

Antiferment Tests - in Mice

Viruses.

- 15. Human Steala - Positive and Negative for Peco Virus.
- Monkey spinal Cords - " " " " " "
- MV Virus in Monkeys

CC22RNT2-
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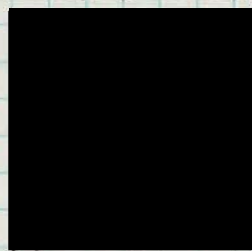
Interference in Mice between a Variety of Poliomyelitis Viruses Recently Recovered in Monkeys from Human Material and [redacted] Virus

Procedure: Method confidentially reported by Lepine

- 1) Inoculate 10 mice (10-12 Gm.) intracerebrally with material known to contain poliomyelitis virus (as a result of previous tests in monkeys)
- 2) Two days later inoculate these mice and 10 controls with 50 PD₅₀ of Lansing virus.

Materials to be tested:

Early passage monkey virus:



use titrated pools

Control Monkey (ord

Rhesus #45 (ex Snyder)

no lesions on ~~the~~ histological exam.

Inoculum: 0.03cc of 20% suspension

Stools of Patients with Poliomyelitis

Positive Stools #



Negative Stools



Stool suspension - 8cc } shake
Ether - 2cc

Centrifuge - 10 min horizontal
Centrifuge - 1 hour on refrigerated
high speed - 13,000

Culture in liquid ?

~~#~~ Inoculum - 0.03cc of final centrif. su. icer

Mice - { 140 mice for preliminary inocul.
20 mice for control inoculation - Lansing

160 mice (10-12 Gm.) - Inoculum 1:60 = 50 PD₅₀

Attempts to Demonstrate Interference in Mice Between Strain of Polio Virus and:-

A. Stools of Patients with Poliomyelitis-

I. Stools of 6 Patients From whom strains of Polio Virus were recovered by monkey inoculation:-

II Stools of 2 Patients From whom strains of Virus could not be recovered by monkey inoculation:-

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Details of Preparation: The blended stool pools of these 8 patients in approximate concentration of 10% (exception Fisher = ±7%), frozen since date indicated, were thawed. - TO 8.0 cc of the ±10% stool blend was added 2.0 cc of anesthetic ether in a centrifuge tube, then vigorously shaken with cork stoppers for 10 minutes. Evaporation of ether was permitted in refrigerator overnight followed by horizontal centrifugation at notch 8 (cut 1) for 10 minutes. The resulting supernatant was centrifuged then in the cold room at 13,000 R.P.M. for one hour, and the resulting supernatant cultured preparatory for use as an inoculum in mice intra-cerebrally; culture 0.2 cc on BAP.

RESULT IN MONKEY	CITY	NAME.	TYPE OF POLIO	AMT SUPT. AFTER 10' HORIZONTAL CENTRIF	AMT SUPT. AFTER 1 HR. AT 13,000 R.P.M.	CULTURE	DATE BLEND WAS FIRST PREPARED	OLFACTORY BULB LESIONS ORIGINAL MONKEYS
P O S I T I V E	B O B A R A C O N S C I C I N G A P O R T A C C I N G	[REDACTED]	NON-PAR.	± 7.0 cc.	6.0 ^{cc}	000	1-13-48	+
			NON-PAR.	" 7.0 cc.	6.0 ^{cc}	000	1-5-48	+
			NON-PAR.	" 6.5 cc.	5.5 ^{cc}	000	12-11-47	+
			PARALYT.	" 7.0 cc.	6.0 ^{cc}	000	11-10-47	not examined.
			PARALYT.	± 7.5 cc	6.5 ^{cc}	###	11-14-47	not examined.
N E G.	A P O R T A C C I N G	[REDACTED]	NON-PAR.	± 6.5 cc	5.5 ^{cc}	000	1-7-48	+
			PARALYT.	± 7.0 cc	6.0 ^{cc}	000	11-10-47	0
			NON-PAR.	± 7.5 cc	6.5 ^{cc}	000	1-7-48	0

* see other side

(over)

Interference Experiment (Cont'd)

and:

B. Early Passage Monkey Virus -

I Potent Polio Monkey Cord Suspensions (20% in saline)

STRAIN.	GEN.	DATE INITIAL TITRATION.
[REDACTED]	II	2/27/48.
[REDACTED]	II	3/10/48.
[REDACTED]	II	4/29/48.
[REDACTED]	II	4/29/48.
[REDACTED]	III	3/10/48.

II Control Monkey Cord.

Rhesus # 45 (Ex-Snyder) - no polio lesions:
20% saline susp. prepared 3-15-48.

Inocula TO Use:

1. STOOL EXTRACTS - 0.03 cc of final supernatant (prepared as described overleaf) inoculated IC intracranially on right side. Remainder of stool extracts frozen in 10 cc vials (location: B-7-9)
2. MONKEY CORD SUSPENSIONS (Virus-containing - and Control).
0.03 cc. of the corresponding 20% in saline cord suspensions.
3. [REDACTED] STRAIN POLIO VIRUS - to be inoculated intracranially into mice given the above stool and monkey cord extracts - 2 days following that corresponding inoculum. - Simultaneously a group of (20) normal mice to be inoc. with [REDACTED] virus for control. - using 0.03 cc of ~~1160~~ virus - \rightarrow 58 P.D. The [REDACTED] virus used is from filtered pools of mouse brains (P.D. 58).

Mice: Maxfield mice (10-12 Gms.) for all mice used.

7/22 Filtration of [REDACTED] Stool: A # small Berkfeld "V" candle (@ 1 inch) prepared by preliminary filtration of 10 cc of broth was used. About 5 cc of clear ultracentrifuged stool extract was filtered within less than 1 minute - @ 4.6 cc recovered.

Culture of filtrate on BAP - ✓✓✓

Interference Experiments with Human Strains of Polio Virus and [redacted] Polio Virus.

A. Stools from Patients Yielding Virus in The Monkey

POSITIVE
STOOL

JUL 22 1948

I [redacted] stool.

	VIRUS:	0	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
STOOL:		0	1	2																										
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7		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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Record 30-60 min.
after inoculation

II [redacted] stool.

	VIRUS:	0	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
STOOL:		0	1	2																										
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6/10

III [redacted] stool.

	VIRUS:	0	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
STOOL:		0	1	2																										
1		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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Interference Experiments - Human Polio and [redacted] Virus (Cont'd)

A. Stools From Patients Yielding Virus in The Monkey (Cont'd)

POSITIVE
STOOLS

JUL 22 1948 IV [redacted] stools.

STOOL	VIRUS																													
	0	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	
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2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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V [redacted] stools. (Berkfeld "V" - filtrate)

STOOL	VIRUS																													
	0	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	
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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VI [redacted] stools.

STOOL	VIRUS																													
	0	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	
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Interference Experiments - Human Polio Virus vs. Lansing Polio Virus (cont'd).

B. Stools from Polio Patients NOT Yielding Virus in the Monkey Not in Steel

JUL 22 1948 [redacted] stool.

	VIRUS.	0	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
<u>STOOL</u>		0	1	2																										
1		-	-	-	-	P-	PP																							
2		-	-	-	-	-	-																							
3		-	-	-	-	P-	PP																							
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II [redacted] stool.

	VIRUS.	0	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
<u>STOOL</u>		0	1	2																										
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2		-	-	-	-																									
3		-	-	-	-																									
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A. Monkey Cord Viruses (Cont'd)

JUL 22 1948 III

8001 55 101

	VIRUS.	0	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
	MONKEY CORD.	0	1	2																										
1		-	-	-	-	PP	D																							
2		-	-	-	-	-	-	-	-	D																				
3		-	-	-	-	-	-	-	-	-	PP	D																		
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7/10

IV Virus.

	VIRUS.	0	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
	MONKEY CORD.	0	1	2																										
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9/10

V Virus.

	VIRUS.	0	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
	MONKEY CORD.	0	1	2																										
1		-	-	-	-	PP	D																							
2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
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9/10

using:

B. Control Monkey Cord Material

I Normal Monkey Cord (From Rh.#45 - & [redacted])

JUL 22 1948

	VIRUS.	0	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
	CONTROL MONKEY CORD.	0	1	2																										
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9/10

JUL 25 1948

* CONTROL WITH [redacted] VIRUS

0.03 cc. I Cor. of 1:100 [redacted] polio (mouse brains) → 150

(8/29/48)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Bx 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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(cont'd) →

(cont'd) →

7:24-48. * Pool III - Pass. 204, 10% in saline [7-6-48] - 2 pool of 2 vials used as virus source for making up the dilutions of [redacted] virus used for all controls and for the 1:100 dilution into test mice. All test and control mice inoculated into left cerebellum.

Bath saline - 12a
7-13-48
7-15-48

- white boy -

(Dr. ^{Ralph B.} Cunningham - friend of family)
(RS 6160) Va 1647

- ONSET - 9-3 - sudden onset in afternoon after meal served in school (other children not sick) with vomiting and generally abdominal ache. In evening headache and fever. No diarrhea.
- 9-4 - admitted to hospital with history of fever to 103 in P.M. and persistence of symptoms of 9/3, to surgery for observation for acute appendicitis.
- P.X. - entirely neg. - no U.R.I.
Throat neg. Abdom. - only upper abd. tenderness; no rigidity - WBC - 8750 - Polys 65
No nuchal or spinal rigidity
"Nissen sign" positive
- 9-5 - Highest temp 102.5 → 99.8.
- 9-6 - Temp 100 → 99; no headache, no vomiting or abd. tenderness.
- 9-7 - Asymptomatic but temp. rose to 102° at 2 P.M. - Appears well and no "meningeal signs", but L.P. done for "academic reasons".
CSF - 150 WBC - no RBC
Pandy neg.
- 9-8 - Blood obtained + swabs + stools started
Temp. coming down very slight neck resistance; ~~no~~ can kiss knees but "Nissen sign" posit. to 90°.
- 9-9 - Temp. normal; ~~temp~~ otherwise OK
- 9-10 - " " ; some spasm of both recti abdominis. Nissen positive to 60°

ADP
ADM

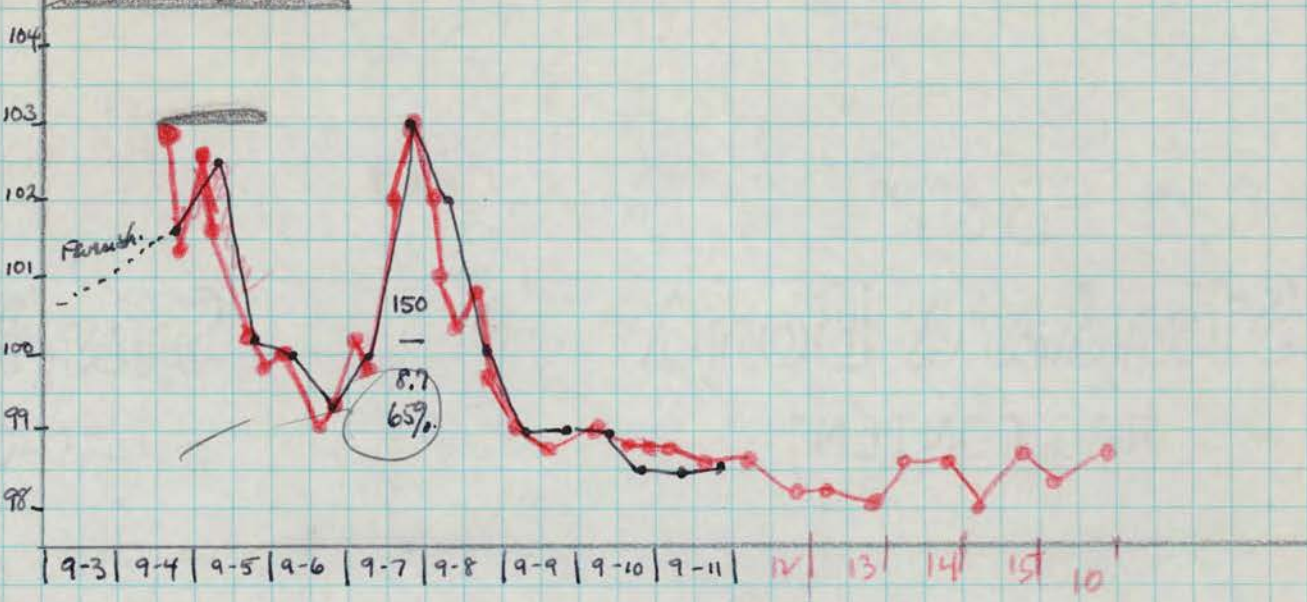
Symptoms -
abdominal pain
Vomiting
Headache

Signs -
abd. tenderness.

