SEMIANNUAL REPORT TO THE NATIONAL FOUNDATION
FOR INFANTILE PARALYSIS

Period of July 1, 1955 - December 31, 1955

FROM: THE CHILDREN'S HOSPITAL RESEARCH FOUNDATION,
UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE

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A. SUMMARY OF SIGNIFICANT NEW FINDINGS DURING PAST 6 MONTHS

In the search for polioviruses possessing minimal or no "spinal activity" in monkeys, 19 additional strains from healthy children were screened, and the behavior of many naturally occurring strains in the relatively refractory Capucin monkey kidney and chick-embryo tissue cultures was studied. The screening of strains from healthy children yielded two Type 1 strains (P2149 and P2226) and one Type 2 strain (P712) with the lowest spinal activity in monkeys of any strain previously tested, except the LSc variant of the Mahoney virus... These newly isolated naturally occurring Types 1 and 2 strains were the ones tested in human volunteers.

Of 3 Type 1, 5 Type 2, and 5 Type 3 naturally occurring strains, tested as first passage cynomolgus monkey kidney culture, only the Type 1 strains multiplied in Capucin monkey kidney tissue culture, but the progeny of two strains resulting from 10 serial passages in Capucin cultures exhibited the same degree of spinal activity in monkeys as the parent virus. None of the strains tested in chick embryo tissue cultures, devoid of interfering Newcastle Disease virus, yielded any evidence of multiplication and accordingly there was nothing to test in monkeys.

To help determine whether monkey-intracerebrally avirulent poliovirus can be the cause of paralytic disease in man, suspensions of the spinal cord and medulla from fatal cases were cultured in cynomolgus monkey kidney cultures and the first passage culture fluid was tested in different concentrations in intracerebrally inoculated monkeys. Five Type 1 and one Type 3 strain tested thus far were all paralytogenic in the large (10^6 to 10^7·7) as well as smaller (10^3 to 10^4·7) doses.
To determine whether paralytogenic activity following subcutaneous inoculation in cynomolgus monkeys can be correlated either with the origin of strains (i.e. from the CNS of fatal cases or from the stools of healthy children in epidemiologically quiescent regions) or with their neurotropic activity as measured by intracerebral inoculation in monkeys, each of 15 strains was tested in 10 cynomolgus inoculated subcutaneously with $10^6 TCD_{50}$. While the 3 intracerebrally avirulent strains all failed to produce paralysis, the incidence of paralysis with intracerebrally virulent strains derived either from fatal cases or from healthy children was often so low, that paralytogenic activity following subcutaneous inoculation in monkeys could not be used as an indicator of virulence.

Five additional attenuated strains were tested intraspinally in 15 chimpanzees in doses of $10^6.3$ to $10^7.5 TCD_{50}$ without producing paralysis in any. This brought the total number of chimpanzees that had been inoculated intraspinally with various attenuated strains or their derivatives to 38 without paralysis in any of them. On the other hand, 6 chimpanzees similarly inoculated with 2 strains derived from fatal human cases, 5 exhibited clinical paralysis (fatal in two) and extensive lesions were present in the sixth.

Further studies on the characteristics of the LSc virus after propagation in the alimentary tract of chimpanzees revealed no change in neurotropism even in spinally inoculated monkeys.

No virus was found in the spinal cord or medulla of 3 chimpanzees, which happened to die of intercurrent disease, either just before the expected time of antibody formation or shortly thereafter, after ingestion of chimpanzee-avirulent virus. However, in one chimpanzee small amounts of virus were found in the superior cervical sympathetic, a pool of thoracic spinal and sympathetic, and in the Gasserian ganglion, while in another only the superior cervical sympathetic ganglia were positive. These data suggest that polioviruses may spread by neural pathways from the alimentary tract. Furthermore quantitative tests for virus in the pharyngeal and intestinal mucosa as well as in the regional lymph nodes showed approximately similar amounts in both. This observation as well as the continued excretion of virus long after antibody has formed are interpreted as indicating that the lymph nodes are not, as has been suggested, the sole site for viral multiplication after ingestion of virus. Indeed, there is really no evidence that they act more than virus filters, for the concentration in the regional lymph nodes seems to be proportional to the amount found in the mucosa. On the other hand, there is no escaping the conclusion that the virus multiplies in the alimentary mucosa.

On September 13 and October 4, additional tests were carried out on 48 volunteers with three type 1 strains (LSc, "P2149" and "P2226"), one Type 2 strain (P712), one Type 3 strain (Glenn), and several combinations ("P2149" + Glenn, P2149 + P712, and P2149 + P712 + Glenn). The following observations may be especially worthy of note:
a) Of the 4 Type 1 strains tested in human volunteers, the LSc produced antibody of lower titer than the others, but also was the only strain which yielded no evidence of the appearance of particles with increased neutropism for the monkey among the progeny in the alimentary tract. While the amount of virus found in the throat was the same for the LSc as for the other strains, the peak titers in the stools were usually more than tenfold less than in the others. Only with strain "P2226" were traces of virus found in the blood (infective dose per 2 ml) of a few volunteers on a single day (the 3rd) after ingestion of single strains. With "P2149" there was no viremia in any of 9 volunteers who ingested it alone (6 men infected) or mixed with "Glenn" virus (3 men infected), but small amounts were found in 3 of 5 men who ingested "P2149" mixed with the Type 2 strain - "P712".

