Detecting Poliovirus in Vaccine Lots Used in Idaho in 1955

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THE TWO LOTS of poliomyelitis vaccine associated with the outbreak of paralytic poliomyelitis among Idaho children in 1955 (*1*) were studied in monkeys to detect virus and compare the relative sensitivity of monkey and man. The method for isolation of poliovirus from monkeys consisted of direct injection of vaccine into those tissues of cortisone-treated animals which were known to support virus growth (*2*, *3*). Vaccine treated with merthiolate and that treated with merthiolate and versene were tested in order to determine the effect of these agents on residual virus.

The data reported in this paper show that the addition of merthiolate to vaccine increases the incidence of animals yielding poliovirus. The data also indicate that monkeys are approximately 500 times more susceptible than man to infection with this agent.

Methods

The monkeys were anesthetized with intravenous sodium pentobarbital before inoculation. Six milliliters of vaccine were injected into each monkey in approximately the following amounts: 0.5 ml. into each thalamic region, 0.6 ml. in the lumbar region of the spinal cord, 0.25 ml. in the region of each tonsil, 1.0 ml. in the brown fat of each axillary region, 0.25 ml. in the region of the inguinal nodes on each side, and 0.5 ml. in the muscles of each thigh.

A special 1¼-inch, 25-gauge needle was used for intraspinal inoculation. The tip was blocked and a small hole was drilled through the shaft about one-eighth inch from the occluded end. The needle was inserted approximately three-fourths inch into 1 of the first 3 lumbar interspaces so that the inoculum was directed into the long axis of the cord. If a general contraction of one leg occurred immediately after the beginning of injection, followed by a general seizure of both hind legs and the back muscles, the inoculation was considered successful; if such seizure did not occur, inoculation was attempted in one of the other interspaces. Some degree of paralysis, usually severe, always followed inoculation, but this did not interfere with the detection of subsequent paralysis caused by poliovirus infection.

All monkeys received large amounts of cortisone intramuscularly, usually 125 mg. at the time of inoculation, 50 mg. on the second, third, and fourth days after inoculation, and then 50 mg. every other day through the 14th day if the monkeys tolerated these amounts, which they usually did. Starting on the fourth day, blood for detection of virus was taken every other day through the 14th day.

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Monkeys with clinical evidence of involvement in all four limbs, either paralysis or weakness, were killed by exsanguination under intravenous sodium pentobarbital anesthesia. Monkeys showing no paralysis were sacrificed similarly between the 18th and 21st days after inoculation. From all monkeys, the tonsils, deep retropharyngeal lymph nodes, axillary brown fat, axillary, inguinal, and abdominal lymph nodes, and portions of the cervical and lumbar cord were removed with sterile instruments for attempted virus isolation. The central nervous system was subjected to thorough histological examination.

In the isolation technique, essentially the same as previously reported from the Rocky Mountain and other laboratories, monkey kidney cells were used in all attempted isolations, and, in most instances, KB carcinoma cells were used in parallel tests (*4*). The monkey kidney cells were incubated approximately 7 days to produce confluent monolayers of cells before inoculation, and the KB carcinoma cells, about 3 days to produce small islands of confluent cells. All cultures were made in 2-oz. prescription bottles containing 10 ml. of medium.

Ten percent suspensions of organs in tissue-culture medium were employed as inoculums. These varied in toxicity to the tissue-culture cells. The following amounts of inoculums were tolerated: blood serum, 1 ml.; tonsil suspension, 0.2 ml.; spinal cord suspension, 0.2 ml.; and lymph node suspension, 0.4 ml. When suspensions of axillary brown fat were tested, the tissue-culture medium was removed

Table 2. Isolations of type 2 poliovirus from various tissues of monkeys inoculated by multiple routes with lot 6039

Species and date of inoculation (1956)	Number of monkeys	Brown fat	Mesenteric nodes	Cord	Total monkeys with virus
Rhesus					
February 16	5	1	0	0	1
February 16 [1]	5	0	0	0	0
May 10	10	0	0	2	2
May 23	10	1	0	0	1
August 27	10	0	0	0	0
African green					
May 23	10	2	1	2	3
Cynomolgus					
August 27	7	1	0	1	2

[1] Diluted 5 times.

