involved consists of following: The pharmacist states that this ~~was~~ (vial tested to-day) is part of the only shipment of 2.0 cc vials of γ glob on hand in March. Dr. J. Little who was the 4 West resident at the time states that only 2.0 cc vials of γ glob. were employed by him, during his stay on the Ward. They had had 2 cases of measles.

Toxo: Single 4 dy PE - fair number of toxo. No pr. clim test.

Test: Dilutions made in NaCl. γ globulin very viscous. & 0.1 cc amts distributed. To this added 0.1 cc PE 1/5 in EB c̄ hep 1/5000. PE used within 10' of withdrawing from mouse. Mixtures incubated one hour @ 37° C & then read. Control poor so not all tubes were used. Toxo not numerous but counted without too much difficulty.

OCT 6 1948

PE 1/5 in EB with hep 1/5000

	Tube #	SERUM SOURCE	DIL.	EFFECT # UNSTAIN	# STAINED	% UNSTAIN	RESULT.	
	1	NaCl	—	13	37	26	—	
4	2	[redacted]	4					
	3		16					
	4		64					
	5		256					
	6		1024	34	16	68	+	1:1024
	7		4096	17	33	34	−	
	8		16384	8	42	16	−	
5	9		64					
	10		256	43	7	86	+	
	11		1024	42	8	84	+	1:1024
	12		4096	6	44	12	−	
1	13	γ glob. (F)	undil	[redacted]			Viscous & seems to need more meth blue.	
	14	"	64	36	14	72	+	Titer?

OCT 8 1948

In Vitro Toxoplasma Tests.

a) γ Globulin: Squibb #83-2. Same vial tested on 6 Oct 48. Refrigerated to date.

Allabelle Ward: 7 Oct 48. Fresh bleeding to check on titer.

[redacted] 48. Fresh bleeding to check on titer.

[redacted]. Still jaundiced at Gen. Hosp. Fresh bleeding.

[redacted] reid this a.m. via air mail from England. ~~Chester~~ infirmary.

Royal Infirmary, Chester, Eng.

No letter or other information than that of ages. Both sera cloudy.

microophthalmus + chorioid degeneration

Rab #363: normal rabbit proven toxo susceptible. Check on titer.

[redacted] reid yesterday in ordinary mail. Undated & unfrozen.

[redacted] d yesterday from Ochsner clinic. Unfrozen. Mother normal. Jules (13 mos.) has chorioretinitis, normal skull. Had fever in early infancy. Ernel is 7 wks old & has been febrile since birth.

Toxoplasma: pooled from 2 four day mice. Rich in toxo. Prelim ok.

Test: All sera except γ glob & Mass sera inactivated 30' @ 56° C. before dilution in saline. 0.1cc serum dil. mixed c̄ 0.1cc PE 1/5 in Buescher serum with heparin 1/5000. Incubated for one hour @ 37° C. then read c̄ fresh meth. blue. Numerous toxo & tests read easily.

OCT 8 1948

all sera inactivated. PE 1/5 in EBC hep 1/5000

Tube #	SERUM SOURCE	Dil.	EFFECT # UNSTAIN	# STAINED	% UNSTAIN.	RESULT.	
1	NaCl —	—	1	49	2	—	
1 2	γ GLOBULIN (unheated) Seibb #82-3	undil	27	23	54	+	
3	"	4	45	5	90	+	
4	"	16	38	12	76	+	
5	"	64	39	11	78	+	
6	"	256	31	19	62	+	256
7	"	1024	15	35	30	−	
8	"	4096	2	48	4	−	
2 9	Alla Belk Ward 9 Oct 48	undil		almost all unst.		+	
10	"	4		"		+	
11	"	16	38	12	76	+	1:16
12	"	64	8	42	16		
3 13	[redacted]	undil		almost all unst.		+	
14		4	30	20	60	+	1:4
15		16	17	33	34	−	
16		64	2	48	4	−	
4 17		undil	6	44	12	−	
18		4			Neg	−	Neg
19		16			Neg.	−	
11 ~~20~~		~~undil~~	0	50	0	−	loaded ⊂ bacteria.
~~21~~		~~64~~	1	49	0	−	fewer bact. Neg
12 ~~22~~		~~undil~~			Neg.	−	loaded ⊂ bact. Neg
~~23~~		64 ~~4096~~			Neg	−	fewer bact.

all sera inactivated before test
PE ½ in EB & kept 1/5000

OCT 8 1948

	Tube #	SERUM SOURCE	DIL.	EFFECT # UNSTAIN.	EFFECT # STAINED	EFFECT % UNSTAIN.	RESULT	
5	24	Rab. #363 -4 Mar 48	undil	32	18	64	+	undil
	25		4	14	36	28	-	
	26		16	5	45	10	-	
	27		64					
6	28	[redacted]	undil	46	4	92	+	
	29		4	38	12	76	+	1:4
	30		16	13	37	26	-	
	31		64	7	43	14	-	
	32		256			neg.		
	33		1024					
7	34		undil	0	50	0	-	neg
	35		64			neg.	-	
8	36		undil			neg	-	neg
	37		64			neg.	-	
9	38		undil			neg	-	mod. # bact.
	39		64			neg.	-	neg

Effect of Temperature of Inactivation Upon Antibody Titers, "Zones," and Specificity?

OCT 12 1948

Obj: To determine whether the antibody titers, presence of pro-zones, apparent non-specific results, and percentage-low reacting sera will ~~cost~~ be affected differently by inactivation at different temperatures (56°, 60°, 65°).