Lab

CBF cells.
~~per day~~
WBC - thousands.
70 poly.

Virus - stools -
throat swab.



DEC 15 1947

A - Stools -

Solids present = ± 122 gms.
Liquid present = 125 cc.
Distilled water Added = 1095 cc.

Blended at low speed for one minute. -

For Centrifugation = ± 300 cc.
For Refreezing = ± 200 cc. (Box "C")
Remainder Discarded.

Volume of First Supernatant = 260 cc.
Ether Added = 52 cc.

12/16 Volume of Untreated Sediment = 35 cc (For Intranasal Use).
Cult: ✓✓ x 1 large colony (slender sm. neg. rods)

3-23-48. Frozen stool blend thawed; - ± 50 cc removed to inc. intranasally
Rh. 235 and Rh. 1631; remainder frozen. -

DEC 15 1947

B. Pharyngeal Swab Material.

Liquid Expressed	2.1 cc.
Distilled Water Added	1.9 cc.
	<hr/>
	4.0 cc.

Ether Added - 1.5 cc.

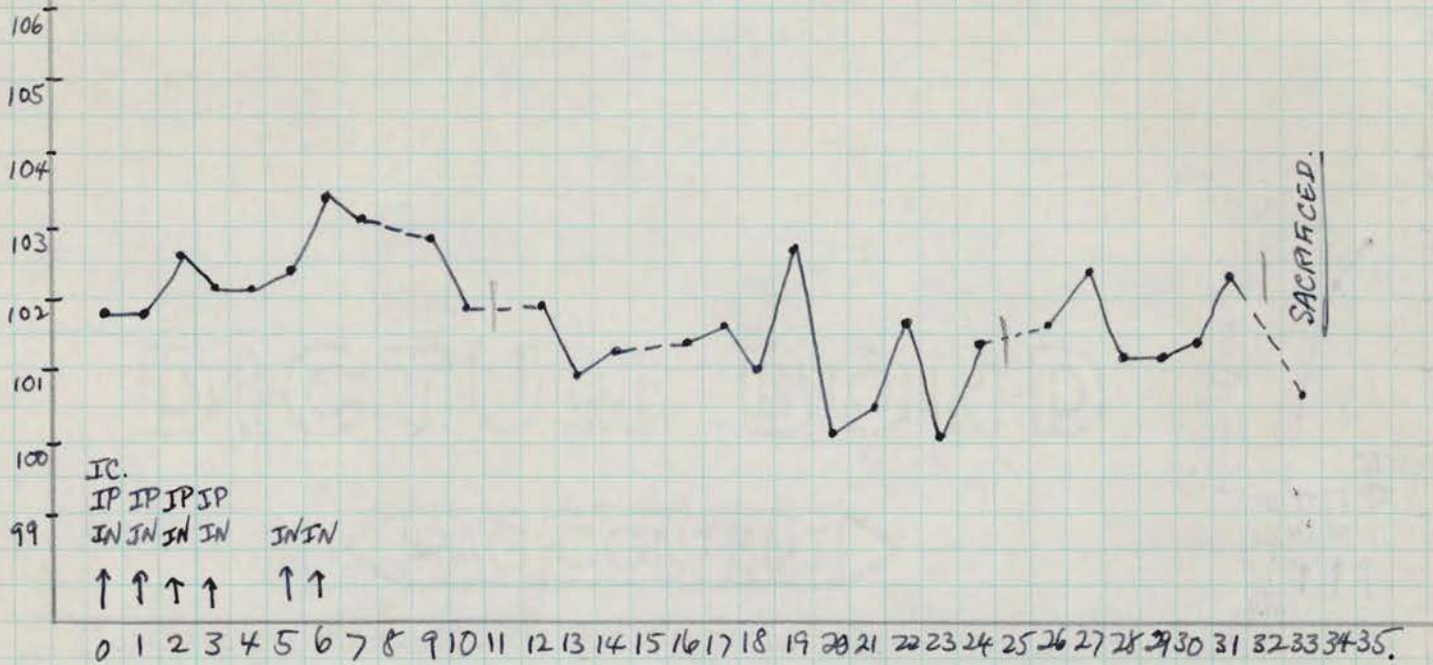
12/16/47. - After Removal of Top Layer = 3.8 c.c.
After Final Centrifugation = 3.8 cc.
Cult. ✓ * 11 large flat colony.

DEC 17 1947

Treated pharyngeal swabs - IC.
Treated stools - IP.
Untreated stools - IN.

Cult.
✓ Col. Staph. aureus
✓ Col. Staph. aureus
30m. only
✓ Col. Staph. aureus

Plesus #55



12/17/47. Inoculated 1.5 cc IC; 20.0 cc IP, and 1 cc per nostril.
1-9-48 - Mod. severe diarrhea.

1-19-48 - Sacrificed (chloroform-exsanguination) for histopathology;

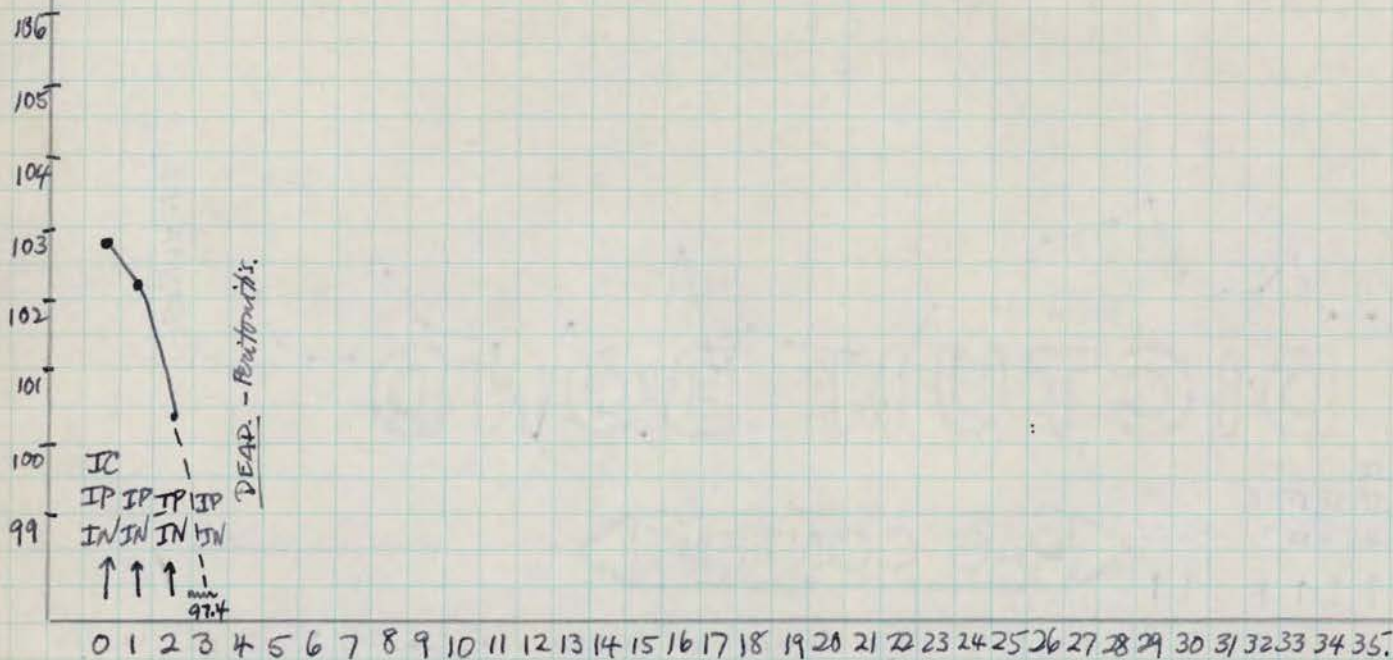
Autopsy: no gross TBc.

TISSUES: olfactory bulbs; thalamus; hypothalamus; medulla and spinal cord levels (2-4.)

DEC 17 1947

Treated pharyngeal swabs - IC
 Treated Stools - JP.
 Untreated Stools - IN.

Chrous #50-



12/17/47. Inoculated 1.5 cc IC; 20 cc IP and 1 cc per nostril.

12/20/47. - Anorectic; abdominal tenderness + distension; given 200,000 μ . of penicillin IP.

12/21/47. DEAD. - Autopsy: entire anterior abdominal wall edematous; peritoneal surfaces bound down by fibrinous strands; omentum plastered to descending colon; \pm 10 cc turbid sanguino-purulent foul fluid in peritoneal cavity; Smear: innumerable pus cells containing "diplococci" with some extracellular rounded forms.

Culture (BAP): - Dense growth - apparently pure culture; on smear = short thick definitely Gram neg. rods, numerous forms of which closely resemble diplococci. - Streaked on SS and MacConkey agars = no suspicious colonies for pathogens - only lactose fermenters.

MAR 23 1948

SECOND TRIAL

Ch. 235 (normal)

untreated Stools IN.

105
104
103
102
101
100

SACRIFICED

IN IN IN IN IN IN IN IN IN IN

↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

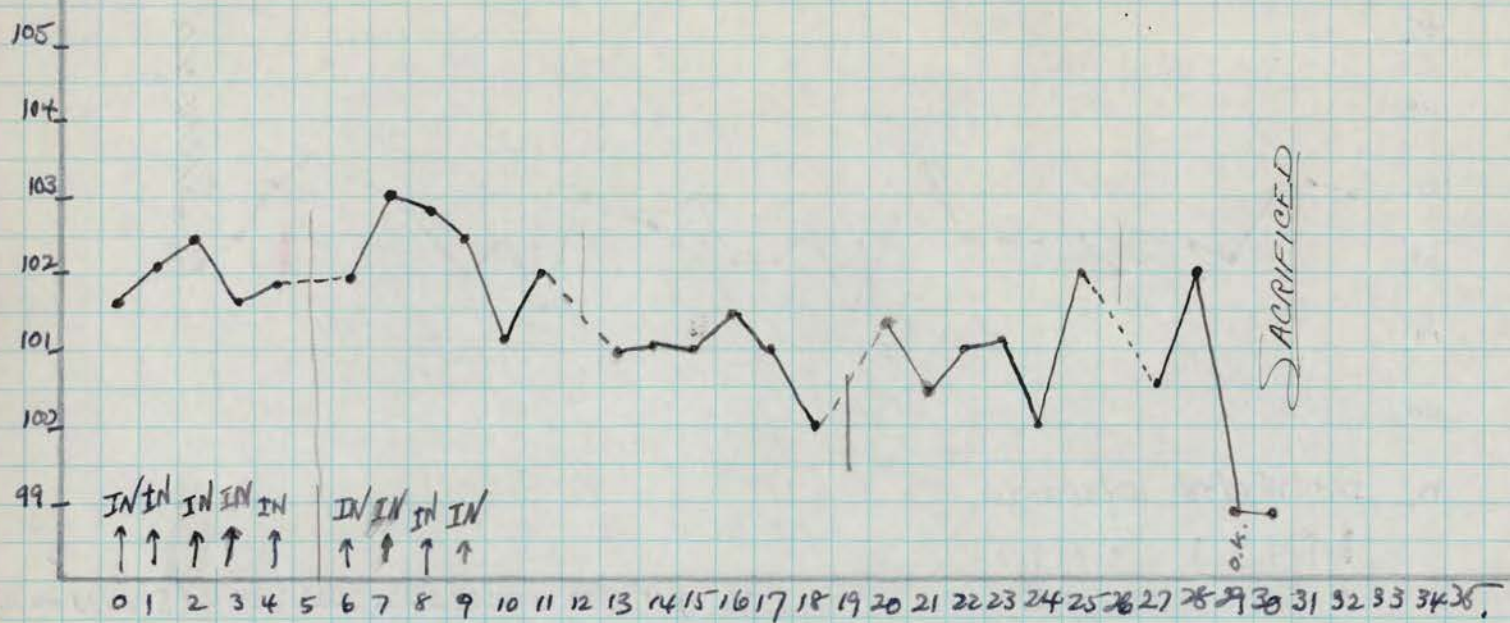
3/23/48. Inoc IN with 1.0 cc per instil - untreated stool blend.