NOTE: No isolations were made from the blood, tonsils, or peripheral nodes.

Table 1. Isolations of type 1 poliovirus from various tissues of monkeys inoculated by multiple routes with lot 6039

Species and date of inoculation (1956)	Number of monkeys	Blood	Brown fat	Peripheral nodes	Tonsils	Cord	Total monkeys with virus
Rhesus							
February 2	5	0	0	0	0	1	1
February 16 [1]	5	1	1	0	0	0	1
May 10	10	0	1	0	0	1	2
May 23	10	2	3	0	0	3	3
August 27	10	3	1	0	0	2	4
African green							
May 23	10	0	0	0	0	0	0
Cynomolgus							
August 27	7	2	1	1	1	1	2

[1] Diluted 5 times.

NOTE: No isolations were made from mesenteric nodes.

Table 3. Isolation of type 3 poliovirus from various tissues of monkeys inoculated by multiple routes with lot 6039

Species and date of inoculation (1956)	Number of monkeys	Cord	Total monkeys with virus
Rhesus			
February 16	5	0	0
February 16 [1]	5	0	0
May 10	10	0	0
May 26	10	0	0
August 27	10	1	1
African Green			
May 23	10	0	0
Cynomolgus			
August 27	7	2	2

[1] Diluted 5 times.

NOTE: No isolations were made from non-nervous tissues.

from cells, replaced with about 3 percent brown-fat suspension, and incubated at 37° C. for 4 to 5 hours. After the cells had thus been exposed to the suspension of brown fat, the suspension was removed and replaced with tissue-culture medium.

Lot 6039 was examined first; it served to determine tissues of value in detecting virus. As shown in table 1, type 1 poliovirus was isolated from tissues of 11 of 40 rhesus monkeys (*Macaca mulatta*). Virus was detected in blood, axillary brown fat, and spinal cord. Since no single tissue yielded virus consistently, all three had to be examined for maximum sensitivity in detecting type 1 virus.

Ten African green monkeys (*Cercopithecus aethiops*) were used on one occasion. Type 1 virus was not isolated although it was detected in 3 of 10 rhesus monkeys inoculated the same day. On one of the occasions when 7 cynomolgus monkeys (*Macaca philippinensis*) were used, type 1 virus was isolated from the blood, brown fat, lymphoid tissue, and spinal cord of 1 and from the blood of another.

Type 2 virus was found in the brown fat of 2 of the 40 rhesus monkeys previously mentioned and in the spinal cord of 2 others (table 2). Type 2 virus was also isolated from the spinal cord, brown fat, and lymphoid tissue of 3 of 10 green monkeys. Of the 7 cynomolgus monkeys, 1 had type 2 poliovirus in brown fat and another in the spinal cord.

Type 3 poliovirus was isolated only from the spinal cord of 1 of the 40 rhesus monkeys and not from any other tissue (table 3). It was not isolated from the green monkeys but was isolated from the spinal cords of 2 of the 7 cynomolgus monkeys.

All cultures were observed for at least 7 days after inoculation for cytopathogenic changes in the cells and passaged once on the original host cell regardless of findings in primary culture.

Antibody response was measured by a metabolic inhibition type of neutralization test similar to one described by Salk and associates (*5*). Trypsinized monkey kidney cells were prepared by a method closely resembling that of Rappaport (*6*). The highest point of serum dilution which sufficiently protected monkey kidney cells against 100 to 300 $TCID_{50}$ of virus to allow the pH to decrease to 7 or below was considered the antibody titer of that

Table 4. Distribution of type 1 poliovirus in monkeys inoculated with vaccine lot 6058 treated with merthiolate or merthiolate plus versene

Vaccine	Blood	Brown fat	Tonsils	Peripheral nodes	Mesenteric nodes	Total, other than nervous tissue	Cord
Merthiolate treated	3/11	4/11	2/11	1/11	0/11	10	3/11
Untreated	0/10	1/10	0/10	0/10	0/10	1	0/10
Merthiolate plus versene treated	1/9	1/9	0/9	3/9	1/9	6	0/9
Untreated	1/10	1/10	0/10	0/10	0/10	2	1/10

NOTE: In fractions, the denominator shows the number of monkeys inoculated, the numerator, the number with type 1 poliovirus.