Sera: Rab #363 - pre-inoc. when titered recently, pos. @ undil only. Animal proven susceptible, therefore this is probably non-specific.

Rab. #363 - 5th day. Serum obtained 5 days after ID inoc.

Rab. 349 - this is the rabbit that was immunized by receiving a non-fatal infection with P-17 toxo. Has the highest zone encountered thus far.

Rab. #365 - 7th day: Bled on 7th day of a fatal toxo infection.

P-15 - an Eden Park pigeon.

[redacted] Park patient. Serum pos. at undil. only in previous test.

[redacted] Cong. toxo. but in previous test ran low percentages at various dilutions.

[redacted] & 7 wks child from Tulane. Baby has hydrocephalus & retinal degeneration;

[redacted] 5 weeks infant. " " , Baby has hydrocephalus, convulsions, cloudy, yellow spinal fluid.

[redacted]

Toxoplasma: pooled from 4, four day mice. Very rich in parasites. Prelim fine.

Test: Sera inactivated, as indicated in protocol, just before test. 30 min @ 56°C & 20 min @ 60° & 65°C. All sera treated @ 65°C dil 1/4 before heating. No coagulation. Dilutions in NaCl. 0.1cc serum dilution & 0.1cc PE 1/5 in Buescher serum = hep 1/5000. Incubated for 1 hr @ 37°C & then read = meth blue. Numerous toxo so tests read rapidly.

OCT 12 1948

PE 1/5 in ETB with hep. 1/5000

	Tube #	SERUM SOURCE		DIL.	TEMP. OF INAC.	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
	1	NaCl		-	—	0	50	0		
1	2	Rab. #363	- 4 MAR. 48	undil	56°	43	7	86	+	
	3	"		4	"	45	5	90	+	4
2	4	Rab. #363	- "	undil.	60°	35	15	70	+	
	5			4	"	36	14	72	+	
3	6	Rab. #~~349~~	- 12-23-47	undil	56°	9	41	18	−	
	7	"		4	"	12	38	24	−	
	8	"		16	"	21	29	42	−?	Zone
	9	"		64	"	39	11	78	+	
	10	"		256	"	43	7	86	+	
	11	"		1024	"	47	3	94	+	
	12	"		4096	"	49	1	98	+	4096
	13	"		16384	"	5	45	10	−	
4	14	Rab. #349	"	undil	60°	11	39	22	−	
	15	"		4	"	12	38	24	−	
	16	"		16	"	15	35	30	−	
	17	"		64	"	35	15	70	+	
	18	"		256	"	45	5	90	+	
	19	"		1024	"	46	4	92	+	
	20	"		4096	"	49	1	98	+	4096

OCT 12 1948

	Tube #	SERUM SOURCE		Dil.	Temp. of Inac.	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
5	~~15~~	Rab. #349	"	~~undil~~	65°					
	63 15	"		4	"	15	35	30	−	Zone
	64 16	"		16	"	31	~~47~~ 37	62	+	
	65 17	"		64	"	45	5	90	+	
	66 18	"		256	"	47	3	94	+	
	67 19	"		1024	"	44	6	88	+	
	68 20	"		4096	"	43	7	86	+	4096
6	21	Rab. 363 (5th dy.)		undil.	56°	48	2	96	+	Titer?
	22			64	"	13	37	26	−	
7	23	Rab. 365 (5th dy.)	12 April 48	undil	"	46	4	92	+	
	24			64	"	29	21	58	+	64?
8	25	P-15		undil	56°	13	37	26	−	
	26			4	"	35	15	70	+	
	27			16	"	40	10	80	+	
	28			64	"	25	25	50	+	64
9	29	P-15		undil	60°	14	36	28	−	
	30			4	"	33	17	66	+	
	31			16	"	43	7	86	+	
	32			64	"	31	19	62	+	64
10	33	P-15		4	65°	36	14	72	+	
	34			16	"	33	17	66	+	
	35			64	"	25	25	50	+	64

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Tube #	SERUM SOURCE	Dil.	Temp. of Inac.	Effect. # unstain.	# stained	% unstain.	Result	
11	36	undil	56°	8	42	16	—	
	37	4	"	5	45	10	—	Neg
12	38	undil	60°	24	45	35	—	
	39	4	"	5	45	10	—	Neg
13	40	undil	56°	37	13	74	+	
	41	4		45	5	90	+	
	42	16		49	1	98	+	
	43	64		38	12	76	+	1:64
	44	256		14	36	28	—	
14	45	undil	60°	38	12	76	+	
	46	4	"	47	3	94	+	
	47	16	"	50	0	100	+	
	48	64	"	43	7	86	+	1:64
	49	256	"	7	43	14	-	
15	50	~~4~~ undil	65°	47	3	94	+	
	51	16	"	45	5	90	+	
	52	64	"	31	19	62	+	1:64
	53	256	"	6	44	12	—	
16	54	undil	56°	46	4	92	+	Titer ?
	55	64	"	5	45	10	—	
17	56	undil	"	5	45	10	—	#1 = 4/50
	57	64	"	4	46	8	—	C.L. = 36/100 — undil.
18	58	undil	"	60	40	60	+	
	59	64	"	43	7	86	+	Titer ?
19	60	undil	"	45	5	90	+	cont.
	61	64	"	42	8	84	+	titer ?
20	62	256	56°	4	46	8	—	

[redacted]

see margin → ROCHESTER

In Vitro Tests – Clinical Samples.

OCT 15 1948

Toxo: Pooled from 5, four day mice. Very rich in toxo. Prelim OK.
also used for tests with Cadez serum to-day.

