

Type II

MAR 23 1959

Rectal Swabs.

Tubes. 1371 of 3/7/59 as attached sheet.

Procedure. 0.4 ml of fluid added to each of 3 tubes.

<u>Date of Rectal Swab.</u>	Tubes B.	3/25 2	3/26 3	3/27 4	3/29 6	3/30 7	3/31 8	<u>Passage</u> 4/7 4/8 4/9 4/10 4/11				
								1	2	3	4	5
2/10/59 One	106	0	0	0	0	0	0					
	107	0	0	0	0	0	0					
	108	0	0	0	0	0	0					
2/12/59 2 days	109	0	0	0	0	0	0					
	110	0	0	0	0	0	0					
	111	0	0	0	0	0	0					
2/14/59 4 days	112	0	0	0	0	0	0					
	113	0	0	0	0	0	0					
	114	0	0	0	0	0	0					
2/16/59 6 days	115	0	0	0	0	?	?	→ 115 0 0 0 0 Saved 6 Pass				
	116	0	0	0	0	0	0					
	117	0	0	0	0	?	?	→ 117 0 0 Saved 6 Pass				
2/19/59 9 days	118	0	0	0	0	0	0					
	119	0	0	0	0	0	0					
	120	0	0	0	0	0	0					
2/23/59 13 days	121	0	0	0	0	?	?	→ 121 0 0 0 0 Saved 6 Pass				
	122	0	0	0	0	?	?	→ 122 0 0 0 0				
	123	0	0	0	0	0	0					
3/3/59 21 days.	124	0	0	0	0	0	0					
	125	0	0	0	0	0	0					
	126	0	0	0	0	0	0					

Type II

Throat Swab.

MAR 23 1959

tubes 1371 of 2/17/59 as overleaf

Procedure 0.4 ml of fluid added to each of 3 tubes.

Date of Throat Swab

tubes
B.3/25
23/26
33/27
43/29
63/30
73/31
8

Passage

4/7 4/8 4/9 4/10
1 2 3 5

2/10/59

1 hr

127

0

0

0

0

0

0

128

0

0

0

0

0

0

129

0

0

0

0

0

0

2/12/59

2 days

130

0

0

0

0

0

0

131

0

0

0

0

0

0

132

0

0

0

0

0

0

2/14/59

4 days

133

0

0

0

0

0

0

134

0

0

0

0

0

0

135

0

0

0

0

0

0

2/16/59

6 days

136

0

0

0

0

0

0

137

0

0

0

0

0

0

138

0

0

0

0

?

138 0 0 0 0

Saved to Pass

2/19/59

9 days

139

0

0

0

0

0

0

140

0

0

0

0

0

0

141

0

0

0

0

0

0

2/23/59

13 days

142

0

0

0

0

0

0

143

0

0

0

0

0

0

144

0

0

0

0

0

0

3/3/59

21 days

145

0

0

0

0

0

0

146

0

0

0

0

0

0

147

0

0

0

0

0

0

APR 29 1959

to test the PRE. Rectal Swab in ERK cells.

No virus isolated in Monkey Kidney

Complete suppression of Polio II multiplication suggests some interferon virus may be present.

Tubes ERK C96 of 4/22/59 Medium added 4/25/59 and changed before use to ERK maintenance medium.

Tubes	5/1	5/2	5/4	
B.	2	2	5	
4	0-1	1-2	3-4	} ERK ₁ of Pre Rectal Swab
5	few	0-1	2-4	
6	few	1-2	2-4	

Passage MAY 4 1959

ERK Tubes C97 of 4/29/59

MK Tubes T380 of 4/28/59

Inoculum 0.2ml / tube of pooled tubes B4-6 above.

B.	5/5	5/6	5/7	5/9	5/11	5/12	
	1	2	3	5	7	8	
ERK.	7	0-1	3-4 off				} ERK ₂ of Pre. Rectal Swab.
	8	0-1	3-4 off				
	9	0-1	3-4 off				
Monkey	10	0	0	0	0	0	0 vac
	11	0	0	0	0	0	0 fac
	12	0	0	0	0	0	0 fac.

The CPE is similar to that observed with the Coxsackie A. group.
Decided to test first for those.

