Aug 4, 1954

Attempt to Propagate Poliomyelites Virus in Chick Embryo Tissue Culture

Purpose - 1.) To determine the extinction curve of poliomyelites virus at 36°c.

2.) To determine if poliomyelites virus ("avirulent" strains produced by rapid passage in homologus kidney tissue culture) will propagate in chick embryo tissue culture.

Procedure - 1.) Chick embryo - 7 day Ancona eggs were obtained from Mt Healthy hatchery. Embryos were harvested aseptically. The heads were discarded and the bodies washed 6x in Hanks ($\frac{1}{3}$ $NaHCO_3$) solution. The tissue was minced and then washed 6x in Hanks $\frac{1}{3}$ solution

2) Preparation of tubes - After placing thin film of chick plasma on $\frac{1}{2}$ of the inner surface of culture tube 12-15 tissue fragments suspended in an equal volume of 1:9 chick embryo extract were placed on the same area. After tissue fragments were securely attached by the plasma clot 2 ml of medium was added.

Series A - 2 ml of Lactalbumin hydrolysate medium

Series B - 2 ml of Lactalbumin hydrolysate medium containing 5% normal horse serum

Materials

1.) 7 day chick embryos - Ancona strain Mt Healthy

2) Chicken Plasma Lot 9 8-3-54
- filtered -

3) Lactalbumin hydrolysate medium - Lot IX 7-10-54
Earles solution Lot VIII 7-16-54 diluted 8-2-54
Simms Lot IX 7-13-54

4) Horse serum - Control # 1462
Microbiological Associates

5) Hanks solution (1/3 $NaHCO_3$)
Lot VI 6-21-54 diluted 7-26-54

6) Chick embryo extract 9-18-53 diluted 1:9
8-4-54

7) 100 units Penicillin 0.1 mg Streptomycin / ml of media

Exp 1 Series A **Check Embryo Tissue Culture** 8-4-54

Lact albumin Hydrolysate Medium

Tube	1	2	3	4	5 (Day)	6	7	9	11
1	Early fibroblastic growth + occ mono.	Mod fibro	Mod fibro		Early fibro degen. Early clot lysis	Mod. fibro degen pH7.2 Clot lysis - patchy	Massive degen of fibro -	Degen. complete	No change pH7.0
2	" "	Early fibro	Early - mod fibro Early clot lysis		Mod fibro degen. Early clot lysis	No change in fibro - Epith sheet??	No change Epith sheet	Fibro degen complete - Early periph Epith degen.	Epith. in good condition No evid. of degen. pH7.2
3	" "	Mod fibro	Mod - good fibro		Mod - good fibro Early clot lysis	Epith sheet - large Early peripheral fibro degen -	Mod fibro degen Slight peripheral epith degen	Massive fibro degen	No change pH7.2
4	" "	Mod fibro	Mod fibro		Early - mod fibro degen Early clot lysis	Marked fibro degen - early clot lysis pH7.2	Large epith sheet Degen fibro almost complete	Complete fibro degen No change in epith	Epith good condition Early peripheral degen. or adsorb cells?? pH7.1
5	" "	" "	" "		Patchy fibro degen Early clot lysis	Early peripheral fibro degen clot lysis	Mod peripheral fibro degen.	Degen. complete	No change pH7.2
6	" "	" "	" "		Patch fibro degen Early clot lysis	Mild - mod peripheral fibro degen - clot lysis	No change - new fibro growth pH7.2	Mod - severe fibro degen	Deg. complete pH7.0
7	" "	" "	" "		Early - Mod fibro degen	Almost complete fibro degen.	No change		No change pH7.4
8	" "	good fibro	good fibro pH7.2		Mold	Mold			
9	" "	Mod fibro	Mod fibro		Early fibro degen Early clot lysis	Early - mod peripheral fibro degen. Patchy clot lysis pH7.2	No change	Massive fibro degen - small epith sheet	Epith degen pH7.0
10	" "	" "	Mod fibro		Large clot lysis Patchy fibro degen	Early - mod peripheral fibro degen - clot lysis	Marked fibro degen.	No change	No change pH7.2
11	" "	" "	" "	No change	Early fibro degen Early clot lysis	No change	No change	Extensive fibro degen.	No change pH7.2
12	" "	Mod fibro Mod mononuclear	" " (Epith ?)		Early fibro degen Early clot lysis Small Epith sheet?	Epith sheet Early - mod fibro degen.	No change Epith good condition	Fibro degen. complete	Epith good condition except for peripheral degen?? pH7.1
13	" "	Mod fibro	Mod fibro		No change	pH7.2 good growth Patchy peripheral fibro degen	Mild peripheral fibro degen	Mod. fibro degen.	Extensive fibro degen. pH6.9
14	" "	" "	" "		good fibro growth	Epith sheet? Early peripheral fibro degen -	Early - mod peripheral degen pH7.2	Marked fibro degener	Epith - unchanged pH7.0
15	" "	" "	" "		Mod fibro degen Early clot lysis	pH7.2 No change	Massive fibro degeneration	No change	No change pH7.0
16	" "	" "	" "		Large clot lysis good fibro growth Early fibro degen	Mod. peripheral fibro degen - clot lysis	No change	Degen complete pH7.2	No change pH7.0
17	" + many mononuclears	" "	" "		Early fibro degen	Early - mod peripheral fibro degen - clot lysis pH7.2	Massive destruct of fibroblasts	No change	No change pH7.0
18	Early fibroblastic	" "	" "		Large clot lysis	clot lysis - mod fibro degen -	Massive degen of fibro	Unchang.	No change pH7.3
19	No growth	Early - mod fibro	Early - mod fibro 2 fragments		No change	Early fibro degen Large epith sheet	No change	No change	Periph. degen of epith?! pH7.3
20	Early fibroblastic + occ mono	" "	Mod - good fibro		good fibro growth Patchy fibro degen	Mod peripheral fibro degen - small epith sheet - clot lysis	No change	Epith OK	Massive fibro degen. Epith OK pH7.2

Series B

Chick Embryo Tissue Culture

Lactalbumin Hydrolysate Medium with 5% Normal Horse Serum

0.1 mg Trypsin Incubator

Tube	1	2	3	4	5	6	7	9	11
					Day				
1	Minimal fibro growth	Mod fibro growth	Mod-good fibro ↓		Mod-good fibro Epith sheet early clot lysis	Patchy mod. fibro degen. assoc ± clot lysis	No change	almost complete fibro degener.	Complete degen. pH 7.2
2	Early fibroblastic growth + occ mono	" "	" "		Large clot lysis	Mod. fibro degen assoc ± clot lysis	Marked degen. Fibro degen complete	Degen. complete	No change pH 7.2
3	" "	" "	" "		Mod-good fibro Mild fibro degen Early epith - 3 sheets	Mod. fibro degeneration	2 Epith unchang! 1 Epith peripheral degeneration	2 Epith unchang	No change pH 7.2
4	" "	good fibro	good fibro ✓		Mod fibro degen	Degen almost complete pH 7.2 clot lysis -	No change	No change	Deg. complete pH 7.0
5	" "	" "	" " ✓		Massive fibro degen 3 small sheets epith	Slight epith degen. in pH 7.2 2 fragments	Epith unchanged	No change	Epith. degen only at periphery pH 7.0
6	" "	" "	" " ✓ Mod mono		Massive degen fibro pH 7.2	1 Epith sheet good condition	No change	Epith degener.	Epith degen complete pH 7.0
7	" " in 2 fragments only	Mod fibro	Mod fibro 2 fragments only		" "	Extensive fibro degen.	No change	No change	Small epith sheet Fibro degen complete pH 7.0
8	Early fibroblastic growth + occ mono	Early-mod fibro	Early-Mod fibro		Mod fibro growth Early epith ?	No change 2 sheets epith ?!	No change Epith	Mod. periph. fibro degener.	Complete fibro degen - Mod epith degen - pH 7.2
9	" "	Good fibro many mono	good fibro ✓		Mod fibro degen Many areas of growth	Mod. peripheral fibro degen. pH 7.2 Early clot lysis	No change	No change	Deg complete pH 7.0
10	" "	Mod-good fibro occ mono	Mod fibro		Mild left fibro degen small epith sheet	Patchy peripheral fibro degen. Small clot lysis	Marked fibro degen - assoc ± clot lysis	Complete degener	No change pH 7.1
11	" "	Mod fibro (Early epith ?)	good fibro ✓ Early epith ?	No change	Marked fibro degen	Small sheet epith - Degen. complete	No change	No change	Epith OK except for mild peripheral degen. pH 7.2
12	" "	Mod fibro	Mod fibro Large clot lysis		Mod-good fibro growth large clot lysis	No change	Mild peripheral fibro degen. 1 Small epith sheet	Marked fibrodegen.	Deg complete pH 7.2
13	" "	Good fibro	good fibro ✓		Large clot lysis Mod fibro degen	No change	Deg. complete	Unchang	No change pH 7.2
14	" "	good fibro pH 7.2	" " ✓		Massive fibro degen clot lysis - epith ?	Degen. complete clot lysis pH 7.2 no epith seen	No change	No change	No change pH 7.0
15	" "	Mod. - good fibro pH 7.2	Mod - good fibro		Marked fibro degen	Almost complete destruct. fibro pH 7.2	Early epith sheet	No change	Fibrodegen complete pH 7.0 Epith OK
16	" "	Mod fibro small epith sheet	good fibro ✓ Epith ?		Early fibro degen Epith sheet - small Early clot lysis	Mod. patch fibro degen - clot lysis - Small epith	Peripheral degen of epith ?	Marked degener. epith + fibro	No change pH 7.1
17	" "	Mod fibro	Mod fibro		Mod fibro degen	Deg. almost complete	No change	No change	Deg complete pH 7.2
18	" "	" "	" "		" "	Extensive fibro degen - Clot lysis	No change	Fibro degen complete	No change pH 7.2
19	" "	good fibro	good fibro ✓		Massive fibro degen	Degen almost complete	1 Small epith sheet	Epith - early degener.	No change pH 7.2
20	Minimal fibro growth	Minimal fibro	Poor fibro		Patchy fibro degen + fibro growth	Mod fibro degeneration	No change	- No change -	No change pH 7.3

Chick Embryo Tissue Culture Exp I

Aug 7, 1954

growth in tubes containing added normal horse serum (5%) is generally better than in the tubes containing lactalbumin hydrolysate medium only. Therefore the former types of tubes were selected for the attempt to demonstrate growth of poliomyelitis virus in chick embryo tissue culture.

0.1 mg of soy bean trypsin inhibitor was added to each tube of the lactalbumin hydrolysate with 5% normal horse serum series (20 tubes) to prevent plasma clot lysis during the experiment to follow.

Aug 9, 1954 14 of 20 tubes in series B (with added horse serum) show moderate to marked fibroblastic degeneration.

Only 4 of 20 tubes in series A (no added horse serum) show the same amount of degeneration - Clot lysis is more advanced in the series A (no trypsin inhibitor added to the series)

Aug 10, 1954 Fibroblastic degeneration in series A is much more extensive to-day 11 of 19 tubes showing moderate loss of fibroblasts - In many instances plasma clot lysis appears to be related to the degeneration of fibroblasts. It is entirely possible that the failure to apply a liberal amount of chicken plasma during the preparation of the tubes (plasma applied as in the preparation of cup pedrey tissue culture = small amount of plasma on a limited circumference of the inner tube surface) resulted in a thin fragile plasma clot, the eventual degeneration of which resulted in the observed fibroblastic degeneration?

11 Aug 54 Chick Embryo Tissue Cult. Exp 2

Purpose - To study the growth of chick embryo tissue in roller tubes in preparation for experiments involving an attempt at propagation of polio virus in such tissues -

The 10th attempt at growing chick embryo tissue in roller tubes resulted in a dramatic degeneration of fibroblastic tissue on the 5th - 7th day of cultivation - one possible reason for this phenomenon may have been an inadequate plasma clot. Therefore in this experiment a liberal amount of chicken plasma was applied to the entire inner surface of each tube and trypsin inhibitor was added to the medium.

Procedure - The tubes were prepared in the same manner as described for the exp. of Aug 4, 1954 with the following exceptions.

1. A liberal amount of chicken plasma was applied to the entire inner surface of each tube
2. 0.05 mg of soy bean trypsin inhibitor was added to each ml of medium.

2 series were prepared

Series A - 15 tubes - 2 ml of lactalbumen hydrolysate medium

Series B 15 tubes 2 ml of lactalbumen hydrolysate medium with 5% heated normal horse serum -

Materials

1.) 7 day chick embryos - Ancona strain Mt Healthy

2.) Chicken plasma Lot X 8-5-54

3) Hanks solution (1/3 $NaHCO_3$) 7-24-54 diluted 8-9-54

4) Horse serum - Control No # 1462 Microbiological Assoc. Heated 56°C 1/2 hour

5) Chick Embryo Extract 7-31-54 diluted 8-11-54

6) Soy Bean Trypsin Inhibitor 4-8-54 Worthington Biochem.

7) Lactalbumin Hydrolysate Medium

Earles solution Lot VIII diluted 8-11-54
Simms solution Lot IX 7-31-54
5% Lactalbumin Hydrolysate Lot X 8-3-54

100 units Penicillin 0.1 mg Streptomycin / ml of media

Exp 2 — Series A – Check Embryo Tissue Culture – Lactalbumin Hydr. Lyost Med. — 8-11-54

Day

Tube	1	2	3	4	5	6	7	8	9	11
1	Majority of fragments died not adhere 5 fragments – no growth	Early fibro growth 3 frag.	Early – mod fibro – 3 frag.	Mod growth fibro	pH 7.3 good fibro ✓	Fibro in good condition	pH 7.5 No change	2 frag excellent fibro	No change pH 7.3	pH 7.3 Excell. fibro
2	All fragments on – early fibro growth in 3 instances	Early-mod fibro growth	Mod. fibro	good fibro growth	Mod. fibro degen ✓ pH 7.4	Marked fibro degen. – however many areas of good fibro present	pH 7.4 No change	No change	pH 7.2 good fibro – Patchy perigh degen –	pH 7.2 Good fibro – very mild perigh degen –
3	Most fragments off – 4 fragments on – fibro growth	1 Frag early epith + 1 Frag early fibro	Mod. epith	good fibro – many round cells in fibro sheets	Perigh. fibro degen ✓ pH 7.3	Marked degen Epith Mod degen. fibro	New fibro growth pH 7.3	No change	No change New fibro scanty pH 7.4	Mod fibro pH 7.4
4	All frag. on early fibro growth	Mod fibro growth	Mod fibro sheet epith	Excellent fibro good epith early pro degen round cells	pH 7.2 Epith OK Beg. fibro perigh degen. ✓	No change	pH 7.3 No change	No change	Mod progressive fibro degen – many round cells pH 7.2	Complete fibro degen. pH 7.2 Epith OK
5	4 frag. on slot No growth	Early fibro 1 frag	Early – mod fibro 1 frag Epith – 1 frag	Mod fibro good epith	No change ✓ pH 7.3	No change	Mod fibro degen pH 7.6	No change Epith OK	New fibro growth pH = 7.5	Mod fibro pH 7.5 pH 6.9
6	All frag. on early fibro growth	Mod fibro growth	No change	good fibro growth many round cells in fibro sheets –	No change – pH 7.2	Excellent fibro Very early perigh fibro degen – Epith	No change pH 7.0	Mod fibro degen.	Complete fibro degen – epith degen pH 7.0	3 good epith sheets –
7	All frag. on Some early growth –	Early fibro growth	No change	Mod. growth 4 frag.	No change ✓ pH 7.4	Mod. fibro degen. 2 frag. New fibro appear.	No change pH 7.5	New fibro appear –	Mod new fibro growth pH 7.4	pH 7.3 good fibro – pH 7.4
8	5 frag. on no growth	Early fibro growth	Early – mod fibro? Epith?	No change	Fibro degen ✓ marked pH 7.6 Epith OK	No change	pH 7.6 No change	No change	No change pH 7.4	Near epith No change
9	7 frag. on no growth	Early fibro growth	Early – mod fibro –	Mod fibro growth	Mod – good fibro – pH 7.5 early fibro degen	Mod. fibro degen 2 Epith sheets	pH 7.2 Marked fibro degen Epith – OK	No change	Fibro degen almost complete pH 7.2	Epith OK pH 7.1
10	9 frag. on no growth	1 Epith sheet 1 frag of fibro growth + early	No change	Epith – good growth Fibro – early to mod –	pH 7.3 Fibro – mod	Epith – good Fibro mod	No change pH 7.3	No change	Peripheral epith degen – pH 7.2 Fibro – beg. degen 1 frag pH 7.3	Epith unchanged pH 7.2 Fibro degen complete pH 7.3 – small area new fibro
11	5 frag on early growth	2 Frag – early – mod fibro	2 Frag – mod fibro growth	1 Frag unchanged 1 Frag – good growth	pH 7.4 No change –	Mod. fibro degen.	No change pH 7.4	No change	Fibro degen almost complete – small area new fibro	
12	7 frag. on early fibro growth	Early – mod fibro growth	Mod. fibro growth	Mod fibro	good fibro + pH 7.3	Mod fibro degen 2 fragments	No change 2 frag – good fibro pH 7.2	Mod. degen all frag but one	Marked degen (pH 7.1 all frag but one – mild degen pH 7.1 No change	No change pH 7.1
13	12 frag on early fibro growth	Early – mod fibro growth	Mod fibro	good fibro growth Beg. fibro degen –	pH 7.3 Beg perigh fibro degen	Marked fibro degen – 2 Epith sheets – OK	almost complete fibro degen pH 7.2 Very mild perigh epith degen	No change Epith uncharged		Epith OK pH 7.1 Fibro – complete degen
14	Only 1 frag. on no growth	No growth	Early epith (1 sheet) No growth	No growth	pH 7.3 Discard					
15	All frag off									

Remarks

Fluids of tubes 1, 2, 3, 4, 5, 7, & 8 changed – pH in other tubes → 7.6

Degeneration same as described for series B

Exp 2 — Series B - Chick Embryo Tissue Culture — Lactalbumin Hydrolysate Med. + 5% N. — Horse Serum

Day

Tube	1	2	3	4	5	6	7	8	9	11
1	13 frag on early growth in some	Mod epith growth 3 frag. Minimal fibro growth	Good epith growth Early fibro	Mod fibro Excellent epith	pH 7.2 Good fibro many round cells ✓	Good epith - 4 frag Beg. fibro degen	Mod fibro degen pH=7.3 Mild periph. epith degen - 1 frag	No change	Epith - OK Good fibro with mild degen pH 7.2	Excellent epith Good fibro - mild degen. pH 7.1
2	3 frag on no growth	Early growth 1 Frag.	Early fibro 2 frag	Early - mod fibro - 1 frag	pH 7.2 Mod. fibro ✓	Beg. fibro degen	Mod fibro degen. pH 7.4	No change	New fibro growth - stringy like pH=7.4	Mod fibro - fat vacuoles pH 7.3
3	7 frag on minimal early growth	Early - mod fibro growth	No change	Mod - good fibro	Good fibro (pH 7.2) Beg. peripheral fibro degen? many round cells ✓	Beg. periph fibro degen	2 Sheets Epith Mild patchy fibro degen pH 7.3	No change Epith OK	Good fibro growth Mild periph. degen pH 7.2	Good fibro Epith OK pH 7.1
4	12 frag on early growth	Mod. fibro growth	No change - Early epith?	Excellent fibro -	pH 7.0 many round cells in fibro sheets ⊖	Beg. fibro degen - periph	Mod. fibro degen pH 6.9	Marked fibro degen.	pH 6.9 Complete fibro degen.	pH 6.8 No change
5	4 frag. on minimal early growth -	Epith - 1 frag.	Epith - good growth 1 Frag - early fibro	Epith - good growth Fibro - early to mod	pH 7.2 Epith good fibro - not present ⊖	No change	pH 7.2 No change	No change Epith OK	pH 7.2 No change	pH 7.2 No change
6	1 frag. on no growth	No growth		No growth	No growth discard					
7	all frag. on early growth	Mod fibro growth	Early epith Mod. fibro	Good fibro	pH 7.2 Beg peripheral fibro degen. many round cells ⊖	Mod periph. fibro degen	pH 7.0 No change	Mod - marked fibro degen - Epith OK	No change pH=6.9	pH 6.9 Marked fibro degen -
8	2 Frag. on no growth	Early fibro growth - 1 frag	No change	Mod. fibro 1 frag	pH 7.2 - beg fibro degen periph ?? round cells - ✓	Mod. fibro degen -	pH 7.4 No change	Early new fibro growth	New fibro growth pH 7.3	Fibro - good pH 7.3 fat vacuoles -
9	all frag. on early growth	Early - mod fibro growth 2 epith sheet	No change	Fibro - good growth	Good fibro pH 7.1 Early peripheral fibro degen? Round cells ⊖	Mod. periph. fibro. degen.	No change pH 7.0	No change -	Epith degen 1 frag. 2 frag - fair fibro pH 6.9	pH 6.9 Epith OK - 1 sheet 2 new sheets - fibro degen complete
10	8 frag on early growth	Mod. fibro growth	Epith sheet Mod fibro	Good fibro 2 Epith sheets	Beg. periph. fibro degen? pH 7.2 ✓	Mod fibro degen -	No change pH 7.2	No change	Good fibro with patchy mild degen. pH 7.1 areas of new fibro	Fibro good except (pH 7.1) for 1 frag c̄ mod degen
11	2 Frag on no growth	No growth	1 Frag - growth - vacuolated - ?	No change	No change discard					
12	12 frag on no growth	Early fibro growth several frag. Epith ?	Large epith sheet Early fibro 3 Frag.	Mod fibro 2 frag	pH 7.2 - no change ⊖	Beg. periph. fibro degen	pH 7.1 Marked fibro degen. Mild periph epith degen	No change	Fibro degen almost complete pH 7.0	Deg. complete Epith OK pH 7.0
13	all frag off									
14	1 frag on no growth	No growth	Early fibro	No change	No progression - discard					
15	1 frag on not growth	Minimal fibro growth	No change	No change	Early fibro growth Discard					

Remarks

Fluids of tubes 1, 2, 3, 8, 10 changed pH → 7.6

Degeneration occurs commonly for an entire sheet of fibroblasts at one time. The sheet takes on a "coagulation necrosis" like appearance with rounding of cells → disintegration.

In other instances peripheral cells round up and degenerate - the process extends inward from the periphery.

16 Aug 54 Beginning degeneration of fibroblasts evident to-day (5th day of incubation) -

Fluid withdrawn from selected tubes and replaced with equal volume of new medium containing the same constituents as that of the original medium. An attempt was made to replace fluid from one half of the tubes of each series in such a way that unchanged and changed tubes would present the same spectrum of growth patterns and fibroblastic degeneration. In this way the effect of replacing the fluid medium at 5 days on the degeneration of fibroblastic tissue could be evaluated.

Tubes - fluid medium replaced

CE - Lactalb. Medium Only	CE-H Lactalb. Medium with 5% Heated Normal Horse Serum
1, 2, 3, 4, 5, 7, 8	1, 2, 3, 8, 10

The degeneration seen in tubes prepared with a liberal amount of chicken plasma eliminates the explanation that an inadequate plasma clot and plasma clot retraction & lysis were responsible for the observed degeneration of chick embryo tissue fibroblastic outgrowth.

23 Aug 54 Effect of replacing fluid on degeneration of chick embryo fibroblasts in roller tube tissue cultures.

Fluid Replaced 5th day	No of tubes showing degeneration 5th day of incubation Series A	Series B	No of tubes showing degeneration 11th day Series A	Series B
	4/7	3/5	3/7 (mild-Marked)	2/5 (mild-mod)
Fluid Not Changed	2/6	2/4	6/6 (Marked)	4/4 (Marked)

Series A: Lactalb. Hydrolysate Series B: Lactalb. Hydrolysate + 5% N. Horse Serum

Conclusion - Change of fluid at the 5th day appears to have resulted in a decreased incidence of chick embryo fibroblast degeneration - Since degeneration was already

in about one half of the tubes
present at the 5th day the ~~effect of~~ fluid
change at that time could not be expected
to exert its maximal effect in preventing degeneration.
Degeneration when it was observed in tubes which
had a fluid change at 5 days was of a lesser
degree than that observed in the tubes in which
fluid was not changed. The effect of fluid
change earlier than the 5th day should be tested!

17 Aug 54

Chick Embryo Tissue Cult. Exp 3

Purpose - 1.) To the effect of a large amount of horse serum (40%) in the medium upon the gowth and "stebulety" of chick embryo fibroblasts grown in roller tube tissue culture -

2) To determine th effect of periodic changes of fluid medium (every 3 days) upon the stabulety of chick embryo fibroblasts -

Procedure Th tubes were prepared in th same manner as described fr the experiments of 11 Aug 54. A liberal amount of chicken plasma was applied to the entire inner surface of the tube - 12-15 fragments were then placed in each tube.

2 series were prepared -

Series A - 2ml of Lactalbumin hydrolysate medium containing 5% normal heated horse serum and 0.05 mg of trypsin soybean inhibitor/ml (feral pH of medium = 8.2)

Series B - 2ml of 40% normal heated horse serum + 40% Earles solution + 20% chick embryo extract (1:20 in Hanks complete) and 0.05 mg of trypsin soybean inhibitor/ml. (feral pH of medium = 8.1)

Materials

1. 8 day chick embryos — Ancona strain Mt Healthy
2. Chicken plasma — 8-5-54 — Lot X
3. Hanks solution (1/3 usual amount $NaHCO_3$) — 7-24-54 — diluted 8-17-54
4. Horse serum — Control No 1462 — Microbiol. Assoc. — Heated 56°C 1/2 hour
5. Chick embryo extract — 7-31-54 — diluted 8-17-54
6. Soy bean trypsin inhibitor — 4-8-54 — Worthington Biochem. — Stock 4°C — S-5293
7. Lact albumin hydrolysate medium

   Earles solution — Lot VIII — diluted 8-17-54
   Simms solution — Lot IX — 7-31-54
   Lact albumin hydrolysate — Lot X — 8-3-54

   100 units penicillin — 0.1 mg streptomycin / ml
8. Earles solution Lot VIII diluted 8-17-54 used for preparation of medium in series B.

   100 units penicillin 0.1 mg streptomycin / ml in fluid ½ medium in series B.

Exp 3 — Chick Embryo Tissue Cult Series B — 40% Heated Normal Horse Serum, 40% Earles Sol., 20% Chick Embryo Extract (1:2)

Day

Tube	1	2	3	4	5	6	7
1	Early fibro 2 frag	Early fibro growth 2 frag	Mod-good (V) fibro - 4 frag	good fibro some vacuoles	No change	Good fibro some clot retract.	No change
2	2 frag or No growth	1 frag early fibro	Good Epith (−) Early fibro 2 frag	Mod fibro 2 frag	Many round cells - fibro OK	Mod fibro	Mod fibro
3	Early fibro growth	Early fibro	Mod fibro (stringy fibro) (−)	Mod fibro stringy	No change - plasma clot retracting in certain areas -	Epith. sheet small	Progressive clot lysis + retraction
4	5 frag or No growth	Very early fibro growth	Early fibro 1 frag (−) Mod fibro 1 frag	2 frag early-mod fibro 1 frag mod fibro	1 frag early fibro 1 frag mod fibro 1 frag good fibro Early fibro - 2 frag	Pounding up of fibro in frag good growth	No change - Fibro is good frag in good condition although rounded + fat vacuoles
5	No growth	No growth	Early - mod fibro 2 frags (V)	No change - isolated cells -	Plasma clot retraction - fibroblasts isolated from fragment	No change	1 patch - early - mod
6	Alk: No growth	Early fibro 2 frag	Alk discard				1 patch mod fibro
7	No growth	Early fibro growth	4 frags c early to mod fibro (V)	Mod fibro - stringy - many isolated cells	Mod - good fibro	good fibro -	No change
8	" "	No growth	Early stringy fibro (V)	Early - mod fibro stringy - many isolated single cells	Mod fibro plasma clot poor in several areas	Mod - good fibro plasma clot retract. in several areas	No change -
9	2 frag or No growth	No growth	Very early fibro 1 frag (V)	Early - mod fibro - detached from frag	No change	Early - mod fibro - stringy -	New fibro between frag and detach fibro
10	Early - mod fibro growth	Mod fibro growth	good fibro (−)	No change	No change	No change Many fat vacuoles	No change
11	No growth	No growth	Early - mod fibro 5 frag (−)	No change	Mod fibro 3 frag	No change	Two frag - fibro degen. assoc c plasma clot retraction Remaining fibro - mod - good
12	" "	Early fibro 1 frag	Early fibro (V)	No change	Early mod fibro - web like	Mod fibro	Mod - good fibro
13	" "	Early - mod fibro	Mod - good (V) fibro	good fibro stringy	No change	No change	good fibro many fat vacuoles in fibro
14	5 frag or No growth	1 frag - Epith	1 frag early fibro (−)	Early - mod fibro	No change	No change	Mod fibro
15	No growth	No growth	No growth Discard				

Remarks

Fibroblasts are darkly pigmented and some areas tend to have a stringy or web like instead of sheet arrangement

Many fatty vacuoles in fibroblast cells

Fluid changed Tubes 1, 5, 7, 8, 9, 12, 13

Exp 3 ● Series A. Check Embryo Tissue Cult ● Lactalbumen Hydrolysate Med + 5% Heated Normal Horse Serum ●

Day

Tube	1	2	3	4	5	6	7
1	Very early fibro growth	Early-mod fibro 1 Epith sheet	pH 7.3 Mod-good fibro ⊖	good fibro many roundcells	Early periph degen fibro + epith pH 7.0	pH 7.0 No change	Mod peripheral fibro degen pH 7.0 } Epith OK
2	7 Frag. on No growth	No growth	Early-mod fibro 1 frag pH 7.2 ⊖	Mod-good fibro	pH 7.2 No change	Good fibro pH 7.2	Beginning periph fibro degen pH 7
3	Early fibro growth 1-2 frags	3 frag - early to mod fibro	pH 7.3 5 frag Mod-good fibro ✓	good fibro -	No change pH 7.4	No change pH 7.2	No change pH 7.2
4	No growth	Early-mod growth 3 frag	Mod fibro pH 7.2 ⊖	good fibro	Early periph fibro degen pH 7.0 1 frag many roundcells	No change pH 7.0	pH 7.0 Early-mod periph fibro degen -
5	3 frag on No growth	No growth	No growth Discard				
6	Early fibro growth	Mod. fibro growth	good fibro pH 7.2 ✓	No change	No change pH 7.4	No change pH 7.2	No change pH 7.2
7	Early fibro growth	Mod fibro 2 Epith sheet	good fibro pH 7.3 ⊖	No change	No change pH 7.0	pH 7.0 Beg. fibro degen peripheral, surrounding cells	pH 7.0 Beg-mod fibro degen -
8	Early fibro growth 1 frag	Early-mod fibro 3 frag	Mod-good fibro pH 7.3 ✓	good fibro	No change pH 7.4	No change pH 7.4	No change pH 7.3
9	No growth	Early-mod fibro good Epith 1 frag	Early-Mod fibro ✓ 2 frag pH 7.4	No change	No change pH 7.4	pH 7.4 No Change	pH 7.2 Fat vacuoles Early-mod fibro
10	No growth	Mod fibro growth	good fibro ⊖ growth pH 7.2	No change	pH 7.0 No change	No change	Beg. periph fibro degen apparent clot reaction in some instances pH 7.0
11	No growth	Early fibro growth 3 frag	Mod fibro pH 7.2 ✓	Mod fibro 2 frag pH 7.5	No change pH 7.3	No change pH 7.2	No change pH 7.2
12	7 Frags or No growth	No growth	pH 7.2 Early-mod fibro 2 frag ✓	Early Mod fibro - beg. degen? pH 7.6	No change No degen pH 7.4	Epith sheet Early-mod fibro pH 7.4.	No change -
13	No growth	1 frag. early fibro growth	Early-mod fibro pH 7.4 1 frag ✓	Early fibro pH 7.6	Epith - pH 7.4 Early fibro - 3 frag	pH 7.4 No change	pH 7.4. No Change
14	5 frags or No growth	Early-mod fibro growth	pH 7.2 Mod fibro growth ⊖	Mod good fibro	No change pH 7.0	Early periph degen pH 7.0	pH 7.0 Early periph. fibro degen
15	No growth	Very early fibro 1 frag	pH 7.2 No change discard				

Remarks

Fluids changed
Tubes 3, 6, 8, 9, 11, 12, 13

24 Aug 54

Effect of changing tissue culture fluid and of composition of tissue culture fluid on degeneration of chick embryo fibroblasts in roller tube tissue culture

No of tubes showing degeneration 7th day

	Lact albumen Hydrolysate Medium + 5% N. Horse Serum	40% N. Horse Serum 40% Earles 20% CEE Medium
Fluid replaced 3rd day	0/7	0/7
Fluid not replaced	6/6	1?/7

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24 Aug 1954

Preliminary experiment to detect a mutant of Poliomyelitis virus capable of multiplication in chick embryo tissue

Procedure 1.) ~~12~~ 15 ml of Lactalbumin Hydrolysate medium (containing penicillin 100 units and streptomycin 0.1 mg /ml and 0.05 mg soy bean trypsin inhibitor /ml) + ~~3~~ 7.5 ml of undiluted tissue culture fluid containing Type I, Type II or Type III Poliomyelitis virus

2.) 3 ml of the final mixture added to 7 tubes - 3 tubes in series A, 3 tubes in series B, and 1 tube in series C.

Series A. Tube containing chick embryo tissue fragments embedded in a plasma clot film - tubes from chick embryo tissue cult exp. 3 of 17 Aug 54 used.

Series B Tube containing 20 minced 7-8 day chick embryo tissue fragments - no plasma clot -

Series C - no tissue in tube

The remaining 1 ml of the virus containing fluid placed in a rubber stoppered test tube and stored at -30°c for titration at a future date

3) Tubes placed on a roller drum air incubator and allowed to rotate for 35 days.

4) At the end of 35 days 1 ml of fluid aspirated from each tube to perform titration for poliomyelitis virus on cynomolgus kidney tissue culture. Remainder of aspirated fluid stored at -30°C.

Series A -	titrate	10^{0} -	10^{-3}
Series B	titrate	"	"
Series C	titrate	10^{0} -	10^{-1}
Original virus mixture	titrate	10^{4} -	10^{-7}

frozen

Note: Before being used the tubes of series A → fluid aspirated - tube filled with Hanks 1/3 solution - then aspirated - tubes then treated as described in paragraph 2.

Materials Tubes of series A - from Chick Exp 3 8-17-54

Type I virus	1.	Tube 1	40% Horse serum series
	2.	Tube 7	" " " "
	3.	Tube 3	Lactalbumin Hydrolysate +5% Horse Serum series
Type II virus	1.	Tube 8	40% Horse serum series
	2.	Tube 10	" " " "
	3.	Tube 6	Lactalbumin Hydrolysate +5% Horse Serum series
Type III virus	1.	Tube 12	40% Horse serum series
	2.	Tube 13	" " " "
	3.	Tube 8	Lactalbumin hydrolysate +5% Horse serum series

Series B

6 8day Ancona chick embryos harvested asepticallly - tissue minced to 1mm size with sterile scissors - tissue washed 6X in Hanks solution with 1/3 the usual amount of $NaHCO_3$. [The whole embryo with the exception of the eyes was used.] Twenty tissue fragments were placed in each tube of series B.

Lact albumin hydrolysate medium 8-24-54
pH 8.1
Soy bean Trypsin Inhibitor 4-8-54 Worthington Biochem
stored 4°C

Viruses

Mahoney KP 35 of 4-19-54
10^8 TCD50/ml Stored -30°C

YSK KP 54 of 5-1-54
10^8 TCD50/ml Stored -30°C

Leon KP 36 of 4-19-54
$10^{-7.5}$ TCD50/ml Stored -30°C

-29 Sept 54- 36 days in incubator

Type	Series	Tube	pH	Microscopic
I	A	1	6.9	Clot retraction - no viable outgrowth degen. original fragments + many degen. cells -
		2	6.9	no viable outgrowth - degen. oregenal frag. many degen. cells.
		3	6.8	" " "
	B	1	6.9	- 1 small frag stuck on glass - large outgrowth - cells appear degen -
		2	6.9-7.0	2 small frag. stuck on glass - degen. cells - no outgrowth
		3	6.9-7.0	
	C		7.6 or >	
II	A	1	6.9	clot retraction - no viable outgrowth - degen of original frag - degen cells -
		2	6.9	clot retraction - no viable outgrowth - degen of oregenal frag and large sheets of cells -
		3	6.9	clot retraction - no viable outgrowth - degen frag. & cells -
	B	1	6.8	several large frag stuck on glass - epith outgrowth - cells visible granular appearance
		2	6.9-7.0	
		3	6.8	1 frag on glass - degen cells
				5 " " " " "
	C		7.6 or >	
III	A	1	6.9	Small area of epith outgrowth - otherwise no viable outgrowth - degen cells -
		2	6.9	- No viable outgrowth - degen of outgrowth + cells -
		3	6.8	" " "
	B	1	6.8	
		2	6.8	
		3	6.9-7.0	
	C		7.6 or >	

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B&P "NOTEAR"

30 Sept 54

Titration of C E Tissue cultures after 37 days of incubation

Procedure Fluids ~~were~~ removed from each tube – ~~the~~ fluids from tubes in the same series ~~were~~ pooled – Then centrifuged at 2000 rpm for 10 minutes – Supernatent fluid titrated ~~on~~ test – Tenfold dilutions from 10^{-1} to 10^{-3} prepared with Hanks solution containing full amount of $NaHCO_3$ – 0.4ml inoculated per tube – 5 tubes for undelated and 2 for 10^{-1}, 10^{-2}, & 10^{-3}.

Materials

1.) Fluids from tissue culture tubes Chick Embryo Tissue Culture Exp 3.

2) Hanks Solution with full amount $NaHCO_3$

3.) Monkey kidney epithelium tissue culture tubes series 61) prepared 9-23-54

Series		Tube	2	3	4	6	8	10
I	A	10^{0} 1	3-4	4off				
		2	3-4	4off				
		3	3	4off				
		4	3	4off				
		5	2-3	4off				
		10^{-1} 6	0	3	4off			
		7	0	1-2	4off			
		10^{-2} 8	0	0	3	4off		
		9	0	0	0	0	4off	
		10^{-3} 10	0	0	0	4off		
		11	0	0	0	0	0	0
	B	10^{0} 12	3-4	4off				
		13	3-4	4off				
		14	1-3	4off				
		15	1-2	4off				
		16	2-3	4off				
		10^{-1} 17	0	0?	4			
		18	0	1	4			
		10^{-2} 19	0	0	0	4off		
		20	0	0	0	0	0	0
		10^{-3} 21	0	0	0	0	0	0
		22	0	0	0	0	0d	0d

Pooled 10-4-54

Pooled 10-4-54

30 Sept 54

Series		Tube	2	3	4	6	8	10
I	C	10^0	23	0	0	0	0	0	0
			24	0	0	0	0	0	0
			25	0	0	0	0	0	0
			26	0	0	0	0	0	0
			27	0	0	0	0	0	0
		10^{-1}	28	0	0	0	0	0	0
			29	0?	0	0	0	0d	0d
		10^{-2}	30	0	0	0	0	0d	0d
			31	0	0	0	0	0	0
		10^{-3}	32	0	0	0	0	0d	0d
			33	0	0	0	0	0	0
II	A	10^0	34	2	4off				
			35	2	4off				
			36	3	4off				
			37	3-4	4off				
			38	4	4off				
		10^{-1}	39	0	4	4off			
			40	1-3	4off				
		10^{-2}	41	0	0	0	0	1	4
			42	0	0	0	4		
		10^{-3}	43	0	0	0	0	0	0
			44	0	0	0	0	0	0
	B	10^0	45	3-4	4off				
			46	4	4off				
			47	1-2	4off				
			48	3-4	4off				
			49	2	4off				
		10^{-1}	50	0	4off				
			51	0	0-?	4			
		10^{-2}	52	0	0	0	0	0d	0d
			53	0	0	0	0	0	0
		10^{-3}	54	0	0	0	0	0	0
			55	0	0	0	0	0	0

Pooled 10-4-54

Pooled 10-4-54

30 Sept 54

Series		Tube	2	3	4	6	8	10
II C	10^{0}	56	0 Foamy Dig.?	0	0	0	0	0
		57	0	0	0	0	0	0
		58	0	0	0	0	0d	0
		59	0	0	0	0	0	0
		60	0	0	0	0	0	0
	10^{-1}	61	0	0	0	0	0	0
		62	0	0	0	0	0d	0d
	10^{-2}	63	0	0	0	0	0	0
		64	0	0	0	0	0d	0d
	10^{-3}	65	0	0	0	0	0d	0d
		66	0	0	0	0	0d	0d
III A	10^{0}	67	1	4*	4off Mold			
		68	1-2	4	4off			
		69	0-1	4	4off			
		70	0-?	4	4off			
		71	0-?	4	4off			
	10^{-1}	72	0	0	0-?	4	4off	
		73	0	0	4			
	10^{-2}	74	0	0	0	4	4off	
		75	0	0	0	0	4off	
	10^{-3}	76	0	0	0	0	0	0
		77	0	0	0	0	0	0
B	10^{0}	78	0-2	4	4off			
		79	0-2	3-4	4off			
		80	0-1	4	4off			
		81	0-2	4	4off			
		82	0-?	3-4	4off			
	10^{-1}	83	0-?	0-?	4			
		84	0	0	0	4	4off	
	10^{-2}	85	0	0	0	4	4off	
		86	0	0	0	0	4off	
	10^{-3}	87	0	0	0	0	0	0
		88	0	0	0	0	0	0d

Pooled 10-4-54

Pooled 10-4-54

30 Sept 54

Series			Tube	2	3	4	6	8	10
III	C	10^{0}	89	0	0	0	0	0	0 d
			90	0	0	0	0	0	0
			91	0	0	0	0	0	0
			92	0	0	0	0	0 d	0 d
			93	0	0	0	0	0	0
		10^{-1}	94	0	0	0	0	0	0
			95	0	0	0	0	0	0
		10^{-2}	96	0	0	0	0	0	0
			97	0	0	0	0	0	0
		10^{-3}	98	0	0	0	0	0	0
			99	0	0	0	0	0	0

CE.

Titers

	TCD_{50} per 0.4 ml	TCD_{50} per 1 ml
I plasma clot series	$10^{+3.0}$	$10^{+3.4}$
I suspended minced tissue series	$10^{+2.0}$	$10^{+2.4}$
II plasma clot series	$10^{+2.5}$	$10^{+2.9}$
II suspended minced tissue series	$10^{+1.5}$	$10^{+1.9}$
III plasma clot series	$10^{+2.5}$	$10^{+2.9}$
III suspended minced tissue series	$10^{+2.5}$	$10^{+2.9}$

Quantity of virus in tubes containing suspended minced 8 day chick embryo tissue, chick embryo tissue in plasma clot, and no tissue after 37 days at 36°C

Time after addition of polio. virus	Titer of virus in indicated tubes TCD50/ml								
	Type I			Type II			Type III		
	Suspended tissue series	Tissue in plasma clot series	No tissue	Suspended tissue series	Tissue in plasma clot series	No tissue	Suspended tissue series	Tissue in plasma clot series	No tissue
0 {calculated = amount added	$10^{7.5}$	$10^{7.5}$	$10^{7.5}$	$10^{7.5}$	$10^{7.5}$	$10^{7.5}$	$10^{7.0}$	$10^{7.0}$	$10^{7.0}$
37 days	$10^{2.4}$	$10^{3.4}$	$<10^{0.4}$	$10^{1.9}$	$10^{2.9}$	$<10^{0.4}$	$10^{2.9}$	$10^{2.9}$	$<10^{0.4}$

OCT 5 1954

Titration of CE, K, "CE Variant"

Procedure

On 10-4-54 fluid was removed from tubes in the titration (using cynomolgus kidney epith cultures) of CE, which showed 4+ degeneration. This fluid was centrifuged at 2000 rpm after the tubes of the same series were pooled. After 10 min. centrifugation the supernatant fluid was removed and stored in the deep freeze.

10-5-54 10 fold dilutions prepared in Hanks solution (with full amount of $NaHCO_3$). 0.2 ml of dilated material inoculated. 3 tubes per decimal dilution.

Materials 1.) Pooled fluids from titration in kidney epith
cultures of CE, = CE, K,

CE, K, Type I plasma clot series
" " " " minced tissue "

" " Type II plasma clot series
" " " " minced tissue "

" " Type III plasma clot series
" " minced tissue "

2.) Hanks Solution

3.) Monkey kidney epith tissue culture tubes series 62
Prepared 9-24-54

	Tube	2	4	6	8
Type I plasma Clot series CE, K, 10^{-3}	1	4			
	2	4			
	3	4			
10^{-4}	4	4			
	5	4			
	6	4			
10^{-5}	7	1-2	4		
	8	0-2	4		
	9	0	4		
10^{-6}	10	0	0	0	0
	11	0	4		
	12	0	4		
10^{-7}	13	0	0	0	0
	14	0	0	0	0
	15	0	0	0	0

		Tube	2	4	6	8
Type I minced tissue series CE, K,	10^{-3}	16	4v			
		17	4v			
		18	4v			
	10^{-4}	19	4v			
		20	4v			
		21	4v			
	10^{-5}	22	0-1	4		
		23	0-1	4		
		24	3	4		
	10^{-6}	25	0-?	4		
		26	0	4		
		27	0	4		
	10^{-7}	28	0	0	0	0d
		29	0	0d	0d	0d
		30	0	0	0	0
Type II plasma clot series CE, K,	10^{-3}	31	4v			
		32	4v			
		33	4v			
	10^{-4}	34	4v			
		35	3	4		
		36	1-2	4		
	10^{-5}	37	0	0	4	
		38	0-1	4		
		39	0	4		
	10^{-6}	40	0	0	0	0
		41	0	0-1	4	
		42	0	0	0	0
	10^{-7}	43	0	0	0	0d
		44	0	0	0	0
		45	0	0	0	0
Type II Minced tissue series CE, K,	10^{-3}	46	4v			
		47	4v			
		48	4v			
	10^{-4}	49	1-2	4		
		50	3	4		
		51	2-3	4		
	10^{-5}	52	0	0d	4	
		53	0-1	4		
		54	0	4		

OCT 5 1954

Titration " CE Variant " CE, K,

		Tube	2	4	6	8
Type II Monocal tissue series CE, K, (cont)	10^{-6}	55	0	0	4	
		56	0	0	4	
		57	0	0	4	
	10^{-7}	58	0	0	0	0
		59	0	0	0	0
		60	0	0	0	0
Type III Plasma Clot series CE, K,	10^{-3}	61	4✓			
		62	4✓			
		63	4✓			
	10^{-4}	64	0	4		
		65	0	4		
		66	0	4		
	10^{-5}	67	0	4		
		68	0	4		
		69	0	4		
	10^{-6}	70	0	0	0d	0d
		71	0	0	4	
		72	0	0	0	0d
	10^{-7}	73	0	0	0	0d
		74	0	0	0	0
		75	0	0	0	0
Type III Monocal tissue series CE, K,	10^{-3}	76	4✓			
		77	4✓			
		78	4✓			
	10^{-4}	79	0	4		
		80	0	4		
		81	0	4		
	10^{-5}	82	0	0	0	0
		83	0	0-?	4	
		84	0	0	4	
	10^{-6}	85	0	0	0	0d
		86	0	0	0	0
		87	0	0d	0	0d
	10^{-7}	88	0	0	0	0
		89	0	0	0	0
		90	0	0	0	0

CE, MKi

Titers

	TCD_{50} per 0.2 ml	per 1 ml
I Plasma Clot Series	$10^{+6.3}$	$10^{+7.0}$
I Suspended Minced Tissue Series	$10^{+6.5}$	$10^{+7.2}$
II Plasma Clot Series	$10^{+5.8}$	$10^{+6.5}$
II Suspended Minced Tissue Series	$10^{+6.5}$	$10^{+7.2}$
III Plasma Clot Series	$10^{+5.8}$	$10^{+6.5}$
III Suspended Minced Tissue Series	$10^{+5.3}$	$10^{+6.0}$

Oct 9, 1954

Effect of pH on the Stability of Type III Poliomyelitis Virus at 36°C -

Procedure 3 series

Series 1. Virus at pH 8.2 (the pH of Lactalbumin hydrolysate medium)

Series 2. Virus at pH 7.5

Series 3 Virus at pH 6.8

3 ml of tissue culture fluid virus + 6 ml of Lactalbumin hydrolysate prepared for each series.