Table 5. Distribution of type 2 and type 3 poliovirus in monkeys inoculated with vaccine lot 6058 treated with merthiolate or merthiolate plus versene

Vaccine	Total isolations from non-nervous tissue		Cord	
	Type 2	Type 3	Type 2	Type 3
Merthiolate treated	0	0	1/11	0/11
Untreated	0	0	1/10	0/10
Merthiolate plus versene treated	0	0	0/9	1/9
Untreated	0	0	0/10	1/10

NOTE: In fractions, the denominator shows the number of monkeys inoculated, and the numerator, the number having the designated poliovirus.

serum. Tests of serums collected early in the study were performed in glass tubes; those collected later were tested in cups of disposable plastic panels sterilized by ultraviolet irradiation (7). Nutrient fluid consisted of Hanks' balanced salt solution containing 0.5 percent lactalbumin hydrolysate and 2 percent horse serum. The mixture of serum, virus, cells, and nutrient fluid in each cup of the panel was overlayed with an equal quantity of mineral oil. Each panel was then covered with a sheet of sterile aluminum foil and incubated at 36° C. for 10 to 12 days as determined by behavior of the controls.

Results

Paralytic poliomyelitis from inoculation of the vaccines was limited virtually to monkeys that had a convulsive seizure at the time of inoculation. Of the 20 monkeys that had no seizure, 1, or 5 percent, had typical clinical and microscopic findings compared with 21, or 40 percent, of the 52 with convulsive seizures.

On 4 occasions, 2 different types of virus were isolated from a single rhesus monkey. Twice type 1 virus was isolated from the spinal cord in the same instances that type 2 was isolated from brown fat. Once type 2 virus was isolated from the spinal cord and type 1 from brown fat. In the final instance type 3 virus was isolated from the spinal cord and type 2 from brown fat of a cynomolgus monkey.

Neuropathological findings in two African green monkeys were typical of acute poliomyelitis although virus was not isolated from the spinal cord. In one monkey, paralysis appeared first in the legs and then extended to the arms. The resulting clinical picture was considered typical of poliomyelitis. In the other monkey, general weakness and coarse tremors were observed, but definite paralysis did not occur. In this instance type 2 virus was isolated from brown fat and mesenteric lymph nodes. The interpretation of these lesions is difficult. It may be, however, that since the distribution and character of lesions were typical of poliomyelitis, a true poliovirus infection of the central nervous system can occur in this species although virus cannot be isolated by tissue culture methods.

Effect of Merthiolate in Vaccine

Merthiolate was added to lot 6058 to determine whether it would inactivate residual poliovirus, and this treated preparation was tested only in rhesus monkeys. One aliquot of vaccine was treated with merthiolate to a final concentration of 1:10,000 and stored at 4° C. for 2 months. Another aliquot of vaccine was similarly treated with merthiolate to a final concentration of 1:10,000, and, in addition, versene was added to a final concentration of 7:10,000 in an attempt to prevent the merthiolate action on the virus. This aliquot was also stored at 4° C. for 2 months.

Table 6. Antibody response of monkeys 21 days after inoculation of vaccine lot 6058 treated with merthiolate or merthiolate plus versene

Vaccine	Type 1 response	Type 2 response	Type 3 response
Merthiolate treated	[1] 1/6	1/6	1/6
Untreated	1/10	10/10	1/10
Merthiolate plus versene treated	[1] 1/8	5/8	2/8
Untreated	1/10	10/10	3/10

[1] Monkeys who became paralyzed were generally sacrificed before the appearance of antibodies and are not included in this table.