b) The naturally-occurring Type 2 "P712" strain was tested in amounts ranging from $10^6.4$ to $10^{2.1}$ TCD$_{50}$ (0.1 to 0.000,005 ml of culture fluid). Infection and antibody development occurred in all who received $10^{4.4}$ TCD$_{50}$ or more but was irregular with $10^{3.1}$ TCD$_{50}$ or less - only 1 of 3 on $10^{3.1}$ TCD$_{50}$ and 2 of 3 on $10^{2.1}$ TCD$_{50}$ became infected. Traces of virus (one infective dose per 2 ml) were found in the blood of 3 of the 10 infected individuals on one day (3rd or 5th) after ingestion of the smallest or largest dose of virus. No sore throats or other symptoms could be attributed to infection with this strain. In most individuals antibody appeared on the 7th day and relatively high titers were attained. Intracerebral inoculation of suspensions of the original stools in no instance produced paralysis in monkeys. However, among 57 monkeys inoculated intracerebrally with $10^5$ to $10^5.7$ TCD$_{50}$ of the culture fluids derived from the stools obtained at various times after feeding, there were 6 monkeys with slight paralysis on whom progeny tests are now in progress.

c) The naturally occurring Type 3 Glenn virus did not appear to behave differently from the laboratory-developed Leon KP34. However, while the Leon KP34 virus was suppressed when fed in mixtures with Types 1 and 2 viruses to chimpanzees or when it was administered simultaneously with the other types intramuscularly to monkeys, the "Glenn" virus was not suppressed when it was fed in mixtures with Type 1, Type 2 or Types 1 + 2 to human volunteers.

d) The comparative data obtained on the various strains in tests on monkeys, chimpanzees and human volunteers, indicate that of the known attenuated viruses the LSc, P712, and Leon KP34 strains possess the optimum complement of properties for further progressive investigations on immunization of human beings.
The studies on the nonpoliomyelitis viruses recovered from healthy children in Mexico indicate that the 13 known antigenic types of ECHO viruses and the known cytopathogenic Coxsackie viruses account for not more than 40 per cent of the 261 strains. Thus, the studies on antigenic classification must continue.

Preliminary observations on the possible role of cytopathogenic viruses in the undifferentiated diarrheal syndromes which are encountered in infants and young children during the summer indicated that 43 per cent of rectal swabs from 56 patients under 4 years of age yielded viruses in cynomolgus kidney tissue culture. In addition to encountering such well-established agents as Type 1 poliovirus, a few Coxsackie viruses and a few recognized ECHO viruses, there were at least 12 strains which could not be identified with pools of sera against the 13 known ECHO viruses and the known cytopathogenic Coxsackie viruses.

Among the ancillary studies and incidental findings the following deserve special mention:

1. Quantitative studies on material obtained from the nose, mouth, throat and feces of patients with paralytic and nonparalytic poliomyelitis indicated that as in volunteers who ingested attenuated strains the mouth (and here also the nose) are not significant sources of virus; nor were the amounts of virus per throat swab or per gram of stool significantly different in the patients and in the volunteers. However, the presence of virus in the throat of 75 per cent of the patients suggested that either they were infected by very large doses of virus ($10^6 \text{TCD}_{50}$ or more is required to achieve this incidence in volunteers with attenuated strains) or that, unlike the situation obtaining in the volunteers, viremia in the patients may result in secondary localization in the pharynx.

2. 80 per cent of patients (12/15) developing a homotypic antibody rise did so by 7 days after hospitalization or 9 to 14 days after onset of first symptoms. While this study indicated that convalescent phase sera may be advantageously tested at 1 week after hospitalization, it was also apparent that virus isolation provided a more practical diagnostic procedure than determination of neutralizing antibodies.

3. During the early stages of antibody development as in early convalescent phase sera or in volunteers after ingestion of virus, the antibody titers obtained by the pH test have in many instances proved to be higher than by the cytopathogenic test - sometimes as much as 64 times higher.

4. Further studies with the chimpanzee rhinitis virus have shown that it is an ether resistant virus, 60 to 90 $\mu$ in size, which multiplies in the nasal mucosa of nonimmune chimpanzees but may be detected in
higher concentrations in the stools than in the nasal secretions. Clinical rhinitis has been reproduced for a third time with multiple passaged tissue culture virus in a group of 3 nonimmune chimpanzees and the resistance of another group of 3 chimpanzees that had passed through two previous episodes was established in the same experiment. Sera obtained from infants were devoid of antibody for this virus while older children and young adults exhibited an increasing incidence of positives to about 90 per cent. Further studies with the virus recovered from the outbreak of "steatorrheic enteritis" indicate that it is almost indistinguishable from the chimpanzee rhinitis virus.

B. SEARCH FOR POLIO VIRUS STRAINS POSSESSING MINIMAL OR NO "SPINAL ACTIVITY" IN MONKEYS.

1. Additional Strains from Healthy Children. -- By the middle of 1955, 30 strains from healthy children had been screened for neurotropic activity in monkeys, but none were found among them with less neurotropic activity than the attenuated viruses which were obtained by selective procedures in the laboratory. Accordingly, 19 additional strains (5 Type 1, 5 Type 2, and 9 Type 3) which were derived from the stools of healthy children in the region of New Orleans, studied by Doctors Fox and Gelfand, were submitted to the screening process for neurotropic activity. Two of the Type 1 strains, ("P2149" and "P2226") turned out to have the least neurotropic activity in monkeys with the exception of the LSc variant of the Mahoney virus. Among the Type 2 strains there were 3 that were intracerebrally negative in monkeys and one of these strains (P 712) produced no paralysis on spinal inoculation in monkeys in doses of $10^{5.7}$ TCD$_{50}$ or less, and only minimal paralysis with doses exceeding $10^{6}$ TCD$_{50}$. Among the 9 type 3 strains which were all of relatively low intracerebral virulence in monkeys, none was found to possess less neurotropic activity in spinally inoculated monkeys than the Leon KP 34 strain developed by selective procedures in the laboratory. These additional screening tests, therefore, provided 3 suitable naturally occurring attenuated viruses for the tests in human volunteers to be described in a subsequent section.