Sera: a) [redacted] rec'd from Tulane. Mother of Michael Prattini who is 7 wks old & has hydrocephalus.

[redacted] Baby is 5 wks old, has hydrocephalus, cloudy, yellow [redacted] fluid, fever convulsions.

[redacted] baby about 3-4 months, has [redacted], chorio recentis, poor develop, no mention of calci-[redacted]

[redacted] From Jersey City.
[redacted] 7 months, L&W, sibling of Gladys Johnson. Serum rec'd to-day.

BORN 12 Feb 1948
SERUM = 12 OCT 48
∴ 8 months old

Test: Sera inactivated @ 56° for 30'. ~~[illegible]~~ Then diluted c̄ NaCl.
0.1cc ~~[illegible]~~ mixed c̄ 0.1cc PE 1/5 in Bruscher serum c̄ hep 1/5000.
Incubated for 1 hr @ 37°C & then read c̄ meth. blue.
Very rich in toxo & read quickly.

all sera inactivated
PE 1/5 in EB c̄ hep 1/5000

OCT 15 1948

	Tube #	SERUM SOURCE	DIL.	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN	RESULT.	
	1	NaCl - cont.	—	3	47	6	—	
1	2	[redacted]	undil	43	7	86	+	
	3		4	28	22	56	+	1:4
	4		16	6	44	12	-	
2	5		undil	35	15	70	+	
	6		4			Pos	+	
	7		16			Pos	+	
	8		64			Pos	+	
	9		256			Pos.	+	
	10		1024	38	12	76	+	
	11		4096	31	19	62	+	4096
	12		16384	35	65	35	—	
3	13		undil	41	9	82	+	
	14		4			—		
	15		16			—		
	16		64			—		
	17		256			—		
	18		1024			—		
	19		4096	44	6	88	+	1:4096
	20		16,384	38	62	38	—	
4	21		undil	45	5	90	+	
	22		64	43	7	86	+	Titer? redo
5	23		undil	44	6	88	+	
	24		64	45	5	90	+	Titer? re-do

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Tube #	SERUM		EFFECT.			RESULT	
	Source.	Dil	# contain.	# stained	% contain		
6 25	[redacted]	undil	46	4	92	+	Many bact.
26	[redacted]	64	5	45	10	−	Titer?

Serum Inactivation Effect test
PE 1/5 in EB ≡ Exp 1/5000.

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Tube #	SERUM SOURCE	DIL.	EFFECT # UNSTAIN.	# STAINED	% UNSTAIN	RESULT	
31	NaCl —	—	NOT DONE				
7 32	[redacted]	undil	5	45	10	−	ZONE
33	"	4	31	19	62	+	
34	"	16	40	10	80	+	
35	"	64	45	5	90	+	
36	"	256	40	10	80	+	
37	"	1024	29	21	58	+	1024
38	"	4096	14	36	28	−	
8 39	[redacted]	undil	27	23	54	+	
40	"	4	46	4	92	+	
41	"	16			—		
42	"	64			—		
43	"	256			—		
44	"	1024	25	25	50	+	1024
45	"	4096	13	37	26	−	
~~46~~							

See following sheets of 15 Oct 48.

OCT 15 1948

In The Mechanism Of the "Zone" Reaction.

Obj: Using the same "high-zone" serum (Joseph Codey) to determine: a) the effect of ultra-centrifugation upon the pro-zone; b) will toxoplasma that have absorbed a "zone" serum be fully or incompletely reactivated by the addition of "activator" serum. As a corollary of the latter experiment, what will be the titer & "zone" of serum that has been absorbed with toxoplasma.

Serum used: All of these trials conducted with Joseph Codey serum. In a previous titration this had a titer of 1/1024 & a zone to 1/64.

Toxoplasma: The same pool that was used in to-day's titration of clinical sera.
Activator: Buescher serum used for reactivating to-day's clinical sera.

Procedure:
1) Ultracentrifuged portion: 2.1cc of Codey serum were spun for one hour @ 13,000 rpm. on the Swedish angle (in the cold) yesterday. No noticeable pellet was formed but a marked lipoid layer was formed at the top. The sparkling clear central portion was removed & tubed & stored overnight in the refrigerator. To-day, 0.2cc were inactivated as usual, then diluted c̄ Abs Cl & tested with to-day's PE 1/5 in Buescher serum c̄ hep 1/5000.

2) Absorption: To 0.5cc of Codey serum, untreated except for inactivation for 30' @ 56°C just prior to test, was added 0.5cc of the freshly-drawn, undiluted PE pool used to-day. This was extremely rich in toxoplasma. After mixing, this tube was incubated for 1 hour @ 37°C. Examined at that time, only 1/100 toxo were unstained. This mixture was then transferred to a

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narrow centrifuge tube & spun for 15' @ 2000 on the horizontal centrifuge. At that time the supernatant was carefully withdrawn & saved for titration (see below) and the ~~pellet~~ tube sides washed twice with about 0.5 cc Na Cl. Pellet remained undisturbed and was assumed to measure 0.02 cc. This pellet was then resuspended in 0.99 cc Na Cl. Resuspension was readily accomplished by agitation with a wooden stick. This material was then used as is indicated in the protocol. This then makes a 1/200 dilution of antibody absorbed from the original serum.

3) Supernate of centrifuged absorbed serum: This material diluted 1:2 & four-fold dilutions with Na Cl made from this. ~~diluted four-fold with Na Cl~~; the supernate was considered to be a 1:2 dilution of the serum. The same PE 1/5 in Buescher serum = tap 1/5000 that had been used in the clinical tests & then stored in the refrigerator for 3 hrs. was used for this test.