4-22-48: SACRIFICED: (chloroform-exsanguination) autopsy: No gross tox.
TISSUE FOR SECTION - Hypothalamus, thalamus, midbrain, pons, medulla, cord levels, (2-A)

MAR 23 1948

SECOND TRIAL

Rh. 1631 (splenectomized)



3/23/48. Inoc. IN with 1.0 cc praziquantel - retreated stool O.C.D.

4/22/48: SACRIFICED: (chloroform-exsanguination) Autopsy: No to. TISSUE FOR SECTION - Hypothalamus, thalamus, hindbrain, pons, medulla, cord levels. (Z-A)

Chart # 6630
Ref by Dr. Rittershoffer
Adm. 9/6/47.

Op Swab.

Stool.

C.S.F. (D. Lakey) 9/6/47 - $\frac{\text{crees.}}{1}$ $\frac{\% \text{oh.}}{100\%}$.

9/7 ✓
9/8 ✓
9/9 ✓
9/10 ✓

✓ Enema with soap.
partially + food particles.
formed stool.
✓ stool - semi-solid.
✓ stool - semi-solid.

Onset

Onset: 9/5/47. -

History: Pt. felt feverish in A.M. of 9/5, was listless - held her extremities still + limp. - Went to nursery school but returned complaining of stomach-ache. That same day she had wet the bed at nursery school - unusual for her. - The evening of 9/5 - her temp. was 102°; restless in her sleep. Complained during the night of sore throat - + she coughed several times.

A.M. of 9/6/47 - still coughed occasionally, and the eyes seemed red. --- Since onset there has been poor appetite and pt. has been drowsy + listless.

Adm 9/7/47 (AJS) (see also adm. exam. 9/8/47 by Dr. Lakey).

Exam: - (Not entirely cooperative.) -

- 1) Mild degree of pharyngotonsillar reddening.
 - 2) Small ant. cervical nodes.
 - 3) NO neck rigidity; neg Kernig + Brudzinski signs.
- On testing "Nissen" sign, child resists flexion beyond 45° angle; (I believe this to be voluntary.)

Notes: 9/8/47 - Throat hardly remarkable. Allows complete forward flexion.

9/9/47 - Coughing more frequently - sound productive and she apparently swallows sputum. - X-Ray (AP) of chest show questionable infiltration rt. cardio-phrenic angle; to be checked by pt. lateral film. Can be bent in complete forward flexion.

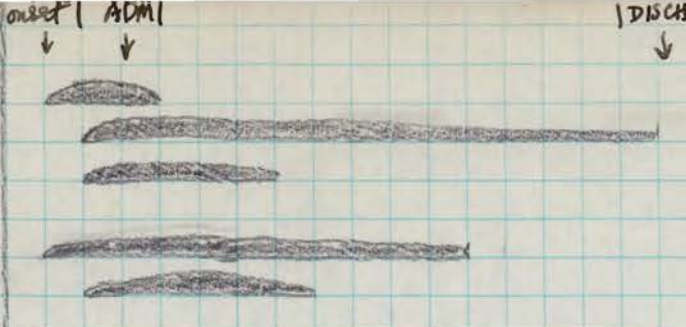
9/10/47. T. went to 101° yesterday afternoon. - Coughing quite considerably, nose shows moist and contains exudate.

9/11/47. T. to 101° yesterday; 100 This A.M.; cough prominent but child seems not toxic or acutely ill. - (Should have cold agglutinin study later. - We plan to

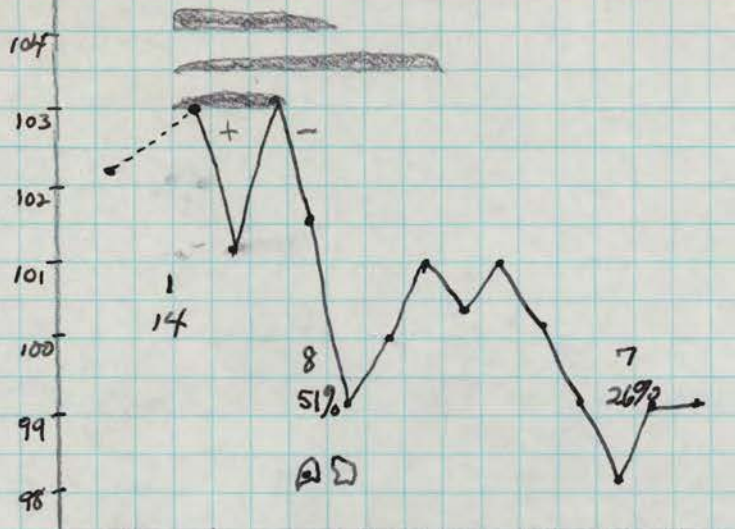
Keep her here until her pneumonia is better; and to do the investigation from the poliomyelitis standpoint.

10/18 - Followup blood serum taken.

Symptoms:
 Abdominal Achc.
 Cough.
 Sore Throat
~~Respiratory~~
 Anorexia
 "Red Eyes"



Signs:
 Pharyngitis.
 Rhinitis.
 Lung signs (R.H.)
 Nissen sign.



LAB:
 ESF cells.
 myel. protein
 WBC (Thousands)
 Polys (%)

X-Ray chest:-

Virus Study:-

Sample	9-5	9-6	9-7	9-8	9-9	9-10	9-11	9-12	9-13
Stools			-	-	-	-			
Throat swabs			-	-	-				

9/7/47 Throat Cult: - green strep. - *Ph. sicca*; *M. catarrhalis*; *Staph. albus*.

JAN 20 1948

3Wset

A. Hools.

Solids present - 104gms.
Liquid present - 50 cc.
Saline added - 730 cc. } $\pm 15\%$

Blended at low speed for one minute.

Portion refrozen = ± 230 cc B- 12-16-48 Discarded

Remainder centrifuged 15' in 100 cc tubes.

Portion for intranasal use = ± 40 cc.

Etherization of 200 cc of supernatant with 40.0 cc ether; shaken 10' and refrigerated.

1-21-48. - Shaken 10'; centrifuged 30' on horizontal = 150 cc

Culture: * covered with dense gray, white hemolytic growth.
Sm. + spore forming rods on smear.

1-22-48 - Re-etherized with 30 cc ether. - 1-23-48 - Am't = 100 cc.

Culture: ✓ ✓ ✓
(inoculated to monkeys 1-24-48)

JAN 20 1948

B. Pharyngeal Swab Material -

Liquid Expressed + 0.6 cc Buffer #1 = 3.2 cc
Buffer #2 = 0.6 cc

3.8 cc

Blended at 40°C for 2-3 minutes.
1 cc ether added; shaken 10'; refrigerated overnight

1-21-48. Centrifuged on horizontal for 20 minutes 3.7 cc
Cultured ✓✓

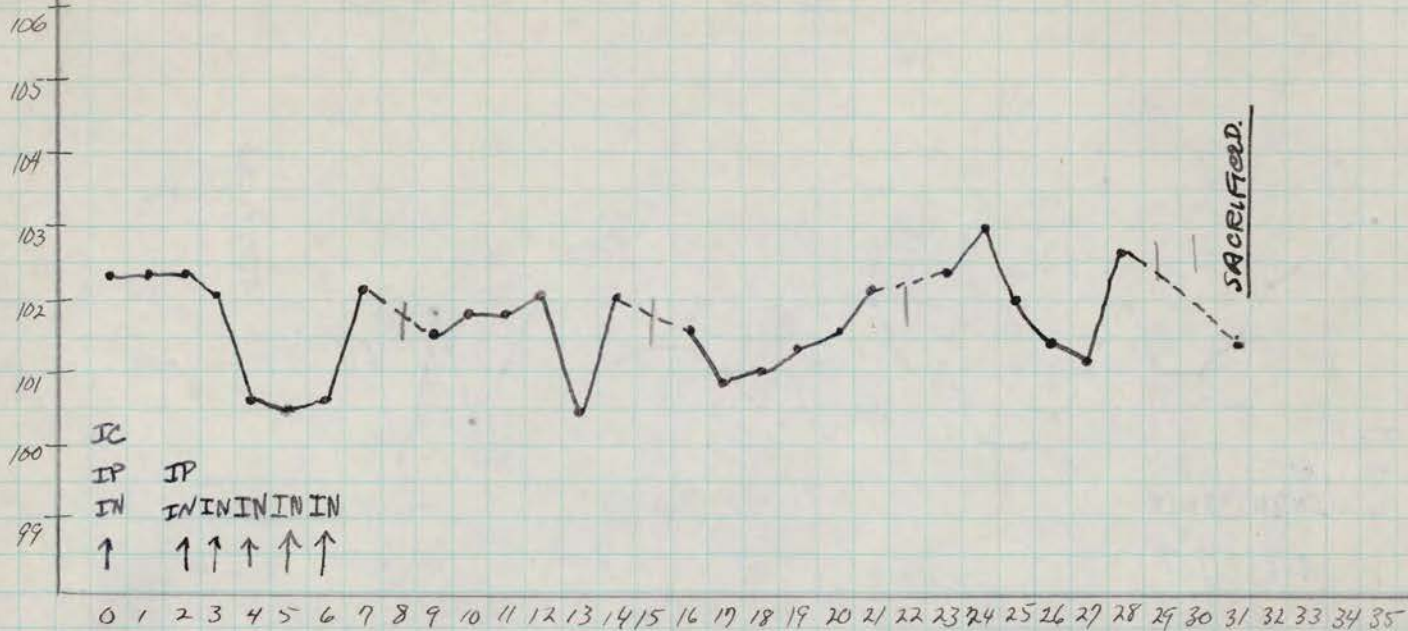
JAN 24 1948

Rhesus 1557 - Splenectomized.