Series 1. Left as is

Series 2 addition of 0.06 ml of N HCl → pH 7.5

Series 3 addition of 0.09 ml of N HCl → pH 6.8

Each series then split in half 4.5 ml in each of 2 tubes

Tubes placed in roller drum in incubator. 36°C

Materials

Virus Leon KP 36 4-19-54 stored in deep freeze.
Titer = $10^{7.5}$ TCD$_{50}$/ml

Medium Lactalbumin hydrolysate medium
10-8-54

Aliquots (1.0 ml each series, 0.5 ml each tube) removed at 5, 10, 15, 20, 25, 30 days and tested for virus in cynomolgus monkey kidney tissue culture -

Oct 10, 1954

pH of tubes

Series 1	pH 8.2	pH 8.2			
Series 2	pH 7.6	pH 7.6	0.02 ml N/10 HCl →	7.5	7.5
Series 3	pH 7.3	pH 7.4	0.02 ml N HCl →	6.8	6.8

Oct 12 pH of tubes

Series 1	pH 8.2	pH 8.2			
Series 2	pH 7.5	pH 7.5			
Series 3	pH 7.0	pH 7.0 →	0.01 ml N HCl →	6.8	6.8

~~Oct~~ 14, 1954

pH of tubes

Series 1	pH 8.2	8.2			
Series 2	pH 7.5	7.5			
Series 3	pH 6.9	6.9 →	0.01 ml N HCl →	6.8	6.8

Oct 16, 1954

pH of tubes

Series 1	pH 8.2	pH 8.2			
Series 2	pH 7.6	pH 7.6	0.005 ml N HCl →	7.5	7.5
Series 3	pH 6.9	6.9	0.01 ml N HCl →	6.8	6.8

Oct 18 pH of tubes

Series 1	pH 8.2	8.2			
Series 2	pH 7.5	7.5			
Series 3	pH 6.9	6.9	0.01 ml N HCl →	6.8	6.8

pH Stability Exp (Cont)

Oct 21, 1954 pH of tubes

Series 1	pH 8.2	pH 8.2
Series 2	pH 7.5	pH 7.5
Series 3	pH 6.9	pH 6.9 $\xrightarrow[N/1\ HCl]{0.005ml}$ pH 6.8 pH 6.8

Oct 25, 1954 pH of tubes

Series 1	pH 8.2	pH 8.2
Series 2	pH 7.5	pH 7.5
Series 3	pH 6.9	pH 6.9 $\xrightarrow[N\ HCl]{0.005\ ml}$ pH 6.8 6.8

Oct 29, 1954 pH of tubes

Series 1	pH 8.2	8.2
Series 2	pH 7.5	7.5
Series 3	pH 6.8	6.8

Nov 3, 1954 pH of tubes

Series 1	pH 8.2	pH 8.2
Series 2	pH 7.5	pH 7.5
Series 3	pH 6.9	pH 6.9 $\xrightarrow[N\ HCl]{0.005ml}$ pH 6.8 pH 6.8

Nov 8, 1954 pH of tubes

Series 1	pH 8.2	8.2
Series 2	pH 7.5	7.5
Series 3	pH 6.8	6.8

Nov 18, 1954 pH of tubes

Series 1 8.2 8.2

Series 2 7.5 7.5

Series 3 6.8 6.8

Persistence of Type III Virus at Indicated pH at 36°C

	— Days —							
	5	10	15	20	25	30	35	40
Series 1 pH 8.2	3/3*	3/3	3/3	0/3	0/3	0/3	0/3	0/3
Series 2 pH 7.5	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3
Series 3 pH 6.8	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3

*3 tubes showing cytopathogenic effect of 3 tubes inoculated with undiluted tissue culture fluid 0.2 ml / tube

Oct 14, 1954 — pH Stability Exp

Test of 5 day aliquots for series

Procedure 0.5 ml removed from each tube of Series 1, 2, & 3. Aliquots from the same series pooled. 0.2 ml of undiluted fluid inoculated into 3 tubes for each series. Remainder of fluid stored in deep freeze (this fluid to be titrated if this is indicated)

Tubes of series 71 used

	Tube No	2
Series 1 (pH 8.2)	71 - 1	4
	71 - 2	4
	71 - 3	4
Series 2 (pH 7.5)	71 - 4	4
	71 - 5	4
	71 - 6	4
Series 3 (pH 6.8)	71 - 7	4
	71 - 8	4
	71 - 9	4

Oct 19, 1954 — Test of 10 day aliquots

Procedure Same as above

Tubes of series 72 used

	Tube No	2	4	6
Series 1 (pH 8.2)	72 - 105	0	3-4	
	106	2	Off	
	107	0	2-3	4 off
Series 2 (pH 7.5)	108	3-4		
	109	4		
	110	4		
Series 3 (pH 6.8)	111	4		
	112	4		
	113	3	Off	

Oct 25, 1954

Test of 16 day aliquots

Procedure same as above

Tubes of series 73 used

	Tube No.	2	4	6
Series 1 (PH 8.2)	73- 43	2-3	4	
	44	0	0	4
	45	0	1	4
Series 2 (PH 7.5)	46	2-3	4	
	47	4		
	48	4		
Series 3 (PH 6.8)	49	4		
	50	3-4		
	51	4		

Oct 29, 1954 Test of 20 day aliquots

Procedure same as above

Tubes of series 77 & 78 used

	Tube No	2	4	6	8	10
Series 1 (PH 8.2)	77-50	0	0	0d	0d	0d
	77-51	0	0	0	0	0d
	78-109	0	0	0	0	0
	78-110	0	0	0	0	0
Series 2 (PH 7.5)	77-52	0	2	4		
	77-53	0	1-2	4		
	78-54	0	0-1	4		
	78-111	1-2	4			
Series 3 (PH 6.8)	77-55	2-3	4			
	77-56	0-?	4			
	78-112	3	4			
	78-113	2	4			

PH Stability Exp

Nov 3, 1954

Test of 25 day aliquots

Procedure same as before

Tubes of trypsinized monkey kidney series No 2 (10/27) used

	Tube No	2	4	6	8	10
Series 1 (pH 8.2)	T_2 - 305	0	0	0	0	0
	306	0	0	0	od	od
	307	0	0	od	od	od
	308	0	0	od	od	od
Series 2 (pH 7.5)	309	0	4			
	310	0	0-1	4		
	311	0	0	4		
	312	0	0	1-2	4	
Series 3 (pH 6.8)	313	2	4			
	314	1-2	4			
	315	1-2	4			
	316	1-2	4			

Nov 8, 1954

Test of 30 day aliquots

Procedure Same as before

Tubes of trypsinized monkey kidney series No. 2 (10/27) used

	Tube No	2	4	6	8	10
Series 1 (pH 8.2)	T_2 - 486	0	0	0	0	0
	487	0	0	0	od	od
	488	0	0	0	0	0
	489	0	0	0	0	0
Series 2 (pH 7.5)	490	0	0	0	0	0
	491	0	0	0	0	0
	492	0	0	0	0	0
	493	0?	0	od	od	od
Series 3 (pH 6.8)	494	2-3	4			
	495	2	4			
	496	3	4			
	497	4				

STANDARD B&P "NOTEAR"
STANDARD B&P "NOTEAR"

Nov 13, 1954 — Test of 35 day aliquots

Procedure same as before

Tubes used Trypanized series No 4 (of 11/4/54)

Series	Tube No	2	4	6	8	10
Series 1 (pH 8.2)	T4-306	0	0	0	0	0
	307	0	0	0	0	od
	308	0	0	0	0	0
	309	0	0	0	0	0
Series 2 (pH 7.5)	310	0	0	0	0	0
	311	0	0	0	0	0
	312	0	0	0	0	0
	313	0	0	0	0	0
Series 3 (pH 6.8)	314	3	4			
	115	4				
	116	4				
	117	3	4			

Nov 18, 1954 — Test of 40 day aliquots

Procedure - same as before

Tubes used - Trypanized series No 5 (of 11-5-54)

Series	Tube No	2	4	6	8	10
Series 1 (pH 8.2)	T5-94	0	0	0	0	0
	T5-96	0	0	0	od	od
	T5-97	0	0	0	0	0
Series 2 (pH 7.5)	T5-98	0	0	0	0	0
	T5-99	0	0	0	0	0
	T5 100	0	od	od	od	od
Series 3 (pH 6.8)	T5-101	0	0	0	1	4
	T5-102	0	0-?	4		
	T5-103	0	od	od	od	od

STANDARD
OCT 7 1954

Identification of Viruses Surviving in Chick Embryo Cultures and Subsequently Propagated in Monkey Kidney Cultures

Purpose - to make certain that the viruses which survived so long in the chick embryo cultures are actually types 1, 2, and 3

Viruses Used: The plasma-clot series of each type harvested 10-4-54 was used.

Type 1 - $CE_1 MK_1$ - 2 day titer - $10^{-5.3}/0.2$ ml
" 2 - " " - " " " - $10^{-4.8}/0.2$ ml
" 3 - " " - " " " - $10^{-3.5}/0.2$ ml

Therefore for Type 1 use 1:1000
" 2 use 1:1000
" 3 use 1:100

Procedure - Mix 0.4 ml of standard monkey typing sera 1:5 & 0.4 ml of indicated dilution of virus
After 1 hour at room temp - 0.2 ml / tube.

Culture Tubes Used: Series 63

Mixture Tested	Tube No.	2	4	6	8
Type 1 + Type I serum	.63-83	0	0	0d	0
	.63-84	0	0	0d	0
	.63-85	0	0d	0d	0 vac
Type 1 + Type II+III serum	.63-86	4			
	.63-87	4			
	.63-88	4			
Type 2 + Type II serum	.63-89	0	0d	0d	0 vac
	.63-90	0	0	0	0
	.63-91	0	0	0	0 vac
Type 2 + Type I+III serum	.63-92	4			
	.63-93	4			
	.63-94	4			
Type 3 + Type III serum	.63-95	0	0	0	0
	.63-96	0	0	0	0
	.63-97	0	0	0	0

(over)

Mixture Tested	Tube No.	2	4	6	8
Type 3 + Type II + II serum	.63-98	4			
	.63-99	4			
	.63-100	4			

Tests to Determine Significance of Survival of all 3 Types of Poliomyelitis Virus in Media Containing Chick Embryo Tissue Long Past Period of Complete Decay in Same Medium without Tissue

Observations:

1. When $10^{7.5}$ TCD$_{50}$ of Lon KP36 virus (1 ml) was put into our regular lactalbumin hydrolysate medium (2 ml), pH @ 8.2–8.4, and left in roller drum at 36–37°C, a minimal amount was still demonstrable in 0.5 ml of medium at 14 days, but none was present in 0.8 ml. at 18 days.

2. When 10^{8} TCD$_{50}$ of Type I or II (Mah, KP35 + Yale KP54) and $10^{7.5}$ TCD$_{50}$ of Type III (Lon KP36) were added to 2 ml of:
   a) medium without tissue
   b) medium + @ 20 fragments of chick embryo tissue
   c) Fibroblasts + epithelial cells growing out of chick embryo fragments in plasma clot

   and left in roller drum at 36–37°C for 37 days – no virus was detectable in any of the tubes without tissue, while it was present in the centrifuged fluid in all cases in the tubes containing tissue, in amounts of @ 10^{2} to 10^{3} TCD$_{50}$/ml as measured in monkey kidney tissue cultures.

3. The pH of the fluid in the tubes without tissue was unchanged i.e. about pH 8.0, while in all the other tubes the metabolizing tissue had changed the pH to @ 6.9

(over)

Possible Interpretations:

1) Mutant capable of multiplying in chick embryo tissue was being released from the tissue long after the (original) added virus had decayed

2) There was no multiplication of virus in chick embryo tissue but

   a) virus adsorbed on tissue survives longer and continues to be liberated into culture fluid or

   b) virus survives much longer at pH 7.4 → 6.9 than at a pH 8.0

Evidence that virus multiplied in chick-embryo tissue could be established by following:

1. Demonstration of cytopathogenic effect on fibroblasts or epithelial cells or both growing out of chick embryo tissue by

   a) ~~virus~~ "CE" (chick embryo) virus boosted in titer by one passage through monkey kidney or b) "CE" virus direct

   Note - absence of "CP" effect would not mean absence of multiplication

~~b)~~

2. Demonstration of multiplication either in suspended chick embryo tissue or in plasma clot fragments + outgrowth of either

   a) "CE" virus boosted by one pass. in monkey kidney or

   b) "CE" virus alone

   by means of either

   a) ~~change~~ growth curve of virus in medium or

   b) testing for growth of virus by repeated changes of medium

Polio Virus in Chick Embryo Tissue

"Continued"

3. By demonstration that virus which has been recovered from chick embryo tubes and then propagated in monkey kidney possesses different "virulence" properties from that of "seed" virus as determined by intraspinal inoculation in monkeys and mice.
4. Ultimately by continuous serial passage in chick embryo tissue.

Tests to be performed with "CE" virus boosted by one passage in monkey-kidney:

1. Virus Derived from Plasma Clot C-E Cultures

✓ a) Titer in monkey kidney cultures
b) Add to plasma-clot CE culture + observe for cytopathogenic effect and at 4-day intervals remove fluid ~~+ wash 3x~~ for titration, wash 4x and add additional fluid to test for multiplication of virus
Use 10 ml for washing
After first time – do not wash
c) Inoculate monkeys ~~spinally~~ with undil. + 10⁻¹ (4 each) and 20 mice with undiluted

2. Virus Derived from Suspend "C-E" ~~fragment~~ Tissue Cultures

✓ a) Titer in monkey kidney
✓ b) Add to new suspended "C-E" cultures + to medium without tissue and test for amount of virus at:
2 hrs, 4, 8, 12, 16 and 20 days
Change pH of control tubes q. 2 days as indicated
? Change pH in tubes without tissue to correspond to those with tissue?

Tests to be performed with "C-E" virus from plasma clot cultures:

a) Titer in monkey kidney – done
b) Add to plasma clot culture and observe for cytopathogenic effect and test for multiplication of virus in ~~fluids~~ removed ~~at 4-day intervals after preliminary washing~~
2 ml of medium + 1 ml of virus
3 tubes ~~of test~~ aliquots removed at 4, 8, 12, 16 & 20 days
remove 0.4 ml from each tube each time
Use 0.5 ml for titration and freeze remainder

(over)

Tests to be performed with "CE" virus derived from suspended tissue cultures

a) Titer in monkey kidney – done
b) Add to similar "CE" cultures and to tubes without medium — test for amount of virus in aliquots removed at 4, ~~7~~ 8, 12, 16 and 20 days and longer if results warrant.
0.7 ml virus + 2.8 ml medium — 3 tubes
Remove 0.4 ml from each tube at each interval
Use 0.5 ml for titration + freeze remainder
At 2 day intervals adjust pH of control to that of tubes with tissue

Other Tests to be Performed:

1. Identify "CE_1" MK_2 (plasma clot series) virus by neutralization test ~~c~~ polio antiserum
2. Test for survival and rate of decay of virus in media without tissue at pH of @ 8.3, 7.5, 6.9 at 5, 10, 15, 20, 30, ~~40~~ day intervals – stopping of course, when no more virus is detectable in 1 ml aliquot.

Serial Passage Series

Suspended CE → suspended CE
Plasma clot CE → plasma clot CE

Alternate Chick – Monkey Kidney Passages

Suspended CE → Monk. Kidney → Susp. CE → Monk.
Plasma Clot CE → " " → Plasma Clot → Monk.

Oct 4, 1954

"CE Variant" Exp 1

Purpose To determine if "CE variant" of types I, II, and III poliomyelites virus multiplies in chick embryo tissue culture.

When large amounts of Types I, II, + III poliomyelites viruses were inoculated into chick embryo tissue culture either of the plasma clot rolled tube or free floating minced tissue in roller tube variety and incubated for 37 days approximately 25 – 250 ID_{50}/ml of virus was recovered at the end of the experiment. Control tubes inoculated with virus and fluid medium but no tissue were without demonstrable virus at end of the experiment (37 days). This suggested that by using a large virus inoculum a mutant or variant with growth potential in chick embryo tissue culture had been selected from the high concentration of introduced virus and had multiplied in chick embryo tissue culture. The alternative explanation would seem to involve an adsorption of virus onto tissue particles, a greater stability under this condition, and finally a slow or gradual elution of virus into the surrounding fluid medium. The following experiment, one of three, was performed to answer the question – does the "CE variant" poliomyelites virus actually multiply in chick embryo tissues in tissue culture.

Procedure Two series of tubes were prepared for each type of polio virus.

Series 1. Minced 8 day chick embryo fragments (approx. 20 per tube) suspended free floating in Lactalbumin hydrolysate medium. 3 tubes used for each virus type.

0.3 ml of minced tissue in Hanks solution
2.5 ml of Lactalbumin hydrolysate medium
0.7 ml of CE, K, virus (harvested 10-4-54)

Series 2 No tissue 2.8 ml of Lactalbumin hydrolysate medium
0.7 ml of CE, K, virus (harvested 10-4-54)
3 tubes per virus

At 2 hours, 4 days, 8 days, 12 days, 16 days and 20 days 0.~~3~~ 40 ml aliquots removed from each tube – the fluid from the 3 tubes in each series pooled, and either titrated for virus content immediately or after storage at -20°c.

Tubes of all series placed in roller drum and allowed to rotate at 36°c.

At 2 day intervals the pH in tubes of series 2 adjusted to correspond with ~~that~~ prevailing in tubes with tissue – Series 1. Use 1N HCl for 1st adjustment.

Materials -

1. Virus

Type I CE, K, from minced tissue series } harvested 10-4-54 from tubes 12-18 of the original CE experiment

Type II CE, K, from minced tissue series } harvested 10-4-54 from tubes 45-51 of the original CE experiment

Type III CE, K, from minced tissue series } harvested 10-4-54 from tubes 78-83 of the original CE experiment -

2. Tissue 12 8 day old White Rock Chick embryos harvested aseptically eyes, lenses removed minced into 1 mm size fragments after 3 washings in Hanks (1/3 $NaHCO_3$), washed 6x in Hanks (1/3 $NaHCO_3$) and introduced into each tube of the minced tissue series in quantity of 20 fragments.

3) Medium

Lactalbumin Hydrolysate Medium - with antibiotics
10-1-54

Type I virus Series 1) Tubes 1, 2 3
Series 2) Tubes 4, 5, 6

Type II virus Series 1) Tubes 7, 8, 9
Series 2) Tubes 10, 11, 12

Type III virus Series 1) Tubes 13, 14, 15
Series 2) Tubes 16, 17, 18.

Oct 6, 1954 — CE Variant Exp 1 (cont)

Record of tubes & pH adjustment

	Tube No.	2 days	4 days	6 days	8 days	10 days	12 days	14 days	16 days	18 days	20 days
Type I Tissue	1	7.4	7.2	7.2	7.0	7.0	7.0	7.1	7.1		7.1 7.2
	2	7.4	7.2	7.3	7.2	7.2	7.2	7.2	7.2		7.2 7.2
	3	7.4	7.3	7.3	7.2	7.2	7.2	7.2	7.2		7.2 7.2
No Tissue	4	8.2→7.4	7.5→7.2	7.4→7.3	7.3→7.0	7.3→7.1	7.3→7.0	7.3→7.2	7.3→7.2		7.3→7.2 7.2
	5	8.2→7.4	7.5→7.2	7.4→7.2	7.4→7.2	7.4→7.2	7.4→7.1	7.3→7.2	7.3→7.2		7.3→7.2 7.2
	6	8.2→7.4	7.5→7.3	7.4→7.2	7.3→7.2	7.3→7.1	7.4→7.2	7.3→7.1	7.3→7.2		7.3→7.2 7.2
Type II Tissue	7	7.4	7.2	7.2	7.2	7.2	7.2	7.2	7.2		7.2 7.2
	8	7.3	7.1	7.2	7.1	7.2	7.2	7.2	7.2		7.2 7.2
	9	7.4	7.2	7.2	7.1	7.1	7.1	7.2	7.2		7.2 7.2
No Tissue	10	8.2→7.3	7.4→7.2	7.4→7.2	7.3→7.1	7.3→7.2	7.3→7.2	7.3→7.2	7.3→7.2		7.3→7.2 7.2
	11	8.2→7.4	7.5→7.2	7.4→7.2	7.3→7.1	7.3→7.2	7.3→7.1	7.3→7.2	7.3→7.2		7.3→7.2 7.2
	12	8.2→7.3	7.4→7.2	7.4→7.2	7.3→7.1	7.3→7.1	7.3→7.2	7.3→7.2	7.3→7.2		7.3→7.2 7.2
Type III Tissue	13	7.3	7.0	7.0	7.0	7.0	7.0	7.1	7.2	7.2	7.2
	14	7.4	7.2	7.3	7.2	7.2	7.2	7.2	7.2	7.2	7.2
	15	7.4	7.2	7.2	7.1	7.1	7.1	7.2	7.2	7.2	7.2
No Tissue	16	8.2→7.3	7.4→7.2	7.4→7.0	7.3→7.0	7.3→7.1	7.2	7.3→7.2	7.3→7.2		7.3→7.2 7.2
	17	8.2→7.4	7.5→7.2	7.4→7.2	7.4→7.1	7.3→7.0	7.3→7.2	7.3→7.2	7.2		7.3→7.2 7.2
	18	8.2→7.4	7.5→7.0	7.4→7.2	7.4→7.2	7.4→7.2	7.3→7.1	7.3→7.2	7.3→7.2		7.3→7.2 7.2

Adjustment with ~~1 N HCl~~

1 N HCl 0.02 ml	1 N HCl 0.01 ml	~~1 N HCl~~ 0.005 ml	1 N HCl 0.005 ml	1 N HCl 0.005 ml	1 N HCl 0.005 ml		1 N HCl	N/10 HCl 0.01 ml	N/10 HCl

Oct 4, 1954 — CE Variant Exp 1

Day	Date aliquots removed	Titrated or stored	Date titrated if stored	Dil. of tissue series To be tested 3 tubes/dil	Dil. of control series to be tested 3 tubes per dil.	Total tubes
2 hours	10-4-54	stored deep freeze				
4 Day	10-8-54	stored deep freeze	OCT 11 1954	$10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, + 10^{-6}$ = 15 tubes X 3 = total 45	$10^{-2}, 10^{-3}, 10^{-4}$ $10^{-5}, + 10^{-6}$ = 15 tubes X 3 = total 45	90
8d	10-12-54	stored deep freeze	OCT 14 1954	"	"	90
12	10-16-54	stored deep freeze	OCT 19 1954	$10^{-1} - 10^{-5}$ = 15 tubes X 3 = 45	$10^{-1} - 10^{-5}$ = 15 tubes X 3 = 45	90
16	10-20-54	stored deep freeze	OCT 26 1954	Undil - 10^{-4} 15 X 3 = 45	Undil - 10^{-3} 12 X 2 = 24 + Undil - 10^{-4} 15 = 39	84
21	10-25-54	stored deep freeze	OCT 26 1954	"	"	84
28	11-1-54	stored deep freeze				

CE Variant Exp 1

Quantity of virus in tubes containing suspended minced tissue and tubes containing no tissue after indicated time at 36°C

Time after addition of Poliomyelitis virus	Titer of virus in indicated tubes TCD_{50}/ml					
	Type I virus		Type II virus		Type III virus	
	Suspended tissue series	No tissue	Suspended tissue series	No tissue	Suspended tissue series	No tissue
0 (calculated = amount introduced)	$10^{6.5}$ ($10^{6.8}$ in repeat C trypsin tubes)	$10^{6.5}$	$10^{6.5}$ ($10^{7.0}$ in repeat E trypsin tubes)	$10^{6.5}$	$10^{5.3}$ ($10^{6.8}$ in repeat C trypsin tubes)	$10^{5.3}$
2 hours						
4 days	$10^{6.2}$	$10^{5.5}$	$10^{5.2}$	$10^{5.5}$	$10^{6.0}$	$10^{5.5}$
8 days	$10^{5.2}$	$10^{4.2}$	$10^{4.5}$	$10^{3.5}$	$10^{5.2}$	$10^{4.2}$
12 days	$10^{4.0}$	$10^{2.4}$	$10^{4.2}$	$10^{2.2}$	$10^{4.2}$	$10^{3.5}$
→ Trypsin tubes started						
16 days	$10^{3.5}$	$10^{3.0}$	$10^{3.5}$	$10^{2.0}$	$10^{4.0}$	$10^{2.2}$
20 days	$10^{3.0}$	$10^{2.2}$	$10^{2.2}$	$10^{1.0}$	$10^{3.2}$	$10^{1.7}$
28 days	$10^{2.2}$	$10^{1.0}$	$10^{2.0}$	$< 10^{0.7}$	$10^{2.2}$	$10^{2.0}$
36 days	$10^{1.0}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$

STANDARD B & P "NOTEAR"

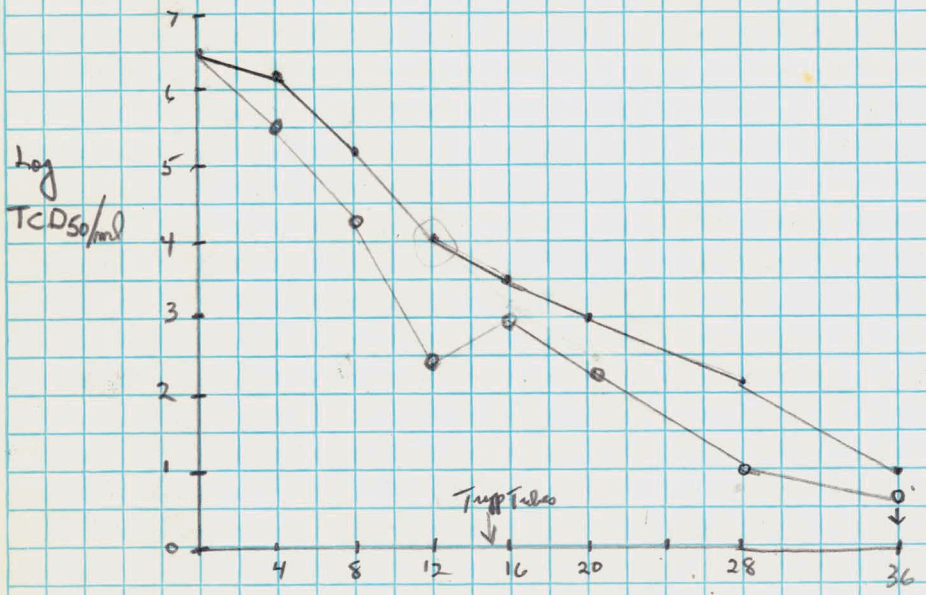
STANDARD B & P "NOTEAR"

CE Variant Exp 1

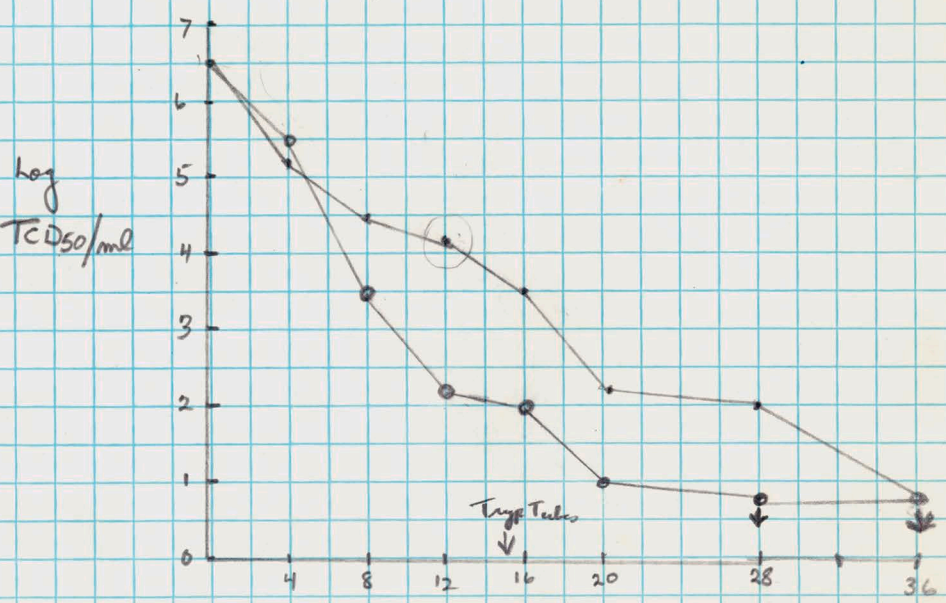
Virus Used – MK→CE→MK! – Suspended tissue series

pH adjusted in controls

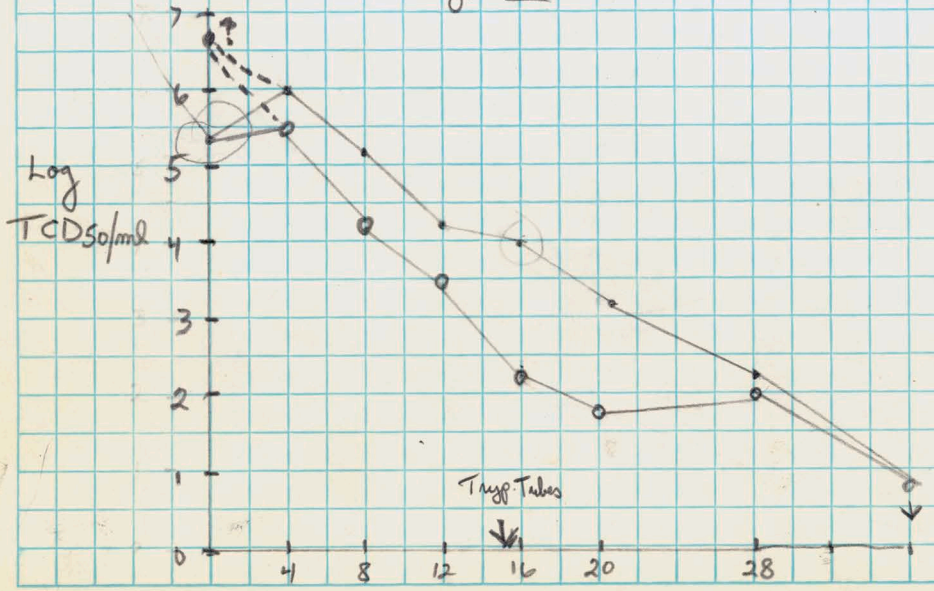
Type I



Type II



Type III



Titer of poliomyelites virus in tissue culture fluid from tubes containing suspended minced chick embryo tissue TCD50/ml

Titer of poliomyelites virus in tissue culture fluid from tubes containing no tissue – medium only TCD50/ml

OCT 11 1954

OCT 11 1954

CE Variant Exp 1 — Titration 4 day aliquots

Viruses – 4 day aliquots – CE variant Exp 1 – aliquots removed 10-8 -54 and stored in deep freeze – [CE, KM, CE,]

Procedure 10 fold dilutions of each virus prepared in Hanks solution (full amt of $NaHCO_3$) – 0.2 ml of diluted material inoculated – 3 tubes / decimal dilution Monkey Kidney Cult. Tubes used = Series 66

Type I		Tube No	2	4	6	8
	10^{-2}	66-1	4			
Series 1		2	4			
		3	4			
Suspended						
minced	10^{-3}	4	4			
tissue		5	4			
		6	3	4		
	10^{-4}	7	2-3	4		
		8	4			
		9	1	4		
	10^{-5}	10	0	4		
		11	0	0	0	0
		12	0	4		
	10^{-6}	13	0	0	4	
		14	0	0	0	0
		15	0	0	0	0
Series 2	10^{-2}	16	4			
		17	4			
No tissue		18	4			
	10^{-3}	19	4			
		20	4			
		21	4			
	10^{-4}	22	0-?	4		
		23	4			
		24	4			
	10^{-5}	25	0	0	0	0
		26	0	0	0	0
		27	0	0	4	
	10^{-6}	28	0	0	0	0
		29	0	0	0	0
		30	0	0	0	0

		Tube No	2	4	6	8	10
Type II	10^{-2}	31	3	4			
		32	4				
Series 1		33	4				
Suspended	10^{-3}	34	2	4			
minced		35	4				
tissue		36	1	4			
	10^{-4}	37	0	4			
		38	0	4			
		39	2	4			
	10^{-5}	40	0	0	0	0	
		41	0	0	0	0	
		42	0	0	0	0	
	10^{-6}	43	0	0	0	0	
		44	0	0	0	0	
		45	0	0	0	0	
Series 2	10^{-2}	46	4				
		47	4				
No tissue		48	2	4			
	10^{-3}	49	0	4			
		50	0	4			
		51	0	4			
	10^{-4}	52	0	2	4		
		53	0	0	3	4	
		54	0	4			
	10^{-5}	55	0	0	0	0	
		56	0	0	0	0	
		57	0	0	0	1	4
	10^{-6}	58	0	0	0	0	
		59	0	0	0	0	
		60	0	0	0	0	
Type III	10^{-2}	61	4				
		62	4				
Series 1		63	4				
Suspended	10^{-3}	64	4				
minced		65	4				
tissue		66	3	4			

OCT 11 1954

CE Variant Exp 1

4 Day Aliquots Titration

6 (Cont'd) 8

		Tube No	2	4		
Type III	10^{-4}	67	0?	4		
		68	2-3	4		
Series 1		69	0-?	4		
Suspend minced tissue	10^{-5}	70	0	0		0
		71	0	4		
		72	0	4		
	10^{-6}	73	0	0	0	0
		74	0	0	0	0
		75	0	0	0	0
Series 2	10^{-2}	76	4			
		77	4			
No tissue		78	4			
	10^{-3}	79	1-2	4		
		80	2-3	4		
		81	2-3	4		
	10^{-4}	82	0-?	4		
		83	1	4		
		84	0	4		
	10^{-5}	85	1	4		
		86	0	0	0	0
		87	0	0	0	0
	10^{-6}	88	0	0	0	0
		89	0	0	0	0
		90	0	0	0	0

OCT 1 4 1954

CE Variant Exp 1 Titration 8 day aliquots

Viruses 8 day aliquots - CE variant Exp 1 - aliquots removed 10-12-54 and stored in deep freeze [CE, MK, CE,]

Procedure 10 fold dilutions of each virus prepared in Hanks solution (full amount of $NaHCO_3$) — 0.2 ml of diluted material inoculated Monkey Kidney

3 tubes per decimal dilution ↓ Culture tubes used =

Series 67 8

		Tube No	2	4	6	8
Type I						
	10^{-2}	67- 1	4			
Series 1		2	4			
		3	4			
Suspended	10^{-3}	4	3	4		
minced tissue		5	4			
		6	4			
	10^{-4}	7	0	4		
		8	2-3	4		
		9	0	4		
	10^{-5}	10	0	0	0d	0
		11	0	0	0d	0
		12	0	0	0d	0
	10^{-6}	13	0	0	0d	0
		14	0	0	0d	0
		15	0	0d	0d	0d
Series 2	10^{-2}	16	4			
		17	4			
No tissue		18	3	4		
	10^{-3}	19	2-3	4		
		20	4			
		21	0	0	0d	0d
	10^{-4}	22	0-?	4		
		23	0	0	0d	0
		24	0	0	0d	0
	10^{-5}	25	0	0	0d	0
		26	0	0	0	0
		27	0	0	0d	0

		Tube No	2	4	6	8
Type I	10^{-4}	27, 28	0	0	0	od
		29	0	0	0	od
Series 2		30	0	0	0	od
No Tissue						
Type II	10^{-2}	31	4			
		32	4			
Series 1		33	3	4		
Suspended	10^{-3}	34	0	4		
minced		35	1-2	4		
tissue		36	0	4		
	10^{-4}	37	0	0	0	
		38	0	3	4	
		39	0	0	0	od
	10^{-5}	40	0	0	od	od
		41	0	0	od	od
		42	0	0	od	od
	10^{-6}	43	0	0	od	od
		44	0	0	od	od
		45	0	0	od	od
Series 2	10^{-1}	46	4			
		47	4			
No tissue		48	4			
	10^{-2}	49	0	4		
		50	0	4		
		51	2	4		
	10^{-3}	52	0	0	od	0
		53	0-?	4		
		54	0	0	od	od
	10^{-4}	55	0	0	od	od
		56	0	0	od	od
		57	0	0	od	od
	10^{-5}	58	0	0	od	od
		59	0	0	od	od
		60	0	0	od	od

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CE Variant Exp 1 — 8 day aliquot titration (cont'd)

Type III

Series 1

Suspended minced tissue

	Tube No	2	4	6	8
10^{-2}	67-61	4			
	62	4			
	63	3	4		
10^{-3}	64	2	4		
	65	1-2	4		
	66	0	4		
10^{-4}	67	0-2	4		
	68	0	0	od	od
	69	0	4		
10^{-5}	70	0	0	od	od
	71	0	0	od	od
	72	0	0	od	3-4
10^{-6}	73	0	0	od	od
	74	0	0	od	od
	75	0	0	od	od

Series 2

No tissue

10^{-2}	76	2-3	4		
	77	0-1	4		
	78	0-?	4		
10^{-3}	79	1-2	4		
	80	0	1	4	
	81	0	2-3	4	
10^{-4}	82	0	0	od	od
	83	0	0	od	od
	84	0	0	od	od
10^{-5}	85	0	0	od	od
	86	0	0	od	od
	87	0	0	od	od
10^{-6}	88	0	0	od	od
	89	0	0	od	od
	90	0	0	od	od

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CE Variant Exp 1 — Titration of 12 day aliquots

Viruses — 12 day aliquots - CE variant Exp 1 - aliquots removed (10-16)-54 and stored in deep freeze [CE, MK, CE,]

Procedure — 10 fold dilutions of each virus prepared in Hanks solution (full amount of $NaHCO_3$) 0.2 ml of dilutes inoculated.

3 tubes per decimal dilution

Monkey kidney culture tubes used = Series 72

		Tube No	2	4	6 (10-25)	8
Type I						
Series 1	10^{-1}	22-15	4			
		16	4			
		17	4			
Suspended minced tissue	10^{-2}	18	0-1	4 off		
		19	0-1	4 off		
		20	0-2	4 off		
	10^{-3}	21	0	0	od	od
		22	0-1	4 off		
		23	0	4 off		
	10^{-4}	24	0	od	od	od
		25	0 fatty degen	od	od	od
		26	0	od	od	od
	10^{-5}	27	0	od	od	od
		28	0	od	od	od
		29	0	od	od	od
Series 2	10^{-1}	30	4			
No tissue		31	0-?	4 off		
		32	3-4			
	10^{-2}	33	0	0	0	od
		34	0	od	od	od
		35	0	od	od	od
	10^{-3}	36	0	0	od	od
		37	0	0	od	od
		38	0	off		
	10^{-4}	39	0	0	0	od
		40	0 fatty degen	od	od	od off
		41	0	0	0	0

		Tube No	2	4	6	8
Type I	10^{-5}					
		72-42	0	od	od	od
Series 2		43	0	od	od	od
		44	0	0	od	od
No tissue						
(cont)						
Type II	10^{-1}	45	4			
		46	4			
Series 1		47	4			
	10^{-2}	48	3-4			
Suspended minced		49	0	4 off		
tissue		50	2-3	4 off		
	10^{-3}	51	0	4 off		
		52	0	4 off		
		53	0	0-?	4	
	10^{-4}	54	0	0	0	0
		55	0	0	0	od
		56	0	0	0	od
	10^{-5}	57	0	0	od	od
		58	0 fatty degen	od	od	od
		59	0	od	od	od
Series 2	Undiluted	60	4			
		61	2-4			
No tissue		62	4			
	10^{-1}	63	2	4 off		
		64	2	4 off		
		65	0	4 off		
	10^{-2}	66	0	od	od	od
		67	0	od	od	od
		68	0	0	od	od
	10^{-3}	69	0	od	od	od
		70	0	0	0	0
		71	0	od	od	od
	10^{-4}	72	0 fatty degen	od	od	od
		73	0	od	od	od
		74	0	0	od	od

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CE Variant Exp 1 — 12 day aliquots titration

Type III

Series 1

Suspended minced tissue

	Tube No	2	4	6 (cont)	8
10^{-1}	75	3	4 off		
	76	3-4			
	77	2	4 off		
10^{-2}	78	0-1	4 off		
	79	0-1	4 off		
	80	0-1	4 off		
10^{-3}	81	0	0	4	
	82	0 fatty degen	od	1-2	4
	83	0	off		
10^{-4}	84	0	od	od	od
	85	0	od marked	od	off
	86	0	0	od	od
10^{-5}	87	0	od	od	od
	88	0	od	od	od
	89	0	od	od	od

Series 2

No tissue

10^{-1}	90	0-?	4 off		
	91	0-2	4 off		
	92	0?	4 off		
10^{-2}	93	0 fatty degen	4 off		
	94	0	od	0	od
	95	0 fatty degen	4 off		
10^{-3}	96	0 fatty degen	od	od	od
	97	0	od	4	
	98	0	0-?	4	
10^{-4}	99	0	od	od	od
	100	0	od	od	od
	101	0 fatty degen	od	od	od
10^{-5}	102	0	od	od	od
	103	0	0	od	od
	104		0	od	od

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CE Variant Exp 1 Titration of 16 day aliquot

Viruses 16 days aliquots - CE variant Exp 1
aliquots removed 10-20-54 and stored in deep freeze [CE, MK, CE1]
Procedure 10 fold dilutions of each virus prepared in Hanks solution - 0.2 ml of diluted material inoculated 3 tubes / decimal dilution
Trypsinized monkey kidney series 1 used

		Tube No	2	4	6	8
Type 1						
Series 1	Undil	T_1-1	4			
Suspended minced tissue		2	4			
		3	4			
	10^{-1}	4	4			
		5	3-4			
		6	3-4			
	10^{-2}	7	1	4 off		
		8	0	4 off		
		9	2	4 off		
	10^{-3}	10	0	0	0	4
		11	0	0	0	0
		12	0	0	0	0
	10^{-4}	13	0	0	0	0
		14	0	0	0	0
		15	0	0 contam mold	0	0
Series 2	Undil	16	1-2	4 off		
		17	2-3	4 off		
No tissue		18	4			
	10^{-1}	19	0	4		
		20	1	4		
		21	1-2	4		
	10^{-2}	22	0	4		
		23	0	2	4	
		24	0	0	0	0
	10^{-3}	25	0	0	0	0
		26	0	0	0	0
		27	0	0	0	0

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		Tube No	2	4	6	8
Type II						
Suspended minced tissue	Undil	28	4			
		29	4			
		30	4			
Series 1	10^{-1}	31	4			
		32	4			
		33	2	4		
	10^{-2}	34	0	4		
		35	0	4		
		36	0-1	4		
	10^{-3}	37	0	0	0	0
		38	0	4		
		39	0	0	0	0
	10^{-4}	40	0	0	0	0
		41	0	0	0	0
		42	0	0	0	0
Series 2 No tissue	Undil	43	1	4		
		44	1	4		
		45	0	4		
	10^{-1}	46	0	0	4	
		47	0	4		
		48	0	0	0	0
	10^{-2}	49	0	0	0	0
		50	0	0	0	0
		51	0	0	0	0
	10^{-3}	52	0	0	0	0
		53	0	0	0	0
		54	0	0	0	0
Type III						
Series 1	Undil	55	4			
		56	4			
		57	4			
Suspended minced tissue	10^{-1}	58	0	4		
		59	1	4		
		60	4			
	10^{-2}	61	0	4		
		62	0	4		
		63	0	4		

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		Tube No	2	4	6	8
Type III Series 1	10^{-3}	64	0	0	0	
		65	0	4		
		66	0	0	4	
	10^{-4}	67	0	0	0	0
		68	0	0	0	0
		69	0	0	0	0
Series 2 NO Tissue	Undil	70	1-2	4		
		71	1	4		
		72	2-3	4		
	10^{-1}	73	2	4		
		74	0-1	4		
		75	0-1	4		
	10^{-2}	76	0	0	0	0
		77	0	0	0	~~0~~
		78	0	0	0	0
	10^{-3}	79	0	0	0	0
		80	0	0	0	0
		81	0	0	0	0
	10^{-4}	82	0	0	0	0
		83	0	0	0	0
		84	0	0	0	0

Titration of 21 day aliquots -

Versus 21 day aliquots removed 10-25-54 - stored in deep freeze -

Procedure same as described above

		Tube No	2	4	6	8
Type I Series 1 Suspended minced tissue	Undil	85	4 off			
		86	4			
		87	4			
	10^{-1}	88	3	4		
		89	1	4		
		90	4			
	10^{-2}	91	0	od (dumpy)	od	od
		92	0	0	4	
		93	0	0	0	4

		Tube No.	2	4	6	8
Type I						
Series 1 (cont)	10^{-3}	94	0	0	0	0
		95	0	0	0	0
		96	0 D.	0d	0d	0d
	10^{-4}	97	0	0	0	0
		98	0	0	0	0
		99	0	0	0	0
Series 2	Undil	100	1-2	4		
		101	2	4		
No tissue		102	0	0	4	
	10^{-1}	103	0	0	0	0
		104	0	4		
		105	No cells poor tube			
	10^{-2}	106	0	0	0	0
		107	bad tube, no cells			
		108	0	0	4	
	10^{-3}	109	0	0	0	0
		110	no cells, bad tube			
		111	0	0	0	0
	~~10^{-4}~~	~~112~~				
		~~113~~				
		114				
Type II						
Series 1	Undil	115	4 off			
		116	4 off			
		117	4 off			
Suspended minced tissue	10^{-1}	118	1-2	4	Pooled stored deep freeze 10-30-54	
		119	1-2	4		
		120	2	4		
	10^{-2}	121	0	0	0	0
		122	0	0	0	0
		123	0	0	0	0
	10^{-3}	124	0	0	0	0
		125	0	0	0	0
		126	0	0	0	0
	10^{-4}	127	0	0	0	0
		128	0	0	0	0
		129	0	0	0	0

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Titration 21 day digests

CE Variant Expt

		Tube No	2	4	6	8
Type II	Undil	130	0	0	0	4
Series 2		131	0	0	4	
No tissue		132	0	0	0	0
	10^{-1}	133	0	0	0	0
		134	0	0	0	0
		135	0	0	0	0
	10^{-2}	136	0	0	0	0
		137	0	0	0	0
		138	0	0	0	0
	10^{-3}	139	0	0	0	0
		140	0	0	0	0
		141	0	0	0	0
Type III	Undil	142	3-4	4 4off		
		143	3-4	4 4off		
		144	3-4	4 4off		
Series 1	10^{-1}	145	0	4		
Suspended minced tissue		146	0-1	4		
		147	1	4		
	10^{-2}	148	0	0-1	4off	
		149	0	0	4off	
		150	0	0?	4off	
	10^{-3}	151	0	0	0	0
		152	0	0	0	0
		153	0	0	0	0
	10^{-4}	154	0	0	0	0
		155	0	0	0	0
		156	0	0	0	0
Series 2	Undil	157	3-4	4		
		158	0-1	4		
No Tissue		159	0	0	0 clumpy degen	4off
	10^{-1}	160	0	0	0	0
		161	0	4		
		162	No cells in tube			
	10^{-2}	163	0	0	0	0
		164	0	0	0	0
		165	0	0	0	0

Type III

Series 2 (cont)

	Tube No	2	4	6	8
10^{-3}	166	0	0	0	0
	167	No cells			
	168	No cells bad tube			
10^{-4}	169	No cells bad tube			
	170	0 clumpy deg.	od	od	od
	171	0 vac. D.	od		od

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CE Variant Expt

Titration 28 day aliquots

Viruses 28 day aliquots CE Variant Expt aliquots removed 10-1-54 and stored in deep freeze.

