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EXPERIENCE WITH PRODUCTION AND CONTROL OF ATTENUATED POLIOVIRUS (SABIN STRAINS) IN HUMAN DIPLOID CELLS (*)

by

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Details of production and control of six lots of live attenuated poliovaccine, Sabin straine, in human diploid cell (WI - 38) are described. The yields of viruses in this cell system were satisfactory. contrary to primary monkey kidney cells which are frequently contaminated with simian or other viruses, WI - 38 cells are thus far free of extraneous agents.

The neurovirulence of all three types of Sabin viruses , developed in WI - 38 cells , and studied in central nervous systems of monkeys , was not found to be greater than that of attenuated poliomyelitis reference viruses received from the National Institute of Health in Hampstead , United Kingdom.

INTRODUCTION

In this report we shall describe the large - scale production of attenuated poliovaccine from three Sabin's strains in the human diploid cells (HDC) WI-38. The advantages of this cell system for use in production of viral vaccines were pointedout by Hayflick (1961).

Since then interest in HDC strains has been developed rapidly .

In the present paper we shall report production of the first six lots of poliovaccine in HDC. This was achieved to show the consistency of our routine production. once ready these lots were re-examined by two competent western control labora tories through WHO and were found satisfactory for human immunization.

(*) Reprinted from WHO Consultative Group on Oral Poliomyelitis vaccine (Sabin Strains) BLG/Polio/77.14, Geneva, 10-12 Oct. 1977.

MATERIALS AND METHODS

Cells

(a) **Human diploid cell cultures :** both WI-38 and MRC-5 cells were received at 10-th and 9th population doubling respectively , from the National Institute for Biololgical Standards and control, Hampstead, london. The serial propagation of cells and preparation of frozen stocks from 17 th - 18 th population doubling were made according to the method of Hayflick & Moorhead (1961) and further developed by Jacobs (1970). Stocks of cells were kept in the liquid phase of liquid nitrogen .

The build-up of 500 to 1000 cultures in Roux flasks at the 29th to 30th population doubling for each batch of production was made over a period of seven to eight weeks . The losses due to poor growth , breakage of flasks or contamination were low , not exceeding %5

To propagate cells , basal medium Eagle (BME) No . G13 (Grand Island Biological Co . ,Grand Island, New York , United States of America) , supplemented with single strength aminoacids and vitamins of BME , 10% unheated calf serum, 50 μg/ml streptomycin and 50 μg/ml neomycin was used, pH adjusted to 6.3 with gas phase carbon dioxide . Filtration was made through millipore 0 . 22 μ .

Blood of calves , less than 1 year old , was received from Teheran slaughter house and serum was separated as soon as possible in order to obtain sterile serum. After millipore (0 . 22 μ) filtration all sera were pretested for the support of the growth of WI - 38 cells through five successive subcultures . Three lots out of 20 lots of 50 litres were found not suitable and were rejected; 10% of cultures were kept uninoculated for two weeke as control cells. The maintenance medium (LEV) contained 0.5% lactalbumin hydrolysate, 0.1% glucose and 0.22% sodium bicarbonate supplemented with 25 μg/ml of neomycin and 90 ml of this medium was added to each Roux flask .

Chromosome monitoring

Chromosome studies were made at cell passage level 30 or 31 and a total of not less than 100 metaphase spreads were carefully examined for the presence of chromosome abnormalities .

A total of 300 divisions were also examined for the detection of both polyploidy and endoreduplication .

(b) Other cell cultures

Human amnion cells (Am 57) , MRC - 5,HDC , primary vervet monkey kindney cells , primary rabbit kidney cells and monkey kidney cell line (Vero) were also prepared in our laboratory by a standard cell culture technique.

VIRUSES

(a) Seed virus vaccine production

After obtaining authorization from professor A . B . Sabin and following his approval, Dr H . Itoh , Chief , Division of laboratories , Japan poliomyelitis Research Institute , generously supplied the following viruses .
Type 1 (SOM + J1 - B) , type 2 (SOM+J1-B) and type 3 (SO+J1-B).

Originally the japanese Institute had received on 26 February 1963 the three seed viruses from professor Sabin. the type 1 (LSc 2ab-KP 3 Which is SOM) and type 2 (P712 ch, 2 ab-KP 3 which is SOM) were treated in Tokyo with anti - SV40 serum and then the above - mentioned seed viruses were prepared . The type 3 (Leon 12 , a , b - KP3) also received from professor Sabin was passed directly into primary monkey kidney cells to prepare type 3 seed virus. type 1 and 2 seed viruses were at second passsage level (SO+2) and type 3 at first passage level (SO+1) .The first seed material received on 5 january 1973 was used in our laboratory as working seed without any further passage . Therefore all six lots of our vaccine were third passage level or SO+3 (Sabin & Boulger , 1972) .