See overleaf.

MAY 7 1959

To identify the virus in the Pre. Rectal Swab.

Virus ERKP₂ of 5/6/59 of Pre Rectal Swab. Diluted 10^{-4}

Serials
Coxsackie A 11
A 13
A 15
A 18.

Tubes ERK Series C 97 of 4/29/59 Medium added 5/2/59 and changed before use to ERK maintenance medium

Mixture	Tubes	5/9	5/11	5/12	5/13	5/15
	C	<u>2</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>8</u>
10^{-4} + equal vol Serum	1	0	3-4	off		
Cox A 11.	2	few	2-3	off		
	3	few	3-4	off		
No TC ₅₀ = 30						
Cox A 13	4	few	2-3	3-4 off		
	5	0	2-3	3-4 off		
	6	few	3-4	off		
Cox A 15	7	few	3-4	off		
	8	few	3-4	off		
	9	few	3-4	3-4 off		
Cox A 18	10	few	2-3	off		
	11	few	2-3	off		
	12	0	1-2	3-4 off		
+ equal vol diluent	13	few	3-4	4 off		
	14	0	0-1	3-4 off		
$\approx 10^{-4.3}$	15	few	3-4	off		
	16	0-1	3-4	off		
$10^{-5.3}$	17	0	1-2	3-4 off		
	18	0	1-2	3-4 off		
	19	0	2-3	4 off		
	20	0	2-3	4 off		
$10^{-5.8}/0.2ml.$						
$10^{-6.3}$	21		0	0?	0?	
	22		0	0	0?	
	23		0	0	0?	
	24		0	0	0?	
$10^{-7.3}$	25		0	0	0?	
	26		0-1	0-1?	0?	
	27		0	0	0?	
	28		0	0	0?	

C 98 5/15/59
Passage in
5/16 5/18
1 2
0 0
0 0
0 0
0 0
0 0
0 0
0 0
0 0
0 0

Nonlytic

MAY 27 1959

MOUSE TEST.

to identify the virus in the Pre Rectal Swab

VirusERKP of RS of 2/10/59 Harvested 5/27/59
Centrifuged sample as used for Ether sensitivity test.New Born miceInoculated { 0.01 i.cer. } into each
{ 0.02 Sub cut in the back. }

new born mouse of 2 litters.

Mice

		5/28 1	5/29 2	5/30 3	5/31 4	6/1 5	6/2 6	6/3 7	6/4 8	6/5 9	6/6 10	6/7 11	6/8 12	6/9 13	6/10 14
①	1	✓	✓	✓	✓	✓	✓								✓
	2	✓	✓	✓	✓	✓	✓								✓
	3	✓	✓	✓	✓	✓	✓								✓
	4	✓	✓	✓	✓	✓	✓								✓
	5	✓	✓	✓	✓	✓	✓								✓
	6	✓	✓	✓	✓	✓	✓								✓
	7	✓	✓	✓	✓	✓	✓								✓
	8	✓	✓	✓	✓	✓	✓								✓
②	1	✓	✓	✓	✓	✓	✓								✓
	2	✓	✓	✓	✓	✓	✓								✓
	3	✓	✓	✓	✓	✓	✓								✓
	4	✓	✓	✓	✓	✓	✓								✓
	5	✓	✓	✓	✓	✓	✓								✓
	6	✓	✓	✓	✓	✓	✓								✓

MAY 11 1959

A virus was isolated in ERK cells from the PRE Rectal swab.

To find if this virus persisted throughout the period of observation

tubes ERK Series C of 5/6/59.

Pate of Rectal Swab.	Tubes B.	5/12 1	5/13 2	5/14 3	5/15 4	5/16 5	5/18 7	
2/12/59 2 days.	4	0	0	0	0	0	?	} Passed.
	5	0	0	0	0	0	?	
	6	0	0	0	0	0	?	
2/14/59 4 days	7	0	few	0-1	1-2	2-3	3-4 off	} Pooled Kept
	8	0	few	?	0-1	0-2	3-4 off	
	9	0?	few	0-1	1-2	2-3	3-4 off	
2/16/59 6 days	10	0	few	0-1	1-2	2-3	3-4 off	
	11	0	few	0-1	1-2	2-3	3-4 off	
	12	0	few	0-1	1-2	2-4	3-4 off	
2/19/59 9 days	13	0	few	0-1	1-2	2-4	3-4 off	
	14	0	0	few	0-1	0-2	3-4 off	
	15	0	0	0	few	few	2-4 off	
2/23/59 13 days	16	0	0	0	0	0	?	} Passed
	17	0	0	0	0	0	?	
	18	0	0	0	0	0	?	
3/3/59 21 days all used.	19	0	0	0	0	0	?	} Passed
	20	0	0	0	0	0	?	
	21	0	0	0	0	0	?	