[CE, MK, CE]

Procedure 10 fold dilutions of each virus prepared in Hanks solution - 0.2 ml of dilutes material inoculated 3 tubes / decimal dilution

Trypsinized monkey kidney series 2 used prepared 10-27-54

		Tube No	2	4	6	8
Type I		T_2 - 200	1-2	4		
Series 1 Suspended minced tissue	Undil	201	1-2	4		
		202	1-2	4		
	10^{-1}	203	0	4		
		204	1-2	4		
		205	1	4		
	10^{-2}	206	0	0	0	0
		207	0	0	0	0
		208	0	0	0	0
	10^{-3}	209	0	0	0	0
		210	0	0	0	0
		211	0	0	0	0
Series 2 No tissue	Undil	212	0	0	2	4
		213	0	0	0	0
		214	0	4		
	10^{-1}	215	0	0	0	0
		216	0	0	0	0
		217	0	0	0	0
	10^{-2}	218	0	0	0	0
		219	0	0	0	0
		220	0	0	0	0
	10^{-3}	221	0	0	0	0
		222	0	0	0	0
		223	0	0	0	0

		Tube No.	2	4	6	8
Type II						
Series 1	Undil	224	2	4		
		225	2-3	4		
Suspended minced tissue		226	2	4		
	10^{-1}	227	0-1	4		
		228	0	0	0	0
		229	0	2	4	
	10^{-2}	230	0	0	0	0
		231	0	4		
		232	0	0	0	0
	10^{-3}	233	0	0	0	0
		234	0	0	0	0
		235	0	0	0	0
	10^{-4}	236	0	0	0	0
		237	0	0	0	0
		238	0	0	0	0
Series 2 No tissue	Undil	239	0	0	0	0
		240	0	0	0	0
		241	0	0	0	0
	10^{-1}	242	0	0	0	0
		243	0	0	0	0
		244	0	0	0	0
	10^{-2}	245	0	0	0	0
		246	0	0	0	0
		247	0	0	0	0
Type III	Undil	248	0-1	4		
		249	0-?	4		
Series 1		250	1-2	4		
Suspended minced tissue	10^{-1}	251	0	4		
		252	0	1	4	
		253	0	4		
	10^{-2}	254	0	0	0	0
		255	0	0	0	0
		256	0	0	0	0
	10^{-3}	257	0	0	0	0
		258	0	0	0	0
		259	0	0	0	0
	10^{-4}	260	0	0	0	0
		261	0	0	0	0
		262	0	0	0	0

11-6-54 Pooled stored deep freeze

CE Variant Exp 1 — 28 Day aliquot titration

Type III

Series 2

No tissue

	Tube No	2	4	6	8
Vndel T_2	263	0	4		
	264	0-?	4		
	265	0-?	4		
10^{-1}	266	0	2	4	
	267	0	2	4	
	268	0	0	0	0
10^{-2}	269	0	0	0	0
	270	0	0	0	0
	271	0	0	0	0
10^{-3}	272	0	0	0	0
	273	0	0	0	0
	274	0	0	0	0

Nov 20, 1954 CE Variant Exp 1

- Virus - 36 day aliquot CE Variant Exp 1
aliquots removed 11- -54 & stored in deep freeze.
[CE, MK, CE.]

Procedure 10 fold dilutions of each virus prepared in Hanks solution - 0.2 ml of / dilutes material inoculated 3 tubes / decimal dilution

Trypsinized monkey kidney series 6 used prepared 11-11-54

		Tube No	2	4	6	8
Type I		T6 - 1	0	0	0	0
Suspended tissue	Undil	2	0-1	4		
		3	0	0d	3	4
	10^{-1}	4	0 (fatty degen.)	0d	0d	0d
		5	0 (" ")	0d	0d	0d
		6	0 (Fatty degen)	0d	0d	0d
	10^{-2}	7	0 (Fatty degen)	0d	0d	0d
		8	0	0	0d	0d
		9	0 (Fatty degen)	0d	0d	0d
No tissue	Undil	10	0	0d	0d	0d
		11	0	0	0d	0d
		12	0 (fatty degen)	0d	0d	0d
	10^{-1}	13	0	0	0d	0d
		14	0	0	0d	0d
		15	0 (fatty degen)	0d	0d	0d
Type II	Undil	16	0	0	0	0
		17	0 (fatty degen)	0d	0d	0d
Suspended tissue		18	0	0	0	0
	10^{-1}	19	0	0	0d	0d
		20	0	0	0	0
		21	0 (fatty degen)	0d	0d	0d
	10^{-2}	22	0 (fatty degen)	0d	0d	0d
		23	0 (fatty degen)	0d	0d	0d
		24	0	0	0d	0d
No tissue	Undil	25	0	0d	0d	0d
		26	0 (fatty degen)	0d	0d	0d
		27	0	0	0	0

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Type III

		Tube No.	2	4	6	8
Suspended tissue	Undil	T6-28	0	0	od	od
		29	0	4		
		30	0	0	od	od
	10^{-1}	31	0	0	od	od
		32	od	od	od	od
		33	od	od	od	od
	10^{-2}	34	0	0	od	od
		35	0	0	0	0
		36	od	od	od	od
No tissue	Undil	37	od (fatty)	od	od	od
		38	0	0	od	od
		39	od	od	od	od
	10^{-1}	40	od	od	od	od
		41	od	od	od	od
		42	0	0	od	od
	10^{-2}	43	od	od	od	od
		44	0	0	od	od
		45	od	od	od	od

Oct 4, 1954 CE Variant Exp 2

Purpose To determine

1. Will "CE variant" Polio virus produce a cytopathogenic effect in chick embryo tissue in plasma clot roller tube cultures?

2. Will "CE variant" polio virus multiply in chick embryo tissue in plasma clot roller tube cultures?

Procedure Chick embryo explants prepared and imbedded in plasma clot roller tubes as previously described. Liberal application of chicken plasma made to the inner surface of the tube before chick embryo fragments added. In this instance the eyes and blendes were removed before the embryos were minced to 1 mm size. The medium used was that described on 17 Aug. 1954 - namely 40% inact. normal horse serum 40% Earle's solution and 20% chick embryo extract (1:20) plus 0.05 mg of soybean trypsin inhibitor / ml + antibiotics 50 tubes were prepared

Materials

1. Chick embryo 8 day White Rocks (Mt Healthy)

2. Medium

40% Normal inact horse serum 56° 30 min
Lot 58L Microbiol. Assoc.

40% Earles solution freshly prepared from 10X Concentrated lot 1X 8-14-54

20% CEE 7-31-54 diluted 1:20 10-4-54

Oct 8, 1954 Total of 15 Tubes selected after 4 days incubation at 36°C Tubes with good tissue outgrowth were picked. The medium was removed and the tube washed 1X with Hanks solution. 10 ml CE, K, virus from plasma clot series was then added in 1 ml amounts to each tube 3 tubes per virus type = 9 total. 2 ml of Lactalbumen hydrolysate medium with 5% added inact normal horse serum added to each tube.

6 control tubes had fluid removed, washed once with 10 ml of Hanks, and 3 of Lactalbumin hydrolysate medium with 5% inact normal horse serum added - no virus!

Tubes observed for CPE.

Materials Medium

Lact albumin hydrolysate medium 10-8-54
Inact Normal Horse Serum Lot # 1462 Microbiol. Assoc.
(No polio antibodies)

Virus - stored in deep freeze -

Type I	CE, K_1	Plasma clot series				10-4-54
Type II	"	"	"	"	"	"
Type III	"	"	"	"	"	"

Oct 12, 1954 Time = 4 days after addition of virus

Fluid removed from tubes and saved for titration if such is indicated. Tubes then washed 4X with 10 ml of Hanks solution - Each washing reduces theoretically the amount of virus in the small liquid film on the glass (calculated at 0.1 ml) by a factor of 10^{-2}. New Lact albumin hydrolysate medium with 5% added inact. normal horse serum then added. 30 minutes later 0.4 ml of fluid removed from each tube - fluids of tubes in same virus series pooled and then either titrated immediately or stored in the deep freeze and titrated at a later date.

At 4 days intervals the fluid removed from each tube, fluid from tubes in the same series pooled and either stored or titrated immediately, and 3 ml of fresh medium added.

Tubes observed daily for CPE.

Materials

Horse serum used Oct 12, 1954 = Lot #2467 Microbiol Assoc.
no polio. antibodies

Lactalbumin Hydrolysate medium 10-11-54

CE Variant Exp 2

STANDARD B & P "NOTEAR" Growth Record and Microscopic Observation STANDARD B & P "NOTEAR"

Days (Turned from addition of virus)

Virus	Tube No	0	1	2	3	4	5	6	7	8	9	10	11	12	13	17	18	19	20
I CE, K,	1	good fibro-2 UN cell sheets pigmented lipid granules	No change	clot lysis + retraction several foci	clot retract 2 discrete areas of fibro degen.	Same as controls	More clot retract.	More retract	More retract fibro OK	No								good fibro	
	2	good fibro 1 UN cell sheet pigments + lipid granules	1 New UN cell sheet	" " otherwise no change	Clot retract - 2 areas of discrete necrosis	" "	No change Excellent fibro	No change	No Change Excellent fibro	" "								Excellent fibro	
	3	good fibro 1 UN cell sheet pigments + lipid granules	No change	" "	" "	" "	More clot retract	" "	Discrete areas of fibro necrosis + retract of clot	" "								Retract remain fibro OK	
II CE, K,	4	good fibro pigment + lipid granules 1 UN cell sheet	3 new UN cell sheets	Small area focal fibro degen. - (same seen in control tube 12 + 15)	Clot retract 1 area of discrete necrosis	" "	" "	" "	No change									Extensive retract remain fibro OK	
	5	Same 2 UN cell sheets	No change	" "	" "	" "	" "	" "	New retract -									Retract remain fibro OK	
	6	Same 5 UN cell sheets	No change	" "	" "	" "	" "	" "	More clot retract									Retract + fibro degen good fibro	
III CE, K,	7	Same 3 UN cell sheet	1 New UN cell sheet	clot retract + lysis several foci	Clot retract 2 areas necrosis	" "	" "	" "	No change except clot retract									good fibro	
	8	Same 3 UN cell sheet	3 New UN cell sheet	" "	" "	" "	No change	" "	No change Fibro OK									good fibro UN cells	
	9	Sam 1 UN cell sheet	No change	Several (4) small areas of focal discrete degen.	Clot retract	" "	No change Excellent fibro	" "	UN cells OK									Retract Fibro degen	
Control (Medium only - no virus)	10	Same 1 UN cell sheet	2 new UN cell sheets	Clot lysis several foci	More retract. of clot 2 discrete areas focal necrosis	More retract many areas stellate left	No Change	No Change	No change									good fibro UN cell degen.	
	11	Sam 3 UN cell sheets	1 new UN cell sheet	2 foci UN cell sheets clot lysis	More retract. Necrosis of fibroblast + sheet	" "	" "	" "	By foamy degen 1 UN cell sheet	No change								Retract only - mod foamy degen in cells	
	12	Same 1 UN cell sheet	4 new UN cell sheets	focal fibro degen. 1 area - clot retract	More retract -	" "	" "	" "	More retract Fibro OK	No change								Extensive retract remain fibro OK	
	13	Same 2 UN cell sheets	2 new UN cell sheets	1 new UN cell sheet clot lysis + retraction	2 areas of discrete fibro necrosis clot retract -	" "	More clot retract.	No Change	advanced Clot retract	No change	No							Extensive retract. remain fibro OK	
	14	Same 2 UN cell sheets	No change	1 new UN cell sheet clot lysis + retraction	More retract - 2 discrete areas of focal necrosis	" "	" "	More clot retract little left fibro	Advanced clot retract UN cells - only peripheral	No change								good fibro UN cells mod fibro degen + retract.	
	15	Same 2 UN cell sheets	1 New UN cell sheet	clot lysis + retract. several foci Small areas of focal degen of fibro	More clot retract -	" "	" "	No change	Clot retract mst tissue off glass Bef. peripheral fibro degen -	No change								Marked fibro degen	

a) Procedures performed

b) Remarks

40° horse serum medium removed after 4 days growth - Replaced with Lactalb. hydrolysate with 5% normal horse serum after 1 washing - Virus added!!

Vast majority of fibro. are normal

above described focal degen. assoc. with clot retraction - areas are discrete and present a picture of tissue bunching up

Tubes washed 4X - New medium added

No significant change

No significant change

No significant change

Attempt to Select a Variant or Mutant of Polio Virus Capable of Multiplication in Check Embryo Tissue By Alternate Passage in Chick Embryo and Monkey Kidney Epithelial Tissue Culture

Type	Passage No.	Material inoculated into chick embryo tissue culture	Duration of virus-tissue contact before washing*	Titer of virus TCD50 per ml			
				Time of virus addition	1/2-1 hour after washing	4 days after washing	37 days
I Valid	1	Mahoney KP35	37 days - no washing	$10^{7.5}$	—	—	$10^{3.4}$
	2	Monkey kidney pass. of 37 day fluid pass 1	4 days	$10^{6.5}$	$10^{1.5}$	$10^{1.7}$	—
Not valid NDV	3	Monkey kidney pass. of 8 day fluid pass 2	2 hours	$10^{6.7}$	$10^{3.0}$	$10^{2.5}$	—
	4	Monkey kidney pass. of 4 day fluid pass 3	4 hours	$10^{5.7}$	$10^{3.2}$	$10^{0.7}$	—
II Valid	1	YSK KP54	37 days - no washing	$10^{7.5}$	—	—	$10^{2.9}$
	2	Monkey kidney pass of 37 day fluid pass 1	4 days	$10^{6.0}$	$10^{3.2}$	$10^{2.5}$	—
Not valid NDV	3 a)	Monkey kidney pass of 8 day fluid pass 2	2 hours	$10^{6.7}$	$10^{2.5}$	$<10^{0.7}$	—
	b)		4 hours	$10^{6.7}$	$10^{3.2}$	$<10^{0.7}$ Trace	—
III Valid	1.	Leon KP36	37 days - no washing	$10^{7.0}$	—	—	$10^{2.9}$
	2	Monkey kidney pass. of 37 day fluid pass. 1	4 days	$10^{6.0}$	$10^{2.5}$	$10^{3.0}$	—
Not Valid NDV	3	Monkey kidney pass. of 8 day fluid pass. 2	2 hours	$10^{6.7}$	$10^{2.7}$	$10^{1.7}$	—
	4	Monkey kidney pass of 4 day fluid pass 3.	4 hours	$10^{5.5}$	$10^{2.2}$	$<10^{0.7}$ Trace	—

Washing = 4 washings of 10 ml of Hank's solution each

CE Variant Exp 2

Oct 8, 1954

Day	Date aliquots removed	Titrated or stored	Date titrated if stored	Dil of plasma clot series to be tested 3 tubes/dil	Total number of tubes
4 day	10-12-54	Stored deep freeze	OCT 18 1954	10^{-2}–10^{-6}	45
4 day – 1/2 hour after 4 washings (10 ml each) of tube and addition of new medium.	10-12-54	Stored deep freeze	OCT 18 1954	Undil – 10^{-3}	36
8 day – fluid	10-16-54	Stored deep freeze	OCT 18 1954	Undil – 10^{-4}	45
12 day fluid	10-20-54	Stored deep freeze	OCT 28 1954	Undil – 10^{-3}	36
17 day fluid	10-25-54	Stored deep freeze	OCT 28 1954	Undil – 10^{-3}	36
21 day fluid	10-29-54	Stored deep freeze	Nov 2, 1954	Undil – 10^{-1}	18

CE Variant Exp 2

Quantity of virus in tissue culture tubes containing chick embryo tissue in plasma clot after indicated incubation at 36°C and after indicated washings and fluid removals.

Time after addition of Poliomyelitis virus.	Manipulation performed	Titer of virus in indicated fluid TCD50/ml		
		Type I	Type II	Type III
0 (= calculated amt. introduced)		$10^{6.5}$	$10^{6.0}$	$10^{6.0}$
4 days	Fluid removed	$10^{5.2}$	$10^{6.0}$	$10^{6.0}$
	4 washings (10ml each) – addition of new medium – aliquot removed 30 min. later	$10^{1.5}$	$10^{3.2}$	$10^{2.5}$
8 days	Fluid removed & tested – new fluid added	$10^{1.7}$	$10^{2.5}$	$10^{3.0}$
12 days	Fluid removed & tested – new fluid added	$<10^{0.7}$, $10^{1.0}$*	$<10^{0.7}$, $<10^{0.7}$ trace*	$<10^{0.7}$, trace, $10^{1.0}$*
17 days	Fluid removed and tested – new fluid added	$<10^{0.7}$, $<10^{0.7}$ trace*	$<10^{0.7}$, $<10^{0.7}$*	$<10^{0.7}$, $10^{1.0}$*
21 days	Fluid removed and tested – new fluid added	$<10^{0.7}$ trace*	$<10^{0.7}$*	$<10^{0.7}$*

* Titration using trypsinized monkey kidney culture tubes

CE Variant Exp 2 [-Valid - No NDV]

Quantity of Polio Virus in Tissue Culture Fluid from Chick Embryo in Plasma Clot Tissue Cultures After Indicated Incubation At 36°C and After Designated Manipulations

Time After addition of virus	Manipulation performed	Fluid tested	Titer of virus TCD50/ml		
			Type I	Type II	Type III
0	None	Not tested - Calculated	$10^{6.5}$	$10^{6.0}$	$10^{6.0}$
4 days	Fluid removed	Removed fluid	$10^{5.2}$	$10^{6.0}$	$10^{6.0}$
	Tubes washed 4x c 10ml Hanks sol. Fluid replaced (3ml)	Not tested			
4 days + ½ hour	None	Aliquot of fluid ½ hour after washing	$10^{1.5}$	$10^{3.2}$	$10^{2.5}$
8 days	Fluid replaced	Removed fluid	$10^{1.7}$	$10^{2.5}$	$10^{3.0}$
12 days	Fluid replaced	Removed fluid	$<10^{0.7}$, $10^{1.0}$ *	$<10^{0.7}$, $<10^{0.7}$ Trace *	$<10^{0.7}$ Trace, $10^{1.0}$ *
17 days	Fluid replaced	Removed fluid	$<10^{0.7}$, $<10^{0.7}$ Trace *	$<10^{0.7}$, $<10^{0.7}$ *	$<10^{0.7}$, $10^{1.0}$ *
21 days	Fluid replaced	Removed fluid	$<10^{0.7}$ Trace *	$<10^{0.7}$ *	$<10^{0.7}$ *

* Titration performed with trypsinized monkey kidney culture tubes - all other titrations performed with explant tubes -

OCT 18 1954

CE Variant Exp 2 — Titration of 4 & 8 day fluids

Purpose Titration of 4 & 8 day fluids to determine if virus multiplication has occured.

Procedure 4 and 8 day fluids CE variant Exp 2 which were removed on 10-12 & 10-16-54 (and stored in the deep freeze) were diluted in 10 fold steps in Hanks solution (with full amount of $NaHCO_3$) - 0.2 ml of diluted material inoculated into each tube — 3 tubes / decimal dilution

Tubes used - Monkey kidney tissue culture series 71

		Tube No.	2	3	5	8 (10-26)
Type I 4 day fluid	10^{-2}	71-1	4			
		2	4			
		3	4			
	10^{-3}	4	4			
		5	2	4		
		6	2	4		
	10^{-4}	7	0	4		
		8	0-1	4		
		9	0	4		
	10^{-5}	10	0	0	0	0
		11	0	0	0d	0d
		12	0	0	0d	0d
	10^{-6}	13	0	0	0	0
		14	0	0	0d	0d
		15	0	0	0	0
4 days after 4 washings Vndl		16	1	4		
		17	0-1	4		
		18	0-1	4		
	10^{-1}	19	0	0	4	
		20	0	0	0	0
		21	0	0	0	0
	10^{-2}	22	0	0	0	0
		23	0	0	0	0
		24	0	0	0	0

		Tube No.	2	3	5	8
Type I						
4 day after 4 washings	10^{-3}	71-25	0	0	od	od
		26	0	0	0	od
		27	0	0	0	od
8 day fluid	Undel	28	0	4		
		29	0	4		
		30	0	2-3	4 off	
	10^{-1}	31	0	0	0d	0d
		32	0	0	od	od
		33	0	0-?	4 off	
	10^{-2}	34	0	0	0	0
		35	0	0	0	0
		36	0ε	0	0d	4
	10^{-3}	37	0	0	0	0
		38	0	0	od	od
		39	0	0	0d	od
	10^{-4}	40	0	0	od	od
		41	0	0	0	0
		42	0	0	0	0
Type II	10^{-2}	43	3	4		
4 day fluid		44	4			
		45	3	4		
	10^{-3}	46	0-2	4		
		47	2	4		
		48	0-1	4		
	10^{-4}	49	0	2	4 off	
		50	0	0	4 off	
		51	0	1-2	4 off	
	10^{-5}	52	0-?	3-4		
		53	0	2	4 off	
		54	0	0	0d	0d
	10^{-6}	55	0	0	0	0d
		56	0	0	0	0d
		57	0	0	0	0d

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		Tube No	2	3	5	8
Type II						
4 day after 4 washings	Undil	58	1	4		
		59	1-2	4		
		60	1	4		
	10^{-1}	61	0	0	4 off	
		62	0	4		
		63	0	3	4 off	
	10^{-2}	64	0	1	4 off	
		65	0	1	4 off	
		66	0 d	0 d	0 d	4
	10^{-3}	67	0	0	0 d	0 d
		68	0	0	0	0 d
		69	0	0	0	0 d
8 day fluid	Undil	70	0	4		
		71	0	4		
		72	0	2-3	4 off	
	10^{-1}	73	0	0	4 off	
		74	0	0	0-1	4
		75	0	0	4 off	
	10^{-2}	76	0	0	0	0 d
		77	0	0 d	0 d	0 d
		78	0	0	0	2-3
	10^{-3}	79	0	0	0	0
		80	0	0	0 d	0 d
		81	0	0	0	0
	10^{-4}	82	0 d	0 d	0 d	0 d
		83	0	0	0 d	0 d
		84	0	0	0	0 d
Type III						
4 day fluid	10^{-2}	85	3	4		
		86	2-3	4		
		87	3	4		
	10^{-3}	88	0-?	4		
		89	0-1	4		
		90	0-1	4		

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Type III

4 day fluid

	Tube No	2	3	5	8
10^{-4}	91	0-1	4		
	92	0	2	4 off	
	93	0	2	4 off	
10^{-5}	94	0	0	4	
	95	0	0	4	
	96	0	0	0	0
10^{-6}	97	0	0	0	0
	98	0	0	0	0
	99	0	0	0	0

4 days after 4 washings

Undil	100	0-2	4		
	101	0	1-2	4 off	
	102	0-?	4		
10^{-1}	103	0	0	4	
	104	0	0	4	
	105	od	od	4 off	
10^{-2}	106	0	0	od	4
	107	od	od	fifty day od	od
	108	0		0	0
10^{-3}	109	0	0	0	0
	110	0	0	od	od
	111	0	0	0	0

8 day

Undil	112	0	0 contaminated	off	
	113	0	4 contaminated		
	114	od	od contaminated	off	
10^{-1}	115	0	0	0-?	4
	116	0	0	0	4
	117	0	0	1-2	4
10^{-2}	118	0	0	0	0
	119	0	1	off	
	120	0	0	0	4
10^{-3}	121	0	0	0	0
	122	0	0	od	od
	123	0	0	0	od
10^{-4}	124	0	0	0	od
	125	0	0	0	od
	126	0	0	od	od

CE Variant Exp 2

Oct 28, 1954

Purpose Titration of 12 & 17 day fluids –

Procedure 12 & 17 day fluids CE Variant Exp 2 which were removed on Oct 20 & 25, 1954 (and stored in the deep freeze) were ~~diluted~~ 10 fold in Hanks solution (with full amount of $NaHCO_3$) 0.2 ml of diluted material inoculated into each tubes –

Tubes used – Monkey kidney tissue culture series 77, 78

		Tube No	2	4	6	8
Type I						
	Undil 77 –	1	0	0	0	od
12 day		2	0	0	0	od
		3	0	0	od	od
	10^{-1}	4	0	od	od	od
		5	od (foamy)	od	od	od
		6	0	od	od	od
	10^{-2}	7	0	od	od	od
		8	od	od	od	od
		9	od	od	od	od
	10^{-3}	10	0	od	od	od
		11	od	od	od (marked)	off
		12	0	0	od	od
17 day	Undil	13	0	od	od	od
		14	od	od	od (marked)	off
		15	0	0	0	od
	10^{-1}	16	0	0	od	od
		17	0	od	od	od
		18	od	od	od	od
	10^{-2}	19	0	0	od	od
		20	0	0	0	0
		21	0	0	od	od
	10^{-3}	22	0	0	od	od
		23	0	0	0	0
		24	od	od	od	od

		Tube No	2	4	6	8
Type II	Undil	25	0	od	od	od
		26	0	0	od	od
12 day		27	0	od	od alk.	
	10^{-1}	28	0	0	od	od
		29	0	0	od	off
		30	0	od	od	off
	10^{-2}	31	0	0	od	od
		32	od	od	od	off
		33	0	0	0	0
	10^{-3}	34	0	od	od	od
		35	od	od	od	od
		36	0	0	od	od
17 day	Undil	37	0	0	od	od
		38	0	0	0	0
		39	0	0	0	0
	10^{-1}	40	0	od	od	od
		41	0	0	od	od
		42	0	0	0	od
	10^{-2}	43	0	0	od	od
		44	od	od	od	od
		78-45	od	od	od	od
	10^{-3}	46	0	od	od	od
		47	0	od	od	od
		78-48	0	0	0	0
Type III	Undil	49	0	0	0	0
		50	od	od	od (foamy)	od
12 day		51	0	0	0	4
	10^{-1}	52	0	0	0	0
		53	0	0	0	0
		54	od (clumpy)	Od	od	od
	10^{-2}	55	0	0	0	0
		56	0	0	0	0
		57	0	0	0	0
	10^{-3}	58	0	0	0	0
		59	0	0	0	0
		60	0	0	0	0

Oct 28, 1954 CE Variant Exp 2 (cont) 12 & 17 day fluids

Type III

#17 day fluid

	Tube No 78-	2	4	6	8
Undil	61	0	0	0	0
	62	0	0	0	0
	63	0	0	0	0
10^{-1}	64	0	0	0	0
	65	0	0	0	0
	66	0	0	0	0
10^{-2}	67	0	0	0	0
	68	0	0	0	0
	69	0	0	0	0
10^{-3}	70	0	0	0	0
	71	0	0	0	0
	72	0	0d	0d	0d

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CE Variant Exp 2 Repeat titration

Purpose Titration of 12 & 17 day fluids

Procedure – 12 & 17 day fluids (CE variant Exp 2 which were removed from deep freeze) were diluted 10 fold in 1/2 Hanks solution (with 1/2 full amount of $NaHCO_3$). 10-20 & 10-25-54 (stored in

0.2 ml of diluted material inoculated into each tube

Tubes used Trypsinized series No. 4 prepared 11-4-54

		Tube No	2	4	6	8
Type I	Undil	- 1	0	4		
		- 2	0	1	4	
12 day		- 3	0	0	0	0
	10^{-1}	- 4	0	0	0	0
		- 5	0	0	0	0
		- 6	0	0	0	0
	10^{-2}	- 7	0	0	0	0
		- 8	0	0	0	0
		- 9	0	0	od	od
17 day	Undil	- 10	0	0	0	0
		- 11	0	4		
		- 12	0	0	0	0
	10^{-1}	- 13	0	0	0	0
		- 14	0	0	0	0
		- 15	0	0	0	0
Type II	Undil	- 16	0	0	0	0
		- 17	0	0	0	0
12 day		- 18	0	3	4	
	10^{-1}	- 19	0	0	od	od
		- 20	0	0	od	od
		- 21	0	0	0	0
	10^{-2}	- 22	0	0	0	0
		- 23	0	0	0	0
		- 24	0	0	0	0
17 day	Undil	- 25	0	0	0	0
		- 26	0	0	od	od
		- 27	0	0	0	0
	10^{-1}	- 28	0	0	0	0
		- 29	0	0	0	0
		- 30	0	0	0	0

Type III

		Tube No.	2	4	6	8
12 day	Undil	- 31	0	0	4	
		- 32	0	0	0	0
		- 33	0	0	2	4
	10^{-1}	- 34	0	0	0	0
		- 35	0	0	0	0
		- 36	0	0	0	0
	10^{-2}	- 37	0	0	0	0
		- 38	0	0	od	od
		- 39	0	0	0	0
17 day	Undil	- 40	0	4		
		- 41	0	4		
		- 42	0	0	0	0
	10^{-1}	- 43	0	0	0	0
		- 44	0	0	0	0
		- 45 ✓	0	0	0	0

[redacted]

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CE Variant Exp 2

Titration of 21 day fluids

Versus 21 day fluids removed from tubes of CE variant Exp 2 - [CE, MK, CE,] fluids stored in deep freeze prior to titration -

Procedure 10 fold dilutions of each virus prepared in Hanks solution (full amt. $NaHCO_3$)

0.2 ml of diluted material inoculated

3 tubes / decimal dilution -

Trypsinized monkey kidney series 2 used prepared 10-27-54

		Tube No.	2	4	6	8	
Type I	Undil	T2-275	0	0	0	0	
		276	0	0	0	0	
		277	0	0	0?	4	Stored deepfreeze
	10^{-1}	278	0	0	0	0	
		279	0	0	0	0	
		280	0	0	0	0	
Type II	Undil	281	0	0	0d	0d	
		282	0	0	0	0	
		283	0	0	0	0	
	10^{-1}	284	0	0	0	0	
		285	0	0	0	0	
		286	0	0	0	0	
Type III	Undil	287	0	0	0	0	
		288	0	0	0	0	
		289	0	0	0	0	
	10^{-1}	290	0	0	0	0	
		291	0	0d	0d	0d	
		292	0	0	0	0	

Nov 20, 1954 — Titration of 28 day effluent Type I

Virus [CE, MK, CE,] CE Variant Exp 2

28 day fluid removed 11- -54 and stored in deep freeze -

Procedure 10 fold dilutions prepared in Hanks Solution - 0.2 ml of diluted material inoculated 3 tubes / decimal dilution - Trypsenized monkey kidney series No 6 of 11-11-54

Type I 28 day fluid

	Tube No:	2	4	6	8 used
Undil	T_6-46	0	od	od	od
	47	0	od	od	od
	48	0	0	0	od
10^{-1}	49	0	0	od	od
	50	0	0	0	od
	51	0	0	od	od

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CE, MK, CE, MK,

2nd Criss Cross Passage of Types I, II, & III Polio Viruses

Criss Cross Passage No 2

Virus - CE, MK, CE, of plasma clot series - fluid removed ~~at~~ 8 days - stored in deep freeze -

Procedure 0.5 ml of fluid inoculated into each of 4 trypsinized monkey kidney culture tubes - Series T2

	Tube No	1	2	3	
Type I	T2-293	0	1	4	Fluid Harvested
	294	0	0?	4	Stored deep freeze 11-5-54
	295	0	1-2	4	
	296	0	1	4	
Type II	297	0	0-?	4	Fluid Harvested
	298	0	1	4	Stored deep freeze
	299	0	0-?	4	11-5-54
	300	0	0-?	4	
Type III	301	0	0-?	4	Fluid harvested
	302	0	0-1	4	Stored deep freeze
	303	0	0	4	
	304	0	0-?	4	11-5-54

Nov 3, 1954

Preparation of chick embryo tissue culture -

8 day White Rock embryos harvested, minced, washed and embedded in a plasma clot in roller tubes as previously described. The medium used was 40% inact normal horse serum (Lot # 2467 Microbiol Assoc) 40% Earles solution 20% Chick embryo extract (Lot # 31-54) (1:20) containing antibiotics and trypsin inhibitor 0.1 mg/ml.

- 50 tubes prepared -

Nov 8, 1954 CE Variant Adsorb. Experiment

Purpose 1. To wash chick embryo cultures after allowing for absorption of virus to occur (2 hours) in order to detect subsequent virus multiplication if such occurs.

2. To study the quantitative aspects of poliomyelitis virus adsorption to chick embryo tissue in roller tube plasma clot cultures.

3. To perform the CE passage portion of the 3rd CE - monkey kidney criss cross passage series.

Procedure - 19 of the best tubes prepared on Nov 3, 1954 were selected for use -

1.) Fluid was removed - replaced with 10 ml of Hanks solution (1/3 $NaHCO_3$) - Hanks solution removed -

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2) Tubes distributed in the following manner.

0 Time

Tube No			
Series 1)	1, 2, 3, 4, 5.	2 ml of Lactalbumin hydrolysate medium (pH 8.2)	+ 1 ml of Type I criss cross passage No 2 CE, MK, CE, MK, harvested Nov 5, 1954.
Series 2)	6, 7, 8, 9, 10	"	+ 1 ml of Type II criss cross passage No. 2 CE, MK, CE, MK, harvested Nov 5, 1954.
Series 3)	11, 12, 13, 14, 15	"	+ 1 ml of Type III criss cross passage No. 2 CE, MK, CE, MK, harvested Nov 5, 1954
Control)	16, 17, 18, 19	3 ml of Lactalbumin hydrolysate medium (pH 8.2)	

3) Tubes were placed in the incubator in the roller drum and allowed to rotate for 2 hours.

2 hours

4.) Entire fluid from each tube removed - → stored in deep freeze ←

5.) Tubes washed 4X with 10 ml of Hanks solution (1/3 $NaHCO_3$).

→ 4th wash fluid stored deep freeze ←

6.) 3 ml of Lactalbumin hydrolysate added to each tube. Tubes returned to roller drum and allowed to rotate for 2 hours.

4 hours 7.) Fluid removed → stored deep freeze ←

3 ml of Lactalbumin hydrolysate medium containing 5% inact. normal horse serum (Lot # 2467) and

Nov 8, 1954

trypsin inhibitor 0.1 mg/ml. added – tubes placed in roller drum.

Tubes 3, 4 Type I
9, 10 Type II
14, 15 Type III

After fluid removed – tissue and clot from tubes of the same series removed with wooden stick – placed in glass tissue grinder (Ten Broeck) and weighed

Type I tissue = 25 mg
Type II " = 28 mg
Type III " = 25 mg

Tissue ground as a 1:40 suspension in Hanks' solution in the glass tissue grinder –
→ Suspension stored in deep freeze

5 hours 8.) After 1 hours rotation in the roller drum the remaining tubes were removed and 0.5 ml of fluid removed. Fluid from the tubes of the same series pooled and → stored in deep freeze ←

4 Day Nov 12 9.) Fluid removed from tubes → stored in deep freeze ←

New fluid added – Lactalbumin hydrolysate medium pH 8.2 containing 5% inact normal horse serum (Lot # 2467) and 0.05 mg/ml trypsin inhibitor per ml. 3 ml of this medium added per tube.

8 Days Nov 16, 1954 10.) Fluid removed → stored in deep freeze ←

Tube No.	1	2	3	4	5	6	7	8
				Fluid Changed				
I 1	Some retract. + degen + disruption of fibro outgrowth - many good areas left	No change	Mod fibro degen - some good areas left	good new fibro		Marked retract + degen		
I 2	Mild degen. + retract of fibro	Mod fibro degen - some good areas left	same	same		" "		
I 3	" "	" "	same - few fibro in good condition	good un cell sheet		" " some good fibro left		
II 6	" "	" "	" "	Same		Marked retract + degen -		
II 7	Moderate retract + fibro disruption	Same	Mod. fibro degen -	Same		" "		
II 8	Mod. disruption + degen of fibro 3 un cell sheets	Same	Un cell degen.	new un cells		" "		
III 11	Mod. disruption + degen of fibro outgrowth	—						
III 12	good fibro c̄ some degen. 1 un cell sheet	Same - good fibro	Mod fibro degen -	Same		" "		
III 13	Mod. disruption of fibro - some degen 2 un cell sheet	Same	un. + fibro degen - mod	Same				
Control 16	Fair fibro 1 un cell sheet some disruption + degen of cell outgrowth - washing?	No change	Fibro degen - good un cells in 1 sheet	Retract + degen un cells		Marked retract + degen.		
Control 17	" " some disruption of cell outgrowth + mod. degen.	mod fibro degen -	marked fibro degen - good sheet un cells	Same		" "		
Control 18	Excellent fibro 1 un cell sheet minimal fibro degen.	mod fibro degen	no change	un cell retract		" "		
Control 19	Considerable disruption of outgrowth - many good areas left.	No change	mod. fibro + un cell degen	Same		" "		

Day

[Note: unused + uninoculated tubes of this series show similar degen at this time]

No significant change

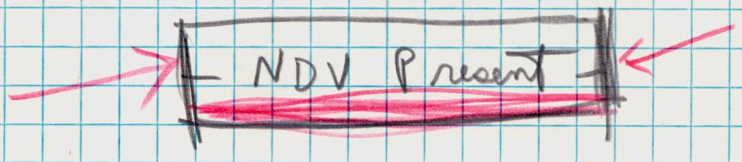
No significant change

Retraction + degeneration marked - metabolism continues as evidenced by pH of 6.8 - 7.3 in all tubes

Chick Embryo Tissue Culture Adsorption Exp

Quantity of Polio Virus Recoverable From ~~Chick~~ Embryo Tissue or Tissue Culture Fluid At Indicated Time and After Designated Manipulations.

Time after addition of virus	Manipulation performed	Fluid or tissue tested	Titer of virus TCD_{50}/ml or gm		
			Type I	Type II	Type III
0	None	Not tested – calculated	$10^{6.7}$	$10^{6.7}$	$10^{6.7}$
2 hours	Fluid removed – Tubes washed 4X w Hanks sol 10ml – Fluid replaced 3ml	Removed fluid	$10^{7.0}$	$10^{6.5}$	$10^{6.5}$
		4th wash fluid	$10^{2.2}$	$10^{2.0}$	$10^{2.5}$
4 hours	Fluid replaced	Removed fluid	$10^{4.0}$	$10^{4.5}$	$10^{4.0}$
	Tissue removed 2 tubes each series	Tissue susp. (wt=25mg)	$10^{3.8}$	$10^{4.1}$	$10^{3.3}$
5 hours	None	aliquot of fluid 1hr after fluid change	$10^{3.0}$	$10^{2.5}$	$10^{2.7}$
4 days	Fluid removed	Removed fluid	$10^{2.5}$	$<10^{0.7}$	$10^{1.7}$



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CE Adsorption Experiment

Quantity of Polio Virus in Tissue or Tissue Culture Fluid

NOV 1 1 1954

Comparative Titration of Monkey Kidney Passage of 2nd Cross Cross Passage of Plasma Clot Series. Trypsinized vs Explant

Viruses CE, MK, CE, MK, harvested 11-5-54 of plasma clot series — Stored in deep freeze.

Procedure 10 fold dilutions made in Hanks solution (full amount $NaHCO_3$). 0.2 ml of diluted material inoculated into each tube.

Tubes used Trypsinized monkey kidney series no. 4 11-4-54

Explant monkey kidney series no. 85 & 86

Type I

	Trypsinized Tube No.	2	4	6	8
10^{-4}	T4- 46	2-3	4		
	47	2-3	4		
	48	3	4		
	49	3	4		
10^{-5}	50	1	4		
	51	1-2	4		
	52	0.?	4		
	53	1	4		
10^{-6}	54	0	4		
	55	0	4		
	56	0	0	4	
	57	0	4		
10^{-7}	58	0	0	0	0
	59	0	0	0	0
	60	0	0	0	0
	61	0	0	0	0
10^{-8}	62	0	0	0	0
	63	0	0	0	0
	64	0	0	0	0
	65	0	0	0	0

$10^{7.2}$ TCD50/ml

	Explant Tube No.				
10^{-4}	85- 1	3	4		
	2	3	4		
	3	3	4		
	4	3	4		

Type I — Explant Tube No.

		2	4	6	8
10^{-5}	- 5	0	od dumpy + nitrite	od	od
	- 6	0	4		
	- 7	1	4		
	- 8	0	od	od	od
10^{-6}	- 9	0	od	od	od
	- 10	0	od	od	od
	- 11	1	4		
	- 12	0	od	od	od
10^{-7}	- 13	0	od	od (foamy)	od
	- 14	0	od	od	od
	- 15	0	od	od	od
	- 16	0	od	od	od
10^{-8}	- 17	0	od	od	od
	- 18	0	od	od	od
	- 19	0	od	od	od
	- 20	0	od	od	od

$10^{5.9}$ TCD_{50}/ml

Type II — Inoculated Tube No.

10^{-4} T4	- 66	2-3	4		
	- 67	2	4		
	- 68	3	4		
	- 69	3	4		
10^{-5}	- 70	0-1	4		
	- 71	0-1	4		
	- 72	0	4		
	- 73	0-1	4		
10^{-6}	- 74	0-?	4		
	- 75	0	0	0	0
	- 76	0 contamin	oc	4	
	- 77	0	0	od	od
10^{-7}	- 78	0	4		
	- 79	0	0	0	0
	- 80	0	2	4	
	- 81	0	0	0	0
10^{-8}	- 82	0	0	0	0
	- 83	0	0	0	0
	- 84	0	0	0	0
	- 85	0	0	0	0

$10^{7.2}$ TCD_{50}/ml

NOV 1 1 1954

Comparative Titration CE, MK, CE, MK, Plasma Clot Series

Trypsinized vs Explant Tubes

Type II		Explant Tube No.	2	4	6	8
10^{-4}	85-	21	3	4		
	-	22	3	4		
	-	23	3	4		
	-	24	4			
10^{-5}						
	-	25	0	4		
	-	26	0.2	4		
	-	27	0	4		
	-	28	0	4		
10^{-6}	-	29	0	od	od	od
	-	30	0	od	od	od
	-	31	0	od	od	od
	-	32	0	od	od	3-4
10^{-7}	-	33	0	od	od	od
	-	34	0	od	od	od
	-	35	0	od	od	od
	-	36	0	od	od	od
10^{-8}	-	37	0	od	od	od
	-	38	0	od	od	od
	-	39	0	od	od	od
	-	40	0	od	od	od

$10^{6.4}$ TCD50/ml

Type III		Trypsinized tube No.				
10^{-4}	Ty	- 86	2	4		
		- 87	1	4		
		- 88	2	4		
		- 89	2	4		
10^{-5}		- 90	1	4		
		- 91	1	4		
		- 92	0-?	4		
		- 93	0-1	4		
10^{-6}		- 94	0	0	0	4
		- 95	0	0	4	
		- 96	0	0	2	4
		- 97	0	0	4	
10^{-7}		- 98	0	0	0	0
		- 99	0	0	0	0
		- 100	0	0	0	0
		- 101	0	0	0	0

$10^{7.2}$ TCD50/ml

Type III

	Trypanized Tube No.	2	4	6	8
10^{-8}	- 102	0	0	0	0
	- 103	0	0	0	0
	- 104	0	0	0	0
	- 105	0	0	0	0
	Explant Tube No				
10^{-4}	- 41	0-?	4		
	- 42	2	4		
	- 43	1	4		
	- 44	0	4		
10^{-5}	- 45	0	od	od	od
	- 46	0	4		
	- 47	0.2	4		
	- 48	0	1	4	
10^{-6}	- 49	0	od	od	od
	- 86-1	0	od	od	od
	- 2	0	od	od	od
	- 3	0	od	od	od
10^{-7}	- 4	0	od	od	od
	- 5	0	od	od	od
	- 6	0	od	od	od
	- 7	0	od	od	od
10^{-8}	- 8	0	od	od	od
	- 9	0	od	od	od
	- 10	0	od	od	od
	- 11	0	od	od	od

$10^{6.0}$ TCD50/ml

STANDARD B&P "NOTEAR"

(1) CE Adsorption Exp. Titration of 2 hour, 4th wash, 4 hour, 5 hour, 4 day fluids and 4 hour tissue susp.

Nov 15, 1954

Also. 3rd cross cross passage of polio types I, II, III viruses –

Viruses 2 hour, 4th wash fluid, 4 hour, 5 hour, and 4 day fluids and 4 hour tissue suspension. Stored in deep freeze.

Procedure 10 fold dilutions made in Hanks solution (full amt. of $NaHCO_3$) 0.2 ml of ~~dilutes~~ material inoculated into each tube 3 tubes / decimal dilution.