2. Strains Propagated in Capucin Monkey Kidney Tissue

Cultures. -- Since Capucin monkey kidney tissue cultures had been reported to be refractory or relatively refractory to the effect of poliomyelitis viruses, it appeared desirable to determine whether among the very large populations of virus particles contained in undiluted inocula of naturally occurring strains one might find small numbers of mutants which might readily be propagated with the
production of a cytopathogenic effect in the Capucin kidney tissue cultures. The intention was to determine whether virus particles which might carry the special genetic determinants for cytopathogenicity in Capucin kidney cells might differ in their neurotropic characteristics for monkeys from the parent strain. Accordingly, 4 Type 1 (P 1553, P 2149, P 2226 and Cleveland "80-4"), 5 Type 2 strains (P 712, P 1818, P 2043, P 720, P 858) and 5 Type 3 strains (P 1850, P 1743, P 1023, P 1749 and Mexico "U.S.V-18") were all tested as first passage cynomolgus kidney culture fluid. None of the Type 2 or Type 3 strains showed any evidence of multiplication in the Capucin monkey kidney cultures. Three of the 4 Type 1 strains multiplied in Capucin monkey kidney tissue cultures with the production of cytopathogenic manifestations which varied in extent in cultures derived from different Capucin monkeys. Tests are still in progress to determine the extent to which the capacity for producing a cytopathogenic change in Capucin kidney cultures may be a property of only a few particles of virus or of almost all. Evidence was obtained that in the epithelial cells derived from the kidneys of some of the Capucin monkeys virus may multiply without producing a cytopathogenic effect, while in similar cultures from other monkeys a cytopathogenic effect would occur even though the level of virus multiplication was not significantly different from that in the cells which remained intact. Two of the Type 1 strains which had been submitted to 10 serial passages in Capucin kidney cultures exhibited the same degree of spinal activity in monkeys as the parent viruses. The one Type 1 strain (Cleveland 80-4) which failed to multiply in the Capucin monkey kidney cells was actually not tested as the first passage cynomolgus kidney culture fluid, but had been submitted to 3 passages in HeLa cells prior to receipt in this laboratory. Whether or not passage in HeLa cells by itself may select against the particles possessing genetic determinants for multiplication in Capucin kidney cells, or whether there are some Type 1 strains which in nature are devoid of this activity, is not presently clear. That 3 passages in HeLa cells do not invariably remove Capucin-active particles is evident from the fact that the "P 1553" strain did not lose this capacity despite having been passaged 3 times in HeLa cells. At any rate, the fact that all 10 Type 2 and Type 3 strains failed to multiply in the Capucin kidney cells indicates that the genetic determinants for multiplication and cytogenitcity in cynomolgus monkey kidney cells are distinct from those for Capucin monkey kidney cells.

3. Attempted Propagation of Naturally Occurring Strains in Chick Embryo Tissue Culture. -- Previous tests with the attenuated strains of the Mahoney, YSK and Leon viruses experimentally selected in the laboratory provided no evidence that demonstrable multiplication occurred in chick embryo tissue cultures devoid of interfering Newcastle disease virus. It appeared possible, however, that only
a very small number of virus particles may possess the capacity for propagation in chick embryo tissue cultures, and that the chances of picking up such virus particles might be greatly enhanced if very large inocula of freshly isolated naturally occurring strains were used. Accordingly, such tests were carried out with 8 Type 1 strains, 3 Type 2 strains, and 4 Type 3 strains. No evidence of interfering Newcastle disease virus was present in the chick embryo cultures that were used, but none yielded any evidence of multiplication with any of the polio strains. All this work was done, not so much to find polio strains which would multiply in chick embryo, but more as a means to determine whether chick embryo tissue cultures might select against virus particles possessing the genetic determinants for neurotropic activity in monkeys.

C. NEUROTROPIC ACTIVITY IN MONKEYS AND CHIMPANZEEs OF STRAINS FROM SPINAL CORD OF FATAL CASES OF HUMAN POLIOMYELITIS

In order to determine the neurotropic activity in monkeys as well as certain other characteristics of polio viruses which produce paralytic disease in man, it appeared desirable to recover virus from the spinal cord and medulla of a certain number of fatal cases by inoculation of monkey kidney tissue cultures. This would provide material of comparable passage history to that obtained from the stools of healthy children. Virus recovered from the nervous system appeared to be preferable to that obtained from the stools of paralytic cases, because many cycles of cultivation in the alimentary tract might modify the character of the original viral population. Accordingly, 10 strains of virus were recovered from fatal cases during the severe 1955 Boston epidemic, 2 strains were obtained from fatal cases in Cleveland, 2 from Cincinnati and one from New York. All 15 strains recovered from these 1955 fatal cases proved to be Type 1. We also had available the spinal cord and medulla from a fatal case in Cincinnati in 1954, and this proved to be a Type 3. In all 16 instances the results indicated that the human nervous system contained virus that is cytopathogenic for cynomolgus kidney epithelial cells. However, in one instance (the Type 1 case from New York City) a definite zone phenomenon occurred in which the 20 per cent suspension of the spinal cord and medulla failed on 2 occasions to exhibit a cytopathogenic effect, while the 2% suspension yielded on each of 2 separate occasions a cytopathogenic effect in 1 out of 3, or 1 out of 4 tubes, but none in a large number of tubes seeded with higher dilutions. The virus recovered in each of these 2 tubes proved to be Type 1 polio. The results suggest that the human nervous tissue contained non-cytopathogenic virus which interfered with the small amount of cytopathogenic virus that was present. Tests are now in progress to determine whether this non-cytopathogenic virus is indeed active and whether it is strictly neurotropic in the monkey.