NOTE: In fractions, the denominator shows the number of monkeys inoculated, the numerator, the number with the designated response.

The merthiolate-treated portion of the vaccine was inoculated into 11 monkeys and an untreated portion into 10 control monkeys. The following day 9 monkeys were inoculated with vaccine treated with merthiolate plus versene, and again, 10 controls were inoculated with untreated vaccine.

Type 1 virus was isolated from 4 of the 11 monkeys that had received vaccine treated with merthiolate (table 4); 10 isolations were made from peripheral tissue and 3 from the spinal cord. In contrast, from the 10 control monkeys, type 1 virus was isolated only once, from brown fat. Also, in monkeys that received vaccine treated with merthiolate plus versene, type 1 virus was isolated from 4 of 9 monkeys; 6 isolations were made from non-nervous tissue and none from nervous tissue. In the control group, type 1 virus was isolated from 3 monkeys, twice from peripheral non-nervous tissue and once from spinal cord. With respect to the presence of type 2 and type 3 polioviruses, all groups were essentially the same (table 5).

Among the monkeys inoculated with lot 6058, two types of virus were isolated from the same monkey on three occasions. Type 3 was isolated from the spinal cord of two monkeys, while type 1 was isolated from brown fat of one, and type 2 from the brown fat of the other. Type 2 was isolated from the spinal cord and type 1 from brown fat of one monkey.

The antibody responses of the four groups of monkeys receiving vaccine lot 6058 possibly explain the effect of merthiolate in aiding the detection of type 1 poliovirus. Inoculation of lot 6039 in rhesus monkeys uniformly produced type 2 antibodies usually in a titer of 1:64 or higher; type 1 or 3 antibodies seldom appeared. With untreated vaccine of lot 6058, a similar antibody response was noted in the two groups of monkeys, but the group receiving merthiolate-treated vaccine had no type 2 antibody response (tables 6 and 7), and the group receiving vaccine treated with merthiolate plus versene had a poor type 2 antibody response. Apparently, merthiolate destroyed the antigenicity of type 2 virus and removed some component which interfered with proliferation of type 1 virus in non-neural tissues.

Table 8. Incidence of paralytic disease following inoculation of lot 6039, among animals with a successful intraspinal inoculation

Species and inoculation date	Number of monkeys	Number with typical clinical and microscopic findings	Virus type isolated from cord
May 23, 1956			
African green	6	3	Type 2 from 2.
Rhesus	4	3	Type 1 from 3.
August 27, 1956			
Cynomolgus [1]	5	3	Type 2 from 1. Type 3 from 2.
Rhesus [2]	5	3	Type 1 from 2. Type 3 from 1.

[1] Type 1 virus was also isolated from brown fat of 1 of the paralyzed monkeys and from the blood of a monkey with no clinical or microscopic findings.
[2] Type 1 virus was also isolated from the blood of a monkey with no clinical or microscopic findings.

Estimate of Paralytic Dose

Inoculation of monkeys with poliomyelitis vaccine to determine its safety for man calls

Table 7. Degree of type 2 antibody response in monkeys receiving vaccine lot 6058 treated with merthiolate or merthiolate plus versene

Vaccine	Serum dilutions							
	0	8	16	32	64	128	256	512
Merthiolate treated	6							
Untreated			[1] 1	1	1	2	4	1
Merthiolate plus versene treated	2	2	1	1		2		
Untreated			[2] 1	3	1	2	1	2

[1] Autopsy 13th day after inoculation.
[2] Autopsy 6th day after inoculation.

for a measure of comparison of the relative sensitivity of monkey and man to residual live virus in a vaccine. The inoculation of lots 6058 and 6039 into 32,000 children in the age group 6 to 8 years resulted in paralytic poliomyelitis in 17 of them (*1*). No extensive antibody surveys were made in Idaho prior to 1955, so the exact proportion of children susceptible to type 1 poliomyelitis is not known. However, in connection with the 1954 poliomyelitis vaccine evaluation study, serums were obtained from children living in the more densely settled areas of Idaho and, of these, 50 percent had type 1 antibodies. These data indicate a paralytic attack rate of approximately 1 in 1,000 from 1 ml. of vaccine inoculated intramuscularly.