Tubes used Trypsinized monkey kidney series 4 11/4/54
+5 11/5/54

Type I

	Tube No.	2	4	6	8
2 hour fluid 10^{-4}	1	4			
	2	4			
	3	4			
10^{-5}	4	1	4		
	5	4			
	6	1	4		
10^{-6}	7	0	0	0	0
	8	0	4		
	9	0	2	4	
10^{-7}	10	0	0	0	0
	11	0 mold	0	0	0
	12	0 mold	0	0	0
4th washing 10^{-1}	13	4			
	14	1	4		
	15	1-2	4		
10^{-2}	16	0	0	0	0 d
	17	0	0	0	0 d
	18	0	0	0	0
10^{-3}	19	0	0	0	0
	20	0	0	0	0
	21	0	0	0	0

STANDARD B&P "NOTEAR"

Type I

	Tube No.		2	4	6	8
4th wash fluid	10^{-4}	22	0	0	0	0
		23	0	0	0	0
		24	0	0	0	0
	10^{-5}	25	0	0	0	0
		26	0	0	0	0
		27	0	0	0	0
4 hour fluid 2 hrs after washing	10^{-1}	28	3	4		
		29	4			
		30	4			
Fluid removed	10^{-2}	31	1	4		
		32	4			
		33	1	4		
	10^{-3}	34	0	4		
		35	0	4		
		36	0	0	0	0
	10^{-4}	37	0	0	0	0
		38	0	4		
		39	0	0	0	0
4 hour tissue suspension	$10^{-1.6}$	40	3	4		
		41	4			
		42	2	4		
2 hrs after washing	$10^{-2.6}$	43	2	4		
		44	2	4		
		45	0	2	4	
	$10^{-3.6}$	46	0	0	od	od
		47	0	0	0	0
		48	0	0	0	0
	$10^{-4.6}$	49	0	0	0	0
		50	0	0	0	0
		51	0	0	od	od
	$10^{-5.6}$	52	0	0	0	0
		53	0	0	0	0
		54	0	0	0	0
	$10^{-6.6}$	55	0	0	0	0
		56	0	0	0	od
		57	0	0	0	od

(2) Nov 15, 1954

CE Adsorption Exp Titration

	Tube No.	2	4	6	8
Type I					
5 hour fluid	10^{-1} - 58	3	4		
	- 59	2	4		
	- 60	3	4		
1 hr after 2nd change of fluid	10^{-2} 61	- 0 mold	0 d	0 deg almost complete	0 d few cells left
	62	- 0	0?	2	4
	63	- 0?	4		
	10^{-3} 64	- 0	0	0	0
	65	- 0	0	0	0
	66	- 0	0	0	0
	10^{-4} 67	- 0	0	0	0
	68	- 0	0	0	0
	69	- 0	0	0	0
4 day fluid	10^{-1} 70	- 0-?	4	Fluid was harvested - pooled & stored deep freeze 11-19-54	
	71	- 1	4		
	72	- 1	4		
	10^{-2} 73	- 0	0	0	0
	74	- 0	0	0	0 d
	75	- 0	4		
	10^{-3} 76	- 0	0	0	0
	77	- 0	0	0	0
	78	- 0 mold	0	0	0
	10^{-4} 79	- 0 mold	0	0	0
	80	- 0	0	0	0
	81	- 0	0	0	0
Type II					
2 hour fluid	10^{-4} 82	- 2-3	4		
	83	- 2	4		
	84	- 3	4		
	10^{-5} 85	- 0	4		
T4	T4 - 118	- 0	4		
	119	- 0	1	4	
	10^{-6} 120	- 0	0	0 d	0 d
	121	- 0	1	4	
	122	- 0	0 d	0 d	0 d
	10^{-7} 123	- 0	0	0 d	0 d
	124	- 0	0	0	0
	125	- 0	0	0	0

Type II

		Tube No.	2	4	6	8
4th washing	10^{-1}	T4-126	0	3	4	
		127	0	od	od	od
		128	0 (contam)	4		
	10^{-2}	129	0	0	od	od
		130	od	od	od	od
		131	0	0	od	od
	10^{-3}	132	0	0	0	0
		133	od	od	od	od
		134	0	0	0	0
	10^{-4}	135	0	0	0	0
		136	0	0	0	0
		137	0	0	0	0
	10^{-5}	138	0	0	0	0
		139	0	0	0	0
		140	0	0	0	0
4 hour fluid	10^{-1}	141	2	4		
		142	2	4		
		143	2	4		
	10^{-2}	144	0?	4		
		145	0?	4		
		146	0	4		
	10^{-3}	147	0	0	4	
		148	od	4		
		149	0	2	4	
	10^{-4}	150	od	od	od	od
		151	0	0	0	0
		152	0	0	4	
4 hour tissue suspension	$10^{-1.6}$	153	1	3	4	
		154	0?	0?	4	
		155	od	od	4	
	$10^{-2.6}$	156	0	0	4	
		157	0	2	4	
		158	0	0?	3	4
	$10^{-3.6}$	159	0	0?	4	
		160	0	0?	od	od
		161	0	0	0	0
	$10^{-4.6}$	162	od	od	od	od
		163	od	od	od	od
		164	0	0	od	od

(3) Nov 15, 1954 CE Adsorption Exp Titration

Type II	Tube No.	2	4	6	8
4 hour Tissue Suspension $10^{-5.6}$	165	0	0	0	0
	166	0	0	0	0
	167	0	0	0	0
$10^{-4.6}$	168	0	0	od	od
	169	0	0	od	od
	170	0	0	0	0
5 hour fluid 10^{-1}	171	0	od	4	
	172	0	0.?	3	4
	173	0	0?	4	
10^{-2}	174	0	0	0	0
	175	0	0	0	0
	176	od	od	4	
10^{-3}	177				
	178	0	0	0	0
	179	0	0	od	od
		0	0	0	0
10^{-4}	180	0	0	0	0
	181	d(contam)	0	0	0
	182	0	od	od	od
4 day fluid 10^{-1}	183	0	od	od	od
	184	0	0	0	od
	185	0	0	0	od
10^{-2}	186	0	0	0	od
	187	0	0	0	0
	188	0	od	od	0
10^{-3}	189	0	0	0	od
	190	0	0	0	od
	191	0	0	0	od
10^{-4}	192				
	193	0	0	0	0
	194	od	od	od	od
		0	0	od	od
Type III					
2 hour fluid 10^{-4}	195	3	4		
	196	2-3	4		
	197	2	4		
10^{-5}	198	0	4		
	199	1	4		
	200	2	4		

Type III	Tube N.		2	4	6	8	9
2 hour fluid	10^{-6}	I4-201	0	0	0	0	
		- 202	0	0	0	2	4
		- 203	0	0	0	0	
	10^{-7}	- 204	0	0	0	0	
		- 205	0	0	0	0	
		- 206	0	0	0	0	
4th washing	10^{-1}	- 207	0-?	4			
		- 208	1-2	4			
		- 209	1	4			
	10^{-2}	210	0	4			
		211	0	0	0	0	
		212	0	0	0	0	
	10^{-3}	213	0	0	0	0	
		214	0	0	0	0	
		215	0	0	0	0	
	10^{-4}	216	0	0	0	0	
		217	0	0	0	0	
		218	0	0	0	0	
	10^{-5}	219	0	0	0	0	
		220	0	0	0	0	
		221	0	0	0	0	
4 hour fluid	10^{-1}	222	2	4			
		223	2	4			
		224	2	4			
	10^{-2}	225	2	4			
		226	1	4			
		227	0	4			
	10^{-3}	228	0	0	0	0	
		229	0	4			
		230	0	4			
	10^{-4}	231	0	0	0	0	
		232	0	0	0	0	
		233	0	0	0	0	
4 hour tissue suspension	$10^{-1.6}$	234	2	4			
		235	0	0?	3	4	
		236	0	3	4		
	$10^{-2.6}$	237	0	3	4		
		238	0	0?	od	od	
		239	0	0	od	od	

(4)

Nov 15, 1954 CE Adsorption Exp titration

			2	4	6	8
Type III	Tube No					
4 hour trivalent	10-3.6	14240	0	0	od	od
suspension		241	0	4		
		242	0	0	0	0
	10-4.6	243	0	0	0	od
		244	0	0	0	0
		245	0	0	0	0
	10-5.6	246	0	0	0	0
		247	0	0	0	0
		248	0	0	0	0
	10-6.6	249	0	od	od	od
		250	0	0	od	od
		251	0	0	0	od
5 hr. fluid	10-1	252	0-?	4		
		253	1	4		
		254	0-1	4		
	10-2	255	0	0	0	0
		256	0	4		
		257	0	od	od	od
	10-3	258	0	0	0	0
		259	0-?	4		
		260	0	0	0	0
	10-4	261	0	0	0	0
		262	0	0	0	0
		263	0	0	0	0
4 day fluid	10-1	264	0	4	Fluids harvested, pooled, stored in deep freeze 11-19-54	
		265	0	4		
		266	0	0	0	0
	10-2	267	0	0	0	0
		268	0	0	0	0
		269	0	0	od	od
	10-3	270	0	0	0	0
		271	0	0	0	0
		272	0	0	0	0
	10-4	273	0	0	0	0
		274	0	0	0	0
		275	0	0	0	0

Nov 18, 1954 Testing of undiluted fluid from 4 day fluids of Type II + III series of adsorb. experiment –

10^{-1} dilution negative on 2nd day of titration – in order to obtain monkey kidney culture virus for passage to check embryo in the cross cross pattern which has been established this test is necessary –

4 day fluid

Type II

undiluted

0.2 ml/tube

Tube No		2	3	4	5	6	7	8
T5 -	86	0	0	0	od	od	od	od
	87	0	0?	0?	od	od	od	
	88	0	0?	0?	od	od	od	
	89	0	0	0	od	od	od	

Type III

undiluted

0.2 ml/tube

T5 -	90	0-?	3	4	} Pooled, stored			
	91	0	0?	0	od	od	od	od
	92	0-1	2-3	4	} in deep freeze			
	93	0-1	3	4	} 11-22-54			

Nov 22, 1954 Repeat titration of 4 day aliquot Type II

4 day fluid II

undiluted

0.2 ml/tube

Tube No.	2	3	5	6	8
T6-1	0	0	od	od	od
-2	0?	0	od	od	od
3	0	0	od	od	od
4	0	0	od	od	od

– Nov 10, 1954 –

Preparation of chick embryo tissue culture

10 8 day White Rock embryos harvested, minced, washed 6x in Hanks (1/3 $NaHCO_3$) and embedded in a plasma clot in roller tubes as described previously. The medium used was 40% inact normal horse serum (Lot # 2467 Microbiol. Assoc) 40% Earles solution and 20% chick embryo extract (1:20 — Lot 7-31-54) containing antibiotics and trypsin inhibitor 0.05 mg/ml

44 tubes prepared –

Nov 16, 1954 6 days after preparations –

1.) tubes examined – good fibro growth – dense undifferentiated cell sheets

2.) 10 tubes discarded because of clot retraction

3.) Remaining tubes – fluid removed – replaced with 2 ml of Lactalbumin hydrolysate medium containing 5% inact. normal horse serum (Lot #2467), antibiotics, and trypsin inhibitor 0.05 mg/ml (Lot of 4-8-54 Worthington S-52193 Biochem)

Nov 26, 1954 4 days after change of medium
10 days after Preparation

Moderate-Marked fibroblastic degeneration –

Series discarded – not used for passage of virus –

Dec 9, 1954 Preparation of Chick embryo tissue cultures

10 9 day White Rock embryos harvested, minced, washed 6X in Hank's solution (1/3 $NaHCO_3$) and embedded in a plasma clot as described previously. The medium used was 40% ~~in~~ normal horse serum (Lot # 2467) 40% Earles solution (freshly prepared) and 20% chick embryo extract (1:20 Lot 7-31-54) The medium contained antibiotics and trypsin inhibitor 0.05 mg/ml (Worthington Biochem Lot S 5300 reconstituted 11-16-54) 2 ml of medium per tube.

- 50 tubes prepared -

In addition 9 tubes had chicken plasma applied - 3 drops of 1:10 CEE then added to clot the plasma - these tubes had the above medium added (2 ml) -

Dec 14, 1954 5 days after preparation

1. Tubes examined

40 tubes - good fibro growth in the same unidentified cell sheets -

10 Tubes - clot retraction - discarded -

10 tubes used in CE [redacted] Polio Exp - see next page

3 tubes used CE variant Exp 4 3rd passage

27 tubes - fluid removed - replaced c 2 ml of Lot ~~#~~ alk. hydrolysate medium pH 8.2 c 5% normal horse serum added (Lot # 2467) plus antibiotics + Trypsin inhibitor

Dec 14, 1954 CE - ~~Polio~~ Adsorption Washing ↓ Exp 2 [Washing After 4 Days for Adsorption]

Purpose 1) To detect mutants of polio verus capable of multiplication in chick embryo tissue culture (grown on plasma clot in roller tube)

2) 4 Day adsorption and multiplication interval to be employed before cultures washed to establish baseline for the demonstration of possible verus multiplication

Procedure 11 of the best tubes prepared on 12-9-54 selected for use - Also 6 of the tubes containing only plasma clot

Washing out of old medium

1.) Fluid was removed - replaced c̄ 10 ml of Hank's solution (1/3 $NaHCO_3$) - Hank's solution removed

2) Tubes distributed in following manner

Tube No	CE.		
Chick Embryo Tissue	1. 2.	2 ml of Lactalb. medium pH 8.2 c̄ antibiotics + trypsin inhibitor	+ 1 ml of Type I Mahoney KP 36 prepared 12-6-54 in trypsinized bottles
	3. 4.	" " " "	+ 1 ml of Type II YSK KP55 prepared 12-6-54 in trypsinized bottles
	5. 6.	" " " "	+ 1 ml of Leon KP 37 prepared 12-9-54 in trypsinized bottles
	7 8 9 10	3 ml " " "	No virus - controls

Dec 17, 1954

Tube No. C.P.
(= Clotted Plasma Only)

1.	2 ml of Lactalb.		+ 1 ml Mahoney KP36
2.	medium pH 8.2		
3	"	"	+ 1 ml YSK KP55
4			
5	"	"	+ 1 ml from KP37
6			

pH of tubes adjusted → 7.2 ± 0.02 ml N HCl

3) Tubes placed in roller drum - allowed to rotate for 4 days -

pH of CP tubes → 6.9 on 12-17-54

CP tubes 5 & 6 Mold on day 7 12-21-54

4) 4th day after virus inoculation -

Dec 18, 1954 Fluid removed from tubes - stored in deep freeze = 4th day fluid

Tubes then washed 4X with Hanks solution (1/3 NaHCO3) - aspiration performed with pipette attached to water suction apparatus -

3 ml of Lactalbumin hydrolysate medium pH 8.2 then added to each tube -

Tubes replaced in roller drum - allowed to rotate for 1 hour - 1 ml aliquots then removed from each tube → stored in deep freeze

pH of CP tubes → 6.9 - 7.0 with N HCl

Dec 22, 1954 8 day after virus inoculation

Fluids removed from tubes - placed in test tubes & stored in deep freeze -

Cultures on Sub. Med.	Before	After Rx
CP 526	+	0
CE 526	+	0
Results after 10 days		

Pooled fluids from tubes CE 526 and CP 526 which were contaminated with mold were treated c nystatin - 20 units per ml added - allowed to react 30 minutes at room temperature. These fluids then centrifuged 2000 rpm for 10 minutes - Supernatant fluids stored in deep freeze

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Observations on Tubes

		1	2	3	4	5	6	7	8
CE									
I	1	good fibro growth	Same	Same	1 un cell sheet Excellent fibro	No change	No change	No change	No change
	2	"	2 un cell sheets	"	Excellent fibro	" "	" "	" "	" "
II	3	Contaminated } Yeast							
	4	" }							
III	5	good fibro growth	Same	Same +1 un cell sheet	Excellent fibro	No change	" "	Mold No change	Mold good fibro
	6	" "	" "	Same 1 un cell sheet	" "	" "	" "	Mold No change	"
Controls	7	" "	" "	Same	Excellent fibro — 2 un sheet	No change	" "	No change	No change
	8	" "	" "	" " (1 un cell sheet)	Excellent fibro	" "	" "	" "	" "
	9	" "	" "	" " (1 un cell sheet)	" "	" "	" "	" "	" "
	10	" "	2 un cell sheets	" "	" "	" "	" "	" "	" "
		pH = 7.3-7.5	pH 7.2-7.3	pH 6.9	↑ 4 washings ℈ 10 ml Hank's sol.	pH 7.4	pH 7.2	pH 7.0-7.1	pH = 6.9-7.0 Fluid removed ~~changed~~

Quantity of Polio Virus in Tissue Culture Fluid in Tubes Containing a.) 9 Day Chick Embryo Tissue in Plasma Clot and b) Plasma Clot Only At Indicated Time and After Designated Manipulations

Time after addition of virus	Manipulation performed	Fluid tested	Titer of virus in indicated tissue culture fluid – TCD_{50}/ml			
			Type I Mahoney		Type III Leon	
			Clotted Plasma Only *	Chick Embryo Tissue	Clotted Plasma Only *	Chick Embryo Tissue
0	None	Not tested – Calculated	$10^{6.7}$	$10^{6.7}$	$10^{6.9}$	$10^{6.9}$
4 days	Fluid removed – tubes washed 4X with 10 ml of Hanks sol. fluid replaced	Removed fluid	$10^{6.5}$	$10^{6.2}$	$10^{6.5}$	$10^{7.0}$
4 days + 1 hour	None	Aliquot of fluid 1 hour after washing	$10^{4.2}$	$10^{4.0}$	$10^{4.0}$	$10^{3.5}$
8 days	Fluid removed	Removed fluid	$10^{3.5}$	$10^{3.5}$	$10^{3.2}$	$10^{3.2}$

* pH of tubes adjusted to that prevailing in chick embryo tissue series

Valid – NDV Not Present – Test of April 26, 1955

Jan 19, 1955 Titration CE Adsorption Washing Exp 2

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Viruses

4 day fluid 12-18-54

1 hour aliquot after washing 12-18-54

8 day fluid 12-22-54

1.) Mahoney KP36 – series with clotted plasma only

2) Mahoney KP36 CE1 series with chick embryo tissue in plasma clot

3) Leon KP37 – series with clotted plasma only

4) Leon KP37 CE1 series with chick embryo tissue in plasma clot

Procedure – 10 fold dilutions made in Hank's sol. (full amt NaHCO3) 0.2 ml inoculated per tube 3 tubes/decimal dilution

Tubes used – Trypsinized Series No #2

			Tube No.	(1-22) 3	(1-24) 5	(1-27) 8
Mahoney – KP36 Clotted plasma series	4 day fluid	10^{-4}	T253	40// ✓		
			– 4	40// ✓		
			– 5	40// ✓		
		10^{-5}	– 6	40// ✓		
			– 7	40// ✓		
			– 8	40// ✓		
		10^{-6}	– 9	0	0	od
			– 10	0	od	od
			– 11	0 ? alk.	4	
Drum B		10^{-7}	– 12	0	0	od
			– 13	0	0	0
			– 14	0	0	0
	1 hour after washing	Undil	– 15	40// ✓		
			– 16	40// ✓		
			– 17	40// ✓		
		10^{-1}	– 18	40// ✓		
			– 19	40// ✓		
			– 20	40// ✓		
		10^{-2}	– 21	40// ✓		
			– 22	40// ✓		
			– 23	40// ✓		
		10^{-3}	– 24	40// ✓		
			– 25	1-3	4	
			– 26	40// ✓		

$10^{6.5}$ TCD50/ml

$10^{4.2}$ TCD50/ml

			Tube No.	(1-22) 3	(1-24) 5	(1-37) 8	
Mahoney KP36 Clotted Plasma series	1 hr after washing	10^{-4}	- 27	0	0	0	
			- 28	0	0	0	
			- 29	0	0	0	
	8 day fluid Undil		- 30	40// ✓			
			- 31	40// ✓			
			- 32	40// ✓			
		10^{-1}	- 33	40// ✓			
			- 34	40// ✓			
			- 35	40// ✓			
		10^{-2}	- 36	40// ✓			
			- 37	40// ✓			$10^{3.5}$ TCD50/ml
			- 38	3-4	4		
		10^{-3}	- 39	0	0	od	
			- 40	0	0	0	
			- 41	40// ✓			
			-				
drum B		10^{-4}	- 42	0	0	od	
			- 43	0	0	od	
			- 44	0	0	0	
Mahoney KP36 CE1 Chick Embryo Series	4 day fluid	10^{-4}	- 45	40// ✓			
			- 46	40// ✓			
			- 47	40// ✓			
		10^{-5}	- 48	40// ✓			
			- 49	40// ✓			
			- 50	0	0	0	$10^{6.2}$ TCD50/ml
		10^{-6}	- 51	3-4	4		
			- 52	0	0	od	
			- 53	0	0	0	
		10^{-7}	- 54	0	0	0	
			- 55	0	0	0	
			- 56	0	0	0	
	1 hour after washing	Undil	- 57	40// ✓			
			- 58	40// ✓			
			- 59	40// ✓			
		10^{-1}	- 60	40// ✓			
			- 61	40// ✓			
			- 62	40// ✓			
		10^{-2}	- 63	3-4	4		
			- 64	40// ✓			
			- 65	40// ✓			

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Jun 19, 1955 Titration – CE Adsorption Washing Exp 2 (cont)

			Tube No.	(1-22) 3	(1-24) 5	(1-27) 8
Mahoney KP36 CE1			T25			
Duck Embryo Series	1 hr after washing	10^{-3}	-66	1-2	4	
			-67	1-3	4	
			-68	0	0	0
		10^{-4}	-69	0-2	0	0
			-70	0-2 alk	od	od
			-71	0	0	0
	8 day fluid	Undil	-72	40///		
			-73	40///		
			-74	40///		
		10^{-1}	-75	40///		
			-76	40///		
			-77	40///		
		10^{-2}	-78	40///		
			-79	3-4	4	
			-80	3-4	4	
		10^{-3}	-81	40///		
			-82	0-2	0	0
			-83	0-2	0	0
		10^{-4}	-84	0	0	0
			-85	0	0	0
			-86	0	0	0
Leon KP37	4 day fluid	10^{-4}	-87	40///		
Clotted plasma series			-88	40///		
			-89	40///		
		10^{-5}	-90	3-4	4	
			-91	3-4	4	
			-92	0-2	4	
		10^{-6}	-93	0	0	od
			-94	0	4	
			-95	0	0	0
		10^{-7}	-96	0	0	0
			-97	0	0	0
			-98	0	0	0
	1 hr after washing	Undil	-99	40///		
			-100	40///		
			-101	40///		
		10^{-1}	-102	40///		
			-103	40///		
			-104	40///		

$10^{4.0}$ TCD_{50}/ml

$10^{3.5}$ TCD_{50}/ml

Drum B

$10^{6.5}$ TCD_{50}/ml

		Tube No	(1-22) 3	(1-24) 5	(1-27) 8	
Leon KP37 Clotted Plasma Series	1 hour after washing	10^{-2} - 105	3-4	4		
		- 106	3-4	4		
		- 107	3-4	4		
		10^{-3} - 108	4 0 //			$10^{4.0}$ TCD50/ml
		- 109	0	4		
		- 110	0	0	0	
		10^{-4} - 111	0-2	0	0	
		- 112	0	0	0	
		- 113	0	0	od	
	8 day fluid Undil	- 114	4 0 //			
		- 115	4 0 //			
		- 116	4 0 //			
		10^{-1} - 117	4 0 //			
		- 118	4 0 //			
		- 119	4 0 //			$10^{3.2}$ TCD50/ml
		10^{-2} - 120	0	0	0	
		- 121	0	1	4	
		- 122	0	4		
		10^{-3} - 123	0	0	0	
		- 124	0	0	0	
		- 125	0-2	4		
		10^{-4} - 126	0	0	0	
		- 127	0	0	od	
		- 128	0	0	od	
Leon KP37 CE1 Chick Embryo Series	4 day fluid	10^{-4} - 129	4 0 //			
		- 130	4 0 //			
		- 131	4 0 //			
		10^{-5} - 132	3-4	4		
		- 133	0	4		
		- 134	1-2	4		$10^{7.0}$ TCD50/ml
		10^{-6} - 135	0	0-?	4	
		- 136	0	0	od	
		- 137	0	4		
		10^{-7} - 138	0	0	0	
		- 139	0	0	0	
		- 140	0	0	0	
	1 hour after washing	Undil - 141	4 0 //			
		- 142	4 0 //			
		- 143	4 0 //			

Quinn B

Jan 19, 1955

Titration CE Adsorption Washing Exp 2 (cont)

Leon WP37 CE 1
Chick Embryo Series

		Tube No.	(1-22) 3	(1-24) 5	(1-22) 8
1 hour after washing	10^{-1}	144	4 0 // ✓		
		145	4 0 // ✓		
		146	4 0 // ✓		
	10^{-2}	147	2-3	4	
		148	1-2	4	
		149	4 ✓		
Drum B	10^{-3}	150	0	0	0
		151	0	0	4
		152	0	0	0
	10^{-4}	153	0	0	0
		154	0	0	0
		155	0	0	0
8 day fluid	Undil	156	4 0 // ✓		
		157	4 0 // ✓		
		158	4 0 // ✓		
Drum F	10^{-1}	159	4 0 // ✓		
		160	4 0 // ✓		
		161	3-4	4	
	10^{-2}	162	1-3	4	
		163	0	4	
		164	0-1	4	
	10^{-3}	165	0	0	0
		166	0	0	0
		167	0	0	0
	10^{-4}	168	0	0	0
		169	0	0	0
		170	0	0	0

$10^{3.5}$ TCD_{50}/ml

$10^{3.2}$ TCD_{50}/ml

Nov 18, 1954

Preparation of chick embryo tissue culture

10 9 day White Rock embryos harvested, minced, washed 6x in Hanks (1/3 $NaHCO_3$) solution, and embedded in a plasma clot in roller tubes as described previously. The medium used was 40% inact normal horse serum (Lot # 2462) 40% Earles solution (freshly prepared) and 20% chick embryo extract (1:20 Lot 7-31-54) - The medium contained antibiotics and trypsin inhibitor 0.05 mg/ml (Worthington Biochem Lot 55300 reconstituted 11-16-54).

- 65 tubes prepared -

Nov 22, 1954 4 days after preparation

1. Tubes examined

   27 tubes - good fibro growth some unidentified cell sheets

   38 tubes - complete retraction of clot - fragments moth off the glass - these tubes discarded -

2. Medium removed - replaced c 2 ml of Lactalbumin hydrolysate medium pH 8.2 containing 5% inact. normal horse serum + trypsin inhibitor (0.05 mg/ml) + antibiotics -

Nov 23, 1954

4th Criss Cross Passage Type I & III in CE.
3rd Criss Cross Passage Type II in CE.

VDV present

Purpose To attempt to demonstrate propagation of poliomyelitis virus in chick embryo explants in plasma clot tissue culture.

The virus used for types I & III has been through 3 previous criss cross "passages". The type II virus is from the 2nd criss cross passage - When the 3rd criss cross passage was performed with CE tissue on Nov 3, 1954 type II virus could not be recovered ~~the~~ 4 days after virus inoculation. For this reason the passage just described is to be repeated in this experiment.

Procedure - 1.) 11 tubes showing the best growth were selected for this experiment. The fluid was removed and the tube washed with 10 ml of Hanks 4/3 solution.

2.) 2 ml of Lactalbumin hydrolysate medium pH 8.2 was added to 6 tubes and 3 ml to 5 tubes. the latter tubes were designated as control tubes.

Time 3) To each of 2 tubes containing 2 ml of medium 1 ml of the following viruses was added.

Tube 1, 2 — Type I poliomyelitis virus CE-MK-CE-MK-CE-MK harvested on 11-19-54 from MK titration of CE-MK-CE-MK-CE of CE Adsorb. Exp. 4 day fluids

Tube 3, 4 — Type II poliomyelitis virus CE-MK-CE-MK harvested 11-5-54 from MK [redacted] passage CE-MK-CE of CE criss cross passage 2 of Nov 2, 1954.

Tube 5, 6 — Type III poliomyelitis virus CE-MK-CE-MK-CE-MK harvested on 11-19-54 from MK titration of CE-MK-CE-MK-CE of CE Adsorb. Exp 4 day fluid

Tubes 7, 8, 9, 10, 11. Controls - Medium only

Nov 23, 1954 4th Cross Cross Passage Types I & III in CE Explants in Plasma Clot

(cont) 3rd Cross Cross Passage Type II in CE Explants in Plasma Clot

4 hours 4.) After 4 hours in the roller drum - the tubes were removed - the fluid removed from each tube - the tube was then washed 4x with 10 ml of Hank's solution ($1/3$ $NaHCO_3$) - The fluid was aspirated in each instance c a capillary pipette attached to a water suction apparatus.

3 ml of lactalbumin medium containing 5% inact. normal horse serum (Lot #2467) and trypsin inhibitor (0.05 mg/ml) added to each tube Tubes returned to roller drum.

5 hours 5) 1 ml aliquots then taken from each tube after 1 hour -

Aliquots from same series pooled - stored in deep freeze -

4 Days 6) Fluid removed — stored in deep freeze - 11-27-54 replaced c Lactalb. hydrolysate pH 8.2 c 5% normal inact horse serum (Lot #2467) and 0.05 mg of trypsin inhibitor/ml

7.) Fluid removed - stored in deep freeze 12-1-54

Microscopic Observations

	Tube	1	2	3	4	5	6	7	8
I	1	Same as controls	Same as controls	No change	Same as controls	Same as controls	No change	No change	No change
	2	" "	" "	" "	" "	" "	" "	" "	" "
II	3	" " 2 un cell sheets	" "	" "	" "	" "	" "	" "	" "
	4	Same as controls	" "	" "	" "	" "	" "	" "	" "
III	5	" "	" "	" "	" "	" "	" "	" "	" "
	6	" "	" "	" "	" "	" "	" "	" "	" "
Control	7	Excellent fibro + 2 un cell sheets	Early-Mod. fibro degen focally - many good areas of fibro left	No change	Mod fibro degen - many good areas left	Marked fibro degen	" "	" "	" "
	8	Excellent fibro minimal peripheral fibro degen -	" "	" "	" "	" "	" "	" "	" "
	9	Excellent fibro minimal peripheral fibro degen	" "	" "	" "	" "	" "	" "	" "
	10	Excellent fibro minimal peripheral fibro degen	Early fibro degen - many good fibro areas left	" "	" "	" " + many good areas left	" "	" "	" "
	11	Excellent fibro minimal peripheral fibro degen -	Mod fibro degen focally - many good areas left	" "	" "	" "	" "	" "	" "

Metabolism continues as evidenced by pH of 6.8-7.0 in all tubes -

Titration CE 4th Cross Cross Passage in CE I & III
3rd Cross Cross Passage in CE II

NOV 27 1954

NOV 27 1954

Viruses

Inoculum → 1.) CE, MK, - CE, - MK, - CE, - MK, I & III harvested 11-19-54
~~CE, MK, - CE, - MK, II harvested 11-5-54~~

2.) 5 hour aliquots - removed 1 hour after 4 washings

3) 4 day fluids

Procedure - 10 fold dilutions made in Hanks solution (full amt. of $NaHCO_3$) - 0.2 ml of diluted material inoculated into each tube - 3 tubes / decimal dilution -

Tubes used Trypsinized monkey kidney series 8 of 11-18-54

Type I

Inoculum CE-MK-CE-MK-CE-MK

	Tube No	2	4	6	8	
10^{-4}	T8 - 136	1-2	4			
	- 137	2	4			
	- 138	1	4			
10^{-5}	- 139	0	2	4		$10^{6.2}$ TCD_{50}/ml
	- 140	0	2	4		
	- 141	0	0d	0d	4	
10^{-6}	- 142	0	0	0	0	
	- 143	0	0	0	0d	
	- 144	0	0	0	0	
10^{-7}	- 145	0	0d	0d	0d	
	- 146	0	0	0	0d	
	- 147	0	0	0	0	
10^{-8}	- 148	0	0d	0d	0d	
	- 149	0	0d	0d	0d	
	- 150	0	0	0	0	
5 hour aliquot 10^{-1}	- 151	0	3	4		
	- 152	1	4			
	- 153	0-?	4			
10^{-2}	- 154	0	4			$10^{3.2}$ TCD_{50}/ml
	- 155	0	4			
	- 156	0	2	4		
10^{-3}	- 157	0	0d	0d	0d	
	- 158	0d	0d	0d	0d	
	- 159	0	0	0	0	

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Type I

5 hour aliquot

4 day feed

	Tube No.	2	4	6	8
10^{-4}	T8 - 160	0	od	od	od
	- 161	0	od	od	od
	- 162	0	0	0	0
10^{-5}	- 163	0	0	od	od
	- 164	0	0	0	0
	- 165	0	od	od	od
Undil	- 166	od	0.?	od	od
	- 167	0	0	od	od
	- 168	0	0?	0-1	4
10^{-1}	- 169	0	0?	od	4
	- 170	0	0?	od	od
	- 171	0	0	od	od
10^{-2}	- 172	0	0?	od complete	0
	- 173	0	0	0	0
	- 174	0	0	0	0
10^{-3}	- 175	0	0	0	0
	- 176	0	0	0	0
	- 177	0	0	0	od
10^{-4}	- 178	0	0	0	0
	- 179	0	0	0	0
	- 180	0	0	0	0

$10^{0.7}$ / TCID$_{50}$/ml

Type II

5 hour aliquot

10^{-1}	- 181	0	4		
	- 182	0?	4		
	- 183	0?	4		
10^{-2}	- 184	0	4		
	- 185	0	0-?	4	
	- 186	0	2	4	
10^{-3}	- 187	0	od	od	od
	- 188	0	od	od	od
	- 189	0	od	od	od
10^{-4}	- 190	0	0	0	0
	- 191	0	0	0	0
	- 192	0	0	0	0
10^{-5}	- 193	0	0	od	od
	- 194	0	0	0	0
	- 195	0	0	0	0

$10^{3.2}$ / TCID$_{50}$/ml

NOV 27 1954 Titration

CE 4th Cross Cross Passage in CE I + III
3rd " " " " " " II

Type II

	Tube No.	2	4	6	8 NOV 27 1954	
4 day fluid	Undil - 196	0	0?	od	od	
	- 197	0	0?	od	od	
	- 198	0	0?	0-?	+	$< 10^{0.7}$ TCD50/ml
10^{-1}	- 199	0	0?	0	0	
	- 200	0	0?	0	od	Trace
	- 201	0	0?	od	od	
10^{-2}	- 202	0	0	od	od	
	- 203	0	0	0	od	
	- 204	0	0	0	od	
10^{-3}	- 205	0	0	0	od	
	- 206	0	0	0	0	
	- 207	0	0	0	0	
10^{-4}	- 208	0	0	0	0	
	- 209	0	0	0	0	
	- 210	0	0	0	0	

Type III

Inoculum	10^{-4} - 211	0	2-3	4		
	- 212	0	0-1	4		
	- 213	0	4			
CE-MK - CE-MK - CE-MK						$10^{6.0}$ TCD50/ml
1 plasma clot series	10^{-5} - 214	0	0?	4		
	- 215	0	0-?	4		
	- 216	0	0	0	0	
	10^{-6} - 217	0	0	0	od	
	- 218	0	od	od	od	
	- 219	0	0	0	0	
	10^{-7} - 220	0	0	0	0	
	- 221	0	0	0	0	
	- 222	0	0	0	0	
	10^{-8} - 223	0	0	0	od	
	- 224	0	0	0	od	
	- 225	0	0	0	0	
5 hour elapsed	10^{-1} - 226	0	4			
	- 227	0	od	od	od	
	- 228	0	0-	4		
						$10^{2.2}$ TCD50/ml
	10^{-2} - 229	0	4			
	- 230	0	0	od	od	
	- 231	0	0	0	0	

Type III

5 hour aliquot

	Tube No.	2	4	6	8
10^{-3}	232	0	0	0	0
	233	0	od	od	od
	234	0	0	0	0
10^{-4}	235	0	od	od	od
	236	0	od	od	od
	237	0	0	od	od
10^{-5}	238	0	od	od	od
	239	0	0	0	od
	240	0	0	0	od
Undil	241	0	3-4		
	242	0	od	od	od
	243	0	od	od	od
10^{-1}	244	0	0	od	od
	245	0	0	od	od
	246	0	0	od	od
10^{-2}	247	0	od	od	od
	248	0	od	od	od
	249	0	od	od	od
10^{-3}	250	0	0	0	0
	251	0	0	od	od
	252	0	0	0	0
10^{-4}	253	0	0	0	0
	254	0	0	0	od
	255	0	0	0	0

4 day feed

$< 10^{0.7}$ TCD_{50}/ml

Trace

Oct 4, 1954 "CE Variant" Exp 3 ~~Part I~~

Purpose To determine if "CE variant" poliomyelitis virus can be propagated in chick embryo tissue culture by serial passage. Does "CE variant" multiply in chick embryo tissue suspended freely in roller tubes.

Procedure - Two series of tubes were prepared for each type of virus.

Series 1 Minced 8 day chick embryo fragments (approx. 20 per tube) suspended free floating in Lactalbumin hydrolysate medium. 3 tubes used for each virus type.

0.3 ml of minced tissue in Hanks solution
2.5 ml of Lactalbumin hydrolysate medium
0.7 ml of CE, virus Harvested Sept 30, 1954 (stored in deep freeze)

Series 2 2.8 ml of Lactalbumin hydrolysate medium
0.7 ml of CE, virus
No Tissue

3 tubes per virus type.

Tubes placed in roller drum and allowed to rotate at 36°C.

At 4, 8, 12, 16, and 20 days 0.40 ml aliquots of tissue culture fluid removed from each tube - the fluid from 3 tubes of the same series pooled and either titrated for virus content immediately or after storage at -20°C.

Materials

Virus

Type I	CE, from minced tissue series	Harvested Sept 30, 1954 Stored deep freeze
Type II	CE, from minced tissue series	
Type III	CE, from minced tissue series	

Tissue 12 day White Rock chick Embryos harvested aseptically, washed 3x in Hanks (1/3 $NaHCO_3$) after removing eyes & lenses, minced to 1mm sized fragments, washed 6x in Hanks solution (1/3 $NaHCO_3$) and 20 fragments suspended in 0.3 ml Hanks introduced into all tubes of series 1.

Medium Lactalbumin Hydrolysate Medium with antibiotics 10-1-54

STANDARD B&P "NOTEAR"

Type I veins — Series 1 — Tubes 1, 2, 3
Series 2 — " 4, 5, 6

Type II veins — Series 1 — Tubes 7, 8, 9
Series 2 — " 10, 11, 12

Type III veins — Series 1 — Tubes 13, 14, 15
Series 2 — " 16, 17, 18

At 2 day intervals the pH in tubes of series 2 adjusted to correspond with that prevailing in tubes with tissue i.e., series 1. Use 1 N HCl for 1st adjustment.

Record of tubes & pH adjustment

	Tube No.	2d	4d	6d	8d	10d	12d	14d	16d	18d	20d	28d
Type I Tissue	1	7.3	7.2	7.1	7.1	7.1	7.1	7.2	7.2		7.2	7.2
	2	7.5	7.3	7.3	7.2	7.2	7.2	7.2	7.2		7.2	7.2
	3	7.3	7.2	7.1	7.1	7.2	7.2	7.2	7.2		7.2	7.2
No Tissue	4	pH 8.2→7.3	7.4→7.2	7.4→7.2	7.4→7.1	7.3→7.2	7.3→7.1	7.3→7.2	7.3→7.2		7.3→7.2	7.2
	5	pH 8.2→7.3	7.4→7.2	7.4→7.1	7.3→7.2	7.4→7.0	7.3→7.2	7.3→7.2	7.3→7.2		7.3→7.2	7.2
	6	pH 8.2→7.4	7.4→7.2	7.4→7.2	7.3→7.1	7.3→7.1	7.3→7.2	7.3→7.2	7.3→7.2		7.3→7.2	
Type II Tissue	7	pH 7.5	7.3	7.2	7.2	7.2	7.2	7.2	7.2		7.2	7.2
	8	7.4	7.2	7.2	7.2	7.2	7.2	7.2	7.2		7.2	7.2
	9	pH 7.2	7.1	7.0	7.0	7.1	7.1	7.2	7.2		7.2	7.2
No Tissue	10	pH 8.2→7.3	7.4→7.2	7.4→7.1	7.4→7.2	7.3→7.1	7.3→7.2	7.3→7.2	7.3→7.2		7.3→7.2	7.2
	11	pH 8.2→7.4	7.4→7.2	7.4→7.2	7.4→7.0	7.4→7.2	7.4→7.2	7.3→7.2	7.3→7.2		7.3→7.2	7.2
	12	pH 8.2→7.3	7.4→7.3	7.4→7.2	7.4→7.1	7.3→7.1	7.2	7.3→7.2	7.3→7.2		7.3→7.2	7.2
Type III Tissue	13	pH 7.3	7.0	7.1	7.1	7.2	7.2	7.2	7.2	7.2		7.2
	14	pH 7.2	7.0	7.0	7.0	7.1	7.2	7.2				
	15	pH 7.4	7.3	7.2	7.2	7.2	7.2	7.2	7.2	7.2		7.2
No Tissue	16	pH 8.2→7.4	7.4→7.2	7.4→7.1	7.4→7.2	7.4→7.2	7.3→7.2	7.3→7.1	7.3→7.2	7.3→7.2		7.2
	17	pH 8.2→pH 7.2	7.4→7.1	7.4→7.0	7.3→7.0	7.3→7.1	7.3→7.1	7.3→7.2	7.2	7.3→7.2		7.2
	18	pH 8.2→7.4	7.5→7.0	7.4→7.2	7.4→7.0	7.3→7.2	7.3→7.2	7.3→7.2	7.3→7.2	7.3→7.2		7.2
adjustment with		N/1 HCl 0.02 ml	N/1 HCl 0.01 ml	N/1 HCl 0.005 ml	N/1 HCl 0.005 ml	N/1 HCl 0.005 ml	N/1 HCl 0.005 ml	N/1 HCl	N/10 HCl 0.01 ml	N/10 HCl 0.01 ml		

Oct 4, 1954

CE Variant Exp 3

Day	Date Aliquots Removed	Titrated or Stored	Date titrated & stored	Dilutions of tissue series to be tested 2 tubes/dil	Dilutions of control series to be tested 2 tubes/dil	Total Tubes
4	10-8-54	Stored deep freeze	OCT 11 1954	Undil, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} = 10 tubes per series X 3 = 30 total	Undil, 10^{-1}, 10^{-2} and 10^{-3} = 8 tubes per series X3 = 24 total	54
8	10-12-54	Stored deep freeze	Oct 16 1954 OCT 16 1954	Undil, 10^{-1}, 10^{-3}, 10^{-4} = 10 tubes per series X3 = 30	Undil, 10^{-1}, 10^{-2} = 6 tubes per series X3 = 18	48
12	10-16-54	Stored deep freeze	OCT 29 1954	Undil 10^{-1} = 4 tubes X3 = 12	Undil 10^{-1} = 4 tubes X3 = 12	24
16	10-20-54	Stored deep freeze	OCT 29 1954	Undil 10^{-1} = 4 tubes X3 = 12	Undil 10^{-1} = 4 tubes X3 = 12	24
21	10-25-54	Stored deep freeze	OCT 29 1954	Undil 10^{-1} = 4 tubes X3 = 12	Undil 10^{-1} = 4 tubes X3 = 12	24
28	11-1-54	Stored deep freeze				

20

CE Variant Exp 3

Quantity of virus in tubes containing suspended minced tissue and tubes containing no tissue after indicated time at 36°C

[CE2]

Time after addition of poliomyelitis virus	Titer of virus in indicated tubes TCD50/ml					
	Type I virus		Type II virus		Type III virus	
	Suspended tissue series	No tissue	Suspended tissue series	No tissue	Suspended tissue series	No tissue
0 (calculated = amt. introduced)	$10^{1.7}$	$10^{1.7}$	$10^{1.2}$	$10^{1.2}$	$10^{2.2}$	$10^{2.2}$
4 days	$10^{1.7}$	$10^{0.7}$	$10^{0.7}$	$10^{1.2}$	$10^{1.2}$	$10^{1.2}$
8 days	$10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$10^{1.2}$ #1	$< 10^{0.7}$
12 days	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$
16 days	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$
20 days	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$	$< 10^{0.7}$

OCT 11 1954

CE Variant Exp 3 Titration 4 Day Aliquots

OCT 11 1954

Viruses 4 day aliquots - CE variant exp 3 - aliquots removed 10-8-54 and stored in deep freeze [CE2]

Procedure 10 fold dilutions of each virus prepared in Hanks solution (with full amt NaHCO3) - 0.2 ml of diluted material inoculated - 2 tubes / decimal dilution

Monkey Kidney Culture tubes used = Series 66

		Tube No.	2	4	6	8
Type I	Undil	66-91	0-1	4		
Series 1		92	1-2	4		
Suspended minced tissue	10^{-1}	93	0	2-3	4	
		94	0	0	0	0
	10^{-2}	95	0	0	0	0
		96	0	0	0	0
	10^{-3}	97	0	0	0	0
		98	0	0	0	0
	10^{-4}	99	0	0	0	0
		100	0	0	0	0
Series 2	Undil	101	0	0	4	
		102	0	0	0	0
No tissue	10^{-1}	103	0	0	0	0
		104	0	0	0	0
	10^{-2}	105	0	0	0	0
		106	0	0	0	0
	10^{-3}	107	0	0	0	0
		108	0	0	0	0
Type II	Undil	109	0	0	0	0
		110	0	3	4	
Series 1						
Suspended minced tissue	10^{-1}	111	0	0	0	0
		112	0	0	0	0
	10^{-2}	113	0	0	0	0
		114	0	0	0	0
	10^{-3}	115	0	0	0	0
		116	0	0	0	0

		Tube No	2	4	6	8
Type II						
Series 1	10^{-1}	117	0	0	0	0
		118	0	0	0	0
Series 2	Undel	119	0	1	4	
No tissue		120	0	4		
	10^{-1}	121	0	0	0	0
		122	0	0	0	0
	10^{-2}	123	0	0	0	0
		124	0	0	0	0
	10^{-3}	125	0	0	0	0
		126	0	0	0	0
Type III	Undel	127	0-?	4		
		128	0-?	4		
Series 1						
	10^{-1}	129	0	0	0	0
Suspended minced tissue		130	0	0	0	0
	10^{-2}	131	0	0	0	0
		132	0	0	0	0
	10^{-3}	133	0	0	0	0
		134	0	0	0	0
	10^{-4}	135	0	0	0	0
		136	0	0	0	0
Series 2	Undel	137	0	4		
		138	0	0	4	
No tissue						
	10^{-1}	139	0	0	0	0
		140	0	0	0	0
	10^{-2}	141	0	0	0	0
		142	0	0	0	0
	10^{-3}	143	0	0	0	0
		144	0	0	0	0

Oct 16, 1954 "CE Variant" Exp 3 Titration 8 day aliquot

Viruses 8 day aliquots - "CE variant" Exp 3 - aliquots removed 10-12-54 and stored in deep freeze - [CE 2]

Procedure 10 fold dilutions of each virus prepared - Hanks solution (with full amount $NaHCO_3$)
0.2 ml of diluted material inoculated
2 tubes / decimal dilution

Monkey Kidney Culture Tubes Used = Series 69

		Tube No	2	4	6	8
Type I						
	Undil	69-100	0	0	0	0
Series 1		101	0	3	4 off	
Suspended minced tissue	10^{-1}	102	0	od	od	od
		103	0	od	od	od
	10^{-2}	104	0	0	od	od
		105	0	0	od	od
	10^{-3}	106	0	0	0	od
		107	0	0	0	od
	10^{-4}	108	0	0	0	od
		109	0	0	od	od
Series 2	Undil	110	0	0	od	od
		111	0	0	od	od
No tissue	10^{-1}	112	0	0	od	od
		113	0	0	od	od
	10^{-2}	114	0	0	od	od
		115	0	0	od	od
Type II	Undil	116	0	0	od	od
		117	0	0	od	od
Series 1 Suspended minced tissue	10^{-1}	118	0	0	od	od
		119	0	0	od	od
	10^{-2}	120	0	0	od	od
		121	0	0	od	od

		Tube No	2	4	6	8
Type II						
Series 1 (cont)	10^{-3}	122	0	0	od	od
		123	0	0	od	od
	10^{-4}	124	0	0	od	od
		125	0	0	od	od
Series 2	Undil	126	0	0	od	od
		127	0	0	od	od
No tissue	10^{-1}	128	0	0	od	od
		129	0	0	od	od
	10^{-2}	130	0	0	od	od
		131	0	0	od	od Alkaline discarded
Type III	Undil	132	0	0-2	off	
		133	0	2-4	off	
Series 1	10^{-1}	134	0	0	od	od
		135	0	0	od	od
Suspended minced tissue	10^{-2}	136	0	0	od	od
		137	0	0	od	od
	10^{-3}	138	0	0	od	od
		139	0	0	od	od
	10^{-4}	140	0	0	od	od
		141	0	0	od	od
Series 2	Undil	142	0	0	0	od
		143	0	0	od	od
No Tissue	10^{-1}	144	0	0	0	od
		145	0	0	od	od
	10^{-2}	146	0	0	od	od
		147	0	0	od	od

CE Variant Exp 3 — Titration 12, 16, 21 day aliquots

OCT 30 1954

Viruses Aliquots 12, 16, 21 days CE variant Exp 3 aliquots removed 10-16, 10-20, 10-25-54 and stored in deep freeze [CE, 1 MK, CE, 1]

Procedure 10 fold dilution of each virus prepared in Hanks solution 0.2 ml of diluted material inoculated. 3 tubes / decimal dilution

Tissue Culture-monkey kidney series 78, & 79

		Tube No	2	4	6	8
Type I	78	75	0	0	0	0
	Undil	76	0	0	od	od
12 days Series 1	10^{-1}	77	0	0	0	0
		78	0	0	0	0
Series 2	Undil	79	0	0	0	0
		80	0	0	0	0
	10^{-1}	81	0	0	0	0
		82	0	0	0	0
16 days Series 1	Undil	83	0	0	0	0
		84	0	od	od	od
	10^{-1}	85	0	od	od	od
		86	0	0	od	od
Series 2	Undil	87	0	0	0	0
		88	0	0	0	0
	10^{-1}	89	0	0	0	0
		90	0	0	od	od
21 days	Undil	91	0	0	od	od
		92	0	0	od	od
Series 1	10^{-1}	93	0	0	0	0
		94	0	0	0	0
Series 2	Undil	95	0	0	0	0
		96	0	0	od	od
	10^{-1}	97	0	0	0	0
		98	0	0	0	0

		Tube No.	2	4	6	8
Type II	Undel	99	0	0	0	0
		100	0	0	0	0
12 days						
Series 1	10^{-1}	101	0	0	0	0
		102	0	0	od	od
Series 2	Undel	103	0	0	0	0
		104	0	0	0	0
	10^{-1}	105	0	0	od	od
		106	0	0	od	od
16 days	Undel	107	0	0	od	od
		108	0		0	0
Series 1						
	10^{-1}	79-1	0	0	od	od
		-2	0	0	0	0
Series 2	Undel	3	0	0	od	od
		4	0	0	0	0
	10^{-1}	5	0	0	0	0
		6	0	0	0	0
21 days	Undel	7	0	0	0	0
		8	0	0	0	0
Series 1						
	10^{-1}	9	0	0	0	0
		10	0	0	od	od
Series 2	Undil	11	0	0	od	od
		12	0	0	0	0
	10^{-1}	13	0	0	0	0
		14	0	0	0	0
Type III	Undel	15	0	0	0	0
		16	0	0	0	0
12 days						
Series 1	10^{-1}	17	0	0	0	0
		18	0	0	0	0
Series 2	Undel	19	0	0	0	0
		20	0	0	0	0
	10^{-1}	21	0	0	0	0
		22	0	0	0	0

OCT 29 1954

CE Variant Exp 3 12, 16, 21 Day aliquot dilutions (cont'd)

Type III

		Tube No	2	4	6	8
16 days	Undil	79-23	0	0	0	0
		-24	0	0	0	0
Series 1	10^{-1}	25	0	0	0	0
		26	0	0	od	od
Series 2	Undil	27	0	0	od	od
		28	0	0	0	0
	10^{-1}	29	0	0	od	od
		30	0	0	0	0
21 days	Undil	31	0	0	0	0
		32	0	0	0	0
Series 1	10^{-1}	33	0	0	od	od
		34	0	0	od	od
Series 2	Undil	35	0	0	od	od
		36	0	0	od	od
	10^{-1}	37	0	0	0	0
		38	0	0	0	0

Oct 4, 1954

CE Variant Exp 4.