3 wreat

Treated Pharyngeal Swabs IC
Treated stools IP.
untreated stools IN

Cultures
✓✓
✓✓



IC
IP IP
IN INININININ
↑ ↑↑↑↑

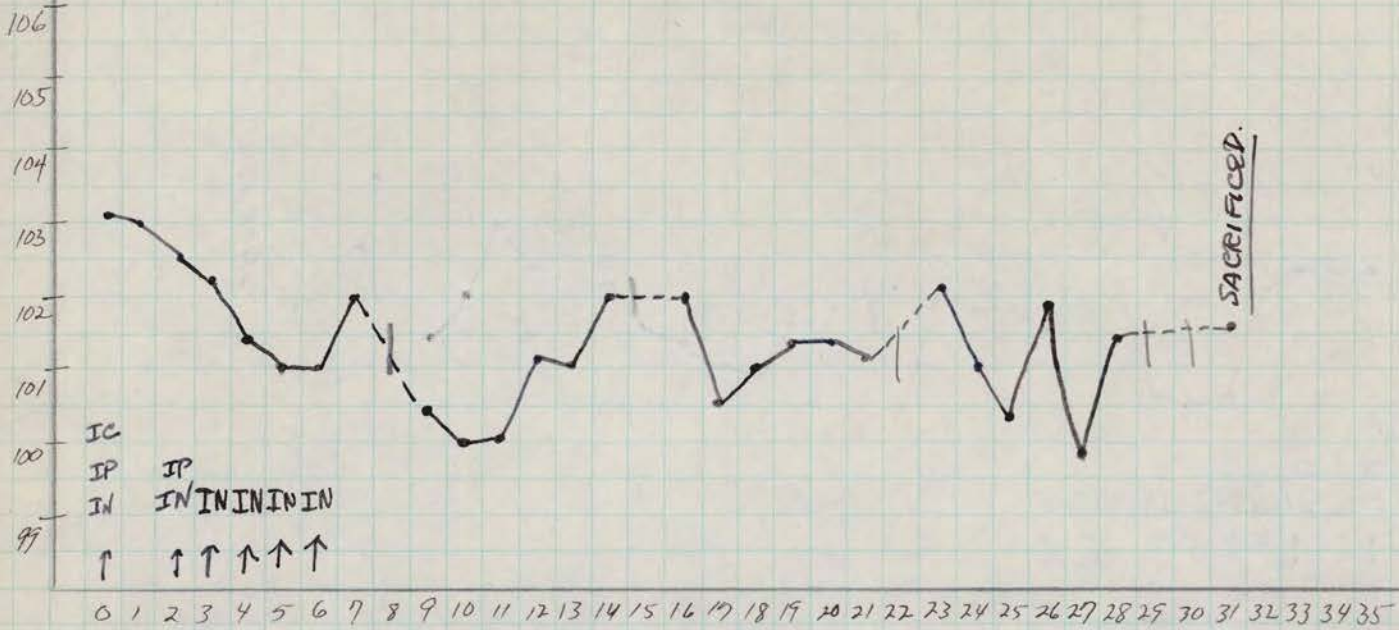
1-24-48. Proc. 1.5 cc IC; 20 cc IP and 1 cc each nostril.
2-24-48. - Sacrificed (chloroform-exsanguination) for histopathology. - Nogross T&E

TISSUES FOR PATHOLOGY: olfactory bulbs (rt. bulb partly macerated); Thalamus; hypothalamus; midbrain; pons; medulla; spinal cord. (2-A).

Histology - No Polio

JAN 24 1948

Rhesus #108



1-24-48. Inoc. 1.5 cc IC; 20 cc IP and 1 cc each nostril.

2-16-48 - Mild facial edema.

2-20-48 - Mild muc. sanguinous diarrhea.

2-24-48 - Sacrificed (chloroform-exsanguination) for histopathology. -
Autopsy: no gross JTB.

TISSUES FOR PATHOLOGY: olfactory bulbs; Thalamus; hypothalamus;
midbrain; pons; medulla; spinal cord - (Z-A)

Histology - No Polio

Lemmus Sore throat

PO210 - 1947.

MONKEY HISTOLOGY.

<u>M.K. #</u>	<u>Lambd.</u>	<u>Thoraci.</u>	<u>Cervicid.</u>	<u>Medulla.</u>	<u>Milbrain.</u>	<u>Thalamus.</u>	<u>Hypothal.</u>	<u>Olf. Bulbs.</u>	<u>DATE NOTED.</u>
Rh. 1557 (AB)	0	0	0	0	0	0	0	0:0.	<u>No.</u>
Rh. 108 (AB)	0	0	0	0	0	0	0	0:0.	<u>No.</u>

- white girl - 5 1/2 yr. old -

ONSET - 8/24 - Headache and fever late at night.
Child also complained of neck stiffness/pain

No nausea or vomiting or sore throat
Mild anorexia for past 2 days

8/25 - 3.A.M. - Temp 103°

10 A.M. - admitted to hospital
Temp. normal - child appeared well.

No nuchal or spinal rigidity

Lumbar puncture done - because this syndrome is comparable to "hundreds" of other similar cases being seen in Cincinnati at this time.

CSF - ~~40~~ cells (65% L - 35% polys)
Pandy +

11 AM - Blood obtained
Throat Swab I

8/26 - Temp. normal - OK.

Throat Swabs II; Stool

8/27 - Throat swabs III

Follow-up:

Condon School to receive
physiotherapy.

UN - 9353 (nurses office)

8216 - Mrs. Heek. 1:30 p.m.

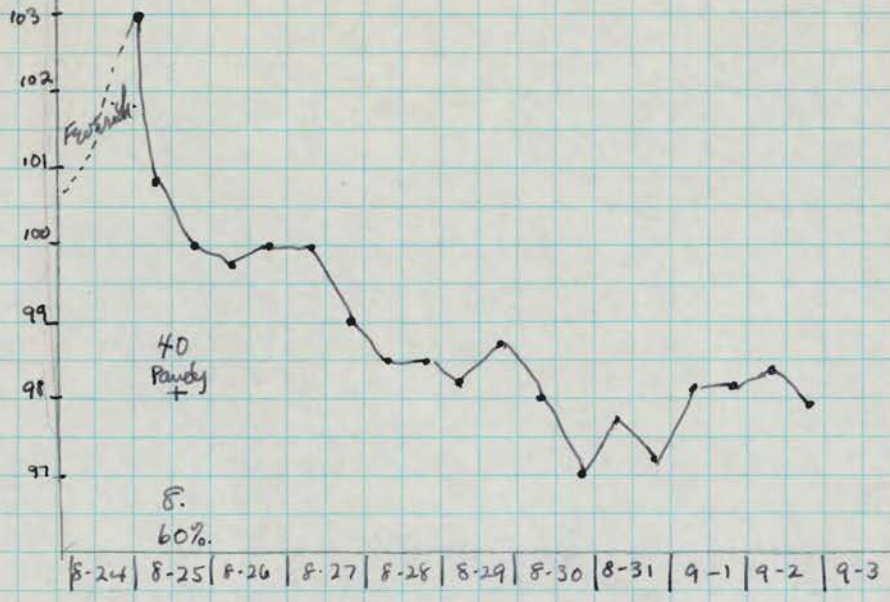
Symptoms -
 Headache
 Anorexia
 Sore Throat

Onset




● Signs - ml.

LAB



LAB:
 CRF - cells.
 Protein - mgms.

WBC - Thousands.
 % Polys.

8-24 | 8-25 | 8-26 | 8-27 | 8-28 | 8-29 | 8-30 | 8-31 | 9-1 | 9-2 | 9-3

Notes on

Case No. 228811
Adm. to Ward H-Contage. 8/25/47

obtained from the Orthopedic Clinic at Cinti, General Hosp.
on September 26, 1947. (By telephone)

"Examination 9/19/47 *Orthopedic Clinic - C.G.H.*

Good muscle power throughout. No corrective therapy at present.

Make arrangements to go to Condon School. To return to clinic in
six months."

A. Stools -

Estimated Solids Present = 73 gm.
 Liquid Present = 75 cc.
 Distilled Water Added = 650 cc.

Blended at low speed for one minute (two runs because of large volume)

For Centrifugation ± 350 cc.
 For Re-Freezing ± 200 cc. (Box "C")
 Discarded ± 180 cc.

First Supernatant = 275 cc.
 Ether Added = 55 cc.

Treated Sediment = 40 cc (for intranasal use)

12/5/47 -

Final Amount of Treated Suspension = 240 cc.

Cult. (Before Final Centrifugation) - * loaded colonies - (pm neg thick rods)
 (After Final Centrifugation) - * " " " (" " " -)

12/6/47 - Re-EMerized - 48.0 cc. ether added.

Cult: ✓ * 6 colonies; large pm. neg. rods.

3-22-48 The frozen stool pool blend thawed; ± 50 cc. removed for a second trial at recovery of virus by intranasal inoculation of Rh. 234 and Rh. 1611

DEC 4 1947

B. Pharyngeal Swab Material -

Liquid Expressed = 2.2 cc.

Distilled water Added = 1.8 cc.

ETAN Added = 4.0 cc.

= 1.5 cc.

12/5/47.

After Removal Top Layer - 4.0 cc.

After Final centrifugation - 3.7 cc.

Culture: - ✓✓

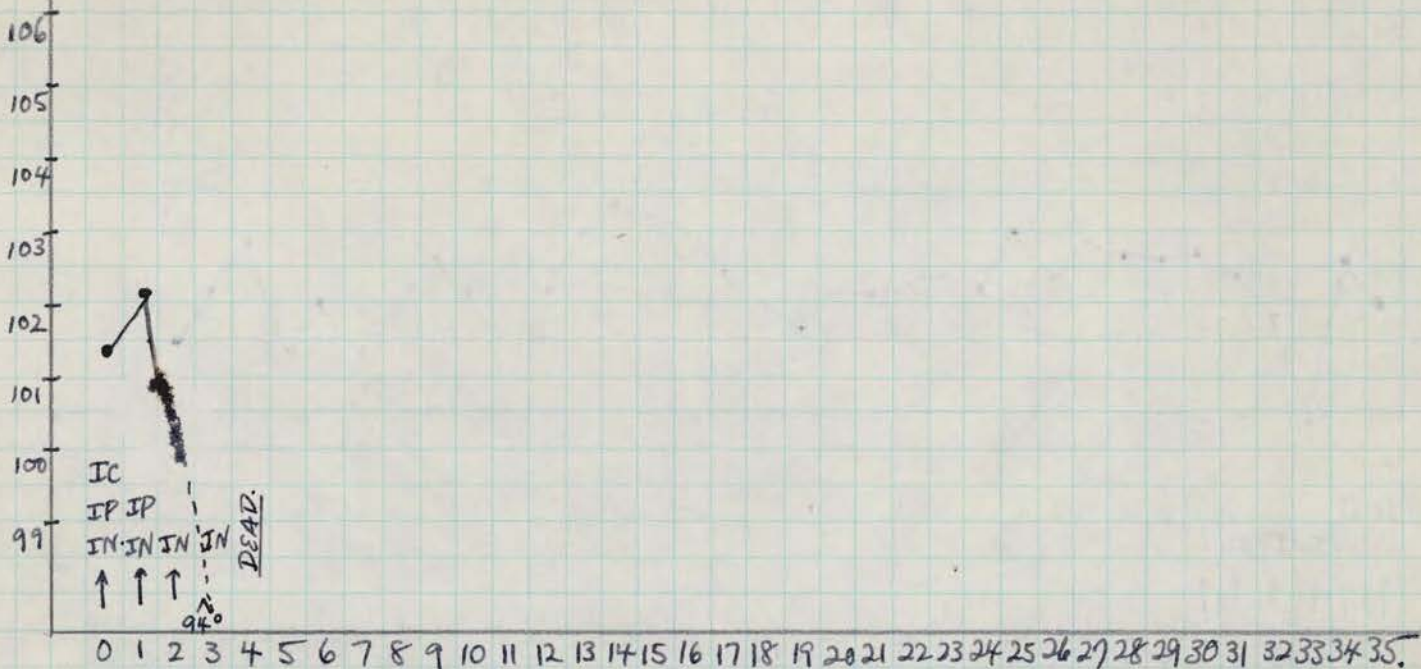
DEC 8 1947



Treated Pharyngeal Swabs IC -
Treated Stools - IP.
untreated Stools - IN

Cults:
✓✓✓
✓x 6 colonies
large than
neg. stools.