Second Passage in ERK. MAY 19 1959 Tubes C99 of 5/13/59.

Inoculum	B	5/21 3	5/23 4	5/25 5	5/26 7	5/27 8	
0.2 ml/tube from tubes above	4	0	0	0	0	0	} Discarded
R.S. 2/12/59	5	0	0	0	0	0	
	6	0	0	0	0	0	
RS 2/23/59	16	0	0	0	0	0	} Discarded
	17	0	0	0	0	0	
	18	0	0	0	0	0	
RS 3/3/59	19	0	0	0	0	0	} Discarded.
	20	0	0	0	0	0	
	21	0	0	0	0	0	

MAY 25 1959

Ether Sensitivity TestTo identify the virus in the Pre Rectal Swab.Virus ERK_{P2} of R.S of 2/10/59. Harvested 5/6/59.Tubes ERK C100 of 5/20/59 Medium added 5/23/59 and changed before use to ERK maintenance mediumTo grow a stock for the ether sensitivity test

<u>Inoculum</u>	<u>Tubes</u>	5/26	5/27	
	X	1	2	
0.2ml / tube	1	3	40//	Frozen & immediately thawed. Pooled. ERK _{P3} of 5/27/59.
ERK _{P2}	2	2-3	40//	
	3	2-3	40//	
	4	2-3	40//	
	5	3	40//	
	6	2-3	40//	

MAY 27 1959

Ether Sensitivity Test

- Procedure
- 1) Centrifuged the above ERK_{P3} of 5/27/59.
 - 2) 4 ml of the S'natant put into a stoppered centrifuge tube with 1 ml Mallinckrodt Analytical Reagent Ether
Control tube 4 ml S'natant
 - 3) Stored 4°C 18 hrs with frequent shaking
 - 4) Centrifuged 2,500 rpm. 10 mins
 - 5) 3ml removed from below ether layer and from the control tube.
 - 6) Centrifuged 1000 rpm. 10 mins in tubes plugged with cotton wool
 - 7) Incubated in sterile petri dishes 30 min at 37°C
 - 8) Titrated.

Tubes ERK C100 of 5/20/59 Medium added 5/23/59 and changed 5/27/59. Changed to ERK maintenance medium before useEther Treated

MAY 28 1959

	<u>Tubes</u>	5/29	5/30	5/31
Undiluted.	B.	1	2	3
	1	2-3	40//	
	2	2-3	40//	
	3	2-3	40//	
10 ⁻¹	4	1-2	40//	
	5	1-2	40//	
	6	0-1	2-3	40//
10 ⁻²	7	0-1	1-3	2-40//
	8	0-1	1-3	2-40//
	9	0-1	1-3	2-3. 3-40//

	tubes	4/29	5/30	5/31	6/1	6/3	6/5	Passage June 8th		
	B	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>6</u>	<u>8</u>	<u>ERK C102</u>		
10^{-3}	10	few	1-2	2-3	3-4 off					
	11	few	1-2	2-3	4 off					
	12	few	1-2	2-3	4 off					
10^{-4}	13	Dark	few	1-2	2-3					
	14	0	few	1-2	2-3			6/9	6/10	6/11
	15	0	0	0-1	2-3			<u>1</u>	<u>3</u>	<u>3</u>
10^{-5}	16		0	0	0	0	?	16	0	0
	17		0	0	0	0	?	17	0	0-1
	18		0	0	0	0	?	18	0	0
10^{-6}	19				0	0	?	19	0	0
	20				0	0	?	20	0	0
	21				0	0	?	21	0	0
10^{-7}	22									
	23									
	24									