The working seeds had the following titres expressed as log10 TCD 50/ml (mean of two tests) .Type 1 , 7 .72 , type 2 , 7 . 74 and type 3 , 7 . 73 .

(b) Reference attenuated viruses for neurovirulence tests

Dr Boulger of the National Institute for Biological Standards and Control Hampstead, London , was kind enough to supply the viruses necessary for the compartaive control of neurovirulence in monkeys. The titres expressed as log10 TCD 50/ml are the mean of four teste were for type 1 (SO+3 , 71/301) 7 . 60 , for type 2 (SO+3 , 71/302) 7 . 80 and for type 3 (SO+3 , 71/303) 8 . 63 .

(c) Reference viruses for in vitro controls

Dr Parkman of the Bureau of Biologics , food and Drug Administration , Bethesda, United states of America, kindly supplied the following viruses. The titres expressed as log 10 TCD 50/ml are the mean of four tests were for type 1 (TA 3) 5.80, for type 2 (TB3) 6.02 and for type 3 (TC3) 5.46.

The following virulent viruses were also received from Dr H . Itoh : type 1 (Mahoney) type 2 (MEF) and type 3 (saukett) .

LABORATORY ANIMALS

Rabbits , guinea - pigs and mice were obtained from our local production and embryonated eggs were supplied by the Department of Avian Diseases .

NEUROVIRULENCE TESTS

Vervet monkeys (cercopithecus aethiops) Weighing 1 . 5 - 3 . 6 Kg were imported from Chad. The monkeys were first subjected to a tuberculin test and were then kept in quarantine for eight weeks . Any mortality or illness occurring during quarantine was recorded and only healthy monkeys were used for the neurovirulence tests . The performance of the tests and evaluation of the results were mainly based on the methods developped by Boulger (1973).

TESTS OF CONTROL CELL CULTURES AND THEIR FLUIDS AND OTHER SAFETY TESTS .

These tests were done in embryonated eggs and in human , monkey and rabbit primary cell cultures according to the WHO Requirements for poliomyelitis Vaccine (oral) (**WHO Technical Report Series '** 1972) .

ABSENCE OF TUMOURIGENICITY OF HUMAN DIPLOID CELLS

Each of 10 suckling mice were inoculated subcutaneously with 0 . 2 ml of HDC containing at least 2 x 10^5 cells . On the day prior to this , on the test day , and on days 1 , 3 and 5 , mice were also inoculated with 0 . 25 ml of antimouse lymphocyte serum. A similar group of suckling mice were treated as controls in the same manner but with 0 . 2 ml of Hep - 2 cells containing 2 x 10 5 cells . All mice were examined on days 5 , 7 , 10 , 14 and 21 for evidence of tumour formation .

CONTROL OF VIRUS SUSPENSIONS

Tests for the detection of haemadsorbing viruses , extraneous agents and safety of virus suspensions were performed in different cell cultures or laboratory animals following the WHO Requirements for poliomyelitis Vaccine (oral) (1972) . Absence of bacteria , fungi and mycoplasma was confirmed by aerobic and anaerobic cultures at different temperatures according to these WHO Requirements for the Sterility of Biological Substances (1973) .

In vitro tests

The property of reproducing at the temperatures of 36°C and 40°C for types 1 and 2 and 36°C and 40. 3°C for type 3 (rct/40) in comparison with reference virus preparation was studied . The sensitivity of reproduction to different concentrations of sodium bicarbonate (d - marker) was also investigated. Both tests were performad by conventional methods according to the WHO Requirements .

RESULTS

Virus yield

As it is evident from Table 1 , WI - 38 cell is a suitable cell system for large - scale production of all three types of Sabin poliomyelitis viruses . With one exception the virus titres of 22 single harvests were between 7. 5-8 . 0 log 10 TCD 50/ml. The mean incubation period at 33°C was 72 hours.

Chromosome aberrations

The results of the chromosome studies are shown in Table 2 . It should be noted that the figures appearing in Table 2 under « polyploidy » refer to both polyploidy and endoreduplication, accounting for relatively higher levels in the cell lots Nos. 52-2,52-3,52-12 and 52-24 than those reported by Jacobs (1970) who has studied the MRC-5 cells in small scale propagation. The other chromosome abnormalities fall within the acceptable limits as stated in the latest standards of karyolong for human diploid cells by Moorhead et al . (1974) .

