Dr. C. H. Stuart-Harris
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My dear Stuart-Harris:

I regret the long delay in replying to your letter of June 25 in which you inquire about my plans for further human tests with our attenuated polioviruses and indicate that you would be in a position to undertake a limited series of feeding tests with such strains next mid-winter. I did not return from Europe until the end of July and since my return have hardly had a free moment.

I should like to give you a chronological account of developments since our meeting in London and Sheffield last November, so that you may understand the reasons for my decisions. By the end of 1955 I had completed a series of human tests with a number of different naturally occurring as well as laboratory-modified attenuated strains and, on the basis of lowest neurotropism for the monkey and greatest stability after propagation in the human alimentary tract, selected the LSc (Type 1), "F712" (Type 2) and modified Leon (Type 3) viruses as the best of the available attenuated strains. These had all originally been submitted to several terminal dilution purifications and there were no indications that the dominant population of each strain

Aug. 31, 1956
was not homogeneous as regards the character of neurotropism.

Before deciding on these 3 strains as the optimum I had also considered the properties of Koprowski's Type 1, "SM" (Sickle-Mahoney) strain and Type 2, "TN" strain, as well as the type 2, chick-embryo adapted "MEF_1" strain of Cox. The type 1 "SM" strain was eliminated on the basis of its greater neurotropism for the monkey and its obvious instability in the human alimentary tract, since as Koprowski first reported (Proc. Soc. Exp. Biol. and Med., 1954, 86, 244) as little as $10^{3.5}$ TCD$_{50}$ of virus in the original stool of a volunteer produced paralysis in both intracerebrally inoculated monkeys. The low yield of both the "TN" strain in rodents and of the MEF_1 strain in chick embryos did not permit a comparison of the spinal paralytogenic activity of $10^5$ and $10^6$ infective doses, but the results reported with the lower concentrations suggested that both were more neurotropic than the Type 2, "P712" strain that I had selected. Furthermore, there were no adequate data on the neurotropism of either the "TN" or the "MEF_1" strains after propagation in the human alimentary tract, because the stools were tested only in mice (in the belief that these strains were not cytopathogenic) which yielded too low a concentration of virus to be significant for further tests in monkeys. Actually I later learned from Dr. Henle that in the Philadelphia tests it was found that the stools of children fed the "TN" strain which had little or no virus when tested in mice yielded fairly high titers when tested in HeLa cells. It is apparent, therefore, that the "TN" strain is a
mixture of noncytopathogenic and cytopathogenic particles, and that the human alimentary tract favors the cytopathogenic component. I have not heard of any tests on the neurotropism of the higher concentrations of "TN" virus grown out from the stools in HeLa cell cultures. The chick embryo, "MEF_1" strain has also been found to be highly cytopathogenic for human amniotic epithelial cells, and thus Cox's criterion that the selected strain should not be cytopathogenic for primate cells does not hold for this strain. Nothing is known of the stability of the chick embryo, "MEF_1" virus in the human alimentary tract.

Before proceeding further with the selected L Sc, "P712" and Leon strains I carried out tests on the neurotropic activity of culture fluids grown in larger amounts and with inocula of different sizes. Having found no significant variations from the low level of neurotropic activity of the seed viruses, each strain was then grown in 20 liter lots, on a "pilot plant" scale at the Sharp and Dohme Research Laboratories. High-titered filtrates (in the range of 10^7 infective doses per ml.) were obtained despite long hours of handling at room temperature, and the tests for neurotropism on monkeys and chimpanzees proved entirely satisfactory. Using high-titered rabbit polio antisera it was also possible to show that the fluids contained no contaminating simian viruses. As an additional precaution, 10% of the total number of tissue culture bottles in each lot were left uninoculated and no cytopathogenic agent was found in them. Other safety tests on mice,
rabbits, guinea pigs and bacterial cultures were also satisfactory. Aliquots of these culture fluids were then used for feeding experiments on 53 volunteers and it is noteworthy that among the hundreds of stool specimens that were tested not a single "nonpolio" cytopathogenic agent was isolated.

The tests on these 53 volunteers begun in April, 1956, yielded the following new information:

1. An oral dose of $10^5$ plaque-forming units (or about 0.01 ml. of culture fluid) of each strain regularly produced an immunogenic alimentary infection in volunteers without homotypic antibody as measured by the more sensitive pH test for antibody of lower avidity.

2. In individuals who lacked 2 types or all 3 types of antibody it was possible to feed $10^5$ P. F. U. of the different types of virus at 3-week intervals not only without interference but often also with some enhancement of the antibody response to the preceding type.

3. Antibody produced by 2 doses of Salk vaccine (at 4-week intervals) did not interfere with the amount and duration of virus excretion after feeding $10^5$ P. F. U. of virus even when the antibody titers prior to feeding were as high as 1:256 by the pH test. In 5 volunteers, originally
lacking all 3 types of antibody prior to injection of
Salk vaccine, the 3 types of virus were fed serially
at 3-week intervals, and each type of virus multiplied
in turn and produced a boost in the level of preexisting
antibody. In 9 volunteers who lacked antibody for only
one type of virus prior to injection of Salk vaccine, there
was also no interference with multiplication when the
corresponding virus was fed.