Control

10^{-4}	25	0	few	1-2	2-3					
	26	0	0	0-1	2-3					
	27	0	0	0-1	2-3					
$10^{4.5}/0.2 \mu 10^{-5}$	28	0	0	0	0	0	?	28	0	0
	29	0	0	0	0	0	?	29	0	0
	30	0	0	0	0	0	?	30	0	0
10^{-6}	31				0	0	?	31	0	0
	32				0	0	?	32	0	0
	33				0	0	?	33	0	0
10^{-7}	34				0					
	35				0					
	36				0					

JUN 1 1959

ERK virus from R.S. of 2/10/59

to identify the virus using Echo & Coxsackie serum pools and single sera

Tubes ERK C101 of 5/26/59 medium added 5/29/59 and changed before use to ERK maintenance medium

Virus ERK 63 of 5/27/59. $10^{-3.5}$ estimated 200 TCID₅₀

Sera listed in detail at front of book.

	<u>Tubes</u>	<u>Serum</u>	6/2 1	6/3 2	6/4 3	6/5* 4
<u>Virus</u>			X			
$10^{-3.5}$ + equal vol Ket	1		few	0-1	2-3	
	2		few alk.	0-2	2-3	
	3		few	0-1	2-3	
	4	E2	few	0-2	2-3	
	5		few	0-2	2-3	
	6		few alk.	0-2	2-4 or 1	
	7	E3	few	0-2	2-3	
	8		few	0-2	2-3	
	9		few	0-1	2-3	
	10	E4	few	0-2	2-4 or 1	
	11		0	0-1	2-3	
	12		0	0-1	2-3	
	13	E5	few	0-2	2-3	
	14		few	0-2	2-3	
	15		few	0-1	2-3	
	16	Echo 40	few	0-2	2-3	
	17		few	0-2	2-3	
	18		few	0-1	2-3	
	19	Echo 17	few	0-2	2-4 or 1	
	20		0 alk.	0-1	2-3	
	21		few	0-2	2-4 or 1	
	22	Echo 20	few	0-2	2-3	
	23		few	0-2	2-4 or 1	
	24		few	0-1	2-3	
	25	Echo 21	few	0-2	2-4 or 1	
	26		0	0-2	2-3	
	27		few	0-2	2-3	

Not Echo 1-24

<u>Virus</u>	<u>Serum</u>	<u> tubes</u>	<u>6/2</u> 1	<u>6/5</u> 2	<u>6/4</u> 3	<u>6/5</u> 4*
$10^{-3.5}$ + equal vol.	Cox B1-5	X				
		28	few	0-2	2-4+//	
		29	few	0-1	2-3	
		30	few	0-2	2-3	

Not Cox B

Cox A Pool 1	31	few	0-2	2-4+//	
	32	few	0-2	2-3	
	33	few	0-2	2-3	

Cox A Pool 2	34	few	1-2	2-3	
	35	few	0-2	2-3	
	36	few	0-2	2-3	

Not Cox A
1-19

Coxsackie A1	37	few	0-2	2-3.	
	38	0	few	0-2	
	39	few	0-2	2-3	

Coxsackie A9	40	few	0-2	2-3	
	41	few	0-2	2-3	
	42	few	1-2	2-3	

Coxsackie A14	43	few	0-2	2-3	
	44	few	0-2	2-4+//	
	45	few	0-2	2-3	

Coxsackie A17	46	few	0-2	2-4+//	
	47	few	0-1	2-3	
	48	few	0-2	2-4+//	

Pett 1:10	49	few	0-2	2-3	
	50	few	0-2	2-3	
	51	few	0-2	2-3	

Not Pett.

Bason 1:10	52	?	0	0	
------------	----	---	---	---	--

Toxic difficult to read 1st day.	53	?	0	0	
Cleaner 2nd day.	54	?	0	0	

Repeated Serum titre
1:55
heat if found 8th

J-V4 1:10	55	few	0-1	2-3	
-----------	----	-----	-----	-----	--

Echo 25	56	few	0-1	2-3	
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Not Echo 25

	57	few	0-1	2-3	
--	----	-----	-----	-----	--

Titration

$10^{-3.5}$ + equal vol diluent	$10^{-3.8}$	58	few	0-1	1-3
		59	0	0-1	2-3
		60	0	0-1	2-3
		61	few	0-1	2-3

170 10950.