Absence of extraneous agents and safety of virus suspenstions

All conventional tests were performed with cell cultures, fluids, cell suspensions or virus suspensions, in several different cell cultures, embryonated eggs, suckling and adult mice , guinea - pigs or rabbit , remain negative showing freedom from extraneous agents in the WI - 38 cells and virus propagated in this cell system was also shown to be free from detectable extraneous agents .

Lack of tumourigenicity of HDC

In repeated experiments none of the mice in the test group showed evidence of progressive tumour formation while the majority of mice in the control group had persistent tumours .

Neurovirulence test

The results of neurovirulence tests for six lots of three types of polioviruses prepared in WI - 38 and their homotype reference are shown in Tables 3 - 8 .

The neurovirulence test of each virus type for test vaccine and its related reference virus was performed within three months by the same operator .

According to these data the neurovirulence of the three types of poliovirus produced in HDC was not greater than that for the homotype reference .

It is important to note that all three virus types used in the vaccine and the homotype references were from the same passage level .

DISCUSSION

As a new manufacturer of live poliomyelitis vaccine we decided to produce the vaccine in HDC in order to avoid difficulties that the manufacturers normally face with primary monkey cells.

In this paper the preliminary results of production of poliviruses, Sabin strains, in WI - 38 cells at stationary Roux flasks are presented. Six lots, each of about 100 litres were produced with an average titre of 7. 5-8, 0 1o g 10 TCD50 / ml. From the large - scale production point of view, these titres are satisfactory. In order to make the production more economic it was logic to find a way to increase the yields of viruses. In a recent study, station-ry cultures were compared with rolling cultures, and it was found that for the same cell surface an increase of 0. 3 to 0. 5 log **10** TCD 50 / ml may be obtained when rolling system is used.

The freedon from extraneous agents, which is the main problem of primary monkey kidney cells, is the attractive advantage in the use of WI-38 cells. Apart from some accidental bacterial or mould contamination, we have not detected any contaminant in repeated investigations. Bacteriophage, which was isolated as an extraneous agent in some commercial virus vaccines used in the United-States of America by Petricciani (1973), was absent in the six lots of live poliovaccine (Petricciani, personal communication).

It is well known that primary monkey kidney cultures are frequently contaminated with different viruses. Apart from great number of simian viruses which may be present in the monkey cells, dangerous viruses such as virus B which causes fatal encephalitis in man, or SV 40, a virus that may give rise to tumours, may be present in monkey cells. In a recent study we have noticed that 14 vervets primary monkey kidney cultures out of 20 cultures were contaminated by viruses, four cultures revealed haemadsorbing agents and only two cultures were apparently free from contaminants. Because of these difficulties there is an increasing trend towards the use of HDC.

Another point which should be mentioned here is that since the stock of WI - 38 cell will soon be exhausted, it was quite normal to search for a suitable cell candidate to replace this cell system. In accordance with Jacobs (1970) we have found that MRC-5 cells, a second HDC, actually used for live vaccine production is easier to propagate and poliovirus yields in this cell were similar to those obtained in WI - 38. The results of a comparative study on the production of poliovirus, Sabin Strains, using both WI - 38 and MRC - 5 cells in roller system will be published later.

It is also worth mentioning that many control tests of the safety of WI - 38 cells were performed in cultures taken at later cell passages but from the same cells as those used to make the virus harvests. By repeated experiment it was

found that these cells were free from contaminants and were not producing tumours in immunosuppressed mice .

The neurovirulence of the six lots of monovalent poliovaccine, Sabin strains, at passage level SO+3 was almost identical to that of the three MRC reference viruses which were also at passage SO+ 3 . The resulte available thus far , obtained in a field trial involving some 5000 children , show that the vaccine was safe and no side effects have been observed during the first 12 months of follow - up . In January , February and March 1977 , about two million children , three months to five years old, were immunized in large cities of Iran by three oral doses of trivalent poliovaccin , made of the above six lote of monovalent vaccines . Each dose (0 . 15 ml) contains 5 . 7 log 10 TCD 50 type 1 , 5 . 0 log 10 TCD 50 type 2 and 5 . 5 log 10 TCD 50 type 3 . The vaccine was stabilized either by succrose or $Mg\ cl_2$. No adverse effect has been reported so far .