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Tube #	CODEY SERUM TREATED	Dil.	% UNSTAIN.	% STAINED	% UNSTAIN.	RESULT	
			EFFECT				
1	Na Cl – same cont. as other chemicals.	—	3	47	6	—	
32	Not-inactivated. No other treatment. Base-line control.	undil	5	45	10	—	Zone.
33		4	31	19	62	+	
34		16	40	10	80	+	
35		64	45	5	90	+	
36		256	40	10	80	+	
37		1024	29	21	58	+	1024
38		4096	14	36	28	—	
39	Ultra-centrifuged. 1 hr @ 43,000	undil	27	23	54	+	NO ZONE BUT
40		4	46	4	92	+	
41		16			—		
42		64			—		
43		256			—		
44		1024	25	25	50	+	1024
45		4096	13	37	26	—	

~~46~~

Tests with resuspended toxoplasma that have "absorbed" Codey serum:

1) 0.1cc resuspended toxo + 0.1cc fresh EB ser. = 107/200 unstain. = 54% unst.

2) 0.1cc " " + 0.1cc heated EB ser. = 5/50 unst. = 10% unst.

3) To test blocking effect:

0.1cc resuspended toxo + 0.1cc Mus. Glover 1:4 dil. + 0.2cc fresh EB ser.

= 69/100 unst = 69% unst.

Each of above incubated for one hour @ 37°

following addition of Bruscher serum.

(over)

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Test for residual antibody in supernate of absorbed Eddy serum:

Tube	Supernate	Dil. of original serum.	# unat. total.	% unat.	
1	Na Cl	cont.	6/50	12	—
2	undil	1:2	12/50	24	—
3	1:2	1:4	15/50	30	—
4	1:8	1:16	37/50	74	+
5	1:32	1:64	49/50	98	+
6	1:128	1/256	46/50	92	+
7	1:512	1:1024	35/50	70	+

Zone.

From these results it is apparent that:

a) The degree of "zoning" of a given serum will vary in different experiments (see control titration) altho the serum titer is unchanged.

b) Ultra-centrifugation altered but did not completely eliminate the "zone"; this treatment did not change the serum titer.

c) whatever is responsible for the zone, was absorbed (partially, at least) by the bacteria so that there was incomplete reactivation following the addition of fresh activator.

d) a serum absorbed under the conditions of this experiment neither had its zone removed, or titer diminished.

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In Vitro Tests — Clinical Samples.

Toxo: Two fourday mice. Very sick in 6 hrs.

Sera: a) [redacted] - dilutions made from yesterday's 1:4 dilution stood overnight in refrigerator.

b) [redacted]: bled this a. m. - 90 days old.

c) [redacted] 1:4 & 1:16 dilutions stood overnight in refrig from yesterday's tests.

d) [redacted] rec'd this a. m. from New Orleans. Baby is 1 yr old, has hydrocephalus, twitchings, poor development, no cerebral calcifications, questionable chorio-retinitis. Sera centrifuged.

Test: [redacted] sera inactivated for 30' @ 56° & then all dilutions made in Ns Cl. 0.1 cc serum dilution plus 0.1 cc PE 1/5 in Buescher serum = Exp 1/5000. incubated 1 hr @ 37° C. & then read as usual. This Buescher serum used yesterday & refrozen.

all sera unitary
PE 1/5 in EB = hyp 1/5000

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	Tube #	SERUM SOURCE		DIL.	EFFECT # UNSTAIN	# STAINED	% UNST.	RESULT	
	1	NaCl	—	—	4	46	8	−	
4	2	[redacted]		64	46	4	92	+	
	3			256	30	20	60	+	Re-titer
	4			1024	45	65	40	−	
	5			4096	4	46	8	−	
	6			16384					
5	7			64	27	73	27	−	
	8			256	24	76	24	−	Re-titer
	9			1024	6	44	12	−	
	10			4096					
	11			16384					
1	12			undil	42	8	84	+	
	13			4	30	20	60	+	1:4
	14			16	17	33	34	−	Re-titer
	15			64	6	44	12	−	
	16			256					
6	17			4	5	45	10	−	
	18			16			Neg.	−	Neg Redo undil
2	19			undil	10	40	20	−	
	20			64			Neg	−	Neg
3	21			undil	40	10	80	+	
	22			64	28	72	28	−	Retite

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In Vitro Tests - Rainey Series and Clinical Samples

Sera:

a) [redacted] the entire series of samples from mother & child. see summary.

[redacted] repeat study. Spec. obt. 12 Oct 48. Child 8 mos. old. Is a younger sibling of Gladys Johnson.

c) [redacted] repeat test. Child about 3 months old. Has ~~no intra-cere. calci~~, but chorio-retinitis, hydrocephalus, poor development. No mention of cerebral calci.

[redacted] repeat. Rec'd from N. Orleans. Baby is one year old, has hydrocephalus, twitchings, poor development, no cerebral calcifications, ~~questionable~~ suggestive chorio-retinitis.

e) [redacted] pt at Cinci. Ch. Hosp. Has bilateral optic atrophy & poor development. Is about 5 months old. No calcifications or hydrocephalus.

Toxo: Pooled from 3, three day mice. Extremely rich in toxoplasma. Prelim test ok.