To attempt to get similar data regarding monkeys, the incidence of paralysis was recorded only on the basis of monkeys receiving a successful intraspinal inoculation. Although a total of 6.0 ml. of vaccine was given to each monkey, only the 0.5 ml. given intraspinally can be considered effective in producing paralysis. With lot 6039 the paralytic rates approach 50 percent for all virus types (table 8). With lot 6058 the rates are approximately one-third that of lot 6039 (table 9), but the results may have been influenced by the fact that lot 6058 was examined 6 to 8 months after studies of lot 6039 had been completed.

Table 9. Incidence of paralytic disease following inoculation of lot 6058, among animals with a successful intraspinal inoculation

Vaccine and inoculation date	Number of monkeys (rhesus)	Number with typical clinical and microscopic findings	Virus type isolated from the cord
February 26, 1956			
Merthiolate treated	11	4	Type 1 from 3. Type 2 from 1.
Untreated	8	1	Type 2.
February 27, 1957			
Merthiolate plus versene treated	5	1	Type 3.
Untreated	7	2	Type 1 from 1. Type 3 from 1.

With both lots of vaccine, the incidence of paralysis was so much greater in monkeys than in children that a vaccine producing no paralysis in monkeys would be unlikely to have deleterious effect on children. If, along with absence of paralysis, no virus can be detected in blood, brown fat, or lymphoid tissue of monkeys, any chance of harmful effects on children appears remote.

Summary

The poliomyelitis vaccine, in two lots, which caused paralytic poliomyelitis in Idaho children in 1955 was studied in monkeys with the objective of detecting virus and comparing the relative susceptibility of children, as determined by observations in the field, and of monkeys, as determined by laboratory experiences.

A method of intraspinal inoculation which assured that the inoculum was actually placed in the spinal cord and inoculations of vaccine directly into lymphoid tissue and brown fat proved to be a most sensitive test for detecting virus in monkeys. Spinal cord, blood, lymphoid tissue, and brown fat all had to be examined for virus. The addition of merthiolate in a concentration of 1: 10,000 to the vaccine aided in the isolation of type 1 poliovirus apparently by removing an interfering effect of type 2 virus.

With the methods of inoculation used, monkeys treated with large amounts of cortisone were at least 500 times more sensitive to the effects of the vaccine than children. Therefore, if virus cannot be detected in monkeys, ill effects in children appear very unlikely.

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Social Security Administration Seeks Eligibles

The Bureau of Old-Age and Survivors Insurance is making an intensive effort to find 400,000 people who may be eligible for payments under the 1958 amendments to the social security law and who must file their claims before benefits can start.

Among the more difficult to find are the estimated 60,000 parents who have survived their children and who were dependent upon them for support. Parents of deceased, insured workers are entitled to benefits even if a surviving spouse or child also gets payments.

Some others sought by the Social Security Administration's bureau are disabled workers 50–65 years of age who failed to qualify for benefits formerly because they did not meet work requirements in effect at that time; dependents of disability insurance beneficiaries; and disabled workers under 50 years of age who may have their earning records frozen to protect their rights to future benefits.

Under the law, a person is considered disabled if he is unable to do "any substantial gainful work," and his condition is expected to continue indefinitely. A person who applies for disability insurance benefits or to have his social security record frozen is requested to obtain a statement on his physical condition from his physician. The physician's clinical findings, along with the applicant's education, experience, and other pertinent factors, are studied by a team of trained people, including a physician, to determine whether the person is disabled and whether he can be rehabilitated. Members of State vocational rehabilitational agencies consider the possibility at that time of providing the disabled person with rehabilitative services.

Social security advisers in district offices will provide detailed information to those interested in filing applications.