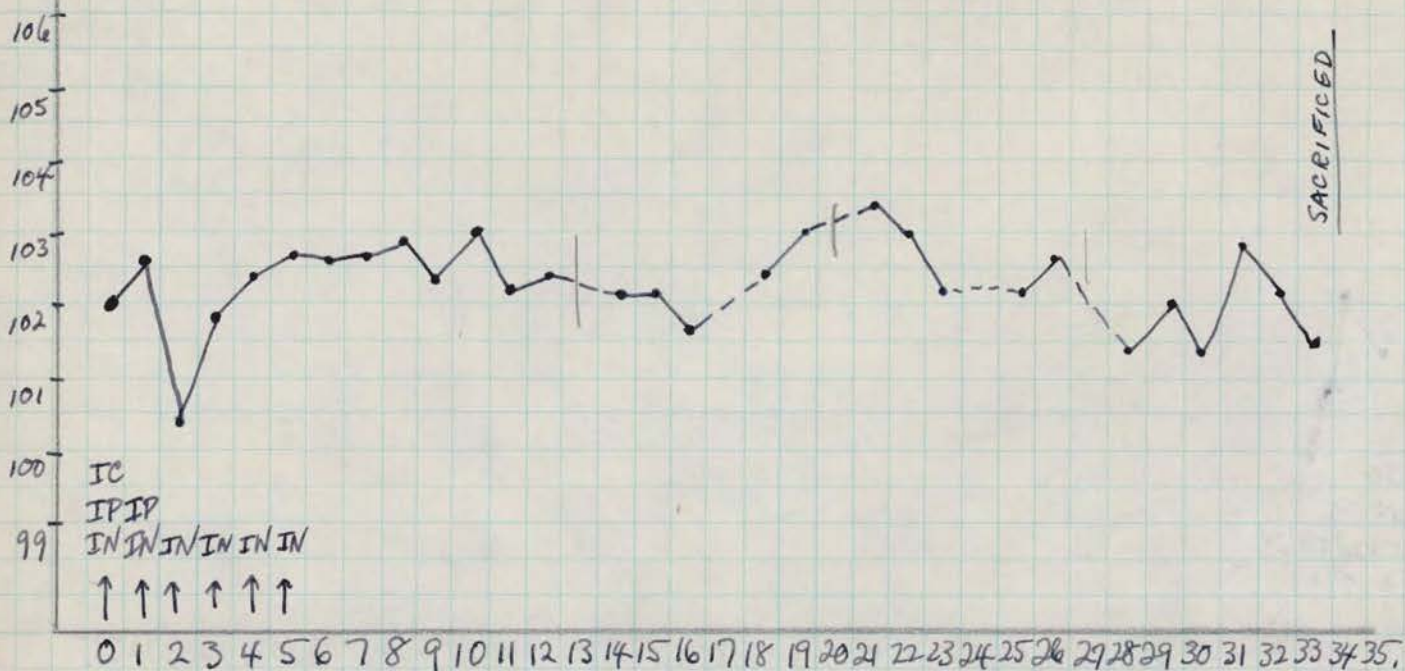
Rhesus # 43.



- 12/8/47. Inoculated 1.5 cc IC; 20 cc IP; 1 cc IN per nostril.
- 12/10/47. T. 100.8; has severe diarrhea; abdomen does not seem tender, not distended.
Given 100,000 units Penicillin IP at 11⁰⁰ A.M.
- 12/11/47. - AM - Prostrate; temp < 94.0; has profuse diarrhea; abdomen scaphoid;
Given 60 cc warm saline; 200,000 u Penicillin; 1 cc epinephrine 1:1000 IP.
P.M. Given 60 cc warm saline IP; still prostrate; continuous streaming diarrhea.
- 12/12/47 - DEAD - Autopsy: No evidence of peritonitis; bowel mucosa (colon) - injected deeply, contents of lower bowel having pea-soup color and consistency; Rt. lower lobe dark + on section is edematous; remainder of autopsy neg; brain + spinal cord not examined. -
Culture of Colonic Content on SS Agar: - No non-lactose fermenting organisms

DEC 8 1947

Rhous #44.



12/8/47. - inoculated 1.5 cc IC; 20 cc IP and 1 cc IN per nostril.

12/10/47. - No diarrhea; but abdomen quite tender; no distension. - Given 100,000 u of Penicillin IP at 11:00 AM.

12/11/47. - No diarrhea noted; abdomen seems more tender than usual; given 100,000 u penicillin IP at 11:00 AM.

12/12/47. - Abdomen not distended; probably not tender; given 100,000 u Penicillin IP - 2:00 pm

12/16/47. - Has mild degree of diarrhea; given 2.0 gms. Sod. Sulfadiazine IP.

1-10-48 - Sacrificed for histopathology (chloroform - excanguination) -

Autopsy: no gross tuberculosis.

TISSUE: cord. Devils; medulla; Thalamus; hypothalamus and olfactory bulbs (1st bulb separate from 2nd tract). (Z-A.)

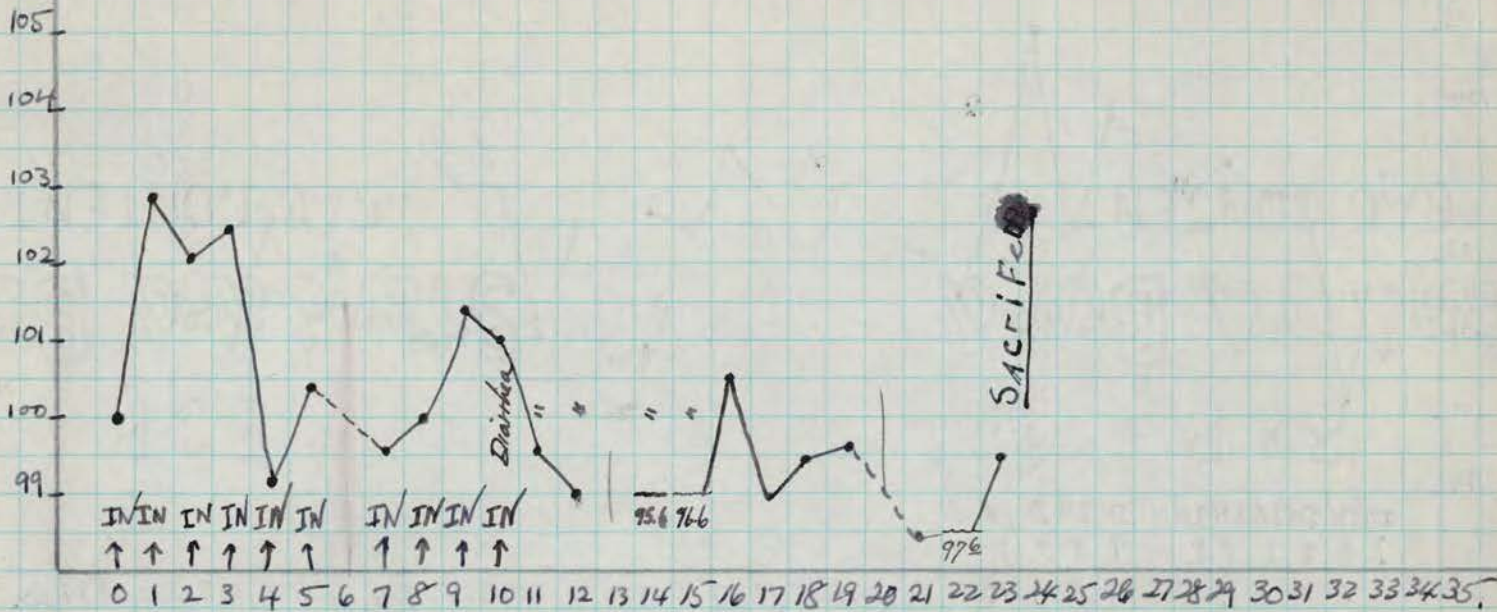
Histology - [faded]

MAR 22 1948

SECOND TRIAL

untreated stools IN.

Rh. # 234 (normal)



3/22/48 - mor. with 1.0 cc per nostril IN. - untreated stool blend.

3-25-48: Severe gaseous abd. distension. - 3/26/48. T=99°; less distension.

3-27-48: Distension gone.

4-6-48: Rapidly becoming severely emaciated.

4-10-48: Feeble; disinterested; ?TB. (see Rh. 1611 overleaf.)

4-14-48: Almost dead in cage. Would very probably not survive until morning. SACRIFICED (chloroform-exsanguination)

Autopsy: Gross ~~find~~ pulmonary tbc. Massive worm infestation. TISSUE FOR SECTION: - Hypothalamus, thalamus, midbrain, pons, medulla, and cord levels. (Z-A) Off. bulbs in several pieces.

TISSUE FOR VIRUS: - One section of lumbar cord and one section of cervical cord taken to be made frozen in a lusteroid tube. Culture: {plate covered & a spreader} (C-1)

4-16-48: Lumbar and cervical cord sections thawed and made in a ± 20% susp. in (4cc) sterile saline. (Cord pieces not weighed). ± 3.5cc frozen in 1-10cc amp. and stored. Culture: ✓✓✓ B-7-3

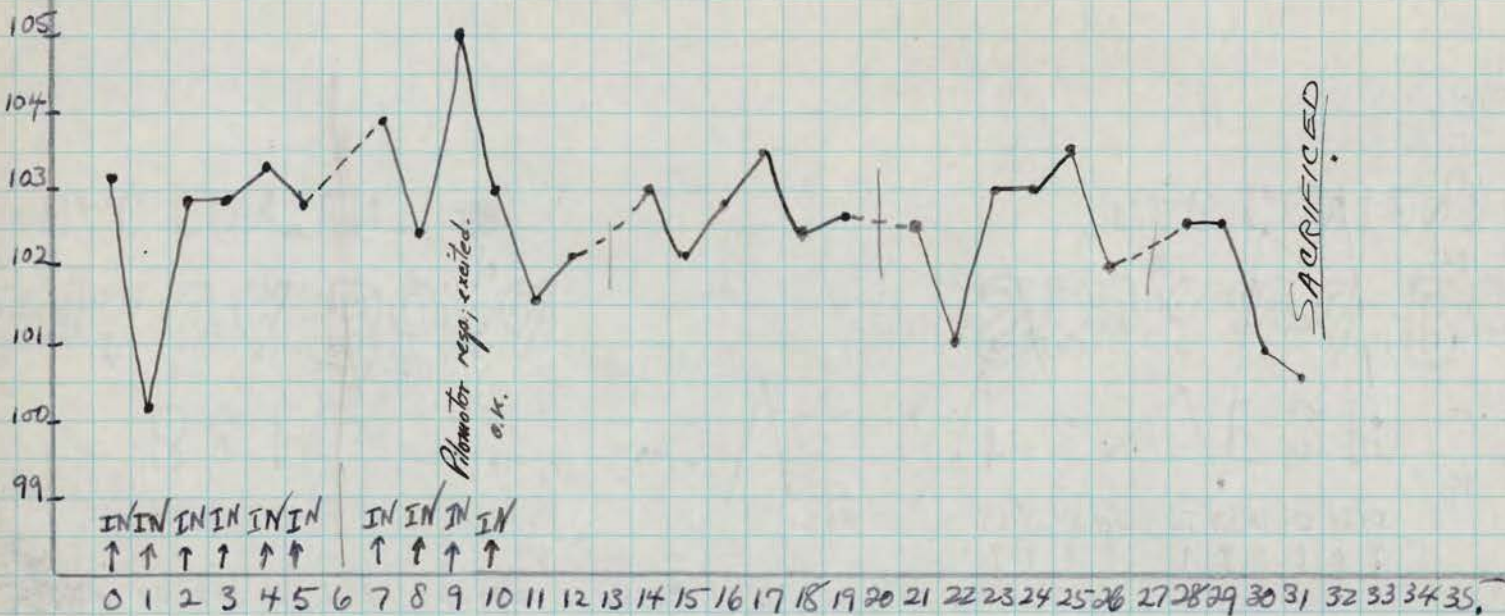
12-17-48 Suspension of Rh # 234 Discarded

MAR 22 1948

SECOND TRIAL

Rh. # 1611 - splenectomized -

untreated stools - IN.