Test: Sera inactivated for 30' @ 56° C just before test. Diluted in NaCl. 0.1cc PE 1/5 in EB (of 19 Oct. 48) c̄ hep 1/5000 & 0.1cc serum dil. incubated for 1 h @ 37°. Then read as usual c̄ meth. blue pH 11.0. Tests read rapidly because of large number of parasites.

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	Tube #	SERUM SOURCE	EFFECT Dil.	# UNST.	# STAINED	% UNST.	RESULT.	
	1	NaCl	—	2	48	4	−	
1	2	[redacted]	undil	42	8	84	+	
	3		4	39	11	78	+	
	4		16	42	8	84	+	
	5		64	46	4	92	+	
	6		256	39	11	78	+	
	7		1024	25	25	50	+	1024
	8		4096	5	45	10	−	
2	9		undil	all unst. all unst.		+	+	
	10		4	43	7	86	+	
	11		16	all unst. all unst.		+	+	
	12		64	49	1	98	+	
	13		256	42	8	84	+	256
	14		1024	15	35	30	−	
	15		4096	4	46	8	−	
3	16		undil	30	20	60	+	undil
	17		4	7	43	14	−	
	18		16			Neg	−	
4	19		undil		Marked	Pos.	+	
	20		4	37	13	74	+	
	21		16	36	14	72	+	1:16
	22		64	13	37	26	−	
	23		256					
5	24		undil	3	47	6	−	
	25		4	2	48	4	−	Neg
	26		16					

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Tube #	SERUM SOURCE	DIL.	EFFECT # UNSTAIN.	# STAIN.	% UNSTAIN.	RESULT.	
#	[redacted]						
6 27		undil	45	5	90	+	
28		4		Pos.		+	
29		16		Pos		+	
30		64		Pos		+	
31		128		Pos.		+	
32		256	40	10	80	+	
33		512	27	23	54	+	512
34		1024	6	44	12	−	
35		2048	3	47	6	−	
36		4096	3	47	6	−	
7 37		undil			Pos.	+	
38		4			Pos.	+	
39		16			Pos.	+	
40		64			Pos.	+	
41		128	37	13	74	+	
42		256	27	23	54	+	256
43		512	6	44	12	−	
44		1024	4	46	8	−	
45		2048					
46		4096					
8 47		undil		N.D.			
48	"	4			Pos.	+	
49	"	16			Pos	+	
50	"	64			Pos	+	
51	"	128	25	22	56	+	128

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Tube #	SERUM SOURCE	DIL.	EFFECT #UNSTAIN.	EFFECT #STAINED	EFFECT %UNSTAIN.	RESULT.	
52	[redacted]	256	15 16	35 34	30 32	−	
53		512	7	43	14	−	
54		1024	2	48	4	−	
55		2048					
56		4096					
9 57		undil			Pos	+	
58		4			Pos	+	
59		16			Pos	+	
60		64			Pos.	+	
61		128	48 23	52 27	48	+	128
62		256	7	43	14	−	
63		512	5	45	10	−	
64		1024					
65		2048					
66		4096					
10 67		undil	48	2	96	+	
68		2	49	1	98	+	
69		4	47	3	94	+	
70		8	38	12	76	+	
71		16	48	52	48	+	16
72		32	20	80	20	−	
73		64	8	43	16	−	
74		128					
75		256					
11 76		undil	14	36	28	−	
76		64	2	48	4	−	16

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Summary of In Vitro Toxoplasma Antibody Titrations of Serial Serum Samples From Mrs. Rainey & Linda Ann Rainey.

May-1947 - [redacted] gave birth to a premature that died shortly thereafter of congenital toxoplasmosis.

DATE	# DAYS. POST-PART.	TITER			C-F TITER	
		MOTHER	CHILD		MOTHER	CHILD
2-23-48	-146	1:512	—		~~16~~ 32	-
7-18-48	0	1:256	1:128	(cord blood = child)	32 16 ~~32~~	16
8-30-48	43	—	1:128		8	8
10-16-48	90	—	1:16		-	4
1 Dec-48	135	1:256	1:4		16	<2

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Titration - Serum of Rab #349 - T40. Immune

Serum: Rabbit bled 29 Oct 48, serum removed on 30 Oct 48 (after overnight refrigeration), tubed & frozen in CO_2. This Rab #349 had rec'd an early passage of P-17 T40 intracutaneously on 15 Oct 47, cleared from infection & then rec'd challenge of "RH" T40. Serum obtained on 23 Dec. 47 was found to contain a titer of 1:4096 against "T" to 1:64 on in vitro test.

T40: Single PE from 3 day mouse. Virus in T40. Preliminary.

Test: Has incubated for 30' @ 56° C, then diluted in NaCl. 0.1 cc of PE 1/5 in Bruecher (19 Oct 48) & heparin 1/5000 mixed & 0.1 cc serum dil, incubated for 1 hr @ 37° C, then read as usual & meth. blue.

Tube #	SERUM SOURCE	DIL.	EFFECT # CONSTAIN.	# STAINED	% UNST.	RESULT:	
1	NaCl	—	1	99	1	−	
2	Rab 349 (29 Oct 48)	undil	4	46	8	−	2048
3	"	4	8	42	16	−	
4	"	16	17	33	34	−	
5	"	64	49	51	49	+	
6	"	256	32	18	64	+	
7	"	1024	32	18	64	+	1024
8	"	4096	8	42	16	−	
9	"	16384	3	47	6	−	
10	[redacted]	16	73	27	73	+	

(over)

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VanThiel Dye

A sample of Grubler meth blue dissolved in absolute alcohol & containing some precipitate was rec'd this a.m. from Prof. VanThiel who claimed that when made up to pH 11.0, it was unsatisfactory for the dye test. It is apparent, however, that his buffer & ours are not the same. In addition, his dye is purplish rather than blue.