4. Among 8 volunteers with varying levels of naturally
acquired antibody (generally as low or lower than those
with antibody produced by Salk vaccine) who were fed
$10^5$ P. F. U. of Type 1 virus there was no evidence of
viral multiplication in 7; in the one man in whom the
virus multiplied the antibody level was so low that it is
possible that it may have resulted from a previous Type
2 and Type 3 infection rather than Type 1. When $10^5$
P. F. U. of the type 2 or type 3 viruses were fed to
volunteers with varying levels of naturally acquired
antibody multiplication occurred in some but not in
others (2 of 5 with Type 2 and 3 of 6 with Type 3).
Both the level of viral multiplication and the duration
of excretion were, however, definitely less in these
than in the volunteers without homotypic antibody or
with relatively high levels of antibody produced
by Salk vaccine.

5. With the exception of 2 men who complained of a
slight sore throat for a day or two, there were no
untoward symptoms.

The original plan was that the 20 liter lots of each of these strains
were to be considered for use in progressively larger numbers of individuals
if the tests turned out as they did. However, I decided against using these lots
of virus because of the following subsequent work on the selected strains.

Early in 1956 I initiated a series of tests on the homogeneity of
the selected strains by preparing progeny from 9 or 10 single, triply puri-
fied plaques of each strain, and testing each one quantitatively by spinal inocu-
lation in large enough numbers of monkeys to yield significant results. These
tests showed that the type 2 and type 3 strains were definitely not homogeneous,
and there was some question about the type 1 virus which was already the most
highly attenuated. Some of the single plaque progeny yielded results comparable
to those of the seed virus, others were definitely more paralytogenic, while a
few were completely nonparalytogenic in doses of about 10^6 TCD_{50} in spinally
inoculated monkeys. Two of the 9 type 3 plaques fell into the nonparalytogenic
category. Both of these are now being studied further by plaque analysis, and
at least on the basis of plaque size (still to be checked by tests for neurotropism) one appears to be more homogeneous than the other. One has already been found to produce alimentary immunogenic infection in chimpanzees, and the other is now being tested. This permits us for the first time to apply the most rigid tests for genetic homogeneity, for there is no assurance that even the progeny from single virus particles are necessarily equally stable. Of the 9 type 2 plaques only one yielded progeny which produced only slight weakness in an occasional monkey inoculated intraspinally with the largest doses, and even here subsequent passages yielded virus which failed to produce paralysis in any of 15 monkeys. This plaque progeny has also been found to produce immunogenic alimentary infections on feeding to chimpanzees, and further studies on its homogeneity and stability in the alimentary tract are now in progress.

One of the 10 type 1, "L Sc" plaques also proved to be superior to all the others. I am also testing single plaques of virus derived from the stools of a volunteer, 15 days after feeding of L Sc virus, because its spinal activity in monkeys was as negligible as that of the best single plaque suggesting a certain stability over many generations during 15 days of propagation in the alimentary tract. Tests on a large number of individual plaque progeny derived from the naturally occurring attenuated Type 1, "P2149" strain has thus far failed to yield anything with the negligible spinal activity of the L Sc derivatives mentioned above. However, I still have to test the progeny of at
least two other plaques which proved to be the smallest of 40 examined. For at least with Type 1 and Type 3 viruses there appears to be some correlation between plaque size and neurotropism. Lepine recently sent me his best attenuated Type 1 and Type 2 viruses and I found both of them to produce extensive to prostrating paralysis in monkeys inoculated intraspinally even with $10^4$ TCD$_{50}$.

In my opinion, studies on progressively increasing numbers of people should be carried out not only with the least neurotropic progeny derived from single plaques but with plaque progeny which can be shown to have the greatest stability on multiplication in vitro and in the alimentary tract. I hope to have a pretty good idea of where we stand on this by the end of this year. If I can make a choice at that time, I shall again prepare 20 liter lots of each selected strain and submit them to further testing in human beings. If the tests are satisfactory, these 20 liter lots may then become the source for progressive tests by many qualified investigators.

Enough work has already been done by Koprowski and myself to show the way various attenuated strains behave on feeding to human beings. In my opinion, the problem now is not of merely repeating these observations with any attenuated strain, but rather to select the best strains on the basis of lowest neurotropism and maximum genetic homogeneity and stability.

I shall be very glad to keep in touch with you and through you with
the M.R.C. committee on polio vaccination. I appreciate your interest and shall be glad to cooperate in any way you may deem desirable. I am sending copies of this "letter" to George Dick and Patrick Meenan, because of their interest in this field of work.

With best wishes and kindest regards.

Sincerely yours,

Albert B. Sabin, M. D.

cc: Dr. George Dick  
    Dr. Patrick Meenan  
    Dr. Thomas B. Turner  
    Dr. Henry W. Kumm