3/22/48. Obs. IN with 1.0 cc per nostril - untreated stool bland.

3-31-48 - A.M. Pilomotor +, excited.

P.M.: Same, no progress.

4-10-48 - Coughing constantly; excited when exercised.

4-22-48: SACRIFICED: (chloroform-exsanguination)

Autopsy: Gross pulmonary & intra-abdominal tbc.
TISSUE FOR SECTION: - Hypothalamus, thalamus, midbrain, pons, medulla, cord levels. (Z-A)

Amesbury Lane Throat

PKND - 1947.

MONKEY HISTOLOGY.

MK. #	Lumbar	Thoracic	Cervical	Medulla	Milobrain	Thalamus (cardiac nucleus)	Hypoth.	Off. Bulbs	DIAGNOSIS
Rh. 44*	0	0	0	0	-	0	0	0 (Missing infilt.)	NEG.
Rh. 234.	0	0	0	0	0	0	0	0:0.	NEG.
Rh. 1611	0	0	0	0	0	0	-	-	NEG.

PRIMARY ISOLATION. (2nd Trial)

* Rh. 44 Serial Sections: Hypothalamus: - (18 slides) District perivasc. infilt. (slides 4 to 17) AB

Off. Bulbs: - (18 slides) Perivasc. infilt. meningeal infilt. with areas ^{to} (\pm 7 sections per slide) of dense infilt. let. glom. and molec. layers. AB

Re-Review (8-18-48) - ABS.

Rh. 44 Serial Sections of Off. Bulbs - 18 slides, average 7 sections per slide.

Initial Sections (4 sections of each per slide) - = meningeal infilt. of one bulb was the only path. finding.

18 Additional Sections: Off. Bulb I - negative throughout.
Off. Bulb II

OFF BULB I - This one which showed only meningeal infilt. at first sectioning showed in the rest half of the serial sections only - Extensive PV infilt. - in outer layer of nerve fibers, & extension bet. the glomeruli. In a few areas of these terminal sections - The area bet. the glomeruli and mitral cell layer exhibited large no. of capillaries with cuffing consisting of generally a single layer of cells, - and only an occ. focus of (interstitial) glial cells.

OFF BULB II - This bulb - (which originally showed nothing) - in the terminal sections exhibited foci of PV infilt. situated about the glomeruli as well as in the mitral cell layer. - scattered glial cells were observed in one area near such a cuffed vessel in the mitral cell layer. (over)

Ph. 44 (cont'd)

DIAGNOSIS: Lesions limited to olfactory bulbs + hippocampus (medulla)

PO-10

Hypothalamus - (18 serial sections) - ^{and definite} original section there was several vessels & marked cuffing - not associated with intrat. glial infiltration or other evidence of neurophagia. - This led to prep. of serial sections (18):

Serial sections revealed many vessels on both sides of 3rd ventricle; - areas of intravascular infiltration as well as several areas of glial aggregates suggestive of old neurophagocytosis.

Review of Other Slides:

Caudate Nucleus Region (no thalamus!): - Cuffed vessel in ant. perf. substance.

Medulla (3 levels): - negative.

Spinal Cord (29 levels) (accompanying roots intact): - Show no evidence of pathological change.

Ph. 234

Cef. Bulbs: (only small torn bits available) - unsatisfactory for suitable exam. - no lesions in these torn bits of tissue.

Hypothalamus (2 levels) - negative

Thalamus (1 level) - negative

Midbrain (sup. colliculi) - negative

Midbrain (inf. colliculi) - "

Pons-Medulla - negative

Medulla (3 levels) - neg.

Spinal Cord (23 levels with roots intact) - negative

DIAGNOSIS:

no polio.

Ph 1011

Cef. Bulbs: - neg.

One section of thalamus + hypothalamus - negative

Pons, Medulla - plus Medulla (4 levels) - all negative.

Spinal Cord (27 levels with roots intact) - all negative.

Midbrain (postcolliculi) - negative

" sup. (incomplete) - "

Diagnosis: no polio.

Ph. 234. Serial sections olfactory bulbs (16 slides). - No infiltration. (ATS)

Ph. 1011 - Serial sections olfactory bulbs (15 slides) No infiltration (ATS).

Chart # 45861. changed to 25462

whits: ♂. 8 yrs

Ref. by Dr. G. Englander.

adm. 9/5/47.

	OP Swab	Stool.	9/5/47 CSF cells.	Protein → 18 mgms. %.
9/5	✓	—	0 (ATS and ABS) - reported as 15 cells - lymphocytes by chamber count - by Dr. Plimmer	
9/6	✓	✓ very effective sucrose solids.		
9/7	✓	✓ formed.		
9/8	—	✓ Effect of lumen = milk solid.		

Onset: 9/4/47.

History: - Awoke A.M. of 9/4/47 complaining of headache. Had temp. 102° and in 2-3 hrs. This dropped to ~~2~~ 99°. Child then complained of chills and malaise. Nose seemed "stuffy".
 Evening of 9/4/47 - Temp. rose to a peak of 101° and pt. continued to have headaches. - No neck or backache; no vomiting, diarrhea, or cough. - Child spoke of stomachache (epigastric) and at night his eyes looked "bloodshot".
 9/5/47 - This morning the pt. seemed to be much better to the mother, and the temp. was 97°; no symptoms.

→ N.B. - 1 month ago child had fever, headache, red eyes, abdominal ache - a picture very much like the current one - and which disappeared in 2 days.

Adm. Exam:
 9/5/47. Mod. enlargement ant. cervical nodes. -
 Pharynx - mod. injected. -
 Nose - sl. amount of yellowish crusting both nares. -
 Soft basal systolic murmur. -
 Mild injection of conjunctivae.
 No meningitic reaction; NTS neg.

9/6/47. - No meningitic reaction. - Nodes as w. - Pharynx if anything is better; mod. yellow dry crusting ant. nares.

9/8/47. No meningitic signs; pharynx entirely normal. To be discharged.

(over)

Follow-up Spinal Puncture:

9/27/47

cells.
0

Quantitated Protein
26 mgms. %.

onset 11 A.M. Disch.

SYMPTOMS:

- Headache
- Chilliness 104
- Malaise
- Epigastric Pain 103
- Stuffy Nose
- "Red eyes" 102

Signs:

- Cervical Adenopathy 101
- Crusted Nose
- Conjunctivitis 100
- Pharyngitis 99

Lab:

CSF cells	0	0
mgm Protein	18	26
wbc (Thousands)	6.4	5.2
Polys %	78	45
	9-4	9-5

Virus Study

Throat Swab	✓	✓	✓			
Stool		✓	✓	✓		

} Polio Virus Isolated.

Note: - one month prior to admission had fever, headache, red eyes for 2 days; mother says illness was very much like this one.

- Throat Cult: 9-5-47 - H. hemolyticus, Staph. aureus (non hem.) + nonhem St. albus, M. catenulatus, gram Strep
- Blood Cult 9-5-47 - Neg - 14 days.
- Urine 9-6-47 - Trace acetone.

JAN 20 1948

3 West

A. Stools.

Solids present. - 180 gms.
Liquid present - 60 cc.
Saline Added 1300 cc. (+15%)

Blended low speed for one minute. -
Portion refrigerated = ± 230 cc. "C"

Remainder centrifuged 15' in 100 cc tubes -

Portion for extra use = ± 35 cc

Etherization of 200 cc. of supernatant with 40.0 cc of ether; shaken for 10';
and refrigerated. -

1-21-48. Shaken 10'; centrifuged 30" on horizontal. = 160 c.c.

Culture. X 3 large spreading colonies almost merged.
Smear = large sm. positive rods.

1-22-48 - Re-etherized with 32.0 cc ether. -

1-23-48 - Am't after centrifugation = ± 100 cc.

Culture: ✓✓

(inoc. to monkeys 1-24-48)

JAN 20 1948

B. Pharyngeal Swab Material -

Fluid expressed + 0.6 cc Buffer #1 = 3.2 cc.

Buffer #2 = 0.6 cc

3.8 cc.

1 cc Ether Added; shaken 10'; refrigerated overnight.

1-21-48: Centrifuged in horizontal for 20 minutes.

3.4 cc

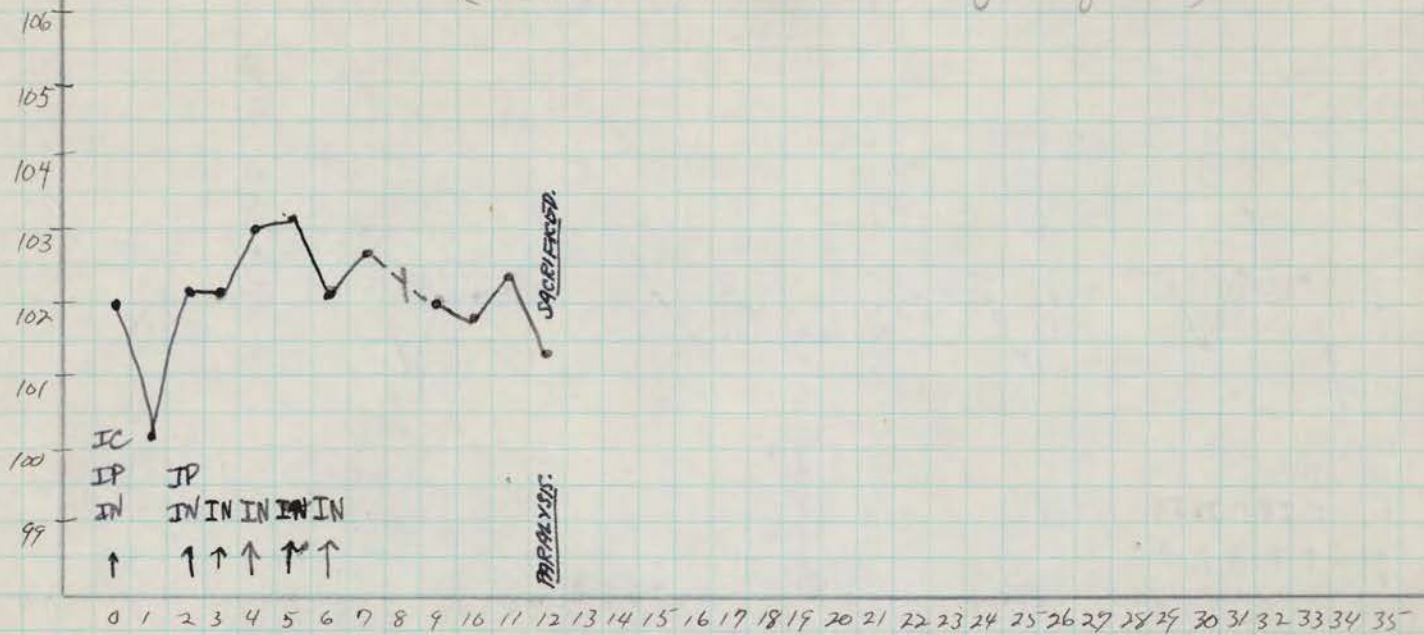
Culture: ✓✓

JAN 24 1948

3 wks
Treated pharyngeal swabs IC. ✓✓
Treated stools IP. ✓✓
Untreated stools IN.