Purpose To determine

1. Is it possible to propagate "CE variant" virus serially in CE plasma clot cultures?

2.) Will "CE variant" virus produce a CPE in chick embryo plasma clot cultures?

3.) Will "CE variant" virus multiply in chick embryo plasma clot cultures?

Procedure Roller tubes containing chick embryo explants embedded in plasma clot were prepared as described in CE Variant Exp 2 of Oct 4, 1954. The 50 tubes prepared for Exp 2 were used for both Exp 2 and the present one. The medium was 40% inact. normal horse serum 40% Earles solution and 20% chick embryo extract (1:20) plus soybean trypsin inhibitor (0.05 mg/ml) and antibiotics.

Materials described in Exp 2.

Oct 8, 1954 15 tubes selected after 4 days incubation at 36°C. Tubes with good tissue outgrowth were selected. The medium was removed and the tube washed 1x with Hanks solution (10 ml) CE$_1$ virus from plasma clot series was then added in 1 ml amounts to each tube. 3 tubes per virus type. 2 ml of lactalbumin hydrolysate medium then added.

6 control tube tubes were treated identically to the virus series except no virus was added — 3 ml of medium added to these tubes.

Tubes observed for CPE.

Materials Medium -

Lactalbumin hydrolysate 10-8-54'
+ 0.05 mg soybean trypsin inhibitor/ml

Virus

Type I CE$_1$ plasma clot series 9-30-54

Type II CE$_1$ plasma clot series 9-30-54

Type III CE$_1$ plasma clot series 9-30-54

At 4 day intervals 0.4 ml aliquots removed from each tube — fluid from tubes in the same series pooled and ~~titrated~~ for virus ~~content~~ either immediately or after storage in the deep freeze.

"CE Variant" Exp 4

Growth Record and Microscopic Observations

Day (Timed from addition of virus)

Virus	Tube No	0	1	2	3	4	5	6	7	8	9	10	11	12	13	17,19	20
I CE1	1	Good fibro 3 unidentifiable cell sheets pigment & lipid granules	No change	Slight clot retract & lysis	1 sheet Vn. cells clot retract	Like controls	Like controls excellent fibro	No change		Beg peripheral fibro degen –	Same Vn cells OK	Mod fibro degen	Marked degen	Same vn cell degen		Same	Degen complete
	2	Same 4 Vn. Cell Sheets	No change	1 sheet of vn. cells focal fibro degen	clot retract	" "	No change	" "		Beg. peripheral fibro degen.	Mod. fibro degen.	No change	Same	Marked degen		Same	" "
	3	Same 1 Vn. Cell Sheet	1 New Vn. Cell sheet	1 sheet of vn. cells clot retract & lysis	clot retract 2 areas of discrete fibro necrosis	" "	More clot retraction –	" "		More clot retract	Beg fibro degen	Mod degen	Same Vn cells OK	Same		Same	" "
II CE1	4	Same 1 UN Cell sheet	No change	Small areas of fibro degen. assoc. c̄ clot retract.	Clot retract. 2 areas decrete fibro necrosis	" "	" "	" "		Clot retract increases	No change	Mod fibro degen.	Marked degen	Same		Same	Mold contamination
	5	Same	No change	clot retract & lysis –	clot retract. 1 area of necrosis of fibro	" "	" "	" "		Beg – mod fibro degen – peripheral	Mod fibro degen	No change Vn cells OK	Marked degen	Same vn cells OK		Same	Degen complete
III CE1	6	Same 2 UN cell sheets	2 new sheets Vn. cells	1 sheet Vn. cell – clot retract & lysis	clot retract.	" "	No change	" "		Beg – mod peripheral fibro degen → rounding	advanced fibro degen	No change	Same Vn cells OK	Beg. peripheral degen vn cells	Change	Marked degen	"
	7	Same 1 UN cell sheet	No change	1 sheet vn. cell – Focal fibro degen assoc c̄ clot retract	more clot retract.	" "	" "	" "		Beg – mod. peripheral fibro degen → rounding	" "	No change	Marked degen	Same		Same	" "
	8	Same 2 UN cell sheets	No change	clot retract & lysis	More retract –	" "	" "	" "		Incr. clot retract.	No change	Beg fibro degen	Same	Same		Marked degen	Degen complete
Control (No virus)	9	Same 2 UN cell sheets	No change	clot retract & lysis – focal area of fibro degen	Clot retract 2 areas of discrete fibro degen –	" "	No change	" "		Mod. peripheral fibro degen → rounding	Mod fibro degen	Marked degen	Same	Same		Same	Degen complete
	10	Same 1 UN cell sheet	2 new UN cell sheets	focal areas of fibro degen – clot lysis & retract –	clot retract. 3 areas of discrete fibro necrosis	More clot retract	More clot retract excellent fibro	No change	No significant change	areas of beg. peripheral fibro degen.	Mod. fibro degen.	No change	Same	Marked degen	No significant	Same	Degen complete
	11	Same 3 UN cell sheet	No change	" "	clot retract	" "	More clot retraction	More clot retraction		No change	Extensive retraction	No change	Beg – mod degen.	Marked degen		Same	Degen complete
	12	Same 1 UN cell sheet	No change	Clot lysis & retraction	No change	No change	No change Excellent fibro	No change		No change	No change good vn cells	Beg. fibro degen.	Mod degen	Same good vn cells		Marked degen	Degen complete
	13	Same 2 UN cell sheets	2 New UN cell sheets	" " + focal degen of fibro	More clot retract	More clot retract	Excellent fibro	No change		Beg. peripheral fibro degen → rounding	Beg. mod fibro degen. vn cells OK	Mod degen –	Same good vn cells	Marked degen.		Same vn cells OK	Degen complete
	14	Same 3 UN cell sheets	1 New UN cell sheet	" "	Clot retract 2 areas of discrete focal fibro degen	" "	More clot retract	More clot retract		More clot retract – degen one sheet vn cells others good	Extensive retract. good vn. cells.	No change	No change	No change			Fibro degen complete vn cells OK
	15	Same 2 UN cell sheets	1 New UN cell sheet	clot lysis & retraction	" "	" "	Excellent fibro	No change		No change	No change	No change	No change	Beg fibro degen – peripheral		Marked fibro degen	Deg complete

Procedures performed

40% horse serum medium removed after 4 days growth – Replaced with Lactalb. hydrolysate medium + virus – 1 washing performed before virus added.

Remarks

focal degen assoc c̄ clot retraction – areas are discrete and present picture of tissue rounding up – Vast majority of fibro are normal

Unidentified cell sheets in good condition

CE Variant Exp 4

Oct 8, 1954

Day	Date clot not removed	Titrated or stored	Date titrated if stored	Dil of plasma clot series to be tested 2 tubes/dil	Total number of tubes
4 day					
4 day	10-12-54	Stored in deep freeze	Nov 5, 1954	Undil - 10^{-4}	30
8	10-16-54	" "	Nov 5, 1954	Undil - 10^{-4}	30
12	10-20-54	" "	Nov 5, 1954	Undil - 10^{-3}	24
17	10-25-54	" "	Nov 5, 1954	Undil - 10^{-2}	18
21	10-29-54	" "	Nov 5, 1954	Undil - 10^{-2}	18
28	11-5-54	" "	Nov 20, 1954	Type III Undil - 10^{-1}	4

10-8-54

CE Variant Exp 4

Quantity of virus in roller tubes containing plasma clot chick embryo tissue cultures after indicated time at 36°C.

[CE2]

Time after addition of poliomyelitis virus	Titer of virus in indicated tubes TCD50/ml		
	Type I virus	Type II virus	Type III virus
0 (calculated = amt. introduced)	$10^{2.9}$	$10^{2.4}$	$10^{2.4}$
4 days	$10^{2.7}$	$10^{2.2}$	$10^{2.2}$
8 days	$10^{2.2}$	$10^{1.7}$	$10^{1.7}$
12 days	$10^{1.7}$	$10^{1.2}$	$10^{1.7}$
17 days	$10^{1.7}$	$10^{1.2}$	$10^{1.7}$
21 days	$< 10^{0.7}$	$< 10^{0.7}$	$10^{0.7}$
28 days			$< 10^{0.7}$

Chicken plasma used not that of NDV contaminated lot of 10-21-54

Nov 5, 1954 CE Exp 4 Titration 4, 8, 12, 17, 21 day aliquots

Viruses 4, 8, 12, 17, 21 day aliquots CE Variant Exp 4 -
aliquots removed 10-12, 10-16, 10-20, 10-25, & 10-29-54
Stored in deep freeze - [CE_2]

Procedure 10 fold dilution of each virus prepared in Hank's solution 0.2 ml of diluted material inoculated - 2 tubes/decimal dilution

Tissue Culture Tubes - Monkey kidney trypsinized series 3
10-27-54

		Tube No.	2	4	6	8
Type I						
4d	Undil	T2- 317	1	4		
		318	1	4		
	10^{-1}	319	0	0	4	
		320	1	4		
	10^{-2}	321	0-1	4		
		322	0	0	0	0
	10^{-3}	323	0	0	0	0
		324	0	0	0	0
	10^{-4}	325	0	0	0	0
		326	0	0	od	od
8d	Undil	327	1	4		
		328	0	4		
	10^{-1}	329	0	0	4	
		330	0	4		
	10^{-2}	331	0	0	0	0
		332	0	0	od	od
	10^{-3}	333	0	0	od	od
		334	0	0	0	0
	10^{-4}	335	0	0	0	0
		336	0	0	od	od

		Tube No	2	4	6	8
Type I	Undel	337	0-?	4		
		338	1	4		
12d	10^{-1}	339	0	4		
		340	0	0	0	0
	10^{-2}	341	0	0	0	0
		342	0	0	0	0
	10^{-3}	343	0	0	0	0
		344	0	0	0	0
17d	Undel	345	0	0	4	
		346	0?	4		
	10^{-1}	347	0	0?	4	
		348	0	0	0	0
	10^{-2}	349	0	0	0	0
		350	0	0	0	0
24d	Undel	351	0	0 (fatty degen.)	od	od
		352	0	0	0	0
	10^{-1}	353	0	0	0	0
		354	0	0	0	0
	10^{-2}	355	0	0	0	0
		356	0	0	0	0
Type II	Undel	357	1	4		
		358	1	4		
4d	10^{-1}	359	0	4		
		360	0	4		
	10^{-2}	361	0	0	0	0
		362	0	0	0	0
	10^{-3}	363	0	0	0	0
		364	0	0	0	0
	10^{-4}	365	0	0	0 molel	0 molel
		366	0	0	0	0
8d	Undel	367	0	4		
		368	0	4		
	10^{-1}	369	0	0 (fatty degen.)	od	od
		370	0	0	1	4

Nov 5, 1954

CE Variant Exp 4

		Tube No	2	4	6	8
Type II	10^{-2}	371	0	0	0	0
8d		372	0	0	od	od
	10^{-3}	373	0	0	0	0
		374	0	0	0	0
	10^{-4}	375	0	0	0	0
		376	0	0	0	0
12d	Undil	377	0	0	2	4
		378	0	4		
	10^{-1}	379	0	0	0	0
		380	0	0	0	0
	10^{-2}	381	0	0	0	0
		382	0	0	0	0
	10^{-3}	383	0	0	0	0
		384	0	0	0	0
17d	Undil	385	0	0	4	
		386	0	0	0	0
	10^{-1}	387	0	0	4	
		388	0	0	0	0
	10^{-2}	389	0	0	0	0
		390	0	0	0	
21d	Undil	391	0	0	0 mold	0 mold
		392	0	0	0 mold	0 mold
	10^{-1}	393	0	0	0 mold	0 mold
		394	0	0	0	0
	10^{-2}	395	0	0	0	0
		396	0	0 (Fatty degen.)	od	0d
Type III	Undil	397	0	4		
		398	1	4		
4d	10^{-1}	399	0	0	4	
		400	0	0	0	0
	10^{-2}	401	0	0	od	od
		402	0	0	0	4
	10^{-3}	403	0	0	0	0
		404	0	0	0	0

Type III		Tube No.	2	4	6	8
4d	10^{-4}	405	0	0	0	0
		406	0	0	0	0
8d	Undil	407	0	4		
		408	0	4		
	10^{-1}	409	0	0	0	0
		410	0	4		
	10^{-2}	411	0	0	0	0
		412	0	0	0	0d
	10^{-3}	413	0	0 (fatty degen.)	0d	0d
		414	0	0 (fatty degen)	0d	0d
	10^{-4}	415	0	0	0	0
		416	0	0	0	0
12d	Undil	417	0	4		
		418	0	0	4	
	10^{-1}	419	0	4		
		420	0	0	0	0
	10^{-2}	421	0	0	0	0
		422	0	0	0	0
	10^{-3}	423	0	0	0	0
		424	0	0	0	0
17d	Undil	425	0	0	4	
		426	0	0	4	
	10^{-1}	427	0	0	4	
		428	0	0 (fatty degen)	0d	0d
	10^{-2}	429	0	0	0	0
		430	0	0 (fatty degen)	0d	0d
21d	Undil	431	0	0	0	0
		432	0	4		
	10^{-1}	433	0	0	0	0
		434	0	0	0	
	10^{-2}	435	0 mold	0 mold	0d mold	0 mold
		436	0	0	0	0

Nov 20, 1954 CE Variant Exp 4

Virus 28 day aliquot Type III [CE$_2$]
fluid removed 11- -54 and stored in deep freeze.

Procedure 10 fold dilution made in Hank's solution
0.2 ml of diluted material inoculated
2 tubes / decimal dilution

Trypsinized monkey kidney series No 6 used prepared 11-11-54

			Tube No. 2	4	6	8
Type III	Undil	T6-52	0	0	0	od
		53	0	0	0	od
28d	10^{-1}	T6 54	0	0	0	od
		55	od (fatty)	od	od	od

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DEC 14 1954 CE Variant Exp 4 Passo 3 in CE Tissue Cult. - Plasma clot

Purpose To determine if virus of CE Variant Exp 4 can be passed in series in plasma clot chick embryo tissue culture?

Procedure Chick embryo tissue cultures in plasma clot prepared 12-9-54 (9 day chick embryo) in the manner described previously were relieved of their fluid - washed 1x ± Hanks solution (1/3 $NaHCO_3$) and 1 ml of Lactalb. hydrolysate medium pH 8.2 (± trypsin inhibitor + antibiotics added) added to tubes

0.5 ml of 4 day fluid from CE variant Exp 4 added to tubes in indicated manner

Tube 1 0.5 ml Type I 4 day aliquot (10-12-54)

Tube 2 0.5 ml Type II 4 day aliquot (10-12-54)

Tube 3 0.5 ml Type III 4 day aliquot (10-12-54)

Tube	Days 1	2	3	4	
1	Good fibro growth	Same	Same	Excellent fibro	→ Passo 4
2	"	"	Good fibro 1 sheet epith cells	" "	→ Passo 4
3	"	"	Beg. peripheral fibro degen.	Same	→ Passo 4

Dec 18, 1954 Fluids harvested 0.5 ml → Passage 4
1.0 ml Stored in deep freeze

Dec 18, 1954 Passage 4

Procedure Same as that just described

Check embryo explant cultures (9 day embryos) prepared on 12-9-54 and fluid changed (Lactalb. pH 8.2 c̄ antibiotics and trypsin inhibitor 0.05 mg/ml) on 12-14-54 had their fluid removed, washed 1X with 10 ml of Hank's solution (1/3 NaHCO3) - following ingredients then added

Tube 1	0.5 ml	Type I	pass 3 harvested 12-18-54	+ 1 ml Lactalb. hydrolysate medium pH 8.2 with antibiotics + trypsin inhibitor
Tube 2	0.5 ml	Type II	" "	0.05 mg/ml " "
Tube 3	0.5 ml	Type III	" "	" "

	1	(Day) 2	3	4
Tube 1	good fibro	No change	No change	No change
Tube 2	" "	"	" "	" "
Tube 3	good fibro + 2 uncell sheets	"	" "	" "

22 Dec 54 Fluids harvested - Stored in deep freeze -

Jan 18, 1955

(54)

Titration of CE - Variant Exp 4 Serial Passage No 3 & 4 in Plasma Clot Cultures of Chick Embryo Tissue -

Viruses Type I Serial Plasma Clot Passage 3 + 4 harvested on 12-18-54 and 12-22-54 (4 day pass. interval)

Type II Serial Plasma Clot Passage 3 & 4 harvested on 12-18-54 and 12-22-54 (4 day pass interval)

Type III - Serial Plasma Clot Passage 3 & 4 harvested on 12-18-54 and 12-22-54 (4 day pass. interval)

Procedure 10 fold dilutions made in Hanks solution (full amt. of $NaHCO_3$) 0.2 ml inoculated per tube 3 tubes / decimal dil

Tubes used - T24 prepared 1-11-55

Fluid replaced c 2 ml of Lactalb- pH 8.0

5 Serums on 1-17-55

(1-21) (1-24) (1-26)

		Tube No.		3	6	8	
Mahoney KP35 CE3 of 12-18-54	Undil	T24	- 138	0-2	4		
			- 139	0-2	4		
			- 140	4 //			$10^{2.0}$ TCD50/ml
	10^{-1}		- 141	0	4		
			- 142	0	0	0	
Drum E			- 143	0	0	4	
	10^{-2}		- 144	0	0	0	
			- 145	0	0	0	
			- 146	0	0	0	
CE 4 of 12-22-54	Undel		- 147	0-2	4		$<10^{0.7}$ TCD50/ml
			- 148	0	0	0	Trace
			- 149	0	0	0	
	10^{-1}		- 150	0	0	0	
			- 151	0	0	0	
			- 152	0	0	0	
	10^{-2}		- 153	0	0	0	
			- 154	0	0	0	
			- 155	0	0	0	
YSK KP54 CE3 of 12-18-54	Undel		- 156	0-	0	0	$<10^{0.7}$ TCD50/ml
			- 157	0	0	0	
			- 158	0	0	0	
	10^{-1}		- 159	0	0	0	
			- 160	0	0	0	
			- 161	0	0	0	

		Tube No	(1-21) 3	(1-24) 6	(1-26) 8	9	
YSK KP54 CE3 of 12-18-54	10^{-2}	T24	0	0	0		
		162	0	0	0		
		163	0	0	0		
		164	0	0	0		
CE4 of 12-22-54	Undil	165	0	0	0		
		166	0	0	0		$< 10^{0.7}$ TCD50/ml
		167	0	0	0		
	10^{-1}	168	0	0	0		
		169	0	0	0		
		170	0	0	0		
	10^{-2}	171	0	0	0		
		172	0	0	0		
		173	0	0	0		
Leon KP36 CE3 of 12-18-54	Undil	174	0	0	0		
		175	0	0	0		
		176	0	0	2-3	4	
	10^{-1}	177	0	0	0d		$< 10^{0.7}$ TCD50/ml
		178	0	0	0		
		179	0	0	0		Trace
	10^{-2}	180	0	0	0		
		181	0	0	0		
		182	0	0	0		
CE4 of 12-22-54	Undil	183	0	0	0		
		184	0	0	0		$< 10^{0.7}$ TCD50/ml
		185	0	0	0		
	10^{-1}	186	0	0	0		
		187	0	0	0		
		188	0	0	0		
	10^{-2}	189	0	0	0		
		190	0	0	0		
		191	0	0			

Attempted Serial Passage of Poliomyelitis Virus in Chick Embryo Tissue Culture - Plasma Clot Series

Passage	Interval	Titer of virus introduced/recovered for indicated passage TCD50 per ml		
		Type I	Type II	Type III
1	3 days	$10^{7.5}/10^{3.4}$	$10^{7.5}/10^{2.9}$	$10^{7.0}/10^{2.9}$
2	4 days	$10^{2.9}/10^{2.7}$	$10^{2.4}/10^{2.2}$	$10^{2.4}/10^{2.2}$
3	4 days	$10^{2.2}/10^{2.0}$	$10^{1.7}/<10^{0.7}$	$10^{1.7}/\underset{\text{Trace}}{<10^{0.7}}$ *
4	4 days	$10^{1.5}/\underset{\text{Trace}}{<10^{0.7}}$ *		$\underset{\text{Trace}}{<10^{0.7}}/<10^{0.7}$

* 1 of 3 tubes inoculated with 0.2 ml of undiluted tissue culture fluid showed specific cytopathogenic change.

March 14, 1955

Preparation of Chick Embryo Tissue Culture

10 8 day White Rock embryos harvested, minced, washed 6X in Hank's solution (1/3 $NaHCO_3$) and embedded in a plasma clot as described previously. The medium used was 40% inact. normal horse serum (Lot 246) 40% Earles solution (freshly prepared) and 20% chick embryo extract (1:20 of Lot 7-31-54). The medium contained antibiotics in usual concentration and trypsin inhibitor 0.05 mg/ml (Worthington Biochem Lot S 5293) 2 ml of medium per tube

— 30 tubes prepared —

5 tubes with plasma clot by CEE only also prepared-
chicken plasma coated tubes + 3 drops 1:10 CEE.

Purpose of experiment addendum to purpose on over side of page.

In previous experiments an attempt was made to wash away residual unadsorbed virus from chick embryo plasma clot tissue culture tubes either 2 or 4 hours or 4 days after addition of virus. After 4 washings with 10 ml of Hank's solution which theoretically reduced any virus present by a dilution of 10^8 level of 200 to 10,000 TCD_{50} were detected following the washing procedure. This level of residual virus after washing made it impossible to determine if subsequent low level virus multiplication occurred. Thus 100 or 1000 TCD_{50} of virus could be produced by chick embryo tissue and after release into the medium its presence could not be detected due to the residual 200-10,000 TCD_{50} which persists following washing — The purpose of the experiment was to determine if 2 series of 4 washings (10 ml each) would lower the level of residual unadsorbed virus to a point where evidence of low level virus multiplication could be detected — i.e.) to levels < 100 TCD_{50} or even < 10 TCD_{50} —

-March 18, 1955 - Double Washing Experiment

Purpose To determine if a double washing procedure will reduce the level of virus in a tube containing chick embryo tissue growing in a plasma clot to a level which will allow the detection of subsequent viral multiplication ?? if such occurs!

Procedure 10 of the chick embryo tubes and 4 of the clotted plasma tubes were treated in the following manner on 3-17-55

Medium removed - tubes washed 1X c 10 ml of Hanks 1/3 - 2 ml of 10% Lactalb hydrolysate in 90% Earles pH 8.2 then added - medium also contained antibiotics + trypsin inhibitor.

On 3-18-55

0 Time To tubes containing 2 ml of Lactalb-Earles pH 8.2 was added

Tube No.		
CE	1, 2, 3	1 ml Type I Mahoney KP36 prepared 12-6-54 in trypsinized bottles Titer $10^{7.2}$ TCD50/ml
	4, 5, 6	1 ml Type III Leon KP37 prepared 12-9-54 in trypsinized bottles Titer $10^{7.4}$ TCD50/ml
	7, 8, 9, 10	1 ml of Lactalb hydrolysate Earles pH 8.2 medium Controls -
CP	1, 2	1 ml Type I Mahoney KP36 as above
	3, 4	1 ml Type III Leon KP37 as above

March 18, 1955 — Double Washing Experiment — Mahoney & Leon

4 Hours — After 4 hours in roller drum – tubes removed – fluid removed + frozen – washed 4 X c 10 ml of Hanks solution (1/3 usual amount of $NaHCO_3$) –

3 ml of Lactalb hydrolysate Earles pH 8.2 medium c trypsin inhibitor added to tubes – tubes returned to roller drum

5 hours / 1 hour after 1st wash — 1 hour after 1st washing – fluid removed + frozen – tubes then washed 4X c Hanks 1/3. 3 ml of Lactalb-Earles pH 8.2 c trypsin inhibitor added to each tube –

6 hours / 1 hour after 2nd wash — 1 ml of fluid removed from each tube – fluids from same series pooled + frozen

CP Series

March 18, 1955 — pH of CP series adjusted → 7.2 c N/1 HCl.

March 19, 1955 — pH of CP series 7.3-7.4 adjusted → 7.2 c N/1 HCl

March 21, 1955 — pH of CP series 7.2-7.3 adjusted → 7.0-7.1 c N/1 HCl

March 23, 1955 — after fluid change pH of CP tubes adjusted → 7.1 c N/1 HCl

March 26, 1955 — pH of CP tubes of 5th day = 7.2-7.3

March 22, 1955 — Fluid removed – stored deep freeze after titration – 3 ml Lactalb-Earles pH 8.2 c trypsin inhibitor added –

4 days

8 days — March 26, 1955 – Fluid removed – stored deep freeze

Microscopic Examination of Tubes

Tube		1	2	3	4	5	6	7	8
CE	1	Excellent fibro no traumatic effect	Excellent fibro 1 mm cell sheet No degen	Excellent fibro early peripheral degen in a few areas	Mod fibro degen many good areas left	Same	Mod fibro degen Some good areas	No change	Marked fibro degen
I	2	Excellent fibro - some traumatic disruption of fibro sheets	Predominately excellent fibro - some early fibro degen	Early fibro degen - most areas still healthy	Same	Mod fibro degen some good areas	" "	" "	Mod fibro degen some good areas left
	3	" "	Early - mod fibro degen many good areas left	Mod fibro degen - some good areas left	Same	Same	" "	" "	Marked fibro degen
	4	" "	" "	" "	" "	" "	" "	" "	" "
III	5	Excellent fibro no traumatic effect	Excellent fibro - no degen	Early fibro degen - most areas healthy	Same	Mod fibro degen many good areas	" "	" "	Same
	6	Excellent fibro + some traumatic effect	" "	Excellent fibro - no degen	Early fibro degen	Same	" "	" "	Mod fibro degen some good areas left
Controls	7	Excellent fibro - some traumatic clot retract + disruption of fibro sheets	Excellent fibro - early fibro degen - peripheral	Early - mod fibro degen - many good areas left	Mod fibro degen Some good areas left	Same	Same	" "	Marked fibro degen
	8	" "	Mod fibro degen many good areas left	Mod fibro degen - some good areas left	Same	Same	" "	" "	" "
	9	" "	Excellent fibro - early peripheral fibro degen	Early - mod. fibro degen - many good areas left	Mod fibro degen - some good areas left	Same	" "	" "	" "
	10	" " 1 mm cell sheet	Mod fibro degen many good areas left	Mod fibro degen many good areas left	" "	" "	" "	" "	" "
		pH = 7.5 - 7.6	pH 7.4 - 7.5	pH 7.3 - 7.4	pH 7.2 - 7.4	7.6	7.5 - 7.6	7.4	7.4
C.P. Series		7.3 - 7.4	7.2 - 7.3	Fluid changed 3 ml Locke's - Early pH 8.2 added 7.1					7.2 - 7.3

Double Washing Exp

Time	Manipulation Procedure	Fluid tested	Titer in indicated tissue culture fluid TCD_{50}/ml			
			Type I Mahoney KP36		Type III Leon KP 37	
			Clotted Plasma Only	Chick Embryo Tissue *	Clotted Plasma Only	Chick Embryo Tissue *
0	None	Not tested - Calculated	$10^{6.7}$	$10^{6.7}$	$10^{6.9}$	$10^{6.9}$
4 hours	Fluid removed - tubes washed 4x ∓ 10ml Hanks sol. - fluid replaced -	Removed fluid	$10^{7.2}$	$10^{7.0}$	$10^{7.0}$	$10^{7.2}$
1 hour after washing	Fluid removed - tubes washed 4x ∓ 10ml Hanks sol. - fluid replaced -	Removed fluid	$10^{5.2}$	$10^{4.2}$	$10^{4.2}$	$10^{4.0}$
1 hour after 2nd washing	aliquot of fluid removed	Fluid aliquot	$10^{3.2}$	$10^{2.5}$	$10^{3.5}$	$10^{3.0}$
4 days after addition of virus	Fluid changed	Removed fluid	$10^{3.0}$	$10^{2.2}$	$10^{3.0}$	$10^{2.2}$

* NDV present — Chick embryo fibroblast degeneration 1st seen on 2nd day —

STANDARD
MAR 22 1955

1) Titration CE Double Washing Experiment

Viruses

a) Fluid 4 hours after addition of virus - 3-18-55 -

b) Fluid 1 hour after 1st washing - 3-18-55 -

c) Fluid 1 hour after 2nd washing -

d) 4 day fluid -

1.) Mahoney KP36 [redacted] - series c clotted plasma only

2) Mahoney KP36 CE, series with chick embryo tissue in plasma clot

3) Lon KP 37 - series with clotted plasma only

4) Lon KP37 CE, - series with chick embryo tissue in plasma clot

Procedure 10 fold dilutions made in Hanks solution (full amt $NaHCO_3$) 0.2 ml per tube 3 tubes/decimal dilution

Tubes used T44

Mahoney KP36

Clotted plasma series

4 hr fluid	Tube No	2	4	6	8
	T44				
10^{-4}	- 1	2	4		
	- 2	2	4		
	- 3	2	4		
10^{-5}	- 4	1	4		
	- 5	1	4		
	- 6	0	4		
10^{-6}	- 7	0	0	od	od
	- 8	0	4		
	- 9	0	0-?	4	
10^{-7}	- 10	0	od	od complete	
	- 11	0-?	4		
	- 12	0	0	0	od
1 hour after 1st washing 10^{-1}	- 13	2	4		
	- 14	3	4		
	- 15	2-3	4		
10^{-2}	- 16	1	4		
	- 17	1	4		
	- 18	1	4		
10^{-3}	- 19	0	4		
	- 20	1	4		
	- 21	0	4		

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Mahoney NP3L

Clotted Plasma Serie

		Tube No	3	4	6	8
	10^{-4}	Tgy – 22	0	2-3	4	
		– 23	0	4		
		– 24	1	4		
	10^{-5}	– 25	0	0	od	od
		– 26	0	0	od	od
		– 27	0	0	od	od
1 hour after 2nd washing	Undil	– 28	4			
		– 29	4			
		– 30	4			
	10^{-1}	– 31	2	4		
		– 32	2	4		
		– 33	1	4		
	10^{-2}	– 34	0	4		
		– 35	0	4		
		– 36	0-?	4		
	10^{-3}	– 37	0	0	od	od
		– 38	0	0	od	od
		– 39	0	0	od	od
	10^{-4}	– 40	0	0	od	od
		– 41	0	0	od	od
		– 42	0	0	od	od
4 day fluid	Undil	– 43	3-4			
		– 44	3	4		
		– 45	3	4		
	10^{-1}	– 46	1-2	4		
		– 47	0-1	4		
		– 48	1	4		
	10^{-2}	– 49	0	4		
		– 50	0	0	od	od
		– 51	0	4		
	10^{-3}	– 52	0	0	od	od
		– 53	0	0	od	od
		– 54	0	0	od	od
	10^{-4}	– 55	0	0	od	od
		– 56	0	0	od	od
		– 57	0	0	od	od

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Mahoney KP36 CE.

		Tube No	2	4	6	8
4 hour fluid	10^{-4}	Tap -58	3	4		
		-59	2-3	4		
		-60	2-3	4		
Chick Embryo Series	10^{-5}	-61	1	4		
		-62	0	4		
		-63	1	4		
	10^{-6}	-64	0	0	od	od
		-65	0	4		
		-66	1	4		
	10^{-7}	-67	0	0	od	od
		-68	0	0	od	od
		-69	0	0	od	od
1 hour after 1st washing	10^{-1}	-70	3	4		
		-71	3	4		
		-72	3-4			
	10^{-2}	-73	0	4		
		-74	2	4		
		-75	0-?	4		
	10^{-3}	-76	0	4		
		-77	0	4		
		-78	1	4		
	10^{-4}	-79	0	0	od	od
		-80	0	0	0	od
		-81	0	0	od	od
	10^{-5}	-82	0	0	od	od
		-83	0	0	od	od
		-84	0	0	od	od
1 hour after 2nd washing	Undil	-85	3-4			
		-86	3-4			
		-87	3-4			
	10^{-1}	-88	1	4		
		-89	1	4		
		-90	3	4		
	10^{-2}	-91	0	0-?	od	od
		-92	0	4		
		-93	0	0?	od	od
	10^{-3}	-94	0	0	od	od
		-95	0	0	od	od
		-96	0	0	0	od

			Tube No	2	4	6	8
Maloney KP36CE, Chick Embryo series	1 hour after 2nd washing	10^{-4}	- 97	0	od	od	od
			- 98	0	0	od	od
			- 99	0	0	od	od
	4 day fluid	Undil	- 100	2	4		
			- 101	1-2	3-4		
			- 102	2-3	4		
		10^{-1}	- 103	0?	4		
			- 104	0?	0?	od	od
			- 105	1	3-4		
		10^{-2}	- 106	0	0	0	od
			- 107	0	0	0	od
			- 108	0-?	3-4		
		10^{-3}	- 109	0	0	0	od
			- 110	0	0	0	od
			- 111	0	0	od	od
		10^{-4}	- 112		0	0	0
			- 113	0	0	0	od
			- 114	0	0	0	0
Lem KP37 Clotted Plasma Series	4 hour fluid	10^{-4}	- 115	1	4		
			- 116	1	4		
			- 117	1	4		
		10^{-5}	- 118	0-?	4		
			- 119	0	4		
			- 120	0-1	4		
		10^{-6}	- 121	0	0	od	od
			- 122	0	1	4	
			- 123	0	4		
		10^{-7}	- 124	0	0	od	od
			- 125	0	0	od	od
			- 126	0	0	od	od
	1 hour after 1st washing	10^{-1}	- 127	1	4		
			- 128	1-2	4		
			- 129	2	4		
		10^{-2}	- 130	0	4		
			- 131	0	4		
			- 132	0	4		
		10^{-3}	- 133	0 alk	4		
			- 134	0	2	4	
			- 135	0	0	4	

3)

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		Tube No	2	4	6	8
Lem KP37 Clotted Plasma Series	1 hour after 1st washing	10^{-4} -136	0	0	od	od
		-137	0	0	od	od
		-138	0	0	od	od
		10^{-5} -139	0	0	od	od
		-140	0	0	od	od
		-141	0	0	od	od
	1 hour after 2nd washing	Undil -142	1	4		
		-143	1-2	4		
		-144	1-	4		
		10^{-1} -145	0	4		
		-146	0	4		
		-147	0	4		
		10^{-2} -148	0	0	od	4
		-149	0	3	4	
		-150	0	4		
		10^{-3} -151	0	4		
		-152	0	od	od	od
		-153	0	od	od	od
		10^{-4} -154	0	0	od	od
		-155	0	0	od	od
		-156	0	0	od	od
Lem KP37 CE, Chick Embryo Series	4 hour fluid	10^{-4} -157	2	4		
		-158	1-2	4		
		-159	2	4		
		10^{-5} -160	0	4		
		-161	0-1	4		
		-162	1	4		
		10^{-6} -163	0	1	4	
		-164	0	0	4	
		-165	0	4		
		10^{-7} -166	0	0	0	0 d
		-167	0	0	0	0 d
		-168	0	0	0	od
	1 hour after 1st washing	10^{-1} -169	1	4		
		-170	2-3	4		
		-171	2	4		
		10^{-2} -172	0	4		
		-173	0	4		
		-174	1	4		

Lewis KP37 CE, Check Embryo Series

		Tube No.	2	4	6	8	9	10
1 hour after 1st washing	10^{-3}	175	0	0	od	4		
		176	0	4				
		177	0	0	0	od		
	10^{-4}	178	0	0	od	od		
		179	0	0	od	od		
		180	0	0	od	od		
	10^{-5}	181	0	0	0	od		
		182	0	0	0	od		
		183	0	0	od	od		
1 hour after 2nd washing	Undil	184	2	4				
		185	2	4				
		186	2	4				
	10^{-1}	187	0	4				
		188	0-1	4				
		189	0?	4				
	10^{-2}	190	0	4				
		191	0	0	od	od		
		192	0	4				
	10^{-3}	193	0	0	od	od		
		194	0	0	0	od		
		195	0	0	od	od		
	10^{-4}	196	0	0	0	od		
		197	0	0	od	od		
		198	0	0	0	od		
4 day fluid	Undil	199	0-1	4				
		200	0-1	4				
		201	0-1	4				
	10^{-1}	202	0?	0	4			
		203	0	0-?	od	2-3	4	
		204	0	0-?	od	od		
	10^{-2}	205	0	0	0	od		
		206	0	0	0	od		
		207	0	0	0	od?	1-2	4
	10^{-3}	208	0	0	od	od		
		209	0	0	0	od		
		210	0	0	0	od		
	10^{-4}	211	0	0	od	od		
		212	0	0	od	od		
		213	0	0	0	od		

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Lem KP37

Clotted Plasma Series

4 day fluid.

		Tube No.	2	4	6	8
	Undil –	214	1-2	4		
	–	215	1-2	4		
	–	216	1	4		
10^{-1}	–	217	1	4		
	–	218	0	4		
	–	219	0	4		
10^{-2}	–	220	0	0	0	od
	–	221	0	3	4	
	–	222	0	3	4	
10^{-3}	–	223	0	0	od	od
	–	224	0	0	0	od
	–	225	0	0	od	od
10^{-4}	–	226	0	0	od	od
	–	227	0	0	od	od
	–	228	0	0	od	od

Double Washing Exp Glenn Type III

Purpose To determine if using the procedure of 4 hour incubation of virus + chick embryo tissue culture and the 2 series of 4 washings (10 ml each) following this period it is possible to demonstrate multiplication of glenn Type III poliomyelitis virus in chick embryo tissue. The glenn strain at the 3rd tissue culture passage is very tested since it is "close to nature" and extensive selection of various mutation variants present in the orgnal virus population is not likely to have occurred. The possibility exists that the process of selection to which the Mahoney, VSK, + Leon viruses were subjected to resulted in a selection against particles with the capacity for multiplication in chick embryo tissue. For this reason the early passage no 3 is being used in this experiment.

March 24, 1955 Preparation of Chick Embryo Tissue culture

10 9 day white Leghorn embryos harvested minced, washed 6X in Hanks' solution (1/3 NaHCO3) and embedded in a plasma clot as described previously. The medium used was 40% inact normal horse serum (Lot 2467) 40% Earles solution (freshly prepared) and 20% chick embryo extract (1:20 in Earles Lot 7-31-54) The medium contained antibiotics in the usual concentration and trypsin inhibitor 0.05 mg/ml (worthington Biochem Lot S 5293) 2ml of medium per tube

30 tubes prepared

5 tubes with plasma clotted by CEE only also prepared Chicken plasma coated + 3 drops 1:10 CEE.

March 29, 1955 good growth 11 tubes - others extensive clot retraction

Medium replaced c 2ml Lactate-Earles pH 8.2 c
20% normal horse serum
Lot 2467

March 30, 1955

4 of the check embryo tubes with the best growth and 2 of the clotted plasma tubes were treated in the following manner.

Medium removed – tubes washed 1 x c 10 ml of Hanks 1/3 – 2 ml of 10% Lactalb Hydrolysate in 90% Earles pH 8.2 then added – medium also contained antibiotics and trypsin inhibitor

0 Time

Tube No. To tubes containing 2 ml of Lactalb-Earles pH 8.2 was added –

CE 1, 2, 3 } 1 ml Type III glenn KP3 of 1-4-55 prepared in hyperimized bottles titer $10^{7.2}$ TCD50/ml

4, 5, 6 } 1 ml Lactalb - Earles pH 8.2 Controls

CP (Clotted Plasma Series) 1, 2 } 1 ml Type III glenn as above

4 hours After 4 hours in roller tubes were removed – fluid removed + frozen – tubes washed 4 x c 10 ml of Hanks 1/3 –

3 ml of Lactalb - Earles pH 8.2 medium + trypsin inhibitor added to tubes – tubes returned to drum –

5 hours – 1 hour after 1st washing – fluid removed – tubes then washed 4 x c Hanks 1/3 – 3 ml Lactalb - Earles

1 hour after 1st wash

March 30, 1955

6 hours

1 hour after 2nd washing

1 hour after 2nd washing - 1 ml of fluid removed from each tube - stored deep freeze

April 2, 1955

4 Days

Fluid removed from tubes - stored deep freeze

2 ml Lactalb- Earles pH 8.2 C trypsin 1:250 added to each tube - tubes replaced in roller drum -

Microscopic Observations

	Day 1	2	3	4	5	6	7	8
CE 1	Excellent fibro 2 un cell sheets	Same	Beg. fibro degen -	Beg - mod fibro degen - many gord areas	Mod fibro degen - some gord areas left	Same	Same	Marked fibro degen.
2	good fibro - some traumatic effect in certain areas	Same	Same	Early fibro degen	Early - mod fibro degen	Same	"	" "
3	Excellent fibro - some traumatic effect 2 un cell sheets	Same	Same	" "	" "	Mod. fibro degen - some gord areas left	"	" "
4	Most fibro excellent some areas traumatic damage	Same	Beg. fibro degen -	Mod. fibro degen	Same	Same	"	
5	" " - 1 un cell sheet -	Same	" "	" "	Same	"	"	
6	Excellent fibro	Early peripheral fibro degen -	" "	" "	Same	"	"	
	pH 7.5	7.4	7.3	7.2-7.3	7.5-7.6	7.4-7.5	7.3-7.4	7.3-7.4
CP (pH of tubes)	7.2-7.3	7.3	7.3 ↓ adjusted 7.2	7.3	7.3-7.4 ↓ 7.2	7.3	7.3	7.3

Fluid changed ↑

Note - Unused tubes of this series in which the fluid* was changed on 3.30.55 - same degen changes present to - day

*fluid = original preparative fluid in the instance

Double Washing Exp — Glenn KP3

Manipulation		Fluid tested	Titer in indicated tissue culture fluid $TCID_{50}$/ml	
Time	Procedure		Clotted ~~Plasma~~ Only	Chick Embryo Tissue *
0	None	Not tested - calculated	$10^{6.7}$	$10^{6.7}$
4 hours	Fluid removed - tubes washed 4x @ 10 ml Hank's sol. - fluid replaced	Removed fluid	$10^{6.0}$	$10^{6.2}$
1 hour after washing	Fluid removed - tubes washed 4x @ 10 ml Hank's sol. - fluid replaced	Removed fluid	$10^{2.7}$	$10^{4.5}$
1 hour after 2nd washing	Aliquot of fluid removed	Fluid aliquot	$10^{1.0}$	$10^{3.0}$
4 days after addition of virus	Fluid changed	Removed fluid	$10^{1.2}$	$10^{2.5}$
8 days after addition of virus	Fluid removed	Removed fluid	$<10^{0.7}$ Trace	$<10^{0.7}$

* NDV present - Chick embryo fibroblast degeneration 1st seen on 2nd or 3rd day

Titration glenn CE Double Washing

April 5, 1955 Versus { glenn KP3 series Clotted Plasma only
glenn KP3 CE, - series c check embryo tissue in plasma clot

Fluids collected a) 4 hours after addition of virus
b) 1 hour after 1st washing
c) 1 hour after 2nd washing
d) 4 days after addition of virus
e) 8 days after addition of virus

Procedure 10 fold dilutions in Hanks complete - 0.2 ml / tube
3 tubes / decimal dilution

Tubes T49 Prepared 3-30-55 0.5 ml 1:200 cell susp. in lactalb-Hanks + 2% calf serum 1.5 ml Lactalb-Earle pH 7.4 added 4-7-55

glenn KP3 Clotted Plasma Series — 4 hr fluid

	Tube No.	2	4	6	8
10^{-4}	1	2	4		
	2	0-2	4		
	3	0-1	4		
10^{-5}	4	0	0	0	0
	5	0-1	4		
	6	0	3	4	
10^{-6}	7	0	0	0	0
	8	0	0	0	0
	9	0	0	0	0
10^{-7}	10	0	0	0	0
	11	0	0	0	0
	12	0	0	0	0
1 hour after 1st washing 10^{-1}	13	0-1	4		
	14	0-2	4		
	15	2	4		
10^{-2}	16	0	0 ?	3-4	
	17	0	0	0	0
	18	0	0	0	0
10^{-3}	19	0	0	0	0
	20	0	4		
	21	0	0	0	0
10^{-4}	22	0	0	0	0
	23	0	0	0	0
	24	0	0	0 vac	0 vac

Glenn KP3

Clotted Plasma Series

		Tube No.	2	4	6	8
1 hr after 1st washing	10^{-5}	25	0	0	0	0
		26	0	0	0	0
		27	0	0	0	0
1 hour after 2nd washing	Undil	28	0	3	4	
		29	0	0	1-2	4
		30	0	0	0	
	10^{-1}	31	0	0	0	0
		32	0	0	0	0
		33	0	0	0	0
	10^{-2}	34	0	0	0	0
		35	0	0	0	0
		36	0	0	0	0
	10^{-3}	37	0	0	0	0
		38	0	od	od vac	od vac
		39	0	0	od vac	od vac
	10^{-4}	40	0	0	0	0
		41	0	0	0	0
		42	0	0	0	0
4 day fluid	Undil	43	0	0	0-1	4
		44	0	0	4	
		45	0	0	0	0
	10^{-1}	46	0	0	0	0
		47	0	0	3	4
		48	0	0	0	0
	10^{-2}	49	0	0	0	0
		50	0	0	od vac	od vac
		51	0	0	0	0
	10^{-3}	52	0	0	0	0
		53	0	0	0	0
		54	0	0	0	0
	10^{-4}	55	0	0	0	0
		56	0	0	0	0
		57	0	0	0	0

Glenn KP3 CE1

									10	12
8 day fluid	Undil	58	0?	od	granular degen. of approx 1/4-1/3 of epith sheet	Same	1/2 Epith sheet remains OK		Same	Same
		59	0	od		"	"	"	"	"
		60	0	od		"	"	"	"	"
	10^{-1}	61	0	0	" "	"	1/3 "	"	"	"
		62	0	0	" "	"	1/2 "	"	"	"
		63	0	0	" "	"	" "	"	"	"

April 8, 1955 Titration Glenn CE Double Washing

Glenn KP3 CE1 8 day fluid ~~Tolled Plasma Series~~

	Tube No.	2	4	6	8	10	12
10^{-2}	64	0	0	"	"	"	Same
	65	0	0	"	"	"	"
	66	0	0	"	"	"	"
10^{-3}	67	0	0 Marked degen granules	"	"	"	"
	68	0	0 "	"	"	"	"
	69	0	0 "	"	"	"	"
10^{-4}	70	0	0	0	0	0	0
	71	0	0 "	"	1/4 granules degen	Same	Same
	72	0	0		"	"	"

Glenn KP3CE1 4 hour fluid

	Tube No.						
10^{-1}	73	0-2	4				
	74	1-2	4				
	75	0-2	4				
10^{-5}	76	0	4				
	77	0-1	4				
	78	0	0	2-3	4		
10^{-6}	79	0	0	0 dvac	od		
	80	0	0	0	0		
	81	0	0	0	0		
10^{-7}	82	0	0	0	0		
	83	0	0	0	0		
	84	0	0	0	0		

1 hour after 1st washing

	Tube No.						
10^{-1}	85	0-1	4				
	86	1	4				
	87	1-2	4				
10^{-2}	88	0	2-3	4			
	89	0-1	3-4				
	90	0-1	4				
10^{-3}	91	0	0	0	0		
	92	0	0	1-2	4		
	93	0	2	4			
10^{-4}	94	0	0	0	0		
	95	0	0 ?	3-4			
	96	0	0	4			
10^{-5}	97	0	0	0	0		
	98	0	0	0	0		
	99	0	0	0	0		

1 hour after 2nd washing Undil

	101	2-3	4				
=	102	2-3	4				

Glenn KP3 CE1

		Tube No	2	4	6	8
1 hour after 2nd washing	10^{-1}	103	0-1	4		
		104	0-1	4		
		105	0-1	4		
	10^{-2}	106	0	0	3-4	
		107	0	0	0	0
		108	0	1-2	4	
	10^{-3}	109	0	0	0	0 dvac
		110	0	0	-0	0
		111	0	0	0	0
	10^{-4}	112	0	0	0	0
		113	0	0	0	0
		114	0	0	0	0
4 day fluid	Undel	115	1-2	3-4		
		116	2-3	3-4		
		117	1-2	4		
	10^{-1}	118	0-1	0 + granular degen of approx	complete degen + ? polio change	→ passage pos
		119	0-1	" 1/2 of epith cell sheet	"	→ passage pos
		120	0-1	complete degen + small residual epith sheet	complete degen	→ passage pos
	10^{-2}	121	0	0 1/3 degeneration - as above	Same + 1-2 polio ?	complete degen → Pass Pos 10
		122	0	0 " " "	No change	1/2 epith sheet remains Same
		123	0	0 " " "	No change	" " " "
	10^{-3}	124	0	0 " " "	" "	" " 1/4 epith sheet remains "
		125	0	0 " " "	" "	" " Same "
		126	0	0 " "	" "	" " " "
	10^{-4}	127	0	0	1/3 granular degen	" " " "
		128	0	0	minimal granular degen	" " " "
		129	0	0	" "	" " " "

Glenn KP3 CP Series

8 day fluid	Undel	130	0	0	0	0
		131	0	3	4	
		132	0	0	0	0
	10^{-1}	133	0	0	0	0
		134	0	0	0	0
		135	0	0	0	0
	10^{-2}	136	0	0	0	0
		137	0	0	0	0
		138	0	0	0	0
	10^{-3}	139	0	0	0	0
		140	0	0	0	0
		141	0	0	0	0
	10^{-4}	142	0	0	0	0
		143	0	0	0	0
		144	0	0	0	0

April 12, 1955 Is the granular type degeneration observed in monkey kidney culture tubes inoculated with the 4 and 8 day fluids from chick embryo cultures tubes inoculated with glenn virus the result of 1.) a toxic substance elaborated by degenerating chick fibroblasts or 2) a specific change induced by polio virus which has multiplied in chick embryo tissue culture or 3) a virus from chick embryo tissue ??