Test:

10 cc of fresh buffer pH 11.0 was divided into two 5 cc aliquots. To one was added 1.5 cc of VanThiel's & to the other 1.5 cc of our dyes. His was definitely purple (as ours usually appears after about 3-5 days in buffer) while ours was blue. The Connelly test (see over) mixture was set up on the same slide using 0.02 cc mixture & 1 loop of VanThiel's & our dye. When examined under the scope the two preparations appeared identical as to numbers of stained & unstained cells. However, both stained = our dye were blue while with VanThiel's they were purplish. Chromatin was stained with both.

Conclusion: When made in our buffer, VanThiel's dye was adequate for use.

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In Vitro Tests With Clinical Sera.

Test Sera: a) [redacted], mother & child from Cleveland Clinic. Baby has chorio-retinitis, convulsions, hydroceph (?). No cerebral calcifications.

[redacted] mother & child: baby has chorio-retinitis, microphthalmos, no cerebral calcifications. Children's, Cinci.

[redacted] mother & child: No cerebral calci., microcephaly, retarded development. Chorio-ret. (?). Cinci. Ch. Hosp.

d) [redacted] mother, father & child. From Bellvue. Child dead @ 18 months. Said to have cerebral calcifi. & chorio-retinitis.

[redacted]: From Yale. 7 mos. old; Microceph., Retard., Cortic atrophy, Optic? No chorioretin or Cerebr. Calcif.

[redacted]milies. From Ochsner. Letters incomplete.

[redacted] Cinci. Ch. Hosp. No cerebral calci.

[redacted] & wife from AMS. Both have positive C-F & skin tests, child dead.

i) [redacted] child, mother, father, & brother. From AMS (Panama). Mother & child have positive C-F

[redacted] AMS. Mother & child have pos. C-F & rabbit. Neg skin tests. Child 11 yr. old c̄ C-R.

Toxo: Pooled from 5 three day mice - Extremely rich in toxo. Prelim OK.

Test: All sera inactivated undil on 12 Nov. & stored in refrig. since. Diluted c̄ NaCl just prior to test. As usual, 0.1cc serum dil. mixed c̄ 0.1cc toxo 1/5 in EB (19 Oct '48) c̄ hep 1/5000. Incubated for 1 hr @ 37° C & then read c̄ meth-blue pH 11.0. Numerous toxo & tests read rapidly.

PE 1/5 in EB 1 leg 1/1000
all sera inactivated

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	Tube #	SERUM SOURCE	DIL.	# UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
	1	Na Cl – cont.	—	2	98	2	—	
1	2	[redacted]	undil			neg	—	
	3		64			neg.	—	Neg
2	4		undil			neg.	—	
	5		64			neg.	—	Neg
✓ 3	6		undil	35	15	70	+	Titer? below 1:64
	7		64			neg.	—	
✓ 11	8		undil					
	9		64					
✓	10		undil					
	11		64					
4	12		undil			neg.	—	
	13		64			neg.	—	Neg
5	14		undil			neg.	—	
	15		64			neg.	—	Neg
6	16		undil	38	12	76	+	Titer? below 1:64
	17		64			neg	—	
7	18		undil			neg	—	
	19		64			neg	—	Neg
8	20		undil			neg	—	
	21		64			neg.	—	Neg
9	22		undil			neg.	—	
	23		64			neg.	—	Neg
	24							

all serum inactivated
PE 1/5 in EB ≈ hp 1/5000

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	Tube #	Serum Source	Dil.	Effect # Unstain.	Effect # Stained	Effect % Unstain.	Result	
10	24	[redacted]	undil			neg	−	neg
	25		64			neg	−	
11	26		undil			neg	−	neg.
	27		64			neg.	−	
12	28		undil	30	20	60	+	
	29		4	40	10	80	+	
	30		16					
	31		64					
	32		256			✓ Pos.	+	
	33		1024	25	25	50	+	1:1024
	34		4096			neg.	−	
	35		16384					
13	36		undil	45	5	90	+	
	37		4					
	38		16					
	39		64	28	22	56	+	1:64
	40		256	10	40	20	−	(1:128 ?)
	41		1024			neg.	−	
	42		4096			neg	−	
	43		16384					
14	44		undil			neg	−	neg
	45		64			neg.	−	
15	46		undil			neg.	−	neg
	47		64			neg.	−	

all sera inactivated
PE 1/5 in EB = dep 1/5000

NOV 13 1948

Tube #	SERUM SOURCE	DIL.	# UNSTAIN.	# STAINED	% UNSTAIN.	RESULT	
16 48	[redacted]	undil	45	5	90	+	
49		4					
50		16					
51		64					
52		256					
53		1024	28	22	56	+	1:1024
54		4096	10	40	20	−	(1:2048?)
55		16384			Neg.	−	
56		65536					
17 57		undil	46	4	92	+	
58		4					
59		16					
60		64					
61		256					
62		1024	43	7	86	+	1:1024
63		4096	41	59	41	±	(1:2048?)
64		16384			Neg.	−	
18 65		undil			Neg	−	
66		64			Neg.	−	
67							