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Table-1 Summary of Production of Live Oral Poliovirus(Sabin strains) in Human Diploid Cells(WI-38)

Polio Type	Bulk No.	Production Batch No.	No of Roux Flasks	Seed Dilution	Incubation Temp.(C°)	Incubation Period (Hours)	Volume of Harvest (Litres)	Titre(TCD/50)Log10 Pre-Filtration	Titre(TCD/50)Log10 Post-Filtration
1	51-T1/1	51-1	226	1:20000	33±0·5	64	23·5	7·84	7·81
		51-2	384	1:30000	〃	69	33·5	8·14	8·05
		51-3	301	1:30000	〃	70	17·35	8·09	8·04
		52-2	119	1:30000	〃	68	8·0	8·08	7·8
		52-7	336	1:30000	〃	70	26·0	7·9	7·84
	51-T1/2	52-3	148	1:30000	〃	72	12·5	7·75	7·65
		52-6	96	1:30000	〃	75	9·0	7·87	7·64
		52-11	377	1:40000	〃	72	37·25	7·54	7·54
		52-12	150	1:30000	〃	76	10·0	7·58	7·58
		52-13	394	1:40000	〃	78	29·4	7·65	7·65
2	52-T2/1	52-14	467	1:30000	〃	72	40	7·47	7·1
		52-15	408	1:30000	〃	78	36	7·95	7·4
		52-17	475	1:30000	〃	80	42	7·25	7·15
	52T2/2	52-18	428	1:30000	〃	72	37·5	7·28	7·22
		52-19	433	1:30000	〃	74	38·5	7·15	7·15
		52-20	471	1:30000	〃	78	41	7·25	7·15
		52-21	241	1:15000	〃	72	29·5	7·25	7·0
3	53-T3/1	52-22	483	1:30000	〃	68	45	7·7	7·45
		52-24	462	1:30000	〃	70	43	8·05	7·75
	53T3/2	52-23	433	1:40000	〃	78	41	7·93	7·8
		53-1	348	1:30000	〃	72	29	6·45	6·24
		53-2	484	1:30000	〃	70	45	7·9	7·65

Table 2 : WI-38 Cells Passaged at Levels 30-31

Passage Level	P 30																			P 31		
Lot No.	54-1	51-3	52-2	52-3	52-6	52-12	52-13	52-15	52-14	52-17	52-18	52-19	52-21	52-20	52-22	52-23	52-24	53-1	53-2	51-2	52-7	52-11
Total	106	107	138	120	117	110	110	106	105	107	107	107	105	104	110	103	106	105	105	107	104	131
Normal (Diploid No.)	97	101	116	99	103	91	99	80	98	101	92	98	104	101	107	98	105	103	105	10	94	116
Sub diploidy %	6	4	10	9	14	6	8	19	8	5	15	8	3	3	2	4	1	3	1	2	12	9
Hyper diploidy %	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0·09	1	–	–	–	–	–	–
Break %	2	3	5	5	8	1	3	3	2	4	4	3	2	2	1	–	–	4	2	4	2	–
Polyploidy %	3	2	12	12	–	13	3	7	8	5·7	4·3	4	5	3·8	4·4	3·4	3·8	4·3	3	4	4	3
Deletion %	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

TABLE - 3

Comparative Intrathalamic Tests of Type1 (Mean of Two Tests)

Lots 51-T1/1 and 51-T1/2 So + 3										Reference Virus (MRC-5) 71/301 So + 3									
TCID50 Inoculated (1ml) Log .10	No. Monkey injected/ No. Valid	Clinical Poliomyelitis				Histology				TCID 50 Inoculated (1ml) Log.10	No. Monkey injected/ No. Valid	Clinical Poliomyelitis				Histology			
						Severity *		Spread **								Severity		Spread	
		W*	P	I	C	Cellular Only	Neuronal + Cellular	Cellular Only	Neuronal Cellular			W	P	I	C	Cellular Only	Neuronal + Cellular	Cellular Only	Neuronal + Cellular
$10^{7.2}$	20/20	-	-	-	-	6***	-	2	0/5	$10^{7.2}$	20/20	-	-	-	-	7	-	0·5	-
$10^{6.2}$	20/20	-	-	-	-	3	-	0·5	-	$10^{6.2}$	20/20	-	-	-	-	4	-	0·5	-
Total	40/40	-	-	-	-	9	-	0·5	0·5	Total	40/40	-	-	-	-	11	-	1	-