A "granular" degeneration affecting monkey kidney epith cells was observed in tubes inoculated with the 4 or 8 day fluid from chick embryo tissue cultures inoculated with glenn virus. This degeneration was not seen in monkey kidney cultures inoculated with the 4 or 8 day fluid from the "clotted plasma only" series inoculated with glenn virus nor with the 1st or 2nd post washing fluids of the chick embryo series. This eliminates the monkey kidney per se as the source of the degeneration effect - It must therefore be the effect of 1.) a degeneration producing substance resulting from chick embryo fibroblast degeneration or 2.) a chick embryo polio variant with a cytopathogenic effect differing from that of the original virus population or 3) Pick up of chick embryo virus -

- Therefore the following experiment performed -

4 day and 8 day fluid of C.E. series
4 day and 8 day fluid from control tubes

titrated in monkey kidney tubes of series T 50.

T50 Prepared 4-5-55 0.5 ml 1:200 cell susp. in Lactalb - Hanks 2% calf serum
1.5 ml Lactalb Earles pH 5.2 added 4-11-55
pH Now = 7.5 - 7.6
- Foamy degen. in this series -

CE Series

glenn

KP3

CE1

	Tube No	1	2	3	4	5	6	8	10	12
4 day fluid Undil	73	0	1-2 polio?	Most epith sheet remains - ghosts of "polio" cells present?	1/2 Epith sheet remains "Swiss cheese"	Same - many round cells	1/3 epith remains	2 small epith sheets remain	No change	Complete degen
	74	0	0-2 polio?	" "	" "	1/3 epith cell sheet remains	1/4 "	" "		" "
	75	0	0-2 polio?	" "	" "	1/2 " " many round cells	1/4 "	1 " "		" "
10^{-1}	76	0	2 polio?	1/2 epith sheet OK - "polio ghosts"?	" "	1/3 " "	1/5 "	Complete degen.		" "
	77	0	0-2 polio?	Same picture as above	1/5 " "	Same	2 small islands	" "		" "
	78	0	0-1 polio?	1-2 + degen - round cells & clumping	1/2 " "	Same	Same	3 small epith sheets		" "
10^{-2}	79	0	0-2 polio	as above	1/4 " " many round cells at periphery	same	2 small islands epith	" "		" "
	80	0	0	as above + cells that look like polio	1/2 epith sheet remains "Swiss cheese"	Same	Same	6 " "		" "
	81	0	0	0	3/4 " " granular degen -	Same	1/2 epith left	Same		3 epith islands left
10^{-3}	82	0	0	0	1/2 " "	Same	Same	"		No change
	83	0	0	0	" " "	"	"	1/3 epith left		" "
	84	0	0	0	3/4 " "	1/2 epith sheet remains	"	" "		Epith islands left
10^{-4}	85	0	0	0	Big degen?	1/2 epith sheet + granular degen	"	" "		Epith islands + vac
	86	0	0	0	0	0	gran degen 1/2 of epith sheet	" "		Same
	87	0	0	od vac	Od vac	od vac	Od vac	Od vac		Od vac
8 day fluid Undil	88	0	0-2 polio?	0-4 polio?	1/2 Epith sheet remains "Swiss cheese"	Same	1/4 epith sheet remains	Same		Complete degen
	89	0	0	1-4 polio?	" " granular degen	" "	Same	"		Small epith islands
	90	0? Polio	0-2 polio?	0-4 polio? 1/2 Epith sheet remains	" " many round cell	1/10 epith sheet left	1 small epith sheet remains	complete degen		Same
10^{-1}	91	0	0	0? polio?	3/4 " " "Swiss cheese" granular degen	Same	Same	1/2 epith remains & definite polio cells		Small epith islands
	92	0	0	0	" "	Same	"	Same		" "
	93	0	0	0	" "	"	"	1/2 epith sheet left		" " + vac
10^{-2}	94	0	0	0	1/2 " "	"	"	Same		Same
	95	0	od vac.	od vac	" " "	1/4 epith sheet remains	"	Several epith sheets left		Complete degen
	96	0	0	0	" " "	1/2 " "	"	1/4 epith sheet left		Small epith islands Large " " + vac
10^{-3}	97	0	0	0	0	granular degen 1/2 epith sheet	"	Same		" " "
	98	0	0	0	Beginning granular degen	" "	"	Same		" " " " "
	99	0	0	0	0	0	granular degen 1/2 epith sheet	Same		" " " " "
10^{-4}	100	0	Od vac	Od vac	Od vac cells mostly good	Same	granular degen 1/2 epith sheets	Same		Complete degen
	101	0	0	Od vac	" "	"	Od vac	Same		Extensive vac - almost complete degen
	102	0	Od vac	od vac	" "	"	od vac	"		" "

April 12, 1955

		1	2	3	4	5	6	8	10	12
CE Control Fluid 4 day	Tube No T50								No change	
Undil	- 103	0	0	Mainly peripheral - Beg. abounding of cells + granular degen	1/2 Epith sheet remains "Swiss cheese"	Same	Same	Same	Same	
	- 104	0	0 Beg granular degen ?	"	" "	Same	Same	" + vac	Epith islands	
	- 105	0	0 "	" "	" "	"	"	"	Same	
10^{-1}	- 106	0	0 d vac	rounding up + granular degen of 1/2 epith sheet mainly peripheral	1/3 epith sheet remains	"	"	"	Same + vac	
	- 107	0	0	" "	1/2 " "	"	"	"	" "	
	- 108	0	0	" "	1/3 " "	"	"	"	Epith islands left	
10^{-2}	- 109	0	0	rounding up + granular degen of 1 + to 2 +	1/2 Epith sheet remains - Swiss Cheese - granular degen	"	"	1/3 epith cells left	" " + vac	
	- 110	0	0	" "	" "	"	"	" "	" " "	
	- 111	0	0	" "	1/2 Epith sheet remains - Swiss Cheese - granular degen → Histology					
10^{-3}	- 112	0	0	0	1/2 " "					
	- 113	0	0	Beg. degen	1/4 " " } Pooled titrated 4+16-56					
	- 114	0	0	0	1/4 " " }					
10^{-4}	- 115	0	0	0	1/4 " " ← Same	many epith islands left	Same	Same		
	- 116	0	0	0	0	1/2 Epith sheet remains - granular degen	Same → Histology			
	- 117	0	0 d retract	0 d retract	1/2 " "	Same	1/3 epith left + retract	Same + vac	Epith Islands left	
8 day Undil	- 118	0	0	1/2 epith sheet → degen -	1/3 " "	Same	Same	Same	Large " "	
	- 119	0	0	" "	1/2 " "	"	"	" + vac	Same + vac	
	- 120	0	0	" "	1/2 " "	"	"	"	" "	
10^{-1}	- 121	0	0	" "	1/2 " "	"	"	"	Large Epith islands	
	- 122	0	0	" "	1/4 " "	"	"	Small islands of epith left	Small " "	
	- 123	0	0 d vac	" "	1/2 " "	"	"	"	" "	
10^{-2}	- 124	0	0	Beg degen ? + retract	1/3 " "	"	"	"	" "	
	- 125	0	0	0	1/2 " "	1/4 epith cells remain	"	" + vac	" " + vac	
	- 126	0	0 d vac	Retract.	1/4 " "	few epith cells remain - retract.	Complete degen - no cells left		Same	
10^{-3}	- 127	0	0	0	0 d vac	0 d vac	Same	Extensive vac	Complete vac degen	
	- 128	0	0	0	0 d vac	0 d vac	"	" "	" "	
	- 129	0	0	0	0	1/2 epith sheet left granular degen	"	Same	Small islands + vac	
10^{-4}	- 130	0	0	0	0	0	"	0	0 vac	
	- 131	0	0	0	0	0	"	0 vac	0 vac	
	- 132	0	0	0	0	0	0 d vac	0 d vac	0 vac - complete	
Uninoc. Controls	133	0	0	0	0	0	0 → Histology			
	134	0	0	0 d retract	0 d vac retract	0 d vac retract	0 d vac	0 d vac retract	0 vac Complete degen	
	135	0	0	0	0	0	0 d vac → Histology		0 "	
	136	0	0	0 d vac	0 d vac	0 d vac	0 d vac	0 d vac	" " "	
	137	0	0 d vac	0 d vac	0 d vac	0 d vac	0 d vac	0 d vac	" " "	

April 15, 1955 Rounding up of cells + granular degeneration mainly peripheral - Also focal areas affected producing "Swiss Cheese" appearance - not like polio! - Some clumping of affected cells - granular cells take on swollen + grotesque appearance

STANDARD B&P "NOTEAR"

"NOTEAR"

Egg Passage of CEA Monkey Kidney Pass 1

Virus CEA KP1 Tissue cult fluid from Tubes 150-112 6

114 of 4-16-55

Inoc 0.1 ml of 10^{-1} into allantoic sac of

10 day old White Leghorn embryos – Inoculum = $10^{4.5}$ TCD50 per egg

10^{-1} dilution prepared in 1 tank complete + 100 units of penicillin + 100 micrograms streptomycin per ml.

1 2 Day

Egg		
1	0	Dead
2	Dead	
3	0	Dead
4	Dead	
5	0	Dead
6	0	Dead
7	0	"
8	0	"
9	0	"
10	0	"
11	0	"
12	0	"
13	Dead	
14	0	"
15	0	"
16	0	"
17	Dead	"
18	0	
19	0	"

April 22, 1955 48 hours after inoc.

embryos that were normal at 24 hrs are all dead –

After chilling for 15 min at deep freeze temperature allantoic fluid harvested – centrifuged 2000 rpm Supernatant fluid tested for HA + remainder stored in deep freeze in lusteroids (24 lusteroids) or in vials + rubber caps in CO_2 box A-3-8 (11 ampules)

All embryos showed hemorrhagic lesions

BAP=0 BAP=0

April 22, 1955 — 0.5% chick rbc in saline; cells of 4-20-55 washed 4-22-55

diluent = M/50 PO_4 Saline pH 7.0

Temp Sed.	Undil	2	4	8	16	32	64	128	256	512	1024	2048	4096	0
40°C	++	++	++	++	++	++	++	+/±	0	0	0	0	0	
27°C	++	++	++	++	+	+	+	+/±	±	0	0	0	0	

after 13 hours at 26°C - (i.e. overnight) patterns all 0 - no evidence of hemagglutination -

April 23, 1955

Tissue Cult Titration of CEA KPI Egg 1

Virus CEA KPI Egg 1 allantoic fluid harvested 4-22-55

Procedure 10 fold dilutions in Hank's complete

0.2 ml per tube 3 tubes/decimal dilution

Tubes T53 prepared 4-14-55 1ml 1:200 cell susp. Lactalb-Hanks
5% calf serum

Medium replaced c̄ Lactalb-Earles pH 8.2
4-22-55

Dilution Tested		Tube No	1	2	3	4	5	7	10
10^{-1}	T53	29	1-2+ gran focal degen	Small epith islands left	Complete degen	Same	Same	Same	Same
		30	2+ " "	1/5 epith left	Epith islands	"	"	"	"
		31	2-3+ " "	epith island left	" "	"	"	"	"
10^{-2}		32	1+ " "	" " "	" "	"	"	"	"
		33	1-2+ " "	1/4 epith left	Same	Epith islands	"	"	"
		34	" " "	1/5 " "	Same	" "	"	"	"
10^{-3}		35	0	2-3+ gran degen	1/5 epith left	" "	"	"	"
		36	0	epith islands left	Same	" "	"	"	
		37	0	2+ gran degen	1/4 epith left	Same	"	"	"
10^{-4}		38	0	1+ " "	1/5 " "	Same	"	Epith islands	"
		39	0	0-1+ " "	" " "	"	"	"	"
		40	0	0-1+ " "	1/3 " "	"	"	"	"
10^{-5}		41	0	0	1/2 epith left	1/4 epith left	"	"	"
		42	0	0	1+ gran degen	1/3 " "	"	Same	Epith islands left
		43	0	0	" " "	" " "	"	1/5 epith left	"
10^{-6}		44	0	0	0	1/2 " "	"	"	"
		45	0	0	1+ gran degen	" " "	"	"	"
		46	0	0	0	1/3 " "	"	"	"
10^{-7}		47	0	0	0	2+ gran degen	1/3 epith left	"	"
		48	0	0	1+ gran degen	1/3 epith left	1/4 "	"	"
		49	0	0	0?	" "	" "	"	"
10^{-8}		50	0	0	0	0	0	0 retract	Almost complete retraction
		51	0	0	0	0	0	"	" " + vac
		52	0	0	0	0	0	"	0 retract

$10^{8.2}$ TCD50/ml

Controls	Tube No:	1	2	3	4	5	8	10
Uninoc.	T53-56	0	0	0	0	0	~~ordract~~	almost complete retract.
	-57	0	0	0	0	~~retract~~ Same	"	"
	58	0	0	0	0	" "	"	"
	59	0	0	0	0	" "	"	"

April 28, 1955

Identification of CEA by Hemagglutination-Inhibition with N D V Immune Serum from Chicken and Rabbit

Hemagglutinin CEA KP1 Egg 1 allantoic fluid of 4-22-55 previous titer = 1:128 using 0.5% chicken rbc

Sera 1.) N.D.V. Immune Rabbit Cg. Strain
18 day #78 # 5-12-52
obtained from Dr Berg 4-28-55

2) NDV Immune Chicken B Strain
14 day 5-8-54
obtained from Dr Berg 4-28-55

3) Normal chicken serum obtained from Dr Berg 4-28-55

4) Inactivated (56°C 30 min) normal rabbit serum Lot 4-20-55

Sera 1#, 2#, & 3# inactivated 56°C 30 minutes before use in test.

Preliminary titration CEA HA

chicken rbc freshly obtained from 1 day old White Rock Chicks washed 3 X in saline and prepared as a 10% stock susp. in saline - from this 0.5% susp. in saline prepared & used in titration -

Diluent = 0.02 M Poy Saline pH 7.0
Sedimentation of rbc at 4°C

4	8	16	32	64	128	256	512	1024	0
++	++	++	++	++	++ /	±	0	0	0
++	++	++	++	++	++ /	±	0	0	

Serum Tested Inact. 56°C 30 Min	Serum Dilution in M/50 PO4 Saline pH 7.0 0.25 ml 5	10	20	40	80	160	320	640	1280	2560	5120	Serum Control	Inhib. Titer
N.D.V. Immune Rabbit	+	+	+	++	++	++	++	++	++	++		+	Chick RBC ● Aggl. Present
Normal Rabbit	0	0	±	±	+	+	++	++	++	++		0	40
N.D.V. Immune Chicken	0	0	0	0	0	0	0	0	+	++		0	640
Normal Chicken	++	++	++	++	++	++	++	++	++	++		0	<5

Simultaneous titration of 1:8 HA

16 units	8	4	2	1	1/2	0
++	++	++	++	++	±	0
++	++	++	++	++	±	

8 units in test

April 14, 1955

Passage & Titration Chick Embryo Agent (CEA) from 1st Monkey Kidney Passage

Viruses

1st Monkey kidney passage of CEA (monkey culture tubes inoc. c̄ 4 day fluid from Glenn Exp. Control Tubes)
Fluid harvested on 4-16-55 4 days after inoc of tubes c̄ original chick embryo culture fluid -

Titration Pooled fluid Tubes T50-112-114 (inoc c̄ 10^{-3})

Passage c̄ undiluted fluid only from indicated tubes of 1st monkey kidney passage

Tubes				
T50	[redacted] -103	inoc c̄	undil fluid
T50	- 108	" "	10^{-1}
T50	- 109	" "	10^{-2}
T50	- 115 [redacted]	" "	10^{-4}

Inoc 0.2 ml per tube -

Tubes T51 Prepared 4-6-55 0.5 ml 1:200 cell susp. in Lactalb Hanks 2% calf serum 1.5 ml Lactalb Earles p H 8.2 added 4-15-55

Inoc c̄ 0.2 ml from	Tube No.	1	2	3	4	5	6	8	10	12
T50-103 (undil)	T51 - 100	0	0	Big. degen	3/4 epith left	1/2 epith left	1/3 epith left	Same	Same	Same
	- 101	0	0	"	" "	" "	Same		"	Same
T50-108 (10^{-1})	T51 - 102	0	0	1/2 Epith degen	Same	Same	1/3 epith left		"	Same + vac
	103	0	Big. peripheral gran. degen.	"	"	"	Same		"	1/3 epith left + vac
T50-109 (10^{-2})	T51 - 104	0	Peripheral gran. degen.	" 1+	"	"	"		"	Epith Islands
	105	0	"	" "	"	"	"		"	Same
T50-115 (10^{-4})	T51 106	0	gran. degen - "Surrs cheese cells	" 1/2 epith left	1/3 epith remains	"	"		"	Same + vac
	107	0	"	3/4 epith left	1/2 epith remains	"	"		"	1/4 epith left
Uninoculated tubes Control	108	0	0	0	0 vac	0 vac	0 vac	0 vac extensive		Complete vac degen
	109	0	0	0	0	0 retract	0 retract	Same		1/2 epith left c̄ vac
	110	0	0	0	0	0*	0	0		0 retract
	111	0	0	0	0	0*	0 retract	Same		Earles p H 7.4
	112	0	0	0 vac	0 vac	0 vac	0 vac	"		0 vac 2+ Lactalb 0 vac 4+

* Fluid removed + replaced c̄

Titration pooled fluid Tubes T50 112 - 114 (inoc c̄ 10^{-3} of check embryo culture fluid)

Dil	Tube No	1	2	3	4	5	6	8	10	12
Undil	T50 113	0	Beg. gran. degen?	gran. degen Swiss cheese	Same	1/3 epith left	Same	Same	Same	Same
	114	0	0	1/2 epith affected " "	Same active degen					
	115	0	0	" "	" "	Same	Same			"
					" "	Same	1/3 epith left		"	"
10^{-1}	116	0	Beg. gran. degen?	" " 1/3 epith left	Same	Same	Same		"	"
	117	0	0	gran. degen epith islands left	} fluids centrifuged 2000 rpm - fluid pooled + stored in deep freeze -					
	118	0	0	" "						
10^{-2}	119	0	0	" "	Same	Same	Same		"	"
	120	0	0	gran. degen - large gran. cells - Swiss cheese 1/4 epith left	" Epith islands left	Same	"		"	"
	121	0	0 retract	" "	Large epith islands left	Same	"		"	"
10^{-3}	122	0	0	gran. degen - 1/2 epith affected	Epith islands left	Same	"		"	"
	123	0	0	" "	1/4 epith left	Epith islands left	"		"	"
	124	0	0	3/4 " "	Epith islands left	Same	"		"	"
10^{-4}	125	0	0	0	gran. degen - 1/4 epith left	Epith islands left *	"		"	"
	126	0	0	0	gran degen - epith islands left	Same *	"		"	"
	127	0	0 vac	Beg. gran degen.	" "	" *	"		"	"
10^{-5}	128	0	0	0 retract	" "	" *	"		"	"
	129	0	0	0	" "	" *	"		"	" + vac
	130	0	0	0	granular degen - 1/3 epith left	Epith islands left *	"		"	"
10^{-6}	131	0	0	0	0	0	0		0 vac	0 vac
	132	0	0	0	Beg gran degen	1/2 epith left	Same		Same	Same
	133	0	0	0	0 retract & vac	0 retract vac	"		0 vac extensive	0 vac
10^{-7}	134	0	0	0	0 vac	0 vac	0 vac		" "	0 vac
	135	0	0	0	0	0	0		0 retract	0 retract + vac
	136	0	0	0	0	0	0		0 "	0

$10^{6.5}$ TCD50/ml

3rd Day April 19, 1955 Effect most marked in tubes inoc c̄ 10^{-1} & 10^{-2} dilution - suggests mixed population of cyto pathogenic and non cytopathogenic virus particles!

5th day April 21, 1955 * Fluid from 10^{-4} & 10^{-5} dilution tubes removed & replaced by 2 ml of Lactalb - Earles pH 7.4 - Tissue cult fluid centrifuged 2000 rpm for 10 min & stored in deep freeze - tested for HA -

April 21, 1955 Test for HA in Tissue Cult Fluid of 2nd Monkey Kidney Passage of Chick Embryo Agent (CEA)

Virus Pooled fluids from Tubes T51 125-130 of CEA KP2 Fluid from tubes inoc c 10^{-4} & 10^{-5} dilution of KP1 Harvested 4-21-55 5 days after inoculation

Procedure
0.5 ml dilutions of CEA KP2 prepared in M/50 PO4 Saline pH7.0 – 0.5 ml of 0.5% chick erythrocytes in saline then added to each tube – Tubes shaken + placed at indicated temperature

Control fluid from Tubes T51 110 & 111 also tested simultaneously.

	Temp. initial sedimentation	Temp. at which resuspended cells sedimented	Undil	2	4	8	16	32	64	128	256	0
CEA	4°C		++	++	++	+	/	0	0	0	0	0	0	0
		37°	0	0	0	0	0	0	0	0	0	0
	26°C		+ ring	+ ring	+ ring	/	±	0	0	0	0	0	0	0
		37°	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0
	37°C		0	0	0	0	0	0	0	0	0	0
		37°	0	0	0	0	0	0	0	0	0	0
Control Fluid	4°C		0	0	0	0						0
		37°	0	0	0	0						0
	26°C		0	0	0	0						0
		37°	0	0	0	0						0
	37°C		0	0	0	0						0
		37°	0	0	0	0						0

[Patterns began to reverse between 40 min & 60 min at 26°]

April 19, 1955 Mon. Kidney Passage 3 of Chick Embryo Agent [CEA]

Also test for CEA in Horse Serum Lot 2467 of Microbiologic Associates - Tested unheated - This serum used to prepare chick embryo cultures which yielded C.E.A.

Virus CEA KP2 pooled tissue cult. fluid from Tubes T50 117 & 118 inoc c̄ 10^{-3} of KP1 harvested 4-19-55

Inoc. 0.2ml of 10^{-1}

Tubes T52 prepared 4-13-55 0.5ml 1:200 cell susp. in Lactalb Hanks 2% calf serum
1.5ml Lactalb Hanks pH7.4 added 4-19-55

	Tube No.	1	2	3	4	6	8	10	12
CEA KP2 10^{-1}	T52-1	0	0	Beg. gran. degen	1/3 epith sheet degen				
	2	0	0	1+ degen - focal gran degen	1/2 " "				
	3	0	0	" "	" " "				
	4	0	0	1/2 epith left gran degen -	" " "				
	5	0	0	2+ degen - focal	" " "				
	6	0	0	" "	" " "				
	7	0	0	" "	" " "				
	8	0	0	2-3+ "	" " "				
	9	0	0	" "	" " "				
	10	0	0	2+ "	" " "				
	11	0	0	2+ "	" " "				
	12	0	0	1-2+ "	" " "				
	13	0	0	2+ "	" " "				
	14	0	0	" "	" " "				
	15	0	0	1-2+ "	" " "				
	16	0	0	2+ "	" " "				
	17	0	0	1-2+ "	" " "				
	18	0	0	2+ "	" " "				
Horse Serum Lot 2467 0.2ml	19	0	0	0	0	0	0	0	0 vac
	20	0	0	0	0	0 vac	0 vac	0 vac	0 vac
	21	0	0	0	0	0	0	0	0 retract
	22	0	0	0	0	0	0	0	0
Uninoculated controls	23	0	0	0	0	0	0	0 retract	same
	24	0	0	0	0	0	0	"	"
	25	0	0	0	0	0	0 retract	"	"

Pooled, centrifuged 2000 rpm 10 min

Titrated in T.C. and for HA

BAP = 0 BAP = 0

20 lusterods (1ml) stored deep freeze

8 ampules 1ml stored A-3-8

April 23, 1955 HA titration of CEA KP3 Tissue Cult Fluid Pool

Virus CEA KP3 Tissue cult fluid from tube, T_{S_2} 1-18
harvested 4-23-55

0.5% chick rbc in saline prepared 4-23-55 from 2 of 4-20-55
10% susp in saline washed on 4-22-55 from

Diluent = M/50 PO_4 Saline pH 7.0

0.5 ml volume of deleted HA 2 cells of -

Temp.	Sedimentation	Dilution HA								
		Undil	2	4	8	16	32	64	128	0
4°		++	++	++	++	±	0	0	0	0
26°		++	++	++	++	+/	0	0	0	0

Read 40 min 26°

60 min 4°C

2 hours after 1st reading all patterns normal - Tubes held at 26°C

April 23, 1955 — Tissue Cult Titration of CEA KP3

Virus CEA KP3 4-23-55 Tissue cult fluid from Tubes T52 1-18

Procedure 10 fold dilutions in Hanks complete

0.2 ml per tube 3 tubes / decimal dilution

Tubes T53 prepared 4-14-55 1 ml 1:200 all susp. Lactalbumin Hanks 5% Calf Serum

Medium replaced ~~C~~ Lactalbumin - Earles pH 8.2 on 4-22-55

	Tube No T53	1	2	3	4	5	7	10
10^{-1}	1	0	Beg. gran. deg	½ epith left	¼ epith left	Same	1/5 epith left	Epith islands
	2	0	1+ " "	⅓ " "	Large epith islands	"	Same	" "
	3	0	0	⅓ " "	Same	"	Large epith islands	" "
10^{-2}	4	0	0-2+ " "	¼ " "	Large epith islands	"	"	" "
	5	0	0	Large islands epith left	Same	"	"	" "
	6	0	0	½ epith left	⅕ epith left	"	Same	" "
10^{-3}	7	0	0	" "	" "	"	"	" "
	8	0	0	Beg. peripheral degen	½ Epith left	"	¼ epith left	" "
	9	0	0	2+ gran degen focal	¼ " "	"	"	" "
10^{-4}	10	0	0	" "	½ " "	¼ epith left	"	" "
	11	0	0	1+ " "	" "	⅓ " "	"	" "
	12	0	0	" "	⅓ " "	"	"	" "
	13	0	0	1+ " "	½ " "	"	"	" "
	14	0	0	1+ " "	¼ " "	"	"	" "
	15	0	0	1-2+ " "	⅕ " "	Epith Islands	left Same	" "
	16	0	0	2+ " "	Large epith islands	"	"	" "
	17	0	0	1+ " "	⅓ epith left	Same	"	" "
	18	0	0	2+ " "	Epith islands	Same	"	" "
	19	0	0	2+ " "	½ epith left	¼ epith left	Epith islands	" "
10^{-5}	20	0	0	0	2+ gran degen	⅓ epith left	"	" "
	21	0	alk	alk	Beg. gran degen	Epith islands	Same	" "
	22	0	0	0 retract	1+ gran degen? retract.	⅓ epith left	"	" "
10^{-6}	23	0	0	0	0 retract	Same	Same	Almost complete retract
	24	0	0	0	0 retract	"	"	" "
	25	0	0	0	0	0 retract	"	" "
10^{-7}	26	0	0	0	0 retract	"	"	" "
	27	0	0	0	" "	"	"	" "
	28	0	0	0	" "	"	"	" "

$10^{6.2}$ TCID50/ml

		1	2	3	4	5	7	10	
10^{-8}	= 53	0	0	0	0	0	0	~~0 retract~~	
	= 54	0	0	0	0	0	0	~~0 retract~~ + inc	
	= 55	0	0	0	0	0	0	~~0 retract~~	

April 29, 1955 — # Identification of CEA by Neutralization in Tissue Culture with N.D.V Immune Serum from Rabbit & Chicken

Virus CEA KP3 4-23-55 Tissue cult fluid Tubes T52 1-18
Titer per 0.2 ml = $10^{5.5}$ ∴ 100 TCD_{50} in 0.2 ml = $10^{-3.5}$
Stored A-3-8 use 1:1600

Serum - 1) NDV Immune Rabbit - Cg Strain 18 day #78
5-12-52 Received from Dr Berg on 4-28-55
2.) Normal Rabbit Serum 4-20-55 Inact. (CHRF)
3) NDV Immune Chicken B Strain 14 day 5-8-54
~~obtained~~ from Dr Berg 4-28-55
4) Normal chicken ~~obtained~~ from Dr Berg 4-28-55
All sera inact. 56°C 30 min before use in test.

Procedure - 0.4 ml serum dilutions + 0.4 ml virus $10^{-3.2}$ } 1 hr room temp. then inoc 3 tubes with 0.2 ml/tube

Tubes T55 Prepared 4-20-55 1 ml 1:200 cell susp. in Lactalb/Hanks 5% calf serum — Medium replaced 4-29-55 ~ 2 ml Lactalb Earles pH 7.4

	Tube No.	1	2	3	4	5	6	8
CEA $10^{-3.5}$	T55-1	0	0	1+ gran degen	1/3 equith left	Same	Same	Equith islands
	2	0	0	0?	1/4 " "	"	Same	" "
	3	0	0	0-1	1/2 " "	"	"	" "
[CEA MK Pass 4] $10^{-4.5}$	4	0	0	0	1-2+ gran degen	1/2 equith short left	1/3 equith left	" "
	5	0	0	0	?0-1+ "	2/3 " "	Same	" "
	6	0	0	0	0?	1+ gran degen	1/2 equith left	" "
$10^{-5.5}$	7	0	0	0	2-3+ gran* degen	1/5 equith left	Same	" "
	8	0	0	0	0	0	0	0 vac marked + retract
	9	0	0	0	2-3+ gran* degen	Equith islands	Same	Same
$10^{-6.5}$	10	0	0	0	0	0	0	0 retract
	11	0	0	0	0	0 vac	0 vac	0 vac + retract
	12	0	0	0	0	0	0	0 vac + retract
$10^{-7.5}$	13	0	0	0	0	0	0	0 retract
	14	0	0	0	vac	0 vac	0 vac	0 vac + retract
	15	0	0	0	0	0 vac	0 vac	0 vac + retract

$10^{5.8}$ TCD_{50}/0.2 ml
∴ 200 TCD_{50} in test

May 3 1955 * Fluid from tubes T55 7-9 removed, pooled, centrifuged 2000 rpm = CEA KP4 — used to initiate KP5 — remainder stored in deep freeze — 2nd Lactalb Earles pH 7.4 replaced in tubes

Serum Titer vs 200TCD50

	Tube No		1	2	3	4	5	6	8	
CEA $10^{-3.5}$ + Normal Rabbit Serum	T54 - 1:2	16	0	0	1-2+ gran degen	Epith islands left	Same	Same	Complete degen	
		17	0	0	0-1 "	2+ gran degen	1/3 epith left	"	Same	<1:2
	~~Undil~~	18	0	0	0-1 " + retract	Epith islands left	Same	"	Complete degen	
CEA $10^{-3.5}$ + NDV Immune Rabbit	~~Undil~~ 1:2	19	0	0	0	0 vac	0 vac	0 vac	0 vac	
		20	0	0	0	0	0	0 vac	0 vac	1:6
		21	0	0	0	0	0	0	0 vac	
	~~10~~ 1:20	22	0	0	0 retract	3+ gran degen	Epith islands left	Same	Complete degen	
		23	0	0	0	" "	" "	"	"	
		24	0	0	0	" "	" "	"	"	
	~~10~~ 1:200	25	0	0	0-1	Epith islands	" "	"	"	
		26	0	0	1+	" "	" "	"	"	
		27	0	0	1+	" "	" "	"	"	
	~~10~~ 1:2000	28	0	0	1+	" "	" "	"	"	
		29	0	0	1+	1/3 epith left.	Same	"	Epith islands	
		30	0	0 vac	1+	" " "	1/5 epith left	"	" "	
CEA $10^{-3.5}$ + Normal chicken serum	~~Undil~~ 1:2	31	0	0	0?	2+ gran degen	1/3 epith left	Same	Complete degen	<1:2
		32	0	0	1+	Epith islands left	Same	"	"	
		33	0	0	1+	1/4 epith left	"	Epith islands left	Same	
CEA $10^{-3.5}$ + NDV Chicken	~~Undil~~ 1:2	34	0	0	0	0	0	0 vac + retract	0 vac + retract	
		35	0	0	0	0	0	0	0 retract	
		36	0	0	0	0	0	0 vac	0 vac retract	
	~~10~~ 1:20	37	0	0	0	0	0 vac	0 vac	0 vac retract	
		38	0	0	0	0	0	0	0 retract	1:400
		39	0	0	0	0 vac	0 vac	0 vac	0 vac retract	
	~~10~~ 1:200	40	0	0	0	0	0	0	0 retract	
		41	0	0	0	0	0	0	0 retract	
		42	0	0	0	0?	3+ gran degen	Epith islands left	Same	
	~~10~~ 1:2000	43	0	0	1-2+	Epith islands left	Same	Same	Complete degen	
		44	0	0	1+	" " "	"	"	"	
		45	0	0	1+	" " "	"	"	"	

By 8th day tubes 5 CEA change showed marked retract, + few cells left

May 3, 1955 CEA KP5

Virus CEA KP4 – Tissue culture fluid from tubes T55 729 of KP4 – harvested 5-3-55 – (these tubes inoc c $10^{-5.5}$ of KP3 on 4-29-55)

Tubes Series T56 prepared 4-27-55 0.5 ml 1:200 cell susp in Lactall Hanks 2% calf serum 1.5 ml Lactall Earle's pH 7.4 added 5-3-55

Tube No T56	1	2	3	4	5	6	7	10
69	0	1-2+ gran degen	1/3 epith left	Same	1/4 up deg	Same	Same	Same
70	0	" "	1/4 " "	"	1/4 left	Same	Same	"
71	0	" "	1/2 " "	"	1/3 up deg	Same	Same	"
72	0	H "	2+ gran	"	1/2 left	Same	Same	"
73	0	0?	gran degen Epith islands	} Pooled, centrifuged 2000 rpm 10 min – Tested for HA – stored in deep freeze				
74	0	0	1/4 epith left					
75	0	0	" " "					
76	0	0	1/4 " "					

Undil ✓ 10^{-2}

May 6, 1955 Test for HA in CEA KP5

Red Cells = 0.5% susp. in saline prepared from 10% susp of washed rbc of 5-6-55 from chick rbc of 5-5-55

Diluent = M/50 PO_4 Saline pH 7.0 Temp Seal = 4°C

Undil	2	4	8	16	32	64	128	0
H	H	+	+/±	0	0	0	0	0

May 20, 1955 CEA KP6

Virus CEA KP5 Pooled centrifuged fluid from tubes T56 73-76 of 5-6-55

Tubes T60 of 5-11-55 0.5 ml 1:200 cell susp. in Lactalb
Hanks 2% calf serum
1.5 ml Lactalb Earles added 5-17-55
Medium replaced c̄ pH 7.4 Lactalb Earles c̄
0.5 % calf serum 2 ml on 5-20-55

Tube T60	1	2	3	4
90	0	1+ gran degen	3/4 epith left	Same
91	0	"	1/2 " "	1/3 epith left
10⁻¹ 92	0	"	3/4 " "	1/2 " "
93	0	"	1/2 " "	Same
94	0	"	1/2 " "	"
95	0	"	3/4 " "	1/2 epith left
96	0	"	1/2 " "	1/3 " "
97	0	"	1/2 " "	Same
98	0	"	1/2 " "	"
99	0	"	1/2 " "	1/3 epith left
100	0	"	1/2 " "	" " "
101	0	"	1/2 " "	Same
102	0	"	1/2 " "	"
103	0	"	1/2 " "	"
104	0	"	1/2 " "	1/3 epith left
105	0	"	1/2 " "	" " "
106	0	"	Mold	
107	0	"	1/2 epith left	" " "
108	0	"	1/2 " "	Same
109	0	"	1/2 " "	1/3 epith left
110	0	"	1/2 " "	Same
111	0	"	1/2 " "	1/3 epith left
112	0	"	1/2 " "	" " "
113	0	"	1/2 " "	" " "
114	0	"	1/2 " "	" " "
115	0	"	1/2 " "	" " "
116	0	"	1/2 " "	" " "
117	0	"	1/2 " "	Same
118	0	"	1/2 " "	1/3 epith left
119	0	"	1/3 " "	" " "
120	0	"	1/2 " "	" " "
121	0	"	1/2 " "	" " "
122	0	"	1/2 " "	" " "
123	0	"	1/2 " "	" " "
124	0	"	1/2 " "	" " "
125	0	"	1/3 " "	1/4 " " "
~~126~~				

Tco-	1	2	3	4
126	0	0-?	1+ gran degen	1/3 epith left
127	0	0	1-2+ " "	1/2 epith left
128	0	0-?	2+ " "	1/4 epith left
129	0	0	1+ " "	Large epith islands

10-4

Virginia controls

130	0	0	0	0 vac
131	0	0	0	0 vac
132	0	0	0	0
133	0	0	0	0
134	0	0	0	0 vac
135	0	0	0	0
136	0	0	0 vac	0 vac
137	0	0	0	0
138	0	0	0	0
139	0	0	0	0
140	0	0	0	0 vac
141	0	0	0	0 vac
142	0	0	0	0
143	0	0	0	0
144	0	0	0	0
145	0	0	0	0
146	0	0	0	0
147	0	0	0	0
148	0	0	0	0
149	0	0	0	0
150	0	0	0	0
151	0	0	0	0
152	0	0	0	0
153	0	0	0	0
154	0	0	0	0
155	0	0	0	0

May 24, 1955 Fluid removed from tubes - replaced with 2 ml Lactalb - Earles pH 7.4 + 0.5 % calf serum.