Ph. 1666 — splenectomized
(not splenectomized)
If Paralyzed, Call
Dr. Schmidt

(Back to Schmidt — if no polio)



1-24-48. Inoc. 1.5 cc IC; 20 cc IP, 1 cc each nostril.

2-5-48. - Animal shows tremors, very easily exhausted with marked weakness of both lower, nt. upper and back; mild bilateral ocular ptosis. (ATS and ABS)

Etherized for following procedures:-

⊙ Cisternal puncture → 50 cells/cu. mm. - (mononuclear)

⊙ cardiac puncture - ± 20 cc blood + 1 mg. Heparin (For Dr. Schmidt)

Sacrificed - chloroform -

Spleen - to Formalin (For Dr. Schmidt)

Autopsy: Extensive tuberculosis rt. middle + lower lobes.

Polio COLON CONTENTS: frozen in tasteroid B-3-6

TISSUE FOR PATHOLOGY: cord levels; medulla; midbrain; off. bulbs. + lung.

TISSUE FOR VIRUS: cord-medulla frozen in tasteroid. B-3-5

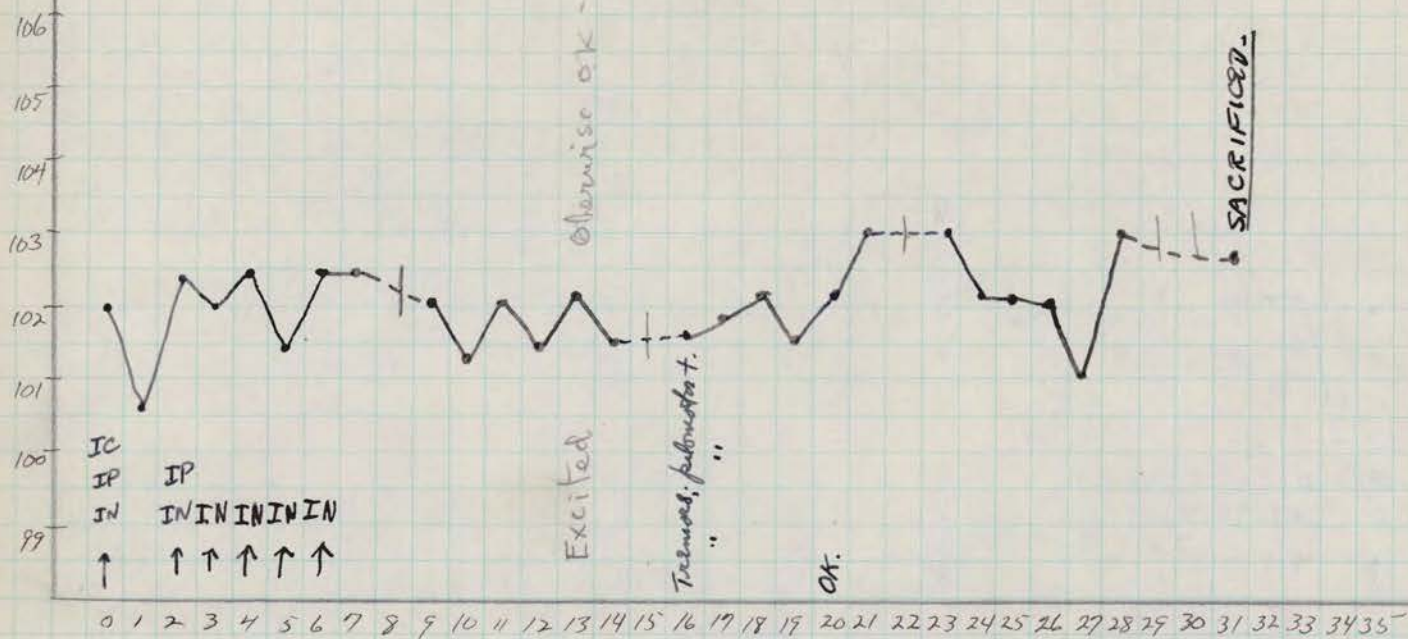
2-17-48 - Cord-medulla thawed; wt. = 4.0 gms; made up as 20% suspension with 16.0 cc saline and this material immediately used for passage in 2 monkeys (Ph. 131 and Ph. 1261); remainder of suspension i.e. ± 10 cc frozen in a 20 cc amp. (B-3-5) Cult: ✓✓

3-2-48. Susp. thawed; 2.0 cc removed to reinoc Ph. 131 and 1261; remainder re-frozen B-3-5

Histology - Polio acute

JAN 24 1948

Ph. 104 - normal.



0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

IC
IP IP
IN INININININ
↑ ↑↑↑↑

1-24-48. Dose 1.5 cc IC; 20 cc IP and 1 cc each control.

2-9-48 - Fine tremors of all extremities, particularly left upper. Seems to have poor coordination of both lower extremities with ? weakness but on re-exam. of this animal with ABS, cannot demonstrate definite weakness; - pilomotor +.

2-10-48 - A.M. - same as 2-9-48; tremors and pilomotor + are the outstanding features.

2-11-48 - Tremors a little less pronounced.

2-13-48 - Seems entirely recovered.

2-24-48 - Sacrificed (chloroform exsanguinating) for histopathology: - No gross ltr.

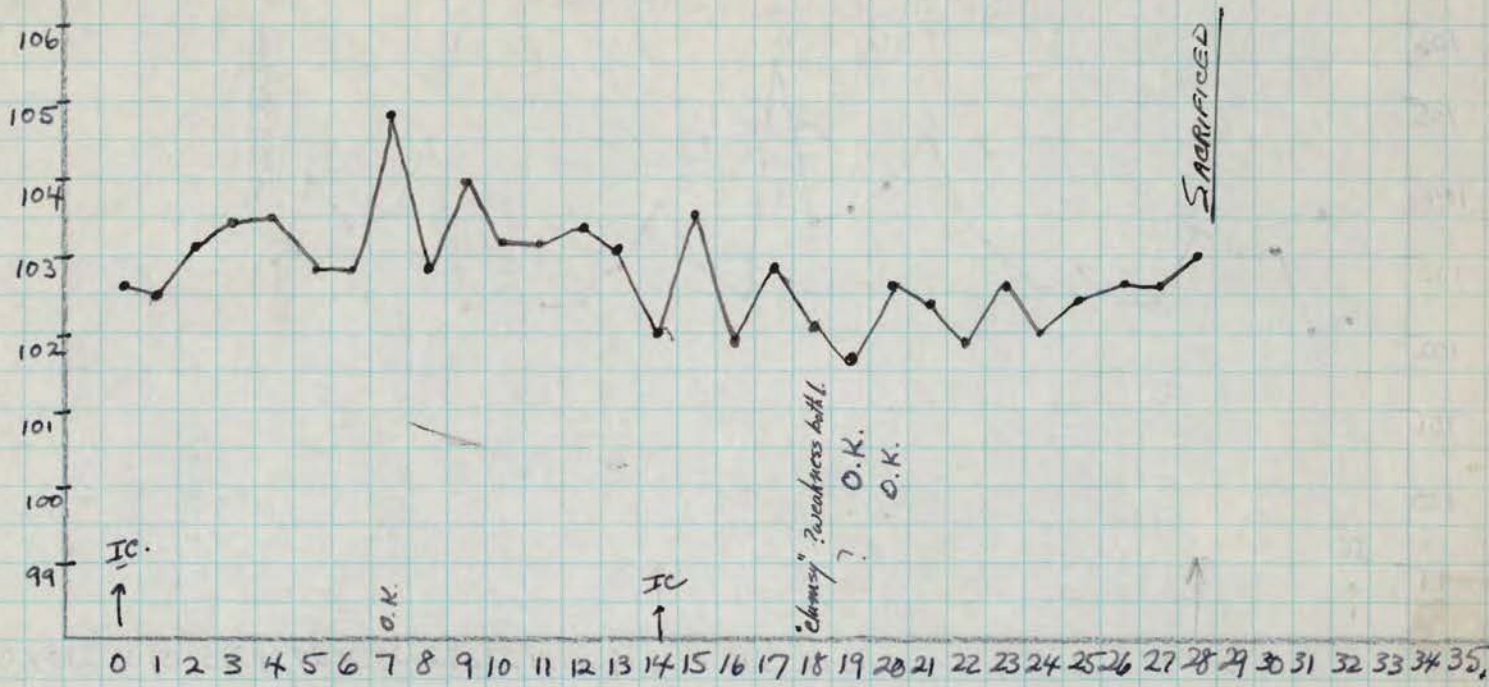
TISSUE FOR PATHOLOGY: olfactory bulbs; Thalamus; Hypothalamus; midbrain, pons, medulla + spinal cord (2-A)

Histology - Polio "old" - N.P.

FEB 17 1948

Generation II

Rhesus #131 - normal.



- 2-17-48: Inoc. IC. with 1.0 cc of 20% in saline of [redacted] virus - gen T.
- 3-2-48: Re-inoc. IC with 1.0 cc of 20% in saline [redacted] virus - gen T
- 3-7-48: O.K.
- 3-16-48: No clinical symptomatology.

Sacrificed (chloroform - exsanguination) Autopsy: no gross obs.
TISSUE FOR SECTION: Cervical, thoracic, and lumbar cord, and medulla (2-A.)

- TISSUE FOR VIRUS: Remainder of cord-medulla, and the basal nuclei pooled with those from Rh #1261 to make 2 separate 20% suspensions in sterile saline as follows:
- 1.) Basal nuclei of Rh #131 and Rh #1261 pooled to make a 20% susp. in (38 cc.) sterile saline. Wt. of the pooled basal nuclei = 9.1 gms. ± 32 cc frozen in 3-20 cc aups (#1, 2, 43) and stored. (B-4-6)
Culture: ✓✓✓
 - 2.) Cord-medulla of Rh #131 + Rh #1261 pooled to make a 20% susp. in (40 cc.) sterile saline. Wt. of pooled cord-medullae = 9.7 gms. ± 33 cc. frozen in 3-20 cc aups and stored. Culture: ✓✓✓ (B-4-6)