All sera inactivated
PE 1/5 in EB c̄ hep 1/5000

NOV 13 1948

	Tube #	SERUM SOURCE	DIL.	EFFECT # UNST.	# STAIN.	% UNST.	RESULT.	
19	67	[redacted]	8					
	68		16	43	7	86	+	
	69		32	37	13	74	+	
	70		64	26	24	52	+	1:64
	71		128	7	43	14	−	
	72		256			Neg	−	
20	73		4					
	74		8					
	75		16	46	4	92	+	
	76		32					
	77		64	33	17	66	+	1:64
	78		128	15	35	30	−	
	79		256			Neg	−	
21	80		undil	4	46	8	−	
	81		4					
	82		16					
	83		64	2	48	4	−	
	84		256			Neg	−	Neg
	85		1024			Neg.	−	
	86		4096			Neg.	−	
	87		16384					
	88		65,536	2	48	4	−	

all sera inactivated
PE 1/5 in EB c̄ hep 1/5000

NOV 13 1948

	Tube #	SERUM SOURCE	Di'L.	EFFECT # UNSTAIN.	# STAIN.	% UNSTAIN.	RESULT.	
22	89	[redacted]	undil	49	1	98	+	
	90		4			Pos.	Pos	
	91		16	17	33	34	±	1:4 (1:8?)
	92		64			Neg.	−	
	93		256	2	48	4	−	
	94		1024					
	95		4096					
	96		16384					
23	97		undil	45	5	90	+	below 1:64
	98		64	3	47	6	−	
	99		1024					
	100		4096					

DEC 8 1948

In Vitro Clinical Tests – Toxoplasma

Sera. [redacted] Baby & mother bled 1 Dec 48 = 135th post-partum day
: Jersey City – baby has chorio-ret., cerebral calci., microphthalmus.
New blood taken 126 days post-partum. Father & grandmother's blood also to be tested because both & the mother had unexplained fever c̄ diarrhea during the 6th month of this pregnancy.

[redacted] St. Louis: Baby born 10-26-48, normal delivery & infant. Onset of hydrocephalus noted 14 Nov. '48. Vomiting began 15 Nov. 48. Head = 41.4 cm. Fundi = areas of depigmentation & increased pigmentation suggestive of chorio-retinitis. Has loud systolic murmur. First LP normal. Others have yellow fluid c̄ prot. of 94 & sugar = 38. Questionable calcification in ventricular areas.

4) [redacted] ha reid from Van Creveld 3 Dec. 48. Unlabelled. Larger bottle assumed to be mother's & smaller, child's. Baby born in May 1946 so the new specimen was drawn at approx. 31 months.

5) γ Glob.: Same material tested previously. Frozen since.

Toxoplasma: PE Pooled from 2 four day & 2 three day mice. Very rich in Toxoplasma. Prelim = 1/80 unst.

Test: All sera (& γ glob.) inactivated @ 56°C for 30' just before test. Then diluted in NaCl. 0.1cc of PE 1/5 in Quescher c̄ hep 1/5000 added to 0.1cc of serum dil. Incubated for 1 hr @ 37°C & then read c̄ meth. blue. Very rich in toxo so read quickly.

all sera inac. for 30' @ 56°
PE 1/5 in EB c̄ hep 1/5000

DEC 8 1948

	Tube #	Serum Source	Dil.	Effect # Unstain.	Effect # Stain.	Effect % Unstain.	Result	
	1	NaCl – cont.	—	2	48	4	—	
1	2	[redacted]	8	45	5	90	+	
	3		16	35	15	70	+	1:16
	4		32	15	35	30	—	
2	5		undil	48	2	96	+	
	6		2	43	7	86	+	
	7		4	34	16	68	+	1:4
	8		8	14	36	28	—	
	9		16	—	—	—		
3	10		128					
	11		256	34	16	68	+	1:256
	12		512	6	44	12	—	
4	13		64					
	14		128					
	15		256	25	25	50	+	1:256
	16		512	9	41	18	—	
5	17		64	~~45~~	~~5~~	~~90~~		
	18		256	45	5	90	+	1:256 (512?)
	19		1024	45	60	42	±	
6	20		4	—	—			
	21		16	—	—			
	22		64	—	—			
	23		256	46	4	92	+	
	24		1024	32	18	64	+	1:1024
	25		4096	12	38	24	—	

DEC 8 1948

	Tube #	SERUM SOURCE	Dil.	EFFECT # UNST.	# STAINED	% UNSTAIN.	RESULT	
7	26	[redacted]	256	46	4	92	+	
	27		1024	34	16	68	+	1:1024
	28		4096	6	44	12	−	
8	29		256					
	30		1024	32	20	64	+	1:1024
	31		4096	6	44	12	−	
9	32		undil	20	30	40	±	
	33		4	27	33	54	+	
	34		16	37	13	74	+	1:16
	35		64	14	36	28	−	
	36		256	7	43	14	−	
	37		1024					
	38		4096					
10	39		undil	44	6	88	+	
	40		4	36	14	72	+	1:4
	41		16	2	48	4	−	
	42		64					
	43		256	3	47	6	−	
	44		1024					
	45		4096					
11	46		128					
	47		256	35	15	70	+	1:256
	48		512	16	34	32	±	
	49		1024					
	~~50~~							

DEC 8 1948

	Tube #	Serum Source	Dil.	Effect % unstain.	Effect % stain.	Effect % unstain.	Result.	
12	50	[redacted]	undil	10	40	20	–	
	51		4					
	52		16					
	53		64			neg	–	neg.
	54		256	11	39	22	–	
	55		1024					
	56		4096	2	48	4	–	
13	57		undil	6	44	12	–	
	58		4					
	59		16					neg.
	60		64			neg	–	
	61		256	8	42	16	–	
	62		1024					
	63		4096	2	48	4	–	
14	64		16					
	65		64					
	66		256	30	20	60	+	1:256
	67		1024	7	43	14	–	
15	68		64					
	69		256	45	5	90	+	1:256 (512?)
	70		1024	19	31	38	±	
	71		4096	1	49	2	–	

DEC 10 1948

In Vitro Toxoplasma Tests - Sheffield, Eng. Sera.