May 24, 1955

Interference Between NDV Virus and Polio Types I, II, and III and Human Enteric Types I, II, III & V in Monkey Kidney Epithelial Cultures

- Procedure - Tubes containing residual epith. cells 4 days after infection with NDV virus (KP5) challenged ⊂ 1000 TCD_{50} of Types I, II, & III Poliomyelitis and Types I, II, III, & V Human Enteric Virus. Uninoculated tubes from same series and treated in same manner as NDV tubes except for NDV inoculation challenged with same viruses

- Viruses for challenge -

Polio. Type I A-4-[redacted] KP35 Lot 4 of 1-4-54 Amp III in 1000 TCD_{50} = 0.2 ml of $10^{3.5}$ - virus 1:3200 0.2 ml

Type II A-4-3 VSK KP53 Lot 1 of 1-4-54 Amp I in 1000 TCD_{50} = 0.2 ml of $10^{3.5}$ - virus 0.2 ml 1:3200

Type III A-4-3 [redacted] KP36 Lot 1 of 1-4-54 Amp I in 1000 TCD_{50} = 0.2 ml of $10^{3.5}$ - virus 0.2 ml 1:320

Human Enteric Type I [redacted] KP3 of 2-21-55 Deep freeze HEB, 1000 TCD_{50} = 0.2 ml of $10^{3.8}$ - virus 0.2 ml 1:6400

Type II [redacted] KP4 of 2-22-55 Deep freeze HEB, 1000 TCD_{50} = 0.2 ml of $10^{3.0}$ - virus 0.2 ml 1:1000

Type III [redacted] KP7 of 2-22-55 Deep freeze HEB, 1000 TCD_{50} = 0.2 ml of $10^{3.2}$ - virus 0.2 ml 1:2600

Type V [redacted] KP5 2-22-55 Deep freeze HEB, 1000 TCD_{50} = 0.2 ml of $10^{3.5}$ - virus 0.2 ml of 1:3200

STANDARD B&P "NOTEAR"
STANDARD B&P "NOTEAR"

Challenge Tube Virus		0	1	2	3	4	5	6	7
Polio I	T60-90	3/4 epith left pH 7.5	Same	Same pH 7.4	Same pH 7.3	1 Small pocs round refractile cells - pH 7.1	Few scattered round refractile cells pH 7.0	Same pH 6.9	0-1 (10%)
Inoc 4 days prior to CNV KPS 10-1	91	1/3 epith left pH 7.5	"	moderate vac. - pH 7.6	Extensive vac. - normal epith islands pH 7.6	Progression of vac. - epith normal pH 7.6	Same pH 7.6	Same pH 7.6	Same
	92	1/2 epith left pH 7.5	"	Same pH 7.4	Same pH 7.3	0-1 (10%) pH 7.0	Few scattered round cells pH 6.9	Same pH 6.9	0-1 (5%)
	93	1/2 epith left pH 7.5	"	Same + early vac pH 7.5	Same early vac pH 7.4	Same - early vac pH 7.2	Few scattered round refractile cells pH 7.0	0-1 (10%) pH 7.0	0-1 (10%)
	94	1/2 epith left pH 7.5	"	Same + early vac pH 7.5	Same early vac pH 7.4	Same early vac pH 7.2	Same early vac pH 7.0	0-1 (5%) pH 6.9	1
Uninoc controls	T60-130	0 vac (not extensive)	0-1 (10%)	4	off				
	132	0	0-1 (5%)	4	off				
	133	0	0-2 (15%)	4	off				
	135	0	0-1 (5%)	4	off				
Polio II	T60-95	1/2 epith left pH 7.5	Same	Same + early vac pH 7.5	No change pH 7.3	Same pH 7.1	Same - early vac pH 6.9	0-1 (10%) pH 6.9	0-1 (5%)
Inoc 4 days prior to CNV KPS 10-1	96	1/3 epith left pH 7.6	Same	Same pH 7.6	Same + early vac pH 7.4	Same early vac pH 7.2	Same pH 7.1	Same pH 7.0	one round refractile cell pH 6.9 } Same
	97	1/2 epith left pH 7.5	"	Vac. - pH 7.5	Mod. vac. - epith normal pH 7.4	Mod vac. - large clusters normal epith pH 7.4	0-1 (10%) pH 7.2	1 heavy extensive pH 7.1	Same
	98	1/2 epith left pH 7.5	"	Same pH 7.5	Same pH 7.4	Same pH 7.3	Same pH 7.0	Few scattered round cells pH 6.9	0-1 (5%)
	99	1/3 epith left pH 7.5	"	Same pH 7.5	Same pH 7.5	Few scattered round refractile cells pH 7.3	Same pH 7.3	Same pH 7.2	1 pH 7.2
Uninoc controls	T60-131	0 vac (not extensive)	0-1 (5%)	4	off				Same
	137	0	0-1 (1%)	3	4 off				Same
	138	0	0	3	4 off				
	139	0	0-1 (5%)	3	4 off				

May 24, 1955

Tube	0	1	2	3	4	5	6	7
Challenge Virus Polio III								
T60-100	1/3 equiv left pH 7.5	Same	Same Early vac pH 7.5	Same pH 7.3	Small focus round refractile cells (< 1%) pH 7.1	Scattered round cells (1%) pH 7.0	0-1 (5%) pH 6.9	0-1 (10%)
101	1/2 equiv left pH 7.5	"	Same pH 7.5	Same pH 7.3	Same pH 7.1	0-1 (5%) pH 7.0	1 pH 6.9	0-1 (10%)
102	1/2 equiv left pH 7.5	"	Same pH 7.5	Same pH 7.3	0-1 (1%) pH 7.1	0-1 (5%) pH 7.0	1 pH 6.9	1
103	1/2 equiv left pH 7.5	"	Same pH 7.5	Same pH 7.3	Few Scattered round refractile cells pH 7.1 (< 1%)	0-1 (10%) pH 7.0	1-2 pH 6.9	1-2
104	1/3 equiv left pH 7.6	"	Same pH 7.6	Small focus round refractile cells ? pH 7.4	Normal pH 7.2	Few scattered round cells pH 7.1	Same pH 7.0	0-1 (1%) pH 6.9 } Same
Inoc 4 days prior to NDV KPS 10-1								
Uninoc controls								
T60-134	0 vac (not extensive)	0-1 (5%)	3	4 off				Same
142	0	0-1 (1%)	2-3	4 off				Same
143	0	0-1 (1%)	2-3	4				
144	0	0	2	4 off				
HE I								
T60-105	1/3 equiv left pH 7.5	Same	Few scattered round refractile cells 0-? pH 7.5	1-2 (30%) pH 7.3	4 with small residual equiv vacuoles (95%) pH 7.3	4 off pH 7.3	Same	
107	1/3 equiv left pH 7.5	"	as above 0-? pH 7.5	1-2 (30%) pH 7.3	4 pH 7.3	Same	Same	
Inoc 4 days prior to NDV KPS 10-1								
108	1/2 equiv left pH 7.6	"	as above 0-? pH 7.6	1 (20%) pH 7.5	3-4 pH 7.4	4 pH 7.4	Same	
109	1/3 equiv left pH 7.5	"	No change pH 7.5	1 pH 7.3	2-3 pH 7.3	4 Few residual small vacuoles pH 7.2 (95%)	4 off	
110	1/2 equiv left pH 7.5	"	Small focus round refractile cells 0-? pH 7.5	1-2 (30%) pH 7.3	3 pH 7.2	4 pH 7.2	Same	
Uninoc controls								
T60-136	0 vac	2+	4	off				
145	0	0-2+ (15%)	4	off				
146	0	0-1 (10%)	4	off				
147	0	1-2 (30%)	4	off				

Challenge Virus	Tube No.	0	1	2	3	4	5	6	7
HE II Inoc 4 days prior c̄ NDV KP5 10^{-1}	T60-111	1/3 epith left pH 7.5	Same	Same + early vac pH 7.4	0-1 (5%) pH 7.2	2 pH 7.0	4 ε small residual islands (95%) pH 7.0	4 off pH 7.0	
	112	1/3 epith left pH 7.5	"	Few scattered round refractile cells + early vac. pH 7.4	1-2 (30%) pH 7.2	2 pH 7.1	4 ε mod. residual islands (90%) pH 7.1	4 off pH 7.1	
	113	1/3 epith left pH 7.5	"	as above pH 7.5	1-2 (30%) pH 7.4	3 pH 7.4	4 ε small residual islands (95%) pH 7.4	4 off pH 7.4	
	114	1/3 epith left pH 7.5	"	as above pH 7.5	1-2 (30%) pH 7.4	4 off - (90%) residual islands pH 7.4	4 ε residual islands (95%) pH 7.4	4 off pH 7.4	
	115	1/3 epith left pH 7.5	"	as above pH 7.5	1 pH 7.3	4 off 1/2 of sheet residual 1-2+ pH 7.2	4 ε residual islands (95%)	4 off pH 7.2	
Uninoc Controls Prior	T60-140	0 vac (not extensive)	2+	4	off				
	148	0	1-2 (30%)	4-	4				
	149	0	2+	3-4	4				
	150	0	1+	3-4	4				
HE III Inoc 4 days prior c̄ NDV KP5 10^{-1}	T60-116	1/3 epith left pH 7.5	Same	Same pH 7.4	No change pH 7.3	No change pH 7.0	No change pH 6.9	No change pH 6.9	Few round refractile cells pH 6.9 — Same
	117	1/3 epith left pH 7.6	"	Same + early vac pH 7.6	Same pH 7.5	Few scattered round refractile cells (<1%) pH 7.4	No change pH 7.4	Large epith islands - cells OK pH 7.4	Same
	118	1/3 epith left pH 7.5	"	Same + early vac pH 7.5	Same pH 7.4	Same pH 7.1	Few scattered round cells pH 7.0	No change pH 7.0	Few round cells pH 7.0
	119	1/3 epith left pH 7.5	"	No change pH 7.5	No change pH 7.4	Same pH 7.2	Same pH 7.1	0-1 (1%) pH 7.0	0-1 (5%) pH 7.0
	120	1/3 epith left pH 7.5	"	No change pH 7.5	No change pH 7.2	No change pH 7.1	No change pH 6.9	No change pH 6.9	0-1 (1%) pH 6.9
Uninoc controls	T60-141	0 vac (not extensive)	0-1 (10%)	3-4	4 off				Same
	151	0	0-1 (5%)	2-3	4				Same
	152	0	0-1 (5%)	2-3	3-4	4			

May 24, 1955

Challenge Virus	Tube No	0	1	2	3	4	5	6
HEV	T60-121	1/3 epith left pH 7.5	Same	0-? few round refractile cells pH 7.5	0-1 (10%) pH 7.2	0-1 (10%) pH 7.1	Few refractile round cells pH 7.0	Large Epith islands left 0-1 (10%) pH 7.0
Inoc 4 days prior to NDV KPS 10^{-1}	122	1/3 epith left pH 7.4	"	No change + early rnc pH 7.4	0-1 (5%) pH 7.1	1 pH 7.1	Same as above pH 6.9	Epith islands 0-1 left (10%) pH 6.9
	123	1/3 epith left pH 7.5	"	0-? as above pH 7.5	1 pH 7.3	4 + large (80%) residual islands pH 7.3	Residual islands (95%) pH 7.2	4 pH 7.2
	124	1/3 epith left pH 7.5	"	0-? as above pH 7.5	1 pH 7.3	4 to 1/2 epith sheet - residual → pH 7.2 (60%)	Residual islands (80%) pH 7.2	Residual islands (90%) pH 7.2
	125	1/4 epith left pH 7.6	"	1 focus round refractile cells 0-? pH 7.6	Epith islands with 0-1 (10%) pH 7.6	4 pH 7.6	Same	Same pH 7.6
Uninoc controls	T60-153	0	1+	3	4			
	154	0	0-1 (10%)	3	4 off			
	155	0	1-2 (30%)	3-4	4 off			
Polio Type I Inoc 4 days prior to NDV KPS 10^{-4}	T60-126	1/3 epith left pH 7.5	Same	No change pH 7.5	No change pH 7.4	Same pH 7.2	0-1 (1%) pH 7.1	0-1 (10%) pH 7.0	0-1 (1%)
	127	1/2 epith left pH 7.5	Same	No change pH 7.3	No change pH 7.2	Same pH 7.0	0-1 (10%) pH 6.9	0-1 (5%) pH 6.9	1
	128	1/4 epith left pH 7.6	Same	Same pH 7.6	No change pH 7.5	Same pH 7.5	Few scattered round refractile cells pH 7.3	0-1 (1%) pH 7.2	0-1 (10%)
	129	Large epith islands pH 7.5	Same	Same pH 7.5	No change pH 7.4	No change pH 7.3	No change pH 7.2	Same pH 7.2	Same

April 14, 1955

Passage from Tubes T50-118, 119, & 120-

On sixth day although degeneration of epith cells in tubes T50-118 & 119 was complete a few round cells suggestions of polymorphs, change were also present. Question of co-existence of two processes in these tubes. Process leading to granular degeneration + polio change?

Tubes T50 series prepared 4-5-55
0.5 ml 1:200 cell susp.
in Lactalb Hanks 2% Calf serum
1.5 ml Lactalb Earles pH 8.2 added 4-11-55

0.2 ml inoc per tube

Inoc	Tube No	1 (20 hours)	2	3	4	5
Contents T50-118	T50-192	0-2 rounding up predominates		4 off - stored deep freeze		
	193	0-2 " "		4 off " " "		
T50-119	194	0-2 " "	Mainly 4 small epith clust & granular degen	Same	off → deep freeze	
	195	0-2 " "	Some + large epith clusts	Same	Same	Few epith threads left → deep freeze
T50 120	196	2-3 " "	4 off	stored deep freeze		
	197	2-3 " "	4 off	" " "		
April 16, 1955	T51-137	0	4 polio	" " "		
Inoc T50-121	138	0?	4 polio	" " "		

Isolation of NDV from Infected Chicken Plasma Used to Prepare Chick Embryo Explant Tubes.

Plasma Lot Used	Experiment	No. of days after prep. of culture Polio virus added	Degen. of C.E. fibroblast		Test for NDV in monkey kid. epith. culture	
			Observed	Day after prep. of cultured	Fluid tested	Result
8-5-54	CE Var. Exp 2	4 day	No		8 day	Neg
					12 day	Neg
10-21-54	CE Adsorb Exp 1	5 day	YES	7	9 day	Pos
					13 day	Pos
	CE Adsorb Exp 2	5 day	No		9 day	Neg
					13 day	Neg
	4th Criss Cross Exp	4 day	YES	6	8 day	Pos
					12 day	Pos
	Double Washing Exp	4 day	YES	6	8 day	Pos
	Glenn Double Washing	6 day	YES	8	10 day	Pos
					14 day	Pos

April 26, 1955 Test for CEA in Tissue Culture Fluids from Various Chick Embryo Tissue Culture Experiments.

Also test for CEA in Chick Embryo Extract and Chicken Plasma used in Chick Embryo Experiment from which CEA was isolated

Fluids or Materials to be Tested NDV

1.) Control fluid from Chick Embryo Variants Exp 2
4 day fluid Oct 12, 1954
8 day fluid Oct 16, 1954 0

2) Control fluid from Chick Embryo Adsorb Exp 1
4 day fluid Nov 12, 1954
8 day fluid Nov 16, 1954 +

3) Control fluid from Chick Embryo Adsorb Exp 2
4 day fluid Dec 18, 1954
8 day fluid Dec 22, 1954 0

4) Control fluid Chick Embryo 4th Cross Cross Passage
4 day fluid 11-27-54
8 day fluid 12-1-54 +

5) Control fluid Chick Embryo Double Washing Exp
4 day fluid 3-22-55 +

6) Chicken Plasma Lot of 10-21-54 stored 4°C in refrigerator +!!

7) CEE Lot of 7-31-54 stored in deep freeze 0

Inoc 0.2 ml of indicated dilution of material to be tested

Tubes Series 154 Prepared 4-19-55 0.5 ml 1:2 cell Beef in Lactalb - Hanks 2% calf serum
1.5 ml Lactalb Earles pH 8.2 added 4-25-55

Tube No.

		TSH	1	2	3	5	7	9	10
Control fluid CE Variant Exp 2 4 day	Undil	1	0	0	0	0 vac	0 vac	0 vac	Same
		2	0	0	0	0	0	0	"
		3	0	0	0	0	0	0	"
	10^{-1}	4	0	0	0	0 vac	0 vac	0 vac	"
		5	0	0	0 vac	0 vac	0 vac	0 vac	"
		6	0	0	0 vac	0 vac	0 vac	0 vac	"
8 day	Undil	7	0	0	0 vac	0 vac	0 vac	0 vac	"
		8	0	0	0	0	0 vac	0 vac	"
		9	0	0	0	0	0	0 retract	"
	10^{-1}	10	0	0	0	0	0	"	"
		11	0	0	0	0 vac	0 vac	0 vac	"
		12	0	0	0	0	0 vac	0 vac	"
Control fluid CE Adsorb Exp 1 4 day	Undil	13	0	Big gran degen	1/4 epith left				
		14	0	2+ " "	Epith islands				
		15	0	" " "	1/4 epith left				
	10^{-1}	16	0	0	" "				
		17	0	H gran degen	" "				
		18	0	" " "	Epith Islands				
8 day	Undil	19	0	2+ " "	Epith islands	Same	Same	Complete degen	
		20	0	" " "	1/4 epith left	"	"	Same	
		21	0	" " "	Epith islands	"	"	"	
	10^{-1}	22	0	0	" "	"	"	"	
		23	0	0	" "	"	"	"	
		24	0	0	" "	"	"	"	
Control fluid CE Adsorb Exp 2 4 day	Undil	25	0	0	0	0	0 vac	0 vac	Same
		26	0	0	0	0	"	"	"
		27	0	0	0	0	"	"	"
	10^{-1}	28	0	0 vac	0 vac	0 vac	"	"	"
		29	0	0	0	0	"	"	"
		30	0	0	0	0 vac	"	"	"
8 day	Undil	31	0	0	0	0	0	0	"
		32	0	0	0 vac	0 vac	0 vac	0 vac	"
		33	0	0	0	0 retract	0 retract	Same	"
	10^{-1}	34	0	0	0	0	0 vac	0 vac	"
		35	0	0	0	0	"	"	"
		36	0	0	0	0	"	"	"

Pooled, centrifuged 2000 rpm stored in deep freeze

April 26, 1955

Test for CEA in Chick Embryo Tissue Cult Fluids

		Tube No.	1	2	3	5	6	7	8	9	10
Control fluid CE 4th Criss Cross Ex 4 day	Undel T54	37	0	3+ gran degen	Complete degen	Pooled, centrifuged 2000 rpm – Stored deep freeze					
		38	0	" "	Epith islands						
		39	0	" "	" "						
	10^{-1}	40	0	1-2+ " "	" "	"	"				
		41	0	2+ " "	" "						
		42	0	" " "	" "						
8 day	Undel	43	0	" " "	" "	Same	Same	Complete degen			
		44	0	" " "	" "	"	"	"			
		45	0	" " "	" "	"	"	"			
	10^{-1}	46	0	1+ " "	" "	"	"	"			
		47	0	0	" "	"	"	Same			
		48	0	1+ gran degen	" "	"	"	"			
Control fluid CE Double Washing Exp	Undel	49	0	1+ gran degen	1/3 epith left	Pooled, centrifuged 2000 rpm Stored deep freeze –					
		50	0	" " "	1/4 " "						
		51	0	" " "	1/5 " "						
4 day	10^{-1}	52	0	0	1/4 " "	"	"				
		53	0	0	1/5 " "						
		54	0	0	Epith islands						
Chicken Plasma Lot 10-2-54	Undel	55	0	0	0	0	0	0	0	0	0
		56	0	0	0	0 retract	Same	Same	Same + vac	Same	
		57	0	0	0	0 vac	0 vac	0 vac	* Same + vac "	→ passage of fluid	
		58	0	0	0	0	0 retract	* 0 retract	Same	Same	
		59	0	0 vac	0 vac retract	Same	Same	Same	Same	"	
Chick Embryo Extract	Undel	60	0	0	0	0 vac	0 vac	0 vac	0 vac	0 vac	
		61	0	0 vac	0 vac	0 vac	0 vac	"	"	"	
		62	0	0	0	0 vac	0 vac	"	"	"	
		63	0	0	0	0 vac	0 vac	"	"	"	
		64	0	0	0	0	0	"	"	"	
Uninoc. Controls		65	0	0	0 → histology						
		66	0	0	0 vac	0 vac	0 vac	0 vac	Same		
		67	0	0	0 → histology						
		68	0	0	0	0	0 retract	0 retract	"		
		69	0	0	0	0 retract	"	"	"		
		70	0	0	0	0	"	"	"		
CEA KP3 10^{-3}		71	0	0	2-3+ gran degen	Epith islands	Same	"			
		72	0	0	" "	→ histology					
		73	0	0	2+ "	1/4 epith left	"	Epith islands			
		74	0	0	" "	→ histology					
		75	0	0	" "	1/4 epith left	"	"			

Mostly good epith growth

* May 3 1955 Retraction suggestive of CEA change – can not exclude this possibility
fluid removed, centrifuged, pooled and inoc into Tubes T56 61-68 (sent frozen) – 2 ml Lactalb pH 2.4 replaced

May 2, 1955 In view of poor quality of tubes T54 55-59 inoc ε chicken plasma Lot 10-28-54 on the 6th day 6 additional tubes inoc ε 0.2 ml of the plasma.

Tubes = T56 prepared 4-27-55 0.5 ml 1:200 cell susp. in Lactalb Hanks 2% calf serum 1.5 ml Lactalb Earles pH 7.2 added 5-2-55

1 ml Lactalb Earles pH 8.2 added

Chicken Plasma Lot 10-28-54 0.2 ml

Tube No.	1	2	3	4	5	6	7	8	9	10	11
T56-55	0	0	0	0	0	0	0	0			
56	0	0	0	0	0	0	0	0			
57	0	0	0	0	0	0	0	0			
58	0	0	0	0	0	0	0	0			
59	0	0	0	0	0?	0	0	0			
60	0	0	0	0	0?	0	0	0	0?	0?	0?

5-10-55. Stored in deep freeze.

2 ml Lactalb Earles pH 7.4 replaced

Store in deep freeze 5-10-55 if no change evident by that time

May 3, 1955 Passage of fluid from Tubes T54 57 & 58 - this represents Chicken Plasma Lot 10-28-54 KP1

Tubes - same as above

Chicken Plasma Lot 10-28-54 ✓ Undil 0.2 ml

Tube No.	1	2	3	4	10
T56-61	0	0?	0-1 gran degen	Same + Fallout of small area of diff cells Sunset change effect	Same
62	0	0?	" " "	"	"
63	0	0?	" " "	"	"
64	0	0?	" " "	"	"
~~65~~					
65	0	0	1/5 with left gran degen.		
66	0	0	" "		
67	0	0	1/4 " "		
68	0	0	" "		

10^{-2} 0.2 ml

Pooled, centrifuged 2000 rpm for 10 min - Tested for HA - remainder stored deep freeze

~~April~~ May 6, 1955

Test for HA in Chicken Plasma Agent KP2

HA = Tissue cult fluid tubes T56-65 to 68 KP1

Cells 0.5% chick rbc in saline prepared from 10% susp. prepared 5-6-55 from chick rbc of 5-5-55

Diluent = M/50 PO_4 Saline pH 7.0

Temp Sed = 40°C

Undil	2	4	8	16	32	64	128	0
+	+	+ / ±	0	0	0	0	0	0

Elution after 30 min at 23°C

May 12, 1955 — Test for CEA virus (NDV) in KP1 of Chicken Plasma Lot 10-21-55 (Repeat isolation)

Virus: 1.) Chicken Plasma Agent KP1 Tissue cult fluid Tubes T56-59 Frozen 5-10-55 Thawed & centrif 5-12-55

2) Chicken Plasma Agent KP1 Tissue cult fluid Tube T56-60 @ 5-12-55

Tubes T58 @ 5-4-55 — 0.5 ml 1:200 cell susp. in Lactalb Hanks 2% calf serum + 1.5 ml Lactalb Earles pH 8.2 added 5-10-55

	Tube No	1	2	3	4	5	6
Chicken Plasma Agent KP1 Fluid tubes T58 55-59		T58					
	Undil	−1	0	0-1 grandegen	2+ grandegen	1/2 epith left	Same*
		−2	0	0-1 " "	" " "	" "	1/4 epith left Same + retract
		−3	0	1+ " "	" " "	1/3 "	" " Same + retract
		−4	0	1+ " "	1+ " "	3/4 "	1/2 " *
	10^{-2}	5	0	0?	2+ grandegen −		
		6	0	0?	1/2 epith left		
		7	0	0	1/3 " " Swiss cheese effect		
		8	0	0?	1/2 " " " " "		
Chicken Plasma Agent KP1 Fluid Tube T56-60	Undil	9	0	1+ grandegen	deg. + 1/3 epith left	Same	Same Same
		10	0	1-2+ " "	" + 1/2 " "	1/3 epith left	" "
		11	0	1+ " "	" + 3/4 " "	1/2 "	" " *
		12	0	1-2+ " "	" + 1/3 " "	Same	" *
	10^{-2}	13	0	0-1 " "	degen + 1/3 epith left		
		14	0	0-1 " "	" " " "		
		15	0	0-1 " "	" 1/4 " "		
		16	0	0-1 " "	" 1/3 " "		
Uninoc controls		17	0	0	0	0	0
		18	0	0	0	0	0 inc
		19	0	0	0	0	0 retract
		20	0	0	0	0	0 inc + retract

May 16, 1955 − fluid pooled, centrifuged 2000 rpm − tested for HA & stored deep freeze

May 16, 1955 − fluid pooled, centrifuged 2000 rpm − tested for HA & stored in deep freeze

May 16, 1955 — Test for HA

0.5 ml dilutions in M/50 PO4 Saline pH 7.0
0.5% check rbc in saline − 10% susp. of 5-15-55 from cells of 5-11-55
Sed. at 4°C

	Undil	2	4	8	16	32	64	0
Chicken Plasma Agent KP2 Tube T58 5-8	++	+ /	±	0	0	0	0	0

T58-13-16

May 18, 1955

Challenge of Resedual Monkey Kidney Epith Cells from NDV Infection with Type I Poliomyelitis Virus

Are resedual cells (from NDV infection) alive or dead — does interference = Tubes challenged I) T58 1, 4, 11, and 12 — polio occur??

Degenerative change of NDV present on 2nd day — 1/3-1/2 epith remained unaffected on 5th day

a) T58 1 and 4 inoc = chicken plasma agent KP1 5 days previously (T56 55-59 series)

b) T58 11 + 12 inoc = chicken plasma agent KP1 5 days previously (T58-60 series)

II T58 17-20 Control uninoculated tubes

Virus Maloney KP35 Lot 4 of 1-4-54; AmpTII in A-4-4

10001 CD_{50} = 0.2 ml of $10^{3.5}$; inoc 0.2 ml of 1:3200

Tubes		Day 0*	1 (24 hr)	2 (48 hr)	3 (72 hr)	4 (96 hr)	5	6
T58	1	1/2 epith left Swiss cheese appearance	No change - No polio change Epith good condition pH 7.3	No change No polio effect	Few scattered refractile round cells - similar to polio change	0-2 polio	Many areas 4 off - 1752 Some areas 1-2+ Small epith islands - 0	Small unaffected epith islands
	4	" "	1/4 epith left - retraction - no polio change pH 7.3	" "	NO change	No polio change retract increases	Few epith islands 5 polio change	Same
	11	" "	Same - Epith good condition No polio change pH 7.3	" "	No change	Few scattered round cells no definite change	Same - Excellent epith	Same
	12	1/3 epith left Swiss cheese appearance	Same - Epith good condition No polio change pH 7.3	" "	No change	No change	No change excellent epith	1 Small focus cluster of round cells otherwise no change
Control Virus	17	0	2+ polio	4+ no normal cells left	off			
	18	0 vac	0-2+ polio	" "	off			
	19	0 retraction	2+ polio	" "	off			
	20	0 vac + retract	retract marked 2+ polio	" "	off			

* Fluid removed - replaced = 2 ml Lactate Earles pH 7.4 = 0.5 % calf serum

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May 13, 1955 Egg Passage of Chicken Plasma Agent KP2

Virus Chicken Plasma agent KP2 Tissue culture fluid from Tubes T56 65-68 of 5-6-55

Inoc 0.1 ml of 10^{-1} in allantoic sac of 10 day old white Leghorn embryo. 10^{-1} dilution prepared in Hanks complete + antibiotics

Egg	1	2	
1	0	Dead	—
2	0	"	—
3	0	"	—
4	Sluggish	"	Not used
5	0	"	—
6	0	"	—
7	0	"	—
8	0	"	—
9	Dead		
10	0	"	—
11	0	"	—
12	Dead		
13	0	"	—
14	0	"	—
15	0	"	—
16	Sluggish	"	Not used
17	0	"	—
18	Dead		

— May 15, 1955 Allantoic fluid harvested —
[embryos had many subcutaneous hemorrhages esp. in the cerebral region —]
Fluid centrifuged 2000 rpm for 10 min — tested for HA — remainder stored in 4 lots in deep freeze —

May 16, 1955 HA Titration

0.5 ml dilutions in M/50 PO4 Saline pH 7.0
0.5% chicken rbc in Saline 10% susp 5-15-55 from cells of 5-11-55
Incubated at 4° C

Undil	2	4	8	16	32	64	128	256	512	0
++	++	++	++	++	++	++	+	±	0	0

complete elution after 30 min at room temp = 23° C

May 13, 1955 Test for NDV Virus in Chicken Plasma Lot 10-21-54

and CEE Lot 7-31-53

Material Test

1) Chicken Plasma Lot 10-21-54 Stored 4°c in refrigerator

2) Chick Embryo Extract Lot 7-31-54 stored in deep freeze.

Inoc 0.1 ml of undil. fluid into allantoic sac of 10 day old White Leghorn Embryos

	Egg	1	2	3
Chicken Plasma 10-21-54	19	0	0	Dead*
	20	0	0	"*
	21	0	Dead*	— Pooled c̄ Egg 26 — tested for HA — HA pos see over side
	22	Dead		
	23	Dead		
	24	0	0	"*
	25	0	Sluggish	"*
	26	0	Dead*	— Pooled c̄ Egg 21 — HA positive
	27	0	0	"*
	28	0	0	"*
	29	0	0	"*
	30	0	0	"*
	31	0	0	"*
	32	0	0	"*
	33	0	0	"*
	34	0	0	"*
CEE 7-31-54	35	0	0	0
	36	0	0	0
	37	0	0	0
	38	0	0	0
	39	0	0	0
	40	0	0	0
	41	Dead		
	42	0	0	0
	43	0	0	0
	44	Dead		
	45	0	0	0
	46	0	0	0
	47	0	0	Dead → no hemorrhagic lesions seen —

*Hemorrhagic lesions present in embryo

May 16, 1955 All remaining eggs dead to-day allantoic fluid harvested, centrifuged 2000 rpm & tested for HA — remainder stored in deep freeze —

May 16, 1955 — Allantoic fluid harvested from individual eggs and tested seperately for HA — 0.5 ml allantoic fluid + 0.5 ml of 0.5 % chick rbc (10% susp. of 5-17-55 from rbc lot of 5-11-55) — all fluids negative for HA after 35 min. sed. at 4°c

May 15, 1955 Allantoic fluid of egg 21 & 26 pooled - centrifuged 2000 rpm 10 min - tested for HA - remainder stored in bisteroid in deep freeze

HA Titration

0.5 ml dilutions in M/50 PO_4 Saline pH 7.0
0.5 % chick rbc in saline 10% susp of 5-15-55 from cells of 5-11-55
Sed. at 4°c.

Undil	2	4	8	16	32	64	128	256	512	0
++	++	++	++	++	++	+ /	±	0	0	0

Complete elution after 30 min at room temp = 23°c

May 16, 1955 Allantoic fluid of eggs dead on 3rd day tested for HA as above - chick rbc from 10% susp. of 5-17-55 from rbc lot of 5-11-55

Undil	2	4	8	16	32	64	128	256	512	0
++	++	++	++	++	++	++	+ /	0	0	0

May 18, 1955 Identification of Chicken Plasma Agent by Hemagglutination-Inhibition with NDV Immune Chicken Serum

Hemagglutinin 1.) Chicken Plasma Agent Egg Pass 1 All. Fluid 5-16-55

2) Chicken Plasma Agent KP2 Egg Pass 1 All. Fluid 5-15-55

Sera 1.) NDV Immune Chicken B Strain 5-8-54

14 day obtained from Dr Bang 4-28-55 stored in deep freeze

2) Normal Chicken - obtained from Dr Bang 4-28-55 stored in deep freeze.

Inactivated 56°C 30 min before use in test

Preliminary titration of hemagglutinins

Chick rbc of 5-17-55 10% susp of 5-18-55 → 0.5% susp in saline

0.5 ml dilutions of HA in M/50 PO_4 Saline pH 7.0

Sediment taken of rbc at 4°C for 45 min.

HA	4	8	16	32	64	128	256	512	1,240	0
Egg Pass 1	++	++	++	++	++	++	+/±		0	0
	++	++	++	++	++	++	+/±		0	0
KP2 Egg Pass 1	++	++	++	++	++	++	+/±		0	0
	++	++	++	++	++	++	+/±		0	0

Serum Tested	HA	Serum dilutions in M/50 PO_4 Saline pH 7.0											Serum Control	Inhib Titer
		5	10	20	40	80	160	320	640	1280	2560	5120		
NDV Immune Chicken	Egg Pass 1	0	0	0	0	0	0	0	0	+/++	++		0	1280
	KP2 Egg Pass 1	0	0	0	0	0	0	0	0	+/++	++		0	1280
Normal Chicken	Egg Pass 1	++	++	++	++	++	++						0	<5
	KP2 Egg Pass 1	++	++	++	++	++	++						0	<5

Chicken Plasma

	32	64	128	256	512	1024	0
Egg Pass 1	++	++	++	+?	0	0	0
	++	++	++	+	0	0	0
KP2 Egg Pass 1	++	++	++	+	0	0	0
	++	++	++	+	0	0	0

— 8 units in both tests —

May 18, 1955 — Test for NDV Virus in Chicken Plasma

Prepared 5-17-55

5-17-55 — 5 Adult roosters bled from heart —

2 ml of 1:10 heparin solution used for each 100 ml blood
(100 units = 1 ml of 1:10 heparin sol.)

blood stored in refrigerator

5-18-55 Blood centrifuged 2000 rpm for 1 hour — plasma of Fowl #6 clotted — 5 other specimens removed with cap. pipette and recentrifuged 2000 rpm for 10 min — then stored in refrigerator

5-18-55 — Test for NDV by Egg Inoc.

Eggs — 10 days old White Leghorn eggs —

Inoc — 0.1 ml plasma into allantoic sac.

	Egg	1	2	3	HA test
Plasma 1#	1	0	0	0	0
	2	0	Dead		0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0		0
	6	0	0	0	0
Plasma #2	7	0	0	0	0
	8	0	0	0	0
	9	0	0	0	0
	10	0	0	0	0
	11	0	0	0	0
	12	0	0	0	0
	#				
Plasma #3	13	0	0	0	0
	14	Sluggish	Dead	— Contaminated	
	15	0	0	0	0
	16	0	0	0	0
	17	0	0	0	0
	18	0	0	0	0
Plasma #4	19	0	0	0	0
	20	0	0	0	0
	21	0	0	0	0
	22	0	0	0	0
	23	0	0	0	0
	24	0	0	0	0

HA test: 0.5 ml of 0.5% chicken rbc + 0.5 ml all. fluid — 40 min — 4°C

	Egg	1	2	3	HA
Plasma #5	25	0	0	0	0
	26	0	0	0	0
	27	0	0	0	0
	28	0	0	0	0
	29	0	0	0	0
	30	0	0	0	0
Horse Serum Lot 2467 Microbiological Associates (unheated) Stored in deep freeze	31	0	0	0	0
	32	0	0	0	0
	33	0	0	0	0
	34	0	0	0	0
	35	0	0	0	0
	36	0	0	0	0

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Sept 14, 1955 Test for NDV Virus in Chicken Plasma of 9-10-55

9-10-55 · 6 adult roosters bled from heart -

1 ml of 1:10 heparin solution used for each 10 ml of blood (100 units = 1 ml of 1:10 heparin solution)

blood centrifuged 2000 rpm for 2 hours - plasma removed and stored in refrigerator

9-14-55 Eggs 8 10 day old white Leghorn (Mt Health)

Inoc. 0.1 ml plasma into allantoic sac.

Egg	Day 9/15	9/16	9/17
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	D		
8	D		

HA test (0.5 ml of all fluid + 0.5 ml of 0.5% chicken rbc - 40 min at 4°C)

HA test - Negative on 9/17/55

Undiluted → 1:256

Nov 12, 1954

Preparation of Trypsinized Chick Embryo Kidney & Lung Cultures - 17day embryo

Procedure - 1.) 13 17day old White Rock chick embryos were harvested aseptically and the kidneys and lungs removed.

Wt of kidneys = 0.4 gm.
Wt of lungs = 1.4 gm.

2) The kidneys & lungs were minced seperately in a watch glass with scissors. The tissue was then washed 6x with PBS pH 7.35 (Dulbecco formula) containing antibiotics.

3.) The minced washed tissue was then placed in a 125 ml flask and 10ml of 0.25% trypsin in PBS added. This mixture was allowed to incubate for 30 minutes at 36°C. The flask and its contents were agitated for 10 minutes. The fluid was withdrawn with care with a cap. pipette and 10 ml of fresh trypsin solution added. The agitation process was repeated 5 times for kidney tissue - at this point the tissue was exhausted, only small fibrous strands remaining. The supernatant fluid contained many tubules

The lung tissue was extracted 8x with trypsin - on microscopic examination of the supernatant fluid only single cells were seen - very few cell clumps.

4) The pooled supernatant fluids from each tissue

were centrifuged at 1000 rpm for 5 minutes - The packed sediment was then washed 3x with 10 ml of PBS. Centrifugation for the washings was at 1000 rpm. The suspensions were packed by centrifugation at 2000 rpm for 3 minutes in a graduated tube

vol. of lung sediment = 0.13 ml -
vol of kidney sediment = 0.05 ml -

The sediments were suspended in Lactalb. hydrolysate medium pH 7.45 containing 10% normal horse serum to make a 1:200 suspension. 0.5 ml of the suspensions then placed in each tube

21 tubes of kidney series
44 tubes of lung series.

Tubes incubated in stationary position in 36° C incubator

Materials

1.) 17 day White Rock chick embryos
2.) Lactalbumen hydrolysate medium pH 7.45 - freshly prepared
3) Normal horse serum - inact 56° 30 mins Lot # 246 > Microbiol Assoc.
4) PBS solution freshly prepared from 3 stock solutions (autoclaved & prepared on 11-3-54)

Trypsinized Lung and Kidney Epith Cell Cult. from 17 day Chick Embryo (cont)

Nov 14, 1954 — 2 days -

1.) good epith growth present to-day in 40 lung cultures

2) good growth of what appears to be epith in 18 kidney cultures

Nov 16, 1954 — 4 Days

1.) Lung Series — 4 tubes discarded - alk or contaminated

1 tube - degeneration of epith - clumpy type

good growth:
- 4 tubes predominately reticulum cell growth & interspersed epith cells
- 35 tubes predominately epith cell growth - some reticulum cells present

2) Kidney Series - no tubes discarded -

good growth:
- 4 tubes - predominately reticulum cell growth + 1 or 2 very small islands of epithelium
- 18 tubes - completely reticulum cell growth

In almost all kidney tubes a portion of the cell sheet has detached from the glass and rolled up upon itself ("window shade" like)

1.5 ml of Lactalb. medium pH 7.4 added to each tube -

Nov 18, 1954 - 6 days -

Lung series

Epith in good condition -

pH of tubes = 7.0 (good metabolism)

in 4 tubes with predominately reticulum cell growth - detachment and rolling up of cell sheets has occured - similar to kidney cultures.

Kidney series -

detachment of cell sheets & rolling up of sheets has progressed in some tubes - At least 50% of the cells in the sheets originally present at 2 days are still in good condition

pH of tubes = 7.0

Nov 20, 1954 - 8 days -

Lung series Epith in good condition

pH of tubes = 6.6 - 7.0

Kidney series Beginning degeneration of reticulum cells - retraction, detachment & rolling up of cell sheets progressing -

pH of tubes 6.6 - 7.0

NOV 29 1954

17 days

Lung - Epith in good condition - fluid removed - replaced & 2 ml of Tact all - medium pH 7.4

Kidney cells retraction complete - degen. of cells discarded.

Nov 22, 1954 CE Lung Exp 1

Purpose 1.) To determine if poliomyelites verses will multiply in check embryo lung epithelial tissue

2) To determine if poliomyelites verses will produce a cytopathogenic effect in check embryo lung epithelial tissue

Procedure - Chick embryo (17 day) lung epithelial cultures prepared by trypsenization on Nov 12, 1954 were treated in the following manner.

1.) Fluid medium was removed - Tubes were washed once with 10ml of Hanks solution (1/3 Na_4CO_3)

2.) 2ml of lactalbumen hydrolysate medium pH 7.4 was added to 6 tubes and 3ml to 5 tubes - the latter tubes were designated as control tubes

3) To each of 2 tubes containing 2ml of medium 1ml of the following verses was added

0 Time

Tube 1, 2 - Type I poliomyelites virus - CE_1-MK, harvested 10-4-54 stored in deep freeze titer in explant tubes = $10^{7.0}$ TCD50/ml

[Polio verses from plasma clot series]

Tube 3, 4 Type II poliomyelites virus CE_1-MK, harvested 10-4-54 stored in deep freeze titer in explant tubes = $10^{6.5}$ TCD50/ml

Tube 5, 6 Type III poliomyelites virus CE_1-MK, harvested 10-4-54 stored in deep freeze titer in explant tubes $\geq 10^{6.5}$ TCD50/ml

Tubes 7, 8, 9, 10, 11 - Controls

4). After 4 hours in the roller drum -
4 hours the tubes were removed - the fluid was removed from each tube - the tube was then washed 4X with 10ml of Hank's solution ($1/3\ NaHCO_3$) - the fluid was aspirated in each instance by a capillary pipette attached to a water suction apparatus.

3 ml of Lact albumin hydrolysate medium then added to each tube - Tubes returned to the roller drum -

5) 1 ml aliquots taken from each tube after 1 hour -
5 hours aliquots from controls, Type I, Type II, & Type III virus series pooled seperately.
- Stored in deep freeze -
Tubes returned to roller drum.

4 days 6) Fluid removed from tubes -
Nov 26, 1954 replaced with 2ml of Lact Alb. hydrolysate medium pH 7.4

Dec 2, 1954 7) Fluid removed - stored in deep freeze
10 days

Day — Microscopic Observations

CE - Lung Exp 1

Tube	1	2	3	4	5	6	7	8	10
I 1	Epith cells in good condition - several epith nests have disrupted	No change	No change	No change	No change	Retract. of fibrous stroma	No change	No change	No change
2	" "	" "	" "	" "	" "	No change	" "	" "	" "
3	" "	" "	" "	" "	" "	" "	" "	" "	" "
II 4	" "	" "	" "	" "	" "	" "	" "	" "	" "
5	" "	" "	" "	" "	" "	" "	" "	" "	" "
III 6	" "	Mod. disruption of many epith. nests - good epith cells left in other areas -	" "	" "	" "	" "	" "	" "	" "
Controls 7	Epith cells in good condition - several epith nests have disrupted -	Disruption of fibrous stroma + epith cell nests in several areas - epith cells otherwise OK	Same	Same	Same	" "	" "	" "	" "
8	"	Epith cells in good condition	" "	" "	Retraction of large portion of tissue & remaining tissue epith cells OK	" "	" "	" "	" "
9	"	Same	" "	" "	good epith cells	" "	" "	" "	" "
10	"	Slight increase in epith cell disruption	No change	" "	No change	" "	Incr. disrupt of epith nests	" "	" "
11	"	Retraction + degen of about 60% of treatment - Some good epith cells left in remaining portion -	No change	" "	Excellent epith cells remaining	" "	No change	" "	" "

CE - Lung Exp 1

Quantity of virus in tissue culture fluid in tubes containing chick embryo (17 Day) lung epithelial tissue after indicated washings and fluid removals.

Time after addition of virus	Manipulation performed	Fluid tested	Titer of virus in indicated tissue culture fluid $TCID_{50}$/ml		
			Type I	Type II	Type III
0	- None	Not tested - calculated	$10^{6.0}$	$10^{7.0}$	$10^{7.0}$
4 hours	Tube washed 4 X with 10 ml of Hanks sol Fluid replaced (3 ml)	Not tested			
5 hours	- None	aliquot of fluid 1 hr after washing	$10^{3.5}$	$10^{4.0}$	$10^{2.2}$
4 days	- Fluid removed	Removed fluid	$10^{3.0}$	$10^{3.5}$	$10^{2.5}$

Titration CE Lung Expt

NOV 26 1954

Viruses 1.) CE, MK, Types I, II & III harvested 10-4-54 inoculum

2.) 5 hour aliquots – removed 1 hour after 4 washings (10ml each)

3.) –4 day fluids –

Procedure 10 fold dilutions made in Hanks solution (full amount of $NaHCO_3$) – 0.2 ml of diluted material inoculated into each tube 3 tubes / decimal dilution

Tubes used Trypsinized monkey kidney series 8 prepared 11-18-54

	Tube No	2	4	6	8
Type I					
Inoculum CE, MK, plasma clot series					
10^{-4}	– T8-1	2	4		
	– 2	3	4		
	– 3	2	4		
10^{-5}	– 4	1	4		6.5 TCD50/ml
	– 5	2	4		10
	– 6	2-3	4		
10^{-6}	– 7	0	4		
	– 8	0	0	0	0
	– 9	0	0	od	od
10^{-7}	– 10	0	0	0	0
	– 11	0	0	0	0
	– 12	od	od	od	od
10^{-8}	– 13	0	od	od	od
	– 14	0	od	od	od
	– 15	0	od	od	od
5 hour aliquot					
10^{-1}	– 16	3	4		
	– 17	1	4		
	– 18	3	4		
10^{-2}	– 19	0-?	4		
	– 20	0	0	4	
	– 21	2	4		
10^{-3}	– 22	0	od	od	od
	– 23	0	0	0	0
	– 24	0	0	0	4

		Tube No.	2	4	6	8
Type I						
5 hour aliquot	10^{-4}	Tg 25	0	0	od	od
		- 26	0	0	0	0
		- 27	0	0	0	0
	10^{-5}	- 28	0	0	0	0
		- 29	0	0	0	0
		- 30	0	0	0	od
4 day fluid	Undil	- 31	3	4		
		- 32	3	4		
		- 33	3	4		
	10^{-1}	- 34	2	4		
		- 35	2	4		
		- 36	2	4		
	10^{-2}	- 37	0	4		
		- 38	0	0	4	
		- 39	0	0	0	0
	10^{-3}	- 40	0	0	0	0
		- 41	0	0	0	0
		- 42	0	0	0	0
	10^{-4}	- 43	od	od	od	od
		- 44	0	0	0	0
		- 45	0	0	0	od
Type II	10^{-4}	- 46	2-3	4		
		- 47	2	4		
		- 48	2	4		
Inoculum	10^{-5}	- 49	1	4		
		- 50	2	4		
CE, MK, of plasma clot pools		- 51	1	4		
	10^{-6}	- 52	0	0-?	4	
		- 53	0	0-?	4	
		- 54	0	0	2	4
	10^{-7}	- 55	0	0	1	4
		- 56	0	od	od	od
		- 57	0	0	od	od
	10^{-8}	- 58	0	0	0	0
		- 59	0	0	0	0
		- 60	0	0	0	0
5 hour aliquot	10^{-1}	- 61	2	4		
		- 62	2	4		
		- 63	2	4		

$10^{7.5}$ TCD50/ml

NOV 26 1954

CE Lung Expt Titration

		Tube No.	2	4	6	8
Type II						
5 hour aliquot	10^{-2}	64	0-?	4		
		65	0	0-1	4	
		66	0	3	4	
	10^{-3}	67	0	0	0	0
		68	0	4		
		69	0	0	1	4
	10^{-4}	70	0	0	od	od
		71	od	od	od	od
		72	0	od	od	od
	10^{-5}	73	0	0	0	0
		74	0	0	0	0
		75	0	0	0	0
4 day fluid	Undil	76	2	4		
		77	2	4		
		78	3			
	10^{-1}	79	1	4		
		80	1	4		
		81	2	4		
	10^{-2}	82	0-1	4		
		83	0-?	4		
		84	0	4		
	10^{-3}	85	0	0	od	od
		86	0	0	0	0
		87	0	2	4	
	10^{-4}	88	0	0	0	0
		89	0	0	0	0
		90	0	0	0	0
Type III	10^{-4}	91	2	4		
		92	1-2	4		
		93	1-2	4		
Inoculum						
CE, MK, of plasma clot cultures	10^{-5}	94	0	4		
		95	0	4		
		96	0	4		
	10^{-6}	97	0-?	4		
		98	0	0-?	4	
		99	0	0	0	3-4
	10^{-7}	100	0	0	0	4
		101	0	0	0	0
		102	0	0	0	0

$10^{7.5}$ TCD50/ml

Type III

		Tube No.	2	4	6	8
Inoculum	10^{-8} T8	103	0	0	0	0
		104	0	0	0	0
		105	0	0	0	0
5 hour aliquot	10^{-1}	106	1	4		
		107	0	4		
		108	2	4		
	10^{-2}	109	0	0	0	0
		110	0	0	0	0
		111	0	0	0	0
	10^{-3}	112	0	0d	0d	0d
		113	0	0	0	0
		114	0	0	0	0
	10^{-4}	115	0	0	0	0
		116	0	0	0	0
		117	0	0	0	0
	10^{-5}	118	0	0	0	0
		119	0	0	0	0
		120	0	0d	0d	0d
4 day fluid	Undil	121	0-?	4		
		122	0	4		
		123	1	4		
	10^{-1}	124	0	2	4	
		125	0?	4		
		126	1	4		
	10^{-2}	127	1	4		
		128	0	0	0	0
		129	0	0	0	0
	10^{-3}	130	0	0	0	0
		131	0	0	0	0
		132	0	0	0d	0d
	10^{-4}	133	0	0	0d	0d
		134	0	0	0	0
		135	Mold			

Nov 19, 1954

Preparation of Trypsinized Lung and Kidney Cultures - 17 day Chick Embryo

Procedure Same as that described Nov 12, 1954

Wt of kidneys (16 chick embryos) = 0.6 gm

Wt of lungs (16 chick embryos) = 1.6 gm

Centrifuge tube containing packed kidney tissue broke in centrifuge -

Volume of packed lung tissue = 0.1 cc.

Materials Same as Nov 12, 1954 17 day White Rock embryos used

33 tubes of lung tissue culture prepared -

Nov 21, 1954 - 2 days -

Early growth of epith present in most tubes

Nov 23, 1954 4 days

pH of tubes = 6.8

Fair growth - not organized into sheets as yet - mainly islands occuring around a central core of degenerated tissue.

1.5 ml of Lactalbumen medium pH 8.4 added to each tube -

NOV 29 1954

10 days

No change - discarded

NOV 26 1954

Trypsinized Lung + Kidney Tissue Culture
17 Day Chick Embryo Tissue

Procedure Same as described Nov 12, 1954

Wt of kidney (19 embryos) = 1.1 gm

Wt of lungs (19 embryos) = 1.7 gm

Vol of packed kidney tissue = 0.2 ml

Vol of packed lung tissue = 0.2 ml

Tissue ~~stored~~ as 1:20 suspension in Lockalb. hydrolysate medium pH 7.4 + 10% normal inact horse serum (Lot ~~#~~ 2467) + overnight at 4°C.

NOV 27 1954

1:20 suspensions ~~diluted~~ to 1:200 in Lockalb. hydrolysate medium pH 7.4 + 10% normal horse serum inact. (Lot #2467).

~~30~~ 50 tubes received 0.5 ml of 1:200 kidney

30 tubes received 0.5 ml of 1:200 lung

NOV 29 1954

2 days

Lung - no or poor growth

Kidney - Fair growth in most tubes - reticulum cell type seen exclusively

DEC 1 1954

5 days

Lung - poor to fair growth - mixed epithelial + reticulum cell growth -

Kidney - Fair to good growth of reticulum cells - Tissue retraction already visible -

DEC 2 1954 1.5 ml of Lockalb medium pH 7.4 added to each tube

6 days

Dec 10, 1954 14 Days

1.) Lung - fair growth - predominately reticulum cell type -

2) Kidney - good growth - ~~retraction~~ + rolling up of tissue sheets - vacuolization also present

Degeneration of reticulum cells - early

Dec. 8, 1954 Preparation of Trypsinized Chick Kidney cultures 1-2 day old chick

Procedure 1) 16 1-2 day old white Rock chicks were anesthetized with chloroform and the kidneys removed aseptically.