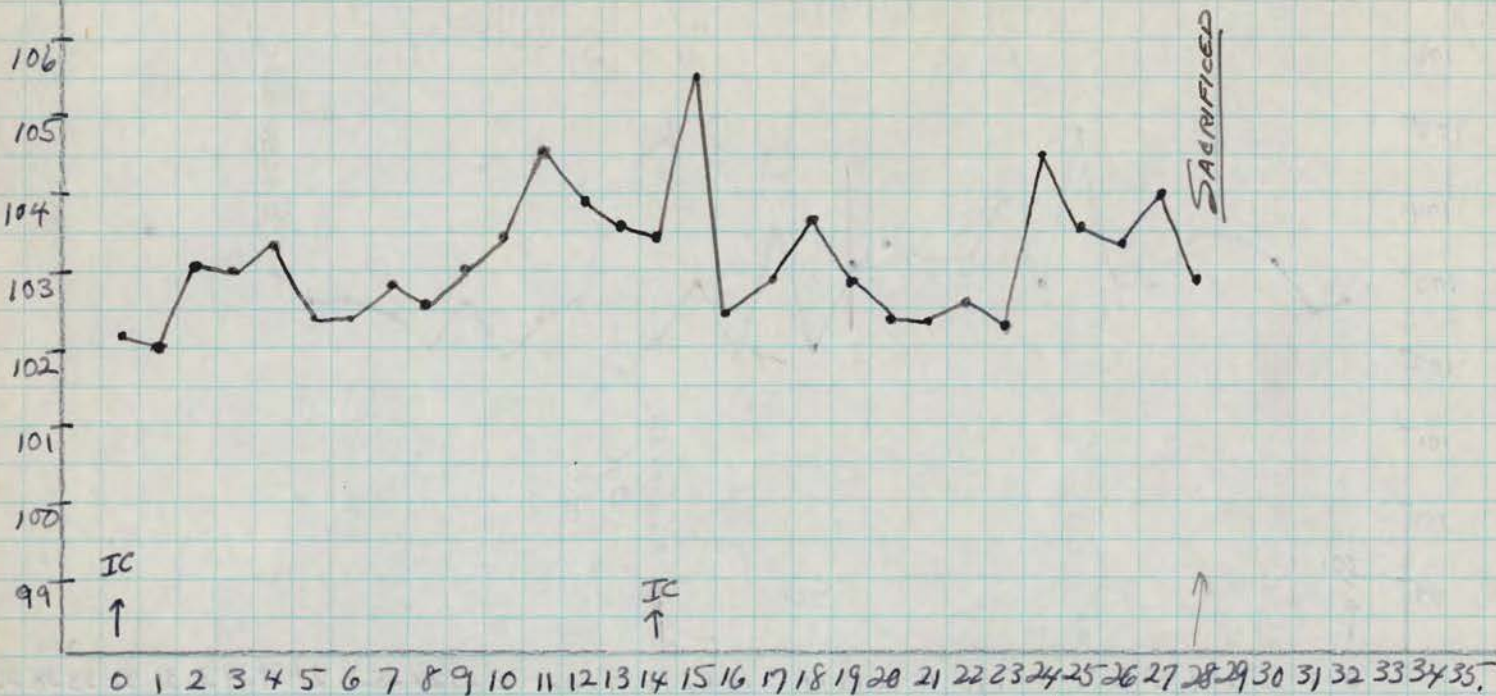
Sacrificed at 28 days in attempt to get virus and histology confirmation

Histology - Non-Paralytic Polio (clumped red neurons)

FEB 17 1948

STRAIN - Generation II

Rhesus #1261 - splenectomized.



2-17-48 - Once IC with 1.0 cc of 20% in saline of virus - Gen I.

3-2-48 - Re-inoc IC with 1.0 cc of 20% in saline of virus - Gen I

3-16-48: No clinical symptomatology.

Sacrificed (chloroform - exsanguination) Autopsies: No gross tbc.

TISSUE FOR SECTIONS: Lumbar, thoracic, cervical, medulla segments (2-A.)

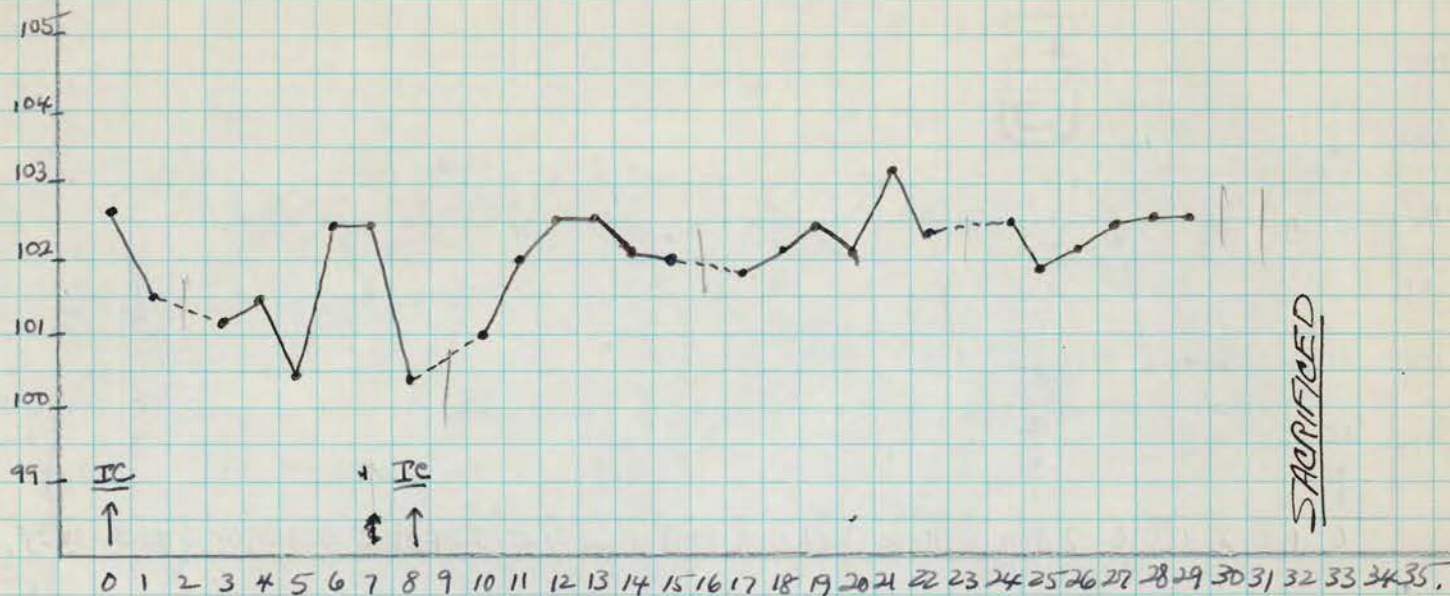
TISSUE FOR VIRUS: Remainder of cord-medulla, and the basal nuclei pooled = Rt #131 to make 2 separate 20% susp. (see overleaf).

Histology - Polio - recent and "old"

APR 30 1948

STRAIN - Generation III

Rhesus # 312



4-30-48 - Material harvested from Gen II of [redacted] (Rh. 131 and Rh. 1261) was suspended in saline as a pool of cords and medullas; and another pool made of basal nuclei of Rh. 131 and Rh. 1261. (g.v.). - These suspensions thawed; 1.0 cc. of basal nuclei pool injected IC (left side); and 1.0 cc of cord-medulla pool injected IC (right side). - Inocula for Rh. 313 (overleaf) given in same manner.

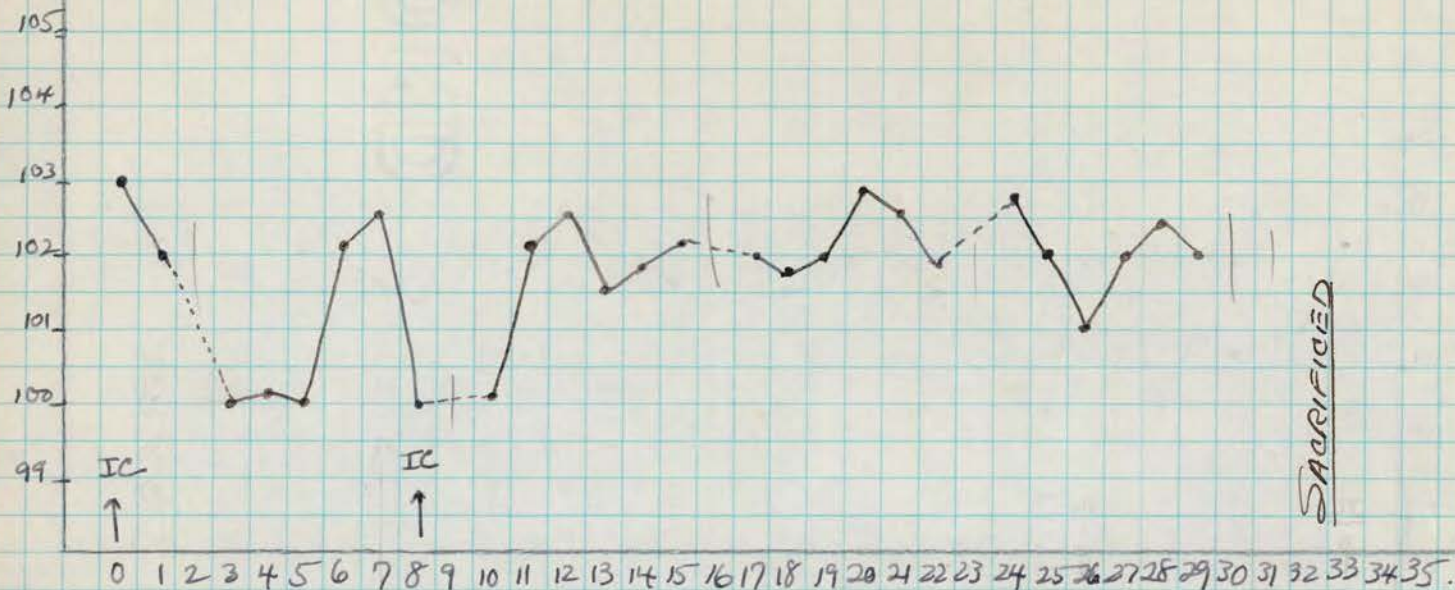
5-8-48 - Identical material and procedure done on 4-30-48 repeated IC.

6-1-48: SACRIFICED (chloroform-exsanguination) Autopsy; No gross the. TISSUE FOR SECTION - Thalamus, midbrain, medulla. single sections of cervical, thoracic, lumbar cord (2A)

APR 30 1948

STRAIN - Generation III

Rhesus # 313



4-30-48. Inoculated bilaterally IC (see Rh. 312 overleaf) for details.

5-8-48 - Identical material and procedure done on 4-30-48 IC.

6-1-48; SACRIFICED, (chloroform-exsanguination)

TISSUE FOR SECTION - Thalamus, midbrain, medulla, single sections of cervical, thoracic, and lumbar cord. (Z-A)

Summer Live Blood

POLIO - 1947. MONKEY HISTOLOGY.

MK.#. Lumb. Cerv. Medulla. Midbrain. Thalamus. Hypothal. Cerebell. Diagnostics

PRIMARY ISOLATION.

MK.#.	Lumb. Cerv. Medulla. Midbrain. Thalamus. Hypothal. Cerebell.	Diagnosis
Rh. 1666 (ABS)	NH; PT	POLIO - Non N.P.
Rh. 1077 (ABS)	NH; P.H.	POLIO - Non N.P.
Rh. 131	NH; P.H. P+	POLIO - N.P. v. infra Polio - N.P. chiefly old
Rh. 1261	sl. sl. ?	POLIO - N.P. v. infra Polio - Recent and old
Rh. 312	sl. sl.	No Polio
Rh. 313	sl. sl.	No Polio

"GENERATION II"

Neuronal loss = round cell replacement. - RD.

NT (acute) RD. Glial fous.

NH (acute) also glial replacement. P+

"GENERATION III"

Round cell fous.

Examined:

(ABS) 8-11-48.

Lumbar Cord: outfall of neurons - medial aspect of 1 ant. horn, with marked deg. change ant. roots of corresp. side. - Area in which many neurons are missing is free of infiltration. Small focus of interstitial infiltr. in lateral aspect of same ant. horn. - There is sparse glial cell infiltration in the area of neuronal loss.

Thoracic Cord: interstitial infiltr. on both sides assoc. with degenerative changes anterior roots. (no cross section available - There is a longitudinal section which is probably cervical cord, This shows interstitial glial infiltr. in grey matter which is diffuse rather than localized in nodules. Lesion strikes one as being old, and slight root changes are present.)

Cervical Cord: ext. glial infiltr. associated with PV infiltr. in lateral portion of the reticular substance (unilateral) - on the same side there are many foci of interstitial glial infiltr. and PV. infiltration - in the nuclei of floor of 4th ventricle. - No present neurophagia.

Medulla: definite PV infiltration; small focus of interstitial glial infiltration.

Hypothalamus: definite PV infiltration; small focus of interstitial glial infiltration.

(over)