* W=Weakness, P=Partial paralysis, I=Incomplete paralysis, C=Complete paralysis

* Severity=Lesions noted in the thalamus

** Spread= Lesions noted in brain and spinal cord

*** Number of Monkeys

TABLE 4-Comparative Intraspinal Tests of Type1 (Mean of Two Tests)

Lots 51-T1/1 and 51-T1/2 So + 3										Reference Virus (MRC-5) 71/301 So + 3									
TCID 50 Inoculated (0·1ml) Log 10	No Monkey injected/ No Valid	Clinical Poliomyelitis				Histology				TCID 50 Inoculated (0·1ml) Log	No. Monkey injected/ No. Valid	Clinical Poliomyelitis				Histology			
						Severity **		Spread ***								Severity		Spread	
		W*	P	I	C	Cellular Only	Neuronal + Cellular	Cellular Only	Neuronal + Cellular			W	P	I	C	Cellular Only	Neuronal + Cellular	Cellular Only	Neuronal + Cellular
$10^{6.3}$	12/12	0·5	-	-	-	3	3	3	0·5	$10^{6.3}$	12/12	-	0·5	-	-	3·5	2	2	-
$10^{5.3}$	12/19	0·5	0·5	0·5	-	2	2	2·5	1	$10^{5.3}$	12/11	-	0·5	1	-	3·5	0·5	2	-
$10^{4.3}$	12/10	-	-	0·5	-	2·5	1·5	2·5	-	$10^{4.3}$	12 11	-	-	1	-	2	0·5	0·5	-
$10^{3.3}$	12/11	-	-	-	-	1·5	1	0·5	-	$10^{3.3}$	12 10	-	-	-	-	2	-	1	-
$10^{2.3}$	12/12	-	-	-	-	0·5	-	-	-	$10^{2.3}$	12/11	-	-	-	-	1	-	-	-
Total	60/57	1	0·5	1	-	9·5	7·5	8·5	1·5	Total	60·55	-	1	2	-	12	3	3·5	-

* W=Weakness, P=Partial Paralysis, I=Incomplete Paralysis, C=Complete paralysis

** Severity= Lesions noted in lumbar enlargement of spinal cord.

*** Spread=Lesions noted in cervical enlargement of spinal cord and brain.

TABLE 5 - Comparative Intrathalamic Tests of Type 2 (Mean of Two Tests)

Lots 52-T2/1 and 52-T2/2 So. 3										Reference Virus (MRC-5 71/302) So. 3									
TCID 50 Inoculated (1ml) Log 10	No Monkey injected / No Valid	Clinical Poliomyelitis				Histology Severity **		Histology Spread ***		TCID 50 Inoculated (1 ml) Log 10	No Monkey injected / No Valid	Clinical Poliomyelitis				Histology Severity		Histology Spread	
		W *	P	I	C	Cellular Only	Neuronal Cellular	Cellular Only	Neuronal Cellular			W	P	I	C	Cellular Only	Neuronal Cellular	Cellular Only	Neuronal Cellular
$10^{7.1}$	20/20	0.5	0.5	-	-	5.5	-	1.5	1	$10^{7.1}$	20/20	-	-	-	-	4.5	-	3	0.5
$10^{6.1}$	20/20	-	-	-	-	5	-	1	0.5	$10^{6.1}$	20/18	-	-	-	-	2.5	-	1	-
Total	40/40	0.5	0.5	-	-	10.5	-	2.5	1.5	Total	40/38	-	-	-	-	7	-	4	0.5

* W=Weakness, P=Partial paralysis, I=Incomplete paralysis, C=Complete paralysis

** Severity= Lesions noted in the thalamus

*** Spread= Lesions noted in the brain and spinal cord

TABLE 6 - Comparative Intraspinal Tests of Type 2 (Mean of Two Tests)

Lots 52-T2/1 and 52-T2/2 So. 3										Reference Virus (MRC) 71/302 So. 3									
TCID 50 Inoculated (0.1ml) Log 10	No Monkey injected / No Valid	Clinical Poliomyelitis				Histology Severity **		Histology Spread ***		TCID 50 Inoculated (0.1 ml) Log 10	No Monkey injected / No Valid	Clinical Poliomyelitis				Histology Severity		Histology Spread	
		W *	P	I	C	Cellular Only	Neuronal Cellular	Cellular Only	Neuronal Cellular			W	P	I	C	Cellular Only	Neuronal Cellular	Cellular Only	Neuronal Cellular
$10^{6.3}$	12/12	0.5	1.5			1.5	4	1.5	3	$10^{6.2}$	12/11		0.5	1		2	3.5	2	3.5
$10^{5.3}$	12/12		0.5	1		0.5	2.5		1.5	$10^{5.2}$	12/12		0.5	0.5	0.5	2	2.5	1	2
$10^{4.3}$	12/11	-	2	-	-	2.5	2	0.5	1.5	$10^{4.2}$	12/12	-	0.5	-	-	-	1	0.5	0.5
$10^{3.3}$	12/11	-	-	-	-	-	2.5	1.5	0.5	$10^{3.2}$	12/12	0.5	-	0.5	-	-	0.5	1	-
$10^{2.3}$	12/11	-	-	-	-	1	0.5	1.5	-	$10^{2.2}$	12/11	-	-	-	-	-	-	-	-
Total	60/57	0.5	4	1	-	5.5	11.5	5	6.5	Total	60/58	0.5	1.5	2	0.5	4	7.5	4.5	6