Screening tests for intracerebral virulence in monkeys have thus far been carried out with 5 of the Type 1 strains (3 from Boston and 2 from Cleveland) and with the 1 Type 3 strain recovered in Cincinnati in 1954. All these strains proved to be paralytogenic in the large ($10^6$ to $10^7$) as well as the smaller
(10^3 to 10^4.7) doses. Two of the Type 1 strains, one from Boston and one from Cleveland, were inoculated intraspinally in doses of 10^6.3 to 10^6.8 in chimpanzees to determine their paralytogenic activity in this species. The 3 chimpanzees which received the Boston strain all developed extensive paralytic polio which proved to be fatal in two. Of the 3 chimpanzees that received the Cleveland strain clinical manifestations were definite in 2 and uncertain in the third, but extensive poliomyelitic lesions were found in all.

The remaining strains will be similarly screened in monkeys to determine in a larger number of cases whether strains which are intracerebrally avirulent in monkeys may occur in the nervous system of fatal cases.

D. PARALYTGENIC ACTIVITY OF VARIOUS STRAINS AFTER SUBCUTANEOUS INJECTION IN CYROMOLGUS MONKEYS.

Effect of Strains of Different Degrees of Neurontropism Derived from Healthy Children and from the Spinal Cord of Fatal Cases. -- Previous tests with the laboratory-selected attenuated strains indicated that after subcutaneous inoculation of doses in the range of 10^6 TCD_{50} in cynomolgus monkeys paralysis was either absent or so rare as to suggest a direct intraneural inoculation in the isolated case. This was true even when strains with high intraspinal activity in monkeys were used. It appeared desirable to determine whether strains which were associated with paralytic infection in human beings (recovered from the spinal cord) would invariably exhibit high paralytic activity after subcutaneous inoculation in monkeys. For comparison it was also of interest to determine the activity of a number of strains which were derived from healthy children and exhibited varying neurontropic activity in monkeys. Accordingly, 6 strains from fatal cases and 9 strains derived from healthy children were all tested for their paralytic activity in cynomolgus monkeys by inoculating a standard dose of 10^6 TCD_{50} subcutaneously in the right forearm. All strains were tested as first cynomolgus kidney passage culture fluid and each strain was inoculated into 10 monkeys. The only strains which were completely non-paralytogenic in these tests were derived from healthy children. Three of these 4 were also dominantly avirulent by the intracerebral route, but one of them, the Mexican strain was intracerebrally virulent in monkeys not only in large but also in small doses. Among this group of 40 monkeys which exhibited no paralysis there were 12 which died of intercurrent infections between 8 and 22 days after inoculation. Tests for virus in the spinal cord of all of these monkeys were negative, indicating the absence of viral invasion.

However, there was a marked variation in the incidence of paralytic infection among the monkeys inoculated with the other strains without reference to whether the strains were derived from fatal cases or from healthy children. The frequency with which paralysis was observed in only one or two out of a group of 10 monkeys was so great that it was obvious that even highly neurontropic polio viruses may be only irregularly paralytogenic in cynomolgus
monkeys after subcutaneous injection of $10^6$ TCD$_{50}$ of virus. Although these results are significant from certain other points of view, the chief bearing which they have on our program is that paralytogenic activity of subcutaneously inoculated virus in cynomolgus monkeys cannot be used as a reliable guide to the virulence of poliomyelitis viruses.

E. STUDIES WITH ATTENUATED STRAINS IN CHIMPANZES

1. Type 1 LSc. -- The LSc virus which possessed the least neurotropic activity in monkeys after spinal inoculation of amounts of $10^6$ TCD$_{50}$ or more, also produced neither paralysis nor lesions after spinal inoculation of $10^6.7$ TCD$_{50}$ in each of 3 chimpanzees.

It was stated in the previous semi-annual report that virus propagated from the stools of chimpanzees which experienced an alimentary infection with the LSc strain exhibited no intracerebral virulence in monkeys. Accordingly, culture fluids from the stools of 3 chimpanzees were inoculated intraspinally in doses of $10^5$ TCD$_{50}$. Only one of 12 inoculated monkeys exhibited a very slight paralysis of one foot which failed to progress. This is about what one might expect if the original culture fluid were used and the indication is that no change in the character of the viral population occurred as a result of its multiplication in the alimentary tract in the chimpanzees.

An extensive quantitative study of the distribution of virus in various tissues, ganglia and CNS of one chimpanzee which died of intercurrent infection prior to development of antibody after feeding of $10^7.4$ TCD$_{50}$ of LSc virus was carried out in order to obtain information not only about the extent and localization of virus multiplication, but also to determine whether any of the virus moves from the alimentary tract to the regional nerve ganglia.