Sera: [redacted] lyophilized specimens. Quantity not indicated but assumed to be from 1.0cc. Reconstituted c̄ 1.0cc dist. H_2O to-day. Specimen dates also not indicated but probably drawn on 19 Nov. 1948 & dried within 24 hrs. Each vial numbered. Equivalent names indicated as follows:

"1" = [redacted] (mother)
"2" = [redacted] (sister)
"3" = [redacted] (patient)

[redacted] on 6 Mar. 1945. Defect of vision for 2 yrs. Two years ago noticed to have transient bi-lateral squints. Ophthalmologist found child "partially blind." Good health but somewhat clumsy. One year ago had a convulsion. None since. Development & mentality normal. Still clumsy.

PK.: now negative except primary optic atrophy of right disc. In temporal areas of both eyes are irregular pale areas with peripheral pigmentation compatible with chorio-retinitis. All lab work neg. Skull plate normal. Rabbit toxo neut. (c̄ peritoneal exudate) equivocal for mother & child.

[redacted] Reconstituted sera not heated. Dil. in Na Cl. 0.1cc mixed c̄ 0.1cc PE 1/5 in EB c̄ hep. 1/5000.

1. Na Cl cont. = 1/50 = 2% unstained.	—		
2. [redacted] undil = 46/50 = 92% "	+	}	mother less than 1/64 (1/32)
3. [redacted] 1/64 = 9/50 = 18% "	—		
4. [redacted] undil = 4/50 = 8% "	—	}	Sibling neg
5. [redacted] 1/64 = 1/50 = 2% "	—		
6. [redacted] undil = 40/50 = 80% "	+	}	Patients titer? (1/1024)
7. [redacted] 1/64 = 43/50 = 86% "	+		

DEC 13 1948

In Vitro Titration – WRIGHT (ENG) SERA.

Sera: Pauline & Mrs. Wright. Recd from Sheffield, England & found positive. Repeated to-day for titration.

Toxoplasma: PE pooled from 2 three day mice. Rich in toxo.

Test: Sera inactivated at 56° C for 30' just prior to test. Then diluted in Na Cl. 0.1cc of PE 1/5 in Bruecher serum = dep 1/5000 mixed with 0.1cc serum dilution. Incubated 1° @ 37° C & then read with freshly prepared meth. blue ptd 11.0. Todo numerous & tests read rapidly.

Tube #	SERUM SOURCE	Dil.	EFFECT # UNST.	# STAINED	% UNST.	RESULT.	
1	Na Cl – CONT.	—	1	49	2	–	
2	[redacted]	undil	43	7	86	+	
3		4	39	11	78	+	
4		8	36	14	72	+	
5		16	30	20	60	+	
6		32	27	23	54	+	1:32
7		64	10	40	20	–	
8		128	1	49	2	–	
9		undil	20	30	40	±	
10		64	43	7	86	+	
11		256	42	8	84	+	
12		1024	29	21	58	+	1:1024
13		4096	8	42	16	–	
14		16384	–	–	–		

DEC 13 1948

In Vitro Titration – Tommy Gibbons

Serum: [redacted] 9 month old wh ♂ admitted because of retarded development. Lower extremities flaccid. Cannot hold head erect. Head normal size. Has bilateral congenital optic atrophy. Spinal fluid & other lab work neg. Encephalograms unsatisfactory but seem to show cerebral atrophy. No calcifications. Serum undated, from 4th Army Area Lab., Brooke General Hosp. Probably obtained in early Dec. '48.

Test: Serum dil. in NaCl. Not inactivated. 0.1cc mixture of fresh PE from a single 3 day mouse dil. 1/5 in Ruegsegger serum = dep. 1/5000 added to 0.1cc of serum dil., incubated for 1 hr @ 37° C & then read = meth. blue. Many tubes.

Results.

1. NaCl – cont. = 1/50 = 2% unstained.
2. [redacted] dil = 3/50 = 6% "
3. " " 1/64 = 2/50 = 4% "
4. " " 1/256 = 4/50 = 8% "

DEC 27 1948

Harvard Plasma — Fa activation & Antibody

Test material: Bottle of dried plasma labelled: Harvard Plasma 4.0cc #33 reid unfrozen on 12-24-48. This bottle was closed with an ordinary rubber stopper. Placed in dry ice on arrival.
Reconstituted = 4.0 cc dist H_2O to-day & then immediately distributed for tests as indicated in protocol.

Toxoplasma: 3 day PE from 2 mice. Mod. with in toxoplasma.
Test: PE made to 1:5 in indicated diluent = keep 1/5000. 0.1cc amts mixed = 0.1cc aleum dil. & then incubated for 1 hr @ 37°C. Read = fresh meth blue. Sera not inactivated.

Tube #	SERUM		Dil.	PE% in	Effect.
1	NaCl	—	—	EB	—
2	[redacted]		1:16	"	+
3			undil	"	+
4			1:4	"	+
5			1:16	"	+
6			1:64	"	—
7			undil	NaCl	—
8			1:16	Plas.	—
9			1:16	NaCl	—

∴ Plasma contains insufficient activator but does have antibody to 1:16