* W=Weakness, P=Partial paralysis, I= Incomplete paralysis, C= Complete paralysis.

** Severity=Lesions noted in lumbar enlargement of spinal cord.

*** Spread=Lesions noted in cervical enlargement of spinal cord and brain

TABLE 7-Comparative Intrathalamic Tests of Type 3 (Mean of Two Tests)

Lots 53-T3/1 and 53-T3/2 So. 3										Reference Virus (MRC-5) 71/303 So. 3									
TCID 50 Inoculated (1ml) Log 10	No Monkey injected / No. Valid	Clinical Poliomyelitis				Histology Severity **		Spread ***		TCID 50 Inoculated (1ml) Log 10	No Monkey injected / No. Valid	Clinical Poliomyelitis				Histology Severity		Spread	
		W *	P	I	C	Cellular Only	Neuronal +Cellular	Cellular Only	Neuronal +Cellular			W	P	I	C	Cellular Only	Neuronal +Cellular	Cellular Only	Neuronal +Cellular
$10^{7.3}$	20/20	-	-	-	-	7	-	1·5	0·5	$10^{7.2}$	20/20	-	-	-	-	6	-	-	-
$10^{6.3}$	20/19	-	-	-	-	3·5	-	1	-	$10^{6.2}$	20/19	-	-	-	-	4·5	-	-	-
Total	40/39	-	-	-	-	10·5	-	2·5	0·5	Total	40/39	-	-	-	-	10·5	-	-	-

* W=Weakness, P= Partial paralysis I= Incomplete paralysis, C=Complete paralysis

** Severity= Lesions noted in the thalamus

*** Spread= Lesions noted in brain and spinal cord

TABLE 8-Comparative Intraspinal Tests of Type 3 (Mean of Two Tests)

Lots 53-T3/1 and 53-T3/2 So. 3										Reference Virus (MRC) 71/303 So. 3									
TCID 50 Inoculated (0·1ml) Log 10	No Monkey injected / No Valid	Clinical Poliomyelitis				Histology Severity **		Spread ***		TCID 50 Inoculated (0·1 ml) Log 10	No Monkey injected / No. Valid	Clinical Poliomyelitis				Histology Severity		Spread	
		W *	P	I	C	Cellular Only	Neuronal +Cellular	Cellular Only	Neuronal +Cellular			W	P	I	C	Cellular Only	Neuronal +Cellular	Cellular Only	Neuronal +Cellular
$10^{6.2}$	12/10	-	1	0.5	-	1.5	3.5	1	1	$10^{6.1}$	12/12	-	-	1	-	1.5	3.5	2	1
$10^{5.2}$	12/12	-	-	1.5	-	1	1.5	0.5	1.5	$10^{5.1}$	12/12	-	0.5	1	-	0.5	3.5	1.5	2
$10^{4.2}$	12/10	-	0.5	-	-	-	-	-	-	$10^{4.1}$	12/12	-	-	-	-	2	0.5	0.5	0.5
$10^{3.2}$	12/12	-	-	-	-	0.5	-	-	-	$10^{3.1}$	12/11	-	-	-	-	0.5	-	-	-
$10^{2.2}$	12/12	-	-	-	-	-	-	-	-	$10^{2.1}$	12/12	-	-	-	-	-	-	-	-
Total	60/59	-	1.5	2	-	3	5	1.5	2.5	Total	60/59	-	0.5	2	-	4.5	7.5	4	3.5

* W=Weakness, P=Partial paralysis, I=Incomplete paralysis, C=Complete paralysis.

** Severity= Lesions noted in lumbar enlargement of spinal cord.

*** Spread=Lesions noted in cervical enlargement of spinal cord and brain.