The results of this study indicated that large amounts of virus were found in the pharyngeal wall as well as in the deep cervical lymph nodes, and that smaller amounts were present at different levels of the small intestine and in the mesenteric lymph nodes. In spite of the absence of demonstrable viremia at any time after feeding of the virus, small amounts were detected in the spleen, but no virus was found in the liver, kidneys, adrenals, inguinal lymph nodes or the central nervous system. Of particular significance is the fact that approximately 100 TCD$_{50}$ of virus were found in the superior cervical sympathetic ganglia and that a trace was also found in the Gasserian ganglia, as well as in a pool of the thoracic spinal and sympathetic ganglia. One half of each of the cervical sympathetic and Gasserian ganglia was examined histologically, but no lesions of any kind were found. These observations suggest that
even a virus which is completely avirulent for the chimpanzee may
nevertheless spread to some extent to the first station of neurons
supplying the alimentary tract. One cannot be certain whether the traces
of virus which were found in the ganglia got there mechanically or by
virtue of very slight multiplication in a few neurons because we are
still uncertain of the mechanism by which neural spread of viruses
takes place. It may be mentioned here that in a similar study of
another chimpanzee which died of intercurrent disease, after the
ingestion of a naturally occurring attenuated Type 1 virus, it was also
possible to demonstrate a trace of virus in the superior cervical
sympathetic ganglia, but not in any of the other ganglia or CNS tissues
tested.

2. Effect of Some Naturally Occurring Attenuated Strains on Spinal
Inoculation in Chimpanzees. -- Two Type 1 (P 2149 and P 2226) strains,
one Type 2 (P 712) strain, and one Type 3 (Glenn) strain, were each
inoculated intraspinally into 3 chimpanzees. The inocula for the different
strains varied from $10^6.3$ TCD$_{50}$ to $10^7.5$ TCD$_{50}$. None of these
chimpanzees exhibited any paralysis. This brings the total number of
chimpanzees to 29 that had been inoculated intraspinally generally with
amounts in excess of $10^6$ TCD$_{50}$ of strains which are intracerebrally
avirulent for monkeys. In addition there were also 9 chimpanzees
which were inoculated intraspinally with culture fluids containing varying
amounts of intracerebrally virulent particles - also with negative
results. This experience with a total of 38 intraspinally inoculated
chimpanzees leaves no doubt of the greater resistance of the chimpanzee
lower motor neurons as compared with those of monkeys.

F. STUDIES WITH ATTENUATED STRAINS IN HUMAN VOLUNTEERS

1. Antibody Levels Six Months After Ingestion of Experimentally Attenuated
Strains. -- Seventeen of the group of volunteers who received either
Type 1, 2 or 3 virus in January, 1955, were tested for the persistence of
antibody six months after ingestion of the virus. In each case, the
antibody level was either of the same order of magnitude found at 3 months,
or somewhat higher. The higher levels were observed in three instances
in which volunteers who ingested Type 1 virus in January but had no
antibody against Type 2 virus had been given Type 2 virus by throat
swabbing in April. The boost in their Type 1 antibody appears to be the
result of a group specific antigenic stimulus resulting from infection
with the Type 2 virus. A similar rise in titer was observed in one
volunteer who received Type 3 virus three months after ingestion of
Type 2 virus.
2. **Effect of Type 1 LSc.** -- The various strains of virus were given by
mouth in a teaspoonful of cherry syrup which was shown to have no
effect on the titer of the virus. Three volunteers received $10^6.4$ TCD$_{50}$
(0.1 ml of culture fluid) and 3 received $10^4.4$ TCD$_{50}$ (0.001 ml of culture
fluid) by mouth. Multiplication of virus occurred in all but one of these
men and this one was found to have a very low level of Type 1 antibody
(1:4) in the serum obtained just before feeding. This would suggest
that a level of antibody which fluctuates from 0 to 1:4 can be associated
with an immunity which will prevent multiplication of $10^4.4$ TCD$_{50}$ of
ingested virus. Not only was there absence of viral multiplication in
this man but there was also no change in his low level of antibody. No
virus was demonstrable in any of the other men in 2 ml amounts of whole
blood taken at 1, 3, 5, 7, 11, and 14 days after ingestion of virus. The
level of viral multiplication in the throat varied in different individuals,
but peaks of $10^4.5$ to $10^5.5$ TCD$_{50}$ per swab were obtained in each of the
4 men who had virus in the throat. It is noteworthy that the one man
who had no virus in his throat complained of a slight sore throat and
transitory headache on the 7th day after ingestion of the virus. Of the
4 men who had demonstrably high levels of virus in their throats, one
complained of a sore throat for one day, 3 days after ingestion of the
virus, and of a slight headache 4 days after the ingestion of the virus.
The peak amounts of virus recovered per gram of stool were about one
to two logs less than the peak amounts recovered per throat swab.
Antibody was first demonstrable between 7 and 11 days after ingestion
of the virus in all but one man. The levels as determined by the
cytopathogenic test were generally lower than those obtained with all
other strains of Type 1 virus. However, very much higher titers were
obtained with the same sera when the antibody was measured by the pH
test (see section H3). Neither the original stools of any of these
volunteers nor $10^5$ to $10^5.5$ TCD$_{50}$ of the culture fluids prepared from
them exhibited intracerebral virulence in monkeys. Intraspinal
inoculation of culture fluids in doses of $10^5$ to $10^6.2$ TCD$_{50}$ into 30
monkeys also indicated that no significant change in neurotropism
occurred in the virus which propagated in the human alimentary tract.
This finding corresponds exactly to that obtained in the tests on virus
recovered from the alimentary tract of chimpanzees.