JUN 1 1959

ERK virus from R.S. 2/10/59

titration

	tubes	6/3	6/4	6/5	6/6	Passage June 5 th		
						ERK C102	6/9	6/10 6/11
$10^{-4.8}$	X	2	3	4*	5			
	62	few	1-3			62	1-2	
	63	0-1	1-3			63	1-2	
	64	0-1	1-3			64	1-2	
	65	0	2-3			65	0-1	
$10^{-5.8}$	66	0	few			66	1-2	
	67	0	few			67	1-2	
	68	0	0			68	0	0 0
	69	few	0-1			69	0-1	
$10^{-6.8}$	70	0	0			70	0	0 0
	71	0	0			71	0	0 0
	72	0	0			72	0	0 0

Other seros. Echo 26, 27, 28.
Cox B6.

*
6/s. tubes disintegrating; impossible to detect CPE from degeneration

JUN 8 1959

ERK virus from R.S. of 2/10/59

To identify the virus using Baront serum.

In the test of 6/1/59 Baront serum diluted 1:10 inhibited the above virus

Tubes. ERK Series C102 of 6/3/59

Virus ERK P₃ of 5/27/59 diluted $10^{-3.5}$ estimated 200 TC₅₀.Serum Baront T. Serum 13645 Titre 1:50 or greater against 30 TC₅₀ of homologous virus in human kidney tissue

Virus	Serum	Tubes	6/9	6/10	6/11	6/12
		X	↓	2	3	4
$10^{-3.5}$ + equal vol. 1:10		1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
30 TC ₅₀						
	1:20	4	0	0	0	0
		5	0	0	0	0
		6	0	0	0	0
	1:40	7	0	0	0	0
		8	0	0	0	0
		9	0	0	0	0
	1:80	10	0	0	0-1	1-2
		11	0	few	0-2	1-2
		12	0	0-1	0-2	1-2
	1:160	13	0	few	0-2	1-2
		14	0	0-1	1-2	1-3
		15	few	0-1	1-2	1-3
	1:320	16	few	0-1	1-2	1-3
		17	few	0-1	1-2	1-3
		18	few	0-1	1-2	1-3
+ equal vol diluent						
	$10^{-3.8}$	19	few	0-1	1-2	1-3
		20	few	0-1	1-2	1-3
		21	few	0-1	1-2	1-3
	$10^{-4.8}$	22	0	0	few	0-1
		23	0	few	0-1	
$10^{-5.3}$ / 0.2 ml.		24	0	0	few	0-1

1:5-5

		Passage 6/16/59							C 103		
		6/10	6/11	6/12	6/13	6/15	6/16	X	6/17	6/18	6/19
		<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>7</u>	<u>8</u>		<u>1</u>	<u>3</u>	<u>3</u>
10 ^{5.8}	X										
	25	0	0	0	0	0?	?	7	0	0	0
	26	0	0	0	0	0?	?	8	0	0	0
	27	0	0	0	0	0?	?	9	0	0	0
10 ^{6.8}	28		0	0	0	0?	?	10	0	0	0
	29		0	0	0	0?	?	11	0	0	0
	30		0	0	0	0?	?	12	0	0	0

JUN 16 1959

ERK virus from R.S of 2/19/59

The ERK virus from the rectal swab was neutralized by Baron virus antiserum

Materials ERK, of R.S of 2/19/59 diluted $10^{-3.5}$

Serum Baron T serum 13645 diluted 1:20

tubes ERK C103 of 6/10/59 medium added 6/13/59 and changed before use to ERK maintenance medium

<u>Virus</u>	<u>Serum</u>	<u>tubes</u>	6/17	6/18	6/19	6/20
		X	1	2	3	4
	Baron	1	0	0	0	0
$10^{-3.5}$	+ equal vol 1:20	2	0	0	0	0
		3	0	0	0	0
	+ diluent	4	0	0	1-2	2-4
		5	0	few	1-2	2-4
		6	0	few	1-2.	2-4.