Wgt. of Kidneys 1.7 gm

2) The kidneys were minced on a watch glass with fine scissors. The tissue was then washed 6X with P.B.S. pH 7.35 (Dulbecco formula) containing antibiotics.

3) The minced washed tissue was then removed to a 125 ml flask and 10 ml of 0.25% trypsin in P.B.S. added. This mixture was allowed to incubate for 30 minutes at 36°C. The flask and its contents was agitated for 10 minutes. The fluid was withdrawn carefully by the use of a capillary pipette and 10 ml of fresh trypsin solution was added. The agitation process was repeated 5 times for kidney tissue - at this point the tissue was exhausted, only small fibrous strands remained. The supernatant fluid contained many tubules.

4) The pooled supernatant fluids were placed in two separate tubes and centrifuge at 1000 rpm for five minutes. The packed sediment was then washed 3X with 10 ml of P.B.S. Centrifugation for the washings was at 1000 rpm respectively. The suspension were packed by centrifugation at 2000 RPM for 3 minutes in graduated tubes.

Vol. of sediment a) 20 ml

The sediment was suspended in Lact. alb. hydrolysate medium PH 7.45 containing 10% normal horse serum to make a 1:200 suspension.

1.0 ml of the suspensions then placed in each tube

39 tubes of kidney series

Tubes incubated in stationary position at 36°C (incubator)

Materials 1.) 8 day old white rock chicks

2) Lactalbumin Hydrolysate medium Ph 7.45 — freshly prepared.

3.) Normal horse serum — inact 560 30 min. Lot # 2467

4) PBS Soln freshly prepared from 3 Stock solutions { autoclaved + prepared 11-30-54

Dec 10, 1954 2 days —

— No growth seen —

Dec 13, 1954 5 Days

Fair - Moderate growth most tubes - good growth in some — Epith sheets & interlaced fibroblasts - What appear to be lipid inclusions are present in some cells —

Dec 14, 1954 6 Days 1.5 ml Lactalb. medium pH 7.4 added to each tube

good epith. growth in 1/2 of tubes - fair to moderate growth in remaining 1/2 -

Dec 16, 1954 Chick Kidney Exp 1

Purpose 1) To determine if poliomyelites verus will multiply and produce a cytopathogenic effect in Chick (1-2 day old) kidney epithelium grown in tissue culture (roller tubes)

Procedure - Chick kidney epithelium cultures prepared on 12-8-54 (by trypsinization) were treated in the following manner.

1.) Fluid removed - tubes washed once c 10 ml of Hanks solution (1/3 $NaHCO_3$)

2) 2 ml of Lactalbumin hydrolysate medium pH 7.4 added to each of 6 tubes and 3 ml of medium to 4 tubes - the latter tubes were designated as control tubes -

3) To each of 2 tubes containing 2 ml of medium 1 ml of the following verses added

Tube 1, 2 Type I Mahoney KP 36 prepared in trypsinized bottles - 12-6-54 stored in deep freeze

3, 4 Type II YSK KP54 prepared in 250 ml centrifuge tubes (explants) - large inoculum - 5-1-54

5, 6 Type III Leon KP 37 prepared in trypsinized bottles 12-9-54

7, 8, 9, 10 Controls

4) After 4 hours in the roller drum the tubes were removed - fluid removed - tubes washed 4 X with 10 ml of Hanks solution (4/3 $NaHCO_3$) - The

fluid was aspirated with a cap. pipette attached to a water suction apperatus.

3 ml of Lactalbumin hydrolysate medium pH 7.4 then added to each tube - Tubes returned to roller drum

5.) 1 ml aliquots taken from each tube after 1 hour - aliquots from controls, Type I, II, & III virus series pooled seperately.

aliquots - Stored in deep freeze.

Roller Tubes - returned to drum in incubator

Dec 20, 1954 Fluid removed from tubes - stored in deep freeze = 4 Day fluid

Replaced w/ 2 ml of Lactalb pH 7.4 medium - Tubes returned to drum -

Dec 24, 1954 Fluid removed - stored in deep freeze = 8 day fluid

Microscopic Observations

Tube		1	2	3	4	5	6	7	8
I	1	Same as controls	Same - no change	Same	More retract.	Same	Same	Same	Same
	2	" "	More retract.	No change	" "	More retract	"	"	"
II	3	" "	" "	More retract	" "	" "	"	"	"
	4	" "	Same	Same	" "	" "	"	"	"
III	5	" "	" "	" "	" "	" "	"	"	"
	6	" "	More retract	More retract	" "	" "	"	"	"
Controls	7	Retract. of tissue - many good epith cells left	More retract.	No change	More retract	" "	"	"	"
	8	" "	" "	" "	" "	" "	"	"	"
	9	" "	" "	" "	" "	" "	"	"	"
	10	" "	" "	" "	" "	" "	"	"	"

Fat globules in most cells

No cytopathogenic change seen - Epithelial cells after 8 days normal except for retraction of epithelial sheets -

Quantity of Virus in Tissue Culture Fluid in Tubes Containing Chick Kidney Epithelial Tissue at Indicated Time and After Designated Manipulations

Time after addition of virus	Manipulation performed	Fluid tested	Titer of polio virus in indicated tissue culture fluid – TCD50/ml		
			Type I	Type II	Type III
0	None	Not tested – Calculated	$10^{6.7}$	$10^{6.9}$	$10^{6.9}$
4 hours	Tubes washed 4X with 10ml of Hank's sol. Fluid replaced (3ml)	Not tested			
5 hours	None	Aliquot of fluid 1 Hour after washing	$10^{3.2}$	$10^{3.7}$	$10^{3.0}$
4 days	Fluid replaced	Removed fluid	$10^{3.0}$	$10^{3.5}$	$10^{2.5}$
8 days	Fluid removed	Removed fluid	$10^{1.2}$	$10^{2.2}$	$10^{2.2}$

135 Jan 17, 1955

Titration - Chick Kidney Exp 1

- Viruses -

[redacted]

KP 36 Chick Kid 1
a) 5 hour aliquot 12-16-54
b) 4 day fluid 12-20-54
c) 8 day fluid 12-24-54

2) YSK KP 54 Chick Kid 1
a) 5 hour aliquot 12-16-54
b) 4 day fluid 12-20-54
c) 8 day fluid 12-24-54

3) [redacted] KP 37 Chick Kid 1
a) 5 hour aliquot 12-16-54
b) 4 day fluid 12-20-54
c) 8 day fluid 12-24-54

Procedure - 10 fold dilutions made in Hank's solution (full amt NaHCO3) 0.2 ml inoculated per tube 3 tubes/decimal dilution

Tubes used - Trypsinized monkey kidney series T24 prepared 1-19-55

		Tube No.		(1-20) 2	4	(1-24) 7	(1-20) 9
[redacted] KP 36 Chick Kid 1							
5 hour aliquot (1 hour after washing)	Undil	T24	1	0?	4 0//		
			2	1	4 0//		
			3	1-2	4 0//		
	10^{-1}		4	0	4 0//		
			5	0-1	4 0//		
			6	0	4 0//		
	10^{-2}		7	0	4 0//		
			8	0	0	4	
			9	0	0	0	4
	10^{-3}		10	0	0	0	0
			11	0	0	0d	0d
			12	0	0	0	0
	10^{-4}		13	0	0	0	0
			14	0	0	0	0
			15	0	0	0	0
	10^{-5}						

$10^{3.2}$ TCD50/ml

Drum A

Drum A

4 day fluid

Undil	16	1	4 0//			
	17	1	4 0//			
	18	1-2	4 0//			

3X10-20 1-200

[redacted] 6

		Tube No.	2	(1-2) 4	7	9
Chick Kid 1	10^{-1} T24	19	1	40//		
4 day fluid		20	1	40//		
		21	0	40//		
	10^{-2}	22	0	0	0	0
		23	0	40//		
		24	0	40//		$10^{3.0}$ TCD50/ml
	10^{-3}	25	0	0	0	0
		26	0	0	0	0
		27	0	0	od	od
	10^{-4}	28	0	0	0	0
		29	0	0	0	0
		30	0	0	0	0
8 day fluid	Undil	31	0	40//		
		32	0	40//		$10^{1.2}$ TCD50/ml
		33	0	40//		
	10^{-1}	34	0	0	0	0
		35	0	0	od	od
		36	0	0	0	0
	10^{-2}	37	0	0	0	0
		38	0	0	0	0
		39	0	0	0	0
	10^{-3}	40	0	0	0	0
		41	0	0	0	0
		42	0	0	0	0
	10^{-4}	43	0	0	0	0
		44	0	0	0	od
		45	0	0	0	0
YSK KP54 Chick Kid 1	Undil	46	4			
		47	4			
		48	4			
5 hour aliquot (1 hour after washing)	10^{-1}	49	2-3	40//		
		50	2	40//		
		51	2	40//		$10^{3.7}$ TCD50/ml
	10^{-2}	52	0-?	40//		
		53	1	40//		
		54	1	40//		
	10^{-3}	55	0	0	0	0
		56	0	0	0	0
		57	1	40//		

Jan 17, 1955

Titration Check Kid Exp 1 (cont)

		Tube No.	2	4 (1-21)	7	9
YSK NP54 Chick Kid 1	10^{-4}	T24 - 58	0	0	od	od
		- 59	0	0	0	0
5 hour aliquot		- 60	0	40		
4 day fluid Undil		- 61	4	} pooled, stored deep freeze 1-19-55		
		- 62	4			
		- 63	3-4			
	10^{-1}	- 64	2	40		
		- 65	2	40		
		- 66	1	40		
	10^{-2}	- 67	1	40		$10^{3.5}$ TCD50/ml
		- 68	1	40		
		- 69	0	40		
	10^{-3}	- 70	0	40		
		- 71	0	0	0	0
Drum		- 72	0	0	0	0
Drum A -	10^{-4}	- 73	0	0	0	0
		- 74	0	0	0	0
		- 75	0	0	0	0
8 day fluid Undil		- 76	1-2	40		
		- 77	1	40		
		- 78	1	40		
	10^{-1}	- 79	1	40		$10^{2.2}$ TCD50/ml
		- 80	0	40		
		- 81	0-1	40		
	10^{-2}	- 82	0	0	0	0
		- 83	0	0	0	0
		- 84	0	0	0	0
	10^{-3}	- 85	0	0	0	0
		- 86	0	0	0	0
		- 87	0	0	0	0
	10^{-4}	- 88	0	0	0	0
		- 89	0	0	0	0
		- 90	0	0	0	0
[redacted]36 Chick Kid 1	Undil	- 91	1	40		
5 hour aliquot		- 92	1-2	40		
		- 93	1-2	40		
	10^{-1}	- 94	0	40		
		- 95	0	40		
		- 96	0	40		

[redacted]
KP36
Chick Kid 1
Shou aliquot

		Tube No (1-21)	2	4	7	9
	10⁻²	97	0	40//		
		98	0	0	od	od
		99	0	1-3	4	
	10⁻³	100	0	0	0	0
		101	0	0	od	od
		102	0	0	0	0
	10⁻⁴	103	0	0	0	0
		104	0	0	0	0
		105	0	0	0	
4 day fluid Undil		106	1	40//		
		107	1	40//		
		108	1	40//		
	10⁻¹	109	0	40//		
		110	0?	40//		
		111	0-?	40//		
	10⁻²	112	0	2-3	4	
		113	0	0	od	od
		114	0	0	od	od
	10⁻³	115	0	0	od	od
		116	0	0	0	0
		117	0	0	0	0
	10⁻⁴	118	0	0	od	od
		119	0	0	0	0
		120	0	0	0	0
8 day fluid Undil		121	0-1	40//		
		122	1	40//		
		123	1	40//		
	10⁻¹	124	0	40//		
		125	0	40//		
		126	0	40//		
	10⁻²	127	0	0	0	0
		128	0	0	0	0
		129	0	0	0	0
	10⁻³	130	0	0	0	0
		131	0	0	0	0
		132	0	0	0	0
	10⁻⁴	133	0	0	0	0
		134	0	0	0	0
		135	0	0	0	0

$10^{3.0}$ TCD$_{50}$/ml

$10^{2.5}$ TCD$_{50}$/ml

$10^{2.2}$ TCD$_{50}$/ml

NOTES ON THE PREPARATION OF TRYPSINIZED MONKEY KIDNEY CELLS

1. Mincing of the kidneys:

Only the capsule and the pelvis of each kidney were removed. The cortex as well as the medulla were minced. I minced the 2 kidneys in one wide-mouthed 50 ml. centrifuge tube using long uterine scissors. It was not very easy to do it and quite a number of the pieces remained approximately 4 to 5 ml. in size. Because of the subsequent residue after trypsinization, I decided that it would be better in the future to mince the kidneys in a petrie dish with small scissors until the pieces are very much smaller.

2. Washing of the minced kidneys:

The washing was performed with Hanks solution containing 1/3 the usual amount of bicarbonate. 6 washings with aproximately 35 to 40 ml. were carried out before a fairly clear supernatant liquid was obtained. A 50 ml. syringe and long spinal needle were used for the washing.

3. Trypsinization:

The minced kidney was transferred to a glass Waring blender and 100 ml. of the prewarmed trypsin solution in phosphate buffer was added. The rheostat was adjusted for very slow operation but constant attention was required to keep the blades running at the proper speed because of a seemingly uneven amount of current coming through the transformer. The first run was for 10 minutes. An attempt was made to draw off the supernatant liquid by suction with a gauze covered tube but that quickly became clogged and this proved to be unsuccessful. After several trials of different methods of drawing off the fluid, it proved best merely to decant the fluid into a 250 ml. centrifuge bottle, pouring the fluid on a gauze covered funnel. Thus any large pieces in the fluid falling on the gauze can then be picked up with forceps and returned to the blender. The subsequent 5 runs were with 100 ml. each of the trypsin solution for approximately 5 minutes. At the end of this time a number of large pieces of kidney were still left as well as a good deal of white connective tissue. I would estimate that the residual kidney tissue was probably about 1 gm.

4. Centrifugation of trypsinized cells:

Approximately 600 ml. of solution contained in three 250 ml. centrifuge bottles were centrifuged at the 6th notch for 5 minutes. The supernatant liquid was quite turbid and the sediment consisted mostly of a kidney red and some whitish material (note: the supernatant liquid which was set aside was subsequently centrifuged at the same speed for another 5 minutes and additional red colored sediment was obtained. This, as well as the final low yield of packed cells, leads me to think that the initial centrifugation was not fast enough.

In the future, therefore, I would centrifuge at the 8th notch for 5 minutes and then recentrifuge the supernatant liquid a second time to make sure that almost all of the red colored sediment or material has been obtained). The sediment from the 3 bottles was taken up in a total of 75 ml. of cold Hanks-1/3 bicarbonate solution and thoroughly shaken in a rubber stoppered centrifuge bottle until it appeared homogenous. This was then centrifuged at the 6th notch for 2 minutes, the supernatant discarded, an additional 50 ml. of Hanks solution was added, shaken, and again centrifuged at the 6th notch for 2 minutes, supernatant again discarded and 50 ml. of Hanks solution added for a third time and centrifuged at the same speed for a third time. My impression is that perhaps here again we may have lost some kidney cells by too slow a centrifugation. The final sediment was shaken in 18 cc. of cold Hanks-1/3 bicarbonate solution and distributed to 2 graduated centrifuge tubes. These were centrifuged at the 6th notch for 5 minutes. The supernatant fluid was fairly clear and the total volume of packed sediment was only 0.9 ml. The supernatant liquid was poured off (note: the sediments in each instance were so well packed that it was possible to get rid of the supernatant fluid by decantation). The 0.9 ml. of sediment was finally taken up in 180 ml. of the lactalbumin hydrolysate Simms ultrafiltrate medium to which was added 2 cc. of heated horse serum of the tested lot 1462 per 100 ml. of medium.

5. Distribution of cells in suspension in tubes:

Part of the cell suspension was distributed in 0.5 ml. amounts and the remainder in 0.7 ml. amounts. The tubes were put in a stationary position in the incubator.

Aug 10, 1954

Preparation of Trypsinized Kidney (Cynomolgus) Cells for Tissue Culture

Solutions -

1. Earles solution (complete - i.e., with $NaHCO_3$ and antibiotics added) -
   1000 ml prepared pH = 8.61
2. 2.5 gm (Difco) Trypsin 1:250 added to 1000 ml of Earles complete
3. Above solution Seitz filtered - stored 4°C
   pH = 8.00

	24	48
BAP =	0	0
BAP =	0	0

Aug 12, 1954

Procedure

Mincing

1.) Cynomolgus monkey # 6814 weighing 2225 gm was sacrificed under chloroform anaesthesia. Kidneys were removed aseptically.

The capsule and pelvis of each kidney removed. The cortex and medulla were minced with small scissors on a watch glass held in a petri dish. After mincing the tissue particles averaged 1-2 mm in size.

2) Washing

Kidney tissue was washed 6X with Hanks solution containing 1/3 the usual amount of $NaHCO_3$. Minced tissue from each kidney was washed seperately, 30 ml of Hanks for each wash. A clear supernatant fluid was obtained after the 3rd wash.

3) Trypsin treatment

The minced kidney tissue was transferred to a 250 ml thick glass pyrex flask and 100 ml

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STANDARD B&P "NOTEAR"

of pre warmed trypsin solution (37°c) in Earles solution was added. An iron magnet sealed in glass was placed in the vessel and agitation of the tissue particles produced by means of the magnetic stirrer. Setting on the machine was between the 1st and 2nd notch. This resulted in a vegorous agitation of the vessel contents without frothing. The run was for 10 minutes. After allowing the tissue fragments to sedirent the supernatant fluid was decanted into a 250 ml centrifuge tube, the fluid being poured through a gauze covered funnel. The subsequent 7 runs were repeated in an identical fashion — 100 ml of trypsin added for each run and 10 minutes of agitation on the magnetic stirrer. At the end of the 5th run the tissue fragments were whitish and generally without color.

4) Washing of trypsinized cells -

The 800 ml of supernatant fluid collected from the 8 trypsin treatments of kidney mince were contained in 4 250 ml centrifuge tubes. The fluid was quite turbid. The material was centrifuged at the 8th notch of the International centrifuge for 5 minutes. The sediment was well packed and consisted mainly of reddish kidney material and some whitish material. After decanting the supernatant fluid 25 ml of cold Hanks solution with 1/3 the usual amount of $NaHCO_3$ was added to each tube, the sediment resuspended and the contents of the 4 tubes pooled. After centrifugation at the 8th notch for 2 minutes the supernatant was decanted and 50 ml of Hanks 1/3 added, the sediment resuspended and the tube again centrifuged at the 8th notch for 2 minutes. After 3 such washings the sediment was resuspended in 20 ml of Hanks and destributed into 2 graduated centrifuge tubes — 10 ml in each tube. After centrifugation at the 8th notch for 5 minutes the clear supernatant fluid was decanted leaving a total volume of 0.8 ml of packed cells.

The 0.8 ml of packed kidney cells was diluted 1:500 in Lactalbumin hydrolysate medium containing 2% heated normal horse serum.

5) Destribution of cell suspension to tubes

1 ml of the 1:500 cell suspension was destributed by means of the Cornwall automatic pipette into 378 tubes. The tubes were placed in a stationary position in the incubator (36°c)

Aug 12, 1954 Trypsinized Cells (cont)

Materials -

Mixed 8-10-54 / Seitz filtered 8-10-54

1. Trypsin - Difco 1:250 -
2. Earles solution 10X conc. Lot VIII } Diluted & Mixed 8-10-54
3. 4.4 % $NaHCO_3$ 8-7-54

Lactalbumin Hydrolysate Medium c̄ 2% heated Normal Horse Serum prepared 8-12-54

4. Earles solution Lot VIII diluted 8-11-54 } Prepared 8-11-54
Simms solution Lot IX 7-31-54
Lactalbumen hydrolysate Lot X 8-3-54
100 units penicillin 100 mg streptomycin/ml
5) Normal horse serum control lot 1462
[Microbiol. associates]
Heated 56° 30 minutes before use -

Aug 19, 1954 After 7 days of incubation in the stationary position in the incubator (36°C) 119 tubes were examined for growth -

15 - contaminated - mold

104

10% 10 - no growth

73% 76 - early growth present - small islands and clusters of viable cells

17% 18 - fair growth - small sheets of epith -

Appreciable amounts of cellular debris was present in each tube -

23 Aug 54

After 18 days incubation in the stationary position in the incubator (36°C) all the tubes were examined for growth -

89 tubes - good growth
large sheets of cells

37 tubes - fair growth -
small sheets of cells

235 tubes - no growth

17 tubes contamination mold -

Interestingly enough the majority of the tubes with good growth (54 out of 90 tubes) came from one rack

Oct 1, 1954 Preparation of Trypsinized Kidney (Cynomolgus) Cells for Tissue Culture -

Solutions

1.) PBS (According to Dulbecco & Youngner)

a) NaCl 8 gm; KCl 0.2 gm; Na_2HPO_4 1.15 gm; KH_2PO_4 0.2 gm } 800 ml H_2O

b) $CaCl_2$ 0.1 gm 100 ml H_2O

c) $MgCl_2 \cdot 6H_2O$ 0.1 gm 100 ml H_2O

Autoclave a, b, & c seperately & mix when cool

2.) 0.25% Trypsin solution

2.5 gm 1:250 (Difco) Trypsin + 1000 PBS

then Seitz filtered

3.) Normal Calf Serum Lot 2268 (Microbiol. Assoc.)

4.) Lactalbumin Hydrolysate Medium Lot 10-1-54

react 56° 30 min -

5) Hanks Solution with 1/3 $NaHCO_3$ Lot 10-1-54

Procedure

Monkey - Cynomolgus # 7417 Wt: 2840

Wt of kidneys = 6 gm 6.15 gm Total 12.15 gm

Monkey was sacrificed with chloroform - Kidneys removed aseptically -

1.) Capsule and pelvis of kidney removed - Cortex & medulla minced with fine scissors - 1-2 mm size

2) Tissue fragments washed 6x in Hanks solution with 1/3 usual amt. of $NaHCO_3$ - Fluid clear after 3rd wash

3) Kidney tissue incubated with 100 ml of 0.25% trypsin solution in PBS for 1 hour at room temp (23°) in a thick Pyrex flask - (500 ml) - This was followed

by 10 minutes agitation on the magnetic stirrer. Stirring was brisk but without frothing - between 1st & 2nd notch - after agitation cloudy supernatant fluid was decanted into 250 ml centrifuge tube - 100 ml of fresh trypsin solution pre heated to 37°C was added - Agitation was in the same manner as just described - 8 periods of agitation were performed - after the 5th stirring the tissue fragments were whitish and without color. The supernatant fluid which was very cloudy after the 1st stirring became progressively less cloudy with subsequent stirrings

4) The 500 ml of supernatant fluid was centrifuged at the 8th notch for 4 minutes - The sediment was well packed - The centrifuge bottle containing the 7th & 8th stirrings containing very little sediment compared to the other 3 centrifuge bottles. after the supernatant fluid was decanted 25 ml of cold Hanks solution with 1/3 the usual amount of $NaHCO_3$ was added to each bottle - the sediment resuspended and the contents of the 4 bottles pooled. after centrifugation at the 8th notch for 4 minutes the supernatant fluid was decanted and the sediment washed 3X in 50 ml of Hanks solution (1/3 $NaHCO_3$). Centrifugation for these washings was at the 8th notch for 4 minutes. After washings were completed the sediment was suspended in 20 ml of Hanks (1/3 $NaHCO_3$) and destributed to 2 graduated centrifuge tubes - 10 ml per tube - After centrifugation at 2000 rpm for 5 minutes the volume of sediment was 1.1 ml.

5) The sediment was diluted to 1:200 in Lactalbumen Hydrolysate medium with 2% added inact. normal calf serum.

6) 0.5 ml of 1:200 cell suspension destributed to 383 tubes by means of the automatic pipette. The tubes were placed in a stationary position in the incubator (36°C)

Oct 10, 1954 — 9 days — 30 tubes examined - no growth - no viable cells seen

Punch to give
[redacted]

Parke, Davis & Company

EXECUTIVE OFFICES AND LABORATORIES, DETROIT 32, MICHIGAN, U. S. A.
JOSEPH CAMPAU AVENUE AT THE RIVER

RESEARCH DEPARTMENT

October 14, 1954

[redacted]
Children's Hospital Research Foundation
Elland-Bethesda Avenues
Cincinnati 29, Ohio

[redacted]

As agreed in our telephone conversation, a bottle containing 50 cc. of trypsinized monkey kidney cells was sent to you by plane last evening. We hope it arrived in good order, and that the cells will be useful and meet your requirements. We will be glad to send more at any time, since it is no great problem for us - at least at the present time - so don't hesitate to call on us.

There is attached an outline for certain procedures of trypsinization, which we feel should improve the yield of cells. We hope you will be able to work it out. If you have any questions about any of the items, just give us a telephone call. If I am not here, please ask for Doctor I. W. McLean, who is Assistant Director and in charge of Virus Research.

So glad to learn that you are well on the road to recovery from the sinus episode. They are certainly miserable, and I can well imagine your relief.

Sincerely yours,

[redacted]

FS/ed
Enclosure

SUGGESTIONS FOR TRYPSINIZATION

1. Remove capsules and vascular material from fresh kidneys. (Do not attempt to separate the medulla and cortical areas.)

2. Mince the tissue.

3. Wash with phosphate buffered saline.

4. Suspend tissue in 0.25% trypsin at 37°C. in Waring Blendor bowl, 20 cc./kidney.

5. Extract at slow speed for 10 minutes.

6. Aspirate off the supernatant through 2 layers of cheese cloth. Ice immediately.

7. Repeat until tissue is exhausted.

8. Centrifuge at 1000 RPM for 5 minutes.

9. Aspirate supernatant and pool the pellets.

10. Wash sediment in approximately 10 volumes of medium D (4% calf serum) in 250 cc. centrifuge bottles.

11. Repeat.

12. Suspend the pellet in 40 cc. medium D/kidney.

13. Shake vigorously.

14. Prepare a 1:4 dilution of 1 cc. of the suspension and add 0.1 cc. glacial acetic acid.

15. Immediately make a direct count of the morphologically intact epithelial cells using hemocytometer and phase scope. Count a clump of cells as only one cell.

16. Dilute the cell suspension with medium D to contain 50,000 cells per cc. and plant in 2 cc. amounts in slant tubes.

OCT 11 1954

SPINAL VIRULENCE IN MICE of MAHONEY VIRUS PASSED THROUGH CHICK EMBRYO TISSUE + BOOSTED IN MONKEY KIDNEY

PURPOSE: 1) Has new variant been obtained?
2) What is its effect intraspinally in mice — has it lost spinal virulence for mice?

Material Used: CE_1 MK_1 — used to-day intraspinally in ~~mice~~ monkeys

Inoculum — 0.02 ml of undiluted culture fluid intraspinally

Mice Used — CHRF Swiss - about 14-16 Gm.

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

Traumatic mouse dead → incorrectly recorded.

Surviving mouse without signs is not traumatic one

OCT 11 1954

Spinal Virulence in Cynomolgus Monkeys of [redacted] Virus Passed through Chick Embryo Tissue and then Boosted in Titer in Monkey Kidney

Purpose – ~~F~~ Has new variant been segregated which is devoid of virulence by spinal route in monkeys?

Material Used – [redacted] CE_1 MK_1 (plasma clot series) – culture fluid harvested and stored in deep freeze.
Monkey Kidney Cytopathogenic Titer – 10^7 TCD_{50}/ml 6th day

Inoculum — 0.1 ml undiluted – 4 monkeys
0.1 ml 10^{-2} — 4 "

Monkeys Used – Cynomolgi from shipment of Aug. 1954

Inoculum	Cynomolgus No.	
0.1 ml – 10^{-2}	✓7189	P_4; K_{28}
10^4 TCD_{50}	✓7190	K_{28}
	✓7191	P_4; K_{28}
	✓7192	P_4; K_{28}
0.1 ml – Undiluted	✓7193	P_4; K_{28}
10^6 TCD_{50}	✓7194	K_{28}
	✓7195	K_{28}
	✓7196	K_{28}

Cyn 7189

DATE	DAY	REMARKS
OCT 11 1954	0	✓
12	1	✓
13	2	✓
14	3	✓
15	4	Partial paralysis left lower – slight
16	5	Same moderate
17	6	Same
18	7	Same
19	8	Extensive L.L.; partial R.L.
20	9	Same
21	10	"
22	11	same
23	12	"
24	13	"
25	14	"
26	15	Same but paralysis somewhat less in both
27	16	Same
28	17	"
29	18	"
30	19	"
31	20	"
11- 1-54	21	"
2	22	Residual partial paralysis in both lowers
3	23	Same
4	24	"
5	25	"
6	26	"
7	27	"
NOV 8 1954	28	" Killed, cus in belly's
	29	
	30	
	31	
	32	
	33	
	34	
	35	

STANDARD B&P "NOTEAR"

STANDARD B&P "NOTEAR"

Cyn. 7190

DATE	DAY	REMARKS
OCT 11 1954	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	✓
NOV 8 1954	28	✓ Killed, cuts in belly's
	29	
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7191

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	//
	3	//
	4	Climbs & runs but clumsy with left thigh muscles had to be taken out to see
	5	Partial paralysis both lowers
	6	Same
	7	Extensive paralysis both lowers
	8	Same
	9	"
	10	"
	11	"
	12	"
	13	"
	14	"
	15	No progression, possibly some improvement.
	16	Same
	17	"
	18	"
	19	"
	20	"
	21	"
	22	Residual partial paralysis both lowers
	23	Same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	" Killed, cuts in belly
	29	
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7192

DATE	DAY	REMARKS
	0	✓
	1	✓
	2	✓
	3	✓
	4	Partial paralysis left lower - mostly thigh - had to be taken out to see
	5	same
	6	Partial paralysis left lower
	7	same
	8	"
	9	"
	10	"
	11	"
	12	"
	13	"
	14	"
	15	"
	16	"
	17	"
	18	"
	19	"
	20	"
	21	"
	22	"
	23	"
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	" Killed, cousin Kelly's
	29	
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7193

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	Partial paralysis left lower
	5	Same
	6	Same
	7	left lower clumsy, paralysis slight.
	8	Same
	9	"
	10	"
	11	"
	12	"
	13	"
	14	"
	15	Very little paralysis, still clumsy in walking
	16	Same
	17	Seems all right in cage
	18	Same
	19	"
	20	"
	21	"
	22	Climbs well, left lower slightly clumsy in walking
	23	Same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	" Killed, cus in belly's
	29	
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7194

DATE	DAY	REMARKS
OCT 11 1954	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	✓
NOV 8 1954	28	✓ Killed, cus in belly's
	29	
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7195

DATE	DAY	REMARKS
OCT 11 1954	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	✓
NOV 8 1954	28	✓ Killed, ears in belly's
	29	
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7196

DATE	DAY	REMARKS
OCT 11 1954	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	✓
NOV 8 1954	28	✓ Killed, cuts in belly &
	29	
	30	
	31	
	32	
	33	
	34	
	35	

OCT 11 1954

Spinal Virulence in Mice of YSk Virus

Passed through Chick Embryo Tissue + Boosted in Monkey Kidney

Purpose: 1) Has new variant been obtained?
2) Has it lost its spinal virulence for mice?

Material Used: $CE_1 MK_1$ – used to-day intraspinally in monkeys

Inoculum — 0.02 ml of undiluted culture fluid intraspinally

Mice Used: CHRF Swiss – about 14–16 Gm.

	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	–	D																										
2	–	D																										
3	–	D																										
4	–	D																										
5	–	D																										
6	–	D																										
7	–	D																										
8	–	D																										
9	–	D																										
10	–	D																										
11	–	D																										
12	–	D																										
13	–	D																										
14	–	D																										
15	–	D																										
16	–	D																										
17	–	D																										
18	–	–	P	P	P	D																						
19	–	–	W	P	P	P	P	P	P	D																		
20	–	–	–	D	P	P	P	P	P	P	P	P	D															

OCT 11 1954

Spinal Virulence in Cynomolgus Monkeys of YSk Virus Passed Through Chick Embryo Tissue and then Boosted in Titer in Monkey Kidney Culture

Purpose – Has new variant been segregated which is devoid of virulence by spinal route in monkeys?

Material Used: YSk CE_1, MK_1 (plasma clot series) – culture fluid harvest of Monkey kidney cytopath. stored in deep freeze. titer – $10^{-6.5}$ TCD_{50}/ml 6th day

Inoculum: 0.1 ml undiluted – 4 monkeys
0.1 ml 10^{-1} – 4 "

Monkeys Used: Cynomolgi from shipment of Aug. 1954

Inoculum	Cynomolgus No.	
0.1ml of 10^{-1}	✓7197	P_6; K_{28}
	✓7198	P_3; K_{28}
$10^{4.5}$ TCD_{50}	✓7199	K_{28}
	✓7200	P_4; K_{28}
0.1ml undilut.	✓7201	P_4; K_{28}
	✓7202	K_{28}
$10^{5.5}$ TCD_{50}	✓7203	K_{27}
	✓7204	P_{13}; K_{29}

Cy-7197

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	✓
	5	
	6	Slight partial paralysis left lower - mostly thigh
	7	Same, must be taken out of cage to see involvement
	8	Same
	9	Same, toes of right foot held inflexion
	10	Same
	11	" * ~~partial paralysis left lower~~
	12	Same
	13	"
	14	"
	15	"
	16	"
	17	"
	18	"
	19	"
	20	"
	21	"
	22	Thigh muscles + right toes same
	23	Same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	" Killed, ens in belly's
	29	
	30	
	31	
	32	
	33	
	34	
	35	

STANDARD B&P "NOTEAR"

STANDARD B&P "NOTEAR"

Cyn 7198

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	
	3	Drags right foot
	4	Slight paralysis - right foot
	5	Same
	6	~~Slight partial~~ paralysis ~~left lower~~ right foot & leg ~~mostly~~ thigh
	7	Same
	8	"
	9	Partial paralysis R.L.
	10	Same
	11	"
	12	"
	13	Extensive paralysis right lower
	14	Same
	15	Moderate paralysis right leg & foot
	16	Same
	17	"
	18	"
	19	"
	20	"
	21	"
	22	Residual partial paralysis right leg & foot
	23	Same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	" Killed, cns in bully's
	29	
	30	
	31	
	32	
	33	
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Cyn 7199

DATE	DAY	REMARKS
OCT 11 1954	0	
	1	
	2	
	3	
	4	
	5	
	6	~~Partial paralysis right lower - mostly foot~~
	7	
	8	
	9	
	10	
	11	
	12	
	13	
	14	
	15	
	16	
	17	
	18	
	19	
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	21	
	22	
	23	
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	26	
	27	
NOV 8 1954	28	Killed, cus in belly's
	29	
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	33	
	34	
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Cyn 7200

DATE	DAY	REMARKS
OCT 11 1954	0	Right foot questionable
	1	Holds right foot questionably - no definite paralysis
	2	"
	3	"
	4	Partial paralysis both lowers
	5	Same but slight on left
	6	Same
	7	Partial paralysis both lowers, less on left
	8	Same
	9	Extensive paralysis both lowers
	10	Same
	11	"
	12	"
	13	"
	14	"
	15	"
	16	"
	17	"
	18	"
	19	"
	20	"
	21	"
	22	Extensive residual paralysis both lowers
	23	Same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	" Pilled, cusin kelly
	29	
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7201

STANDARD B&P "NOTEAR"

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	Slight partial paralysis left lower
	5	Same
	6	Same
	7	"
	8	"
	9	"
	10	"
	11	"
	12	"
	13	"
	14	"
	15	Same, can not be seen while in cage
	16	"
	17	Slight paralysis left lower still evident
	18	Same
	19	"
	20	"
	21	"
	22	Very slight paralysis left lower
	23	Same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	" Killed, cons in kelly's
	29	
	30	
	31	
	32	
	33	
	34	
	35	

STANDARD B&P "NOTEAR"

Cyn 7202

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	✓
	5	✓
	6	✓
	7	✓
	8	✓
	9	✓
	10	Question right foot - Out for run, OK
	11	Can see no involvement of rt. foot.
	12	✓
	13	✓
	14	✓
	15	✓ OK out for run
	16	✓
	17	✓
	18	✓
	19	✓
	20	✓
	21	✓
	22	✓
	23	✓
	24	✓
	25	✓
	26	✓
	27	✓
NOV 8 1954	28	✓ Killed, cus in belly x
	29	
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7203

DATE	DAY	REMARKS
OCT 11 1954	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	✓
NOV 8 1954	28	✓
	29	✓ Killed 1 cow in belly's
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7204

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	✓
	5	✓
	6	✓
	7	✓
	8	✓
	9	✓
	10	✓
	11	✓
	12	✓
	13	Partial paralysis right upper.
	14	Same
	15	Extensive paralysis right upper - out for sum; lowers OK
	16	Same
	17	"
	18	"
	19	"
	20	"
	21	"
	22	Extensive paralysis entire R.U. - otherwise OK
	23	Same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	"
	29	" Killed, cuts in belly's
	30	
	31	
	32	
	33	
	34	
	35	

OCT 11 1954

Spinal Virulence in Mice of Leon Virus

Passed through Chick Embryo Tissue + Boosted in Monkey Kidney

Purpose: 1) Has new variant been obtained?
2) Has it gained capacity to paralyze mice after spinal inoculation?

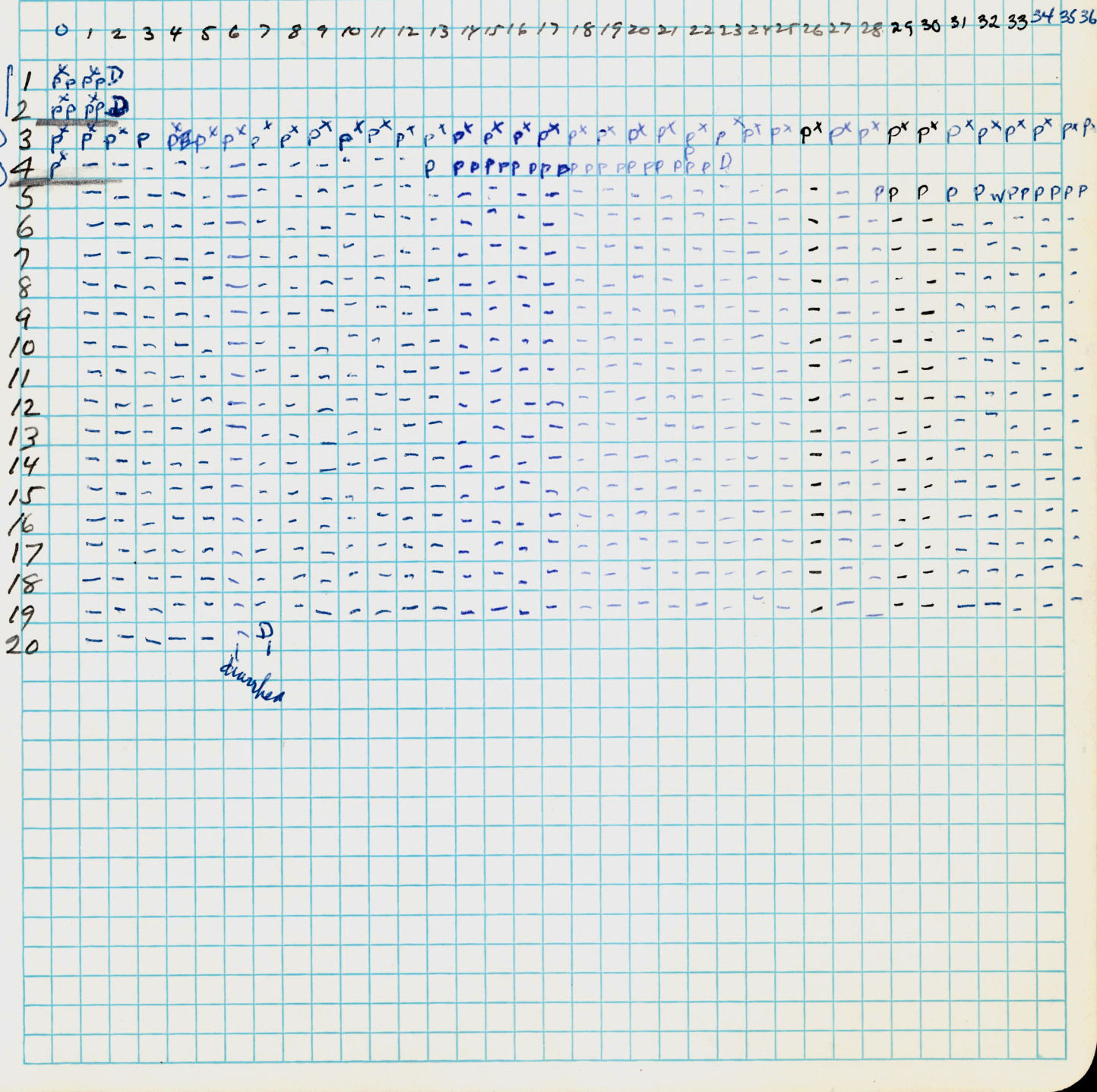
Material Used: $CE_1 MK_1$ – used to-day intraspinally in monkeys

Inoculum — 0.02 ml of undiluted culture fluid intraspinally

Mice Used — CHRF Swiss – about 14–16 Gm

	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	36
1	P^x	P^x	P^x	D																																	
2	P^x	P^x	P^x	D																																	
(2) 3	P^x	P^x	P^x	P	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x	P^x
(1) 4	P^x	–	–	–	–	–	–	–	–	–	–	–	–	P	P	P	P	P	P	P	P	P	P	P	P	P	D										
5		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	P	P	P	P	P	W	P	P	P	P
6		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
20		–	–	–	–	–	–	–	D																												

diarrhea



OCT 11 1954

Spinal Virulence in Cynomolgus Monkeys of [redacted] Virus Passed Through Chick Embryo Tissue and then Boosted in Titer in Monkey Kidney Cultures

Purpose: Has new variant been segregated which is devoid of virulence by spinal route in monkeys?

Material Used: [redacted] ($CE_1 MK_1$ (plasma clot series) – culture fluid harvest of Monkey kidney cytopathog. stored in deep freeze. Titer – $10^{6.5}$ TCD_{50}/ml – 6 days

Inoculum:
0.1 ml undiluted – 4 monkeys
0.1 ml 10^{-1} – 4 "

Monkeys Used: Cynomolgi from shipment of Aug. 1954

Inoculum	Cynomolgus No.	
0.1 ml of 10^{-1} $10^{4.5}$ TCD_{50}	✓ 7205	P_4; D_9
	✓ 7206	P_{10} transit; $K_{7/9}$
	✓ 7209	P_{10}; $K_{7/9}$
	✓ 7208	P_{10} trans.; $K_{7/9}$
0.1 ml of undiluted tr $10^{5.5}$ TCD_{50}	✓ 7209	P_3 D_9
	✓ 7210	$K_{7/9}$
	✓ 7211	P_{14} tr; $K_{7/9}$
	✓ 7212	P_5-slight; $K_{7/9}$

Cyn 7205

DATE	DAY	REMARKS
OCT 11 1954	0	Left foot questionable
	1	✓
	2	✓
	3	✓
	4	Partial paralysis left lower
	5	Extensive paralysis both lowers
	6	Prostrate - complete paralysis lowers & back - partial of uppers
	7	Same
	8	Same; almost complete of uppers also aphonia
	9	Dead A.M. Rigor mortis C'ns in Kelly's
	10	
	11	
	12	
	13	
	14	
	15	
	16	
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NOV 8 1954	28	
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Cyn 7206

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	✓
	5	✓
	6	✓
	7	✓
	8	✓
	9	✓
	10	Partial paralysis right lower
	11	same
	12	Right lower arms improved
	13	Same
	14	"
	15	Completely recovered — out for run OK
	16	Same
	17	"
	18	"
	19	"
	20	"
	21	"
	22	OK
	23	OK
	24	✓
	25	✓
	26	✓
	27	✓
NOV 8 1954	28	✓
	29	✓ Killed, run in belly
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7207

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	✓
	5	✓
	6	✓
	7	✓
	8	✓
	9	✓
	10	Partial paralysis of both lowers & uppers
	11	same
	12	"
	13	"
	14	"
	15	" — moderately severe; left side, 4 months?
	16	Steel closes both eyes - same
	17	~~Same~~
	18	"
	19	"
	20	"
	21	"
	22	Partial paralysis uppers & lowers; no definite facial
	23	same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	"
	29	" Kicked, cuts in belly & - Old healed fracture
	30	right forearm; contracture right hand.
	31	
	32	
	33	
	34	
	35	

Cyn 7208

DATE	DAY	REMARKS
OCT 11 1954	0	Right foot affected
	1	Right lower clumsy - esp. foot
	2	Same
	3	"
	4	"
	5	"
	6	Same
	7	"
	8	"
	9	"
	10	Partial paralysis both lowers & possibly also both uppers
	11	Partial paralysis of all extremities, ataxia,
	12	Same
	13	Same somewhat improved today
	14	Same
	15	Holds head to right; paralysis same; veers to right
	16	Same
	17	"
	18	"
	19	"
	20	"
	21	"
	22	Very little paralysis left; incoordination still present
	23	Same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	"
	29	" Killed, cut in belly's
	30	
	31	
	32	
	33	
	34	
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Cyn 7209

DATE	DAY	REMARKS
OCT 11 1954	0	Right foot questionable
	1	Holds right foot suspiciously; curls toes — nothing definite
	2	Same
	3	Definite paralysis right lower; question left foot
	4	Extensive paralysis both lowers
	5	Same plus paralysis of back - lies down
	6	Prostrate - extensive paralysis lowers + back - partial uppers
10-18-54	7	Dead A.M. Cns in belly's
	8	
	9	
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	11	
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NOV 8 1954	28	
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Cyn 7210

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	✓
	5	✓ Out for run – OK.
	6	✓
	7	✓
	8	✓
	9	✓
	10	✓ OK & Out for run
	11	✓
	12	✓
	13	✓
	14	✓
	15	✓
	16	✓
	17	✓
	18	✓
	19	✓
	20	✓
	21	✓
	22	✓
	23	✓
	24	✓
	25	✓
	26	✓
	27	✓
NOV 8 1954	28	✓
	29	✓ Killed, cns in hilly's
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7211

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	✓
	5	✓ Out for run — OK
	6	✓
	7	✓
	8	✓
	9	✓
	10	✓
	11	✓
	12	✓
	13	✓
	14	Disinclined to climb possible paralysis of deltoids & hip muscles
	15	Partial paralysis of hip muscles + deltoids
	16	Same
	17	Same — can only be seen when out of cage
	18	"
	19	"
	20	"
	21	"
	22	Out for run; paralysis very indefinite
	23	same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	"
	29	" Killed, ears in Kelly's
	30	
	31	
	32	
	33	
	34	
	35	

Cyn 7212

DATE	DAY	REMARKS
OCT 11 1954	0	✓
	1	✓
	2	✓
	3	✓
	4	✓
	5	✓ Out for exam OK; but Randlos right foot questionable
	6	Same
	7	"
	8	Partial paralysis right foot
	9	~~Same~~ Toes held inflexion
	10	Right foot failure - only toes
	11	same
	12	"
	13	"
	14	Entire right involved
	15	Extensive paralysis right lower
	16	Same
	17	"
	18	"
	19	"
	20	"
	21	"
	22	Partial paralysis right lower - residual
	23	Same
	24	"
	25	"
	26	"
	27	"
NOV 8 1954	28	"
	29	" Killed, cut in belly's
	30	
	31	
	32	
	33	
	34	
	35	