3. **Effect of two naturally occurring Type 1 strains (P 2149 and P 2226).** --
Strain "P 2149" was fed in a dose of $10^1$ TCD$_{50}$ (0.1 ml) to 3 men and in a
dose of $10^5$ TCD$_{50}$ (0.001 ml) to another 3. No viremia was demonstrable
in any of these. Viral multiplication occurred in the throat of 5 of the 6
and in the enteric tract in all six. Antibody was demonstrable as early
as the 7th day in at least 4 of the 6 volunteers and high titers developed
in all. In the throat the levels of viral multiplication were no greater
than with the LSc virus, but in the stools peak levels of $10^5$ to $10^5.5$ TCD$_{50}$ per gram were common. Three of the 6 men experienced a subjective sensation of slight sore throat for a period of about 24 hours from 3 to 4 days after the ingestion of the virus. The peak amounts of virus in the throat were found at that time.

Strain "P 2226" was fed in a dose of $10^7.4$ TCD$_{50}$ (0.1 ml) to three men, and $10^5.4$ TCD$_{50}$ (0.001 ml) to another 3 men. A trace of virus was found on the 3rd day in the blood of 3 of the 6 men. The amount was one cytopathogenic dose per 2 ml of whole blood. Viral multiplication occurred in the throat of 5 of the 6 men with peak levels of $10^5.5$ per swab. Viral multiplication occurred in the alimentary tract of all 6 men with peak levels of $10^6$ TCD$_{50}$ per gram of stool. Two of the 6 men experienced slight sore throat for a period of about 24 hours between 2 and 5 days after ingestion of the virus. Antibody of high titer appeared in all men within 7 to 11 days after the ingestion of the virus.

The original stools from 4 volunteers who ingested the "P 2226" virus produced no paralytic infections in intracerebrally inoculated monkeys. However, doses containing approximately $10^5$ TCD$_{50}$ or more of the cultures derived from these stools produced paralytic infections in some of the inoculated monkeys (3/9) in three instances. Among the volunteers who ingested the "P 2149" virus, there were 3 whose stools produced no paralytic infections in intracerebrally inoculated monkeys, either after inoculations as stool suspensions or as first passage kidney culture fluid. The stools of one individual, however, containing virus in high titer ($10^5.5$/gm) as well as the culture fluid derived from it, produced the paralytic infection in some of the intracerebrally inoculated monkeys.

4. Effect of Naturally-Occurring Type 2 Strain (P 712) -- This virus was fed to 13 volunteers in different doses, varying from $10^6.4$ TCD$_{50}$ to $10^2.1$ TCD$_{50}$ (0.1 ml to 0.000,005 ml). Seven volunteers who received either $10^4.4$ TCD$_{50}$ or $10^6.4$ TCD$_{50}$ all developed alimentary infections. One of the 3 who received $10^3.1$ TCD$_{50}$ and 2 of the 3 who received $10^2.1$ TCD$_{50}$ became infected. Those who exhibited alimentary infections also developed high levels of antibody. No antibody appeared in any of the 3 men who exhibited no evidence of viral multiplication in the alimentary tract. Among the 10 men who developed alimentary infection, there were 3 who exhibited traces of virus in their blood (1 infective dose per 2 ml) -- either the 3rd or the 5th day after ingestion of the virus. Neither sore throat nor other symptoms attributable to these infections were exhibited by any of the men.

Suspensions of the original stools from 9 of the men during the period when they were excreting peak quantities of virus ($10^3.7$ to $10^5$ TCD$_{50}$ per gram) yielded no paralytic infections in any of the intracerebrally inoculated monkeys. When the culture fluids from these and other stools
(total of 19) were inoculated intracerebrally in 57 monkeys mild
paralyses were observed in 6 of the animals and progeny tests are
now in progress to determine the character of the associated virus.

5. **Effect of Naturally Occurring Type 3 Strain ("Glenn").** -- This strain
was fed to 6 volunteers, 3 receiving $10^6$ TCD$_{50}$ (0.1 ml) and the other 3
receiving $10^4$ TCD$_{50}$ (0.001 ml). All 6 developed alimentary infections
and prompt antibody responses. The antibody titers in two of the men
who ingested $10^4$ TCD$_{50}$ and had no evidence of multiplication in the
throat were of a low order, but nevertheless persisted at the same
level during the period of observation of 3 months. No viremia was
observed in any of the men. Two of the 3 men who exhibited high titers
of virus in their throats also complained of a soreness of the throat
for a period of 24 hours between the 3rd and 4th days after ingestion of
the virus, which corresponded to the time when peak titers were also
present in the throat. The original stools of none of the 6 men produced
paralysis in intracerebrally inoculated monkeys. The culture fluids of
5 of these stools also failed to produce paralytic infections while the
culture fluid from the stool of a single patient produced paralysis in
2 of 3 monkeys, the nature of which still has to be elucidated by progeny
tests.

6. **Effect of Feeding Various Combinations of Naturally-Occurring Strains
as Mixtures.** --

a) Four men who lacked both Type 1 and Type 2 antibody were fed a
mixture of Type 1 (P 2149) and Type 2 (P 712) viruses - each in a
dose of about $10^6$ TCD$_{50}$. Evidence of multiplication of both types
of virus was obtained in all 4 men, although only Type 2 virus was
recovered from the throat of one of them. In another individual
only Type 1 was recovered from the throat except on one occasion.
The Type 2 virus appeared to be dominant in its antibody response,
but Type 1 antibody also appeared, even though it was delayed for as
long as 4 weeks in one man.

b) A mixture of Type 1 (P 2149) and Type 3 (Glenn) viruses - each in a
dose of $10^6$ TCD$_{50}$ - was fed to 3 men who lacked antibody for Types 1
and 3. Both types of viruses were recovered from the alimentary
tract, although 1 of the men yielded only Type 3 virus in his throat.
There was no delay in antibody formation for either Type 1 or Type 3
virus.

c) A mixture of Type 2 (P 712) and Type 3 (Glenn) viruses - each in a
dose of about $10^6$ TCD$_{50}$ - was fed to 3 volunteers who lacked antibody
for Types 2 and 3. Here the Type 2 virus seemed to dominate. Both
types of virus, however, were recovered from the alimentary tract,
although the throat of one man yielded only the Type 2 virus. Type 2
antibody appeared promptly and in high titer, while the Type 3 antibody was delayed for 3 to 4 weeks, and was generally of a lower titer than that which appeared in the volunteers who ingested the same dose of the Type 3 virus only.

d) Only 1 volunteer who lacked antibody to all 3 types of poliovirus was available and he received a mixture of Type 1 (P 2149), Type 2 (P 712) and Type 3 (Glenn) viruses - each in a dose of $10^6$ TCD$_{50}$. At different times after infection, different types of virus were found in the throat and stools, although during the first two weeks all 3 types were present in the stools simultaneously. Antibody development for all 3 types was delayed to the 3rd week. The levels of the Types 2 and 3 antibody were lower than among the men who ingested the same dose of each type separately.

7. **Effect of Feeding Type 2 (P 712) Virus Three Weeks After Type 1 (LSc).**

One volunteer, who lacked Type 2 antibody and who was still excreting a small amount of virus 3 weeks after ingestion of the Type 1 LSc strain, was given $10^6$ TCD$_{50}$ of Type 2 virus. Within 3 days after ingestion of the Type 2 virus there was no longer any Type 1 virus found in the stool and Type 2 virus was now present both in the throat and in the stools. Antibody of high titer to the Type 2 virus appeared between 7 and 10 days after ingestion, and persisted throughout the period of 3 months during which observations were made.

8. **Selection of Optimal Strains for Further Studies in Human Beings.**

On the basis of all the data accumulated on the behavior in monkeys, chimpanzees and human volunteers, of the attenuated poliomyelitis viruses obtained by selective procedures in the laboratory or from the stools of healthy children, it appeared that the LSc strain possessed the greatest complement of desirable properties among the Type 1 viruses; the naturally occurring "P 712" strain was selected as best Type 2 strain; the "Leon KP34" strain was selected as the more desirable Type 3 strain in preference to the "Glenn" strain, because it is much less neurotropic on spinal inoculation in monkeys.

G. **STUDIES ON ECHO VIRUSES**

1. **Serologic Classification of Mexican Strains** — Among the 233 non-poliomyelitis viruses recovered from healthy children in Mexico City, only 27.4% could be classified as being Cincinnati HE 2, 3, 4, or 5 viruses (ECHO Types 8 to 11). Among the 28 nonpoliomyelitis viruses recovered from healthy children in Vera Cruz only 1 of the Cincinnati Types (HE 5) was found to be represented. Thus of the total number of 261 strains which were recovered from healthy children in Mexico, there remained 195 unidentified strains. When 142 of these were tested with pools of the 8 remaining ECHO antisera and of 6 Coxsackie antisera
(A 9, A 14, and B 1 to B 4), only 11% could be classified. Six strains proved to be Coxsackie viruses (three were A 9, and one each A 14, B 2, and B 3) and 10 belonged to the other ECHO viruses. With only a few exceptions the large number of remaining strains produce a poliomyelitis-like cytopathogenic effect in tissue culture, and further classification will be achieved only by the laborious preparation of antisera for new prototypes and the successive sorting out of the remaining strains.

2. Incidence Among Some Summer Diarrheas in Infants and Young Children in Cincinnati, 1955. -- The possible role of some of the ECHO viruses as etiological agents of the diarrheal diseases in infancy and early childhood was investigated during the summer months of 1955 among patients admitted to two Cincinnati hospitals. Rectal swabs as well as acute and convalescent-phase sera were obtained for study. The following preliminary results of the tests in monkey kidney tissue culture give some idea of the nature of the problem. The rectal swabs from 56 children under 4 years of age (38 under 1 year of age) yielded 24 viruses (43% positive). Among these 24 viruses, 3 were definitely Type 1 poliomyelitis, 3 turned out to be strains of Coxsackie virus (one A 9 and two B 2). Only 2 were classified among the 13 known ECHO viruses (one Type 2, and one Type 8) and 12 strains could not be identified with any of the known ECHO viruses.

H. ANCILLARY STUDIES AND INCIDENTAL FINDINGS

1. Tests for Polio Virus in Nose, Mouth, Throat and Feces of Patients with Paralytic or Nonparalytic Poliomyelitis. -- This study was undertaken to determine whether the localization of polio viruses in patients with clinically recognized poliomyelitis would be similar to that observed among volunteers who swallowed the various attenuated strains. The object was to determine not only the extent to which virus would be present or absent in the mouth, nose, and throat, but also to compare the quantities of virus recovered in these areas as well as in the stools with those obtained in volunteers after ingestion of attenuated strains. Twenty-four patients with paralytic poliomyelitis (22 Type 1 and 2 Type 3) and five patients with nonparalytic poliomyelitis (all Type 1) were included in this study. Only those patients who came to the hospital within a few days and not more than one week after onset of the first symptoms, were investigated. The following points appeared to be especially noteworthy.

a) Although a number of the children had bulbar manifestations with difficulties in swallowing, the nasal swabs were negative in all but one patient in whom only a trace of virus was present. This establishes for the first time the absence of virus in the nasal mucosa of patients at a time when they may yield as much as $10^{4.5}$ TCD$_{50}$ per throat swab, or $10^6$ TCD$_{50}$ per gram of stool.
b) Among the 29 patients there were only 4 with epidemiologically insignificant traces of virus in the swabs which were rubbed over the cheeks, gums, anterior third of the tongue and floor of the mouth. This shows that the absence of virus in the mouth of patients, who have fairly large amounts in their throats, is similar to the situation obtaining in the volunteers who ingested attenuated strains.

c) It was surprising that the throat swabs from 75% of the patients yielded poliomyelitis virus - in different amounts, as one would expect to find at different times after infection. The peak quantity recovered per swab - $10^{4.5}$ TCD$_{50}$ - is not higher than that obtained in volunteers who ingested attenuated strains. The fact that 75% of the patients at such different intervals after infection yielded virus in the throat, strongly suggests that at one time or another all patients with clinically recognizable disease may have virus in their throats. In order to achieve so high an incidence of localization in the throat when attenuated strains are used in volunteers, it has been necessary to feed at least $10^6$ TCD$_{50}$ of virus. There appear, therefore, two possibilities to explain the high incidence of virus in the throats of patients with the clinically recognized disease:

1) either they were all infected with very large doses of virus in the range of $10^6$ TCD$_{50}$; or

2) that in the naturally occurring disease the viremia which may follow the ingestion and multiplication of smaller doses may yield to a secondary localization in the throat - something that does not occur in the volunteers ingesting attenuated strains.

d) The peak titers of virus that were found in the stools was $10^{6.2}$ TCD$_{50}$ per gram. It is, of course, possible that still larger quantities might have been present in the stools of these patients at an earlier stage after infection. Nevertheless, the range of titers found in the patients was not significantly different from that observed in volunteers who were given the attenuated strains.

e) In view of the fact that the statement has often been made that virus disappears from the throat at the time that antibody appears, it was noteworthy that no correlation was found between the presence of antibody in the serum at the time the throat swabs were taken and the amount of virus that was recovered per swab. Thus as much as $10^7$ TCD$_{50}$ of virus was recovered from a throat swab at a time when the homotypic antibody titer in the serum was 1:256.
2. **Diagnostic Efficacy of Neutralization Tests on Acute Phase Sera and Convalescent Specimens Obtained 1 Week after Hospitalization.**

The appearance of antibody as early as 7 days and the development of maximal levels by 14 to 21 days in volunteers ingesting attenuated poliomyelitis viruses suggested that diagnostic rises in neutralizing antibody should be detectable very early after onset of the clinical disease. To test this hypothesis sera were obtained from 16 patients with paralytic poliomyelitis (Type 1 isolated from 14 and Type 3 from 2) and 3 patients with the aseptic meningitis syndrome (Type 1 virus isolated from 2 individuals) on admission to the hospital and again in 7 days. Hospitalization occurred on the 2nd to 9th day of disease, with a mean of 4.5 days. Twelve of the 19 patients studied (63%) developed a 4 fold or greater rise of neutralizing antibody within 7 days after hospitalization. Three individuals (16%) who failed to exhibit a diagnostic antibody rise during their first week in the hospital subsequently developed such a rise. Thus 12 of 15, or 80%, of patients developing an antibody rise did so by the 7th day of hospitalization. Maximal antibody levels were present in 3 patients by the time of hospitalization (5 to 6 days after onset of symptoms) while in 1 patient the maximal response remained so low that a 4 fold rise was not demonstrable.

3. **Difference in Results Obtained by pH and Cytopathogenic Tests for Antibody in Acute and Convalescent Phase Sera of Patients and in Sera of Volunteers at Different Times after Infection with Attenuated Virus.**

A comparison of neutralizing antibody as measured by the cytopathogenic effect in monkey kidney roller tubes and by the pH color technique was made utilizing sera from patients with paralytic poliomyelitis and volunteers fed attenuated viruses. Antibody titers of naturally infected patients and volunteers fed L5c Type 1 virus were generally 2 to 32 fold higher when determined by the pH color test. Higher antibody levels by the pH technique were seen less frequently in volunteers fed Leon virus - 3 of the 5 volunteers tested had comparable antibody titers by both techniques.

In certain individuals studied - 2 of 6 patients with paralytic poliomyelitis and 4 of 5 volunteers fed L5c virus - the disparity between pH color test antibody levels and those determined by cytopathogenic effect was greater in sera collected shortly after onset of clinical symptoms or 2 to 4 weeks after feeding of virus than in sera collected at a later date. Examples of such temporal differences are shown in Table 1; also included are individuals who maintained a constant ratio. Volunteers who developed low levels of antibody to the Leon virus, mainly exhibited the latter pattern.

The occurrence in certain "early" sera of proportionately higher titers of antibody by the pH method than by the cytopathogenic technique suggests a fundamental difference in the character of antibody formed early after antigenic stimulation and that produced at a later date. It is possible
that "early" antibody binds virus less firmly than "late" antibody. If such were the case, a temporary reversible inhibition of virus might be sufficient in the pH test to delay cell destruction long enough for cell metabolism to produce a color change and thus serve as an indication of the presence of antibody. By the cytopathogenic technique such a hypothetical occurrence would be scored as failure to protect against virus infection and thus signify absence of antibody.

4. Further Studies with the Chimpanzee Rhinitis and "Steatorrheic" Enteritis Viruses. -- See end of SUMMARY, Section A.

PUBLICATIONS


IN PRESS


January 18, 1956

Albert B. Sabin, M.D.
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**TABLE I**

**EXAMPLES OF DIFFERENCES BETWEEN "EARLY" AND "LATE" ANTIBODY IN pH AND CYTOPATHOGENIC TESTS**