8th June, 1971

Dr. Albert B. Sabin,
President,
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Dear Dr. Sabin,

I wish to thank you for your letter of 31st May containing the memorandum on the history of your poliovaccine strains. I am extremely grateful that you took the trouble to make the necessary alterations, especially as the use of "cynomolgus" and "inoculate" have caused me concern for many years. I have found the monkey species spelt in three ways viz., "cynomolgus", "cynomolgous" and even "cynamolgus". As to "inoculate" and "inject", I have never been able to find a satisfactory directive and I have tended to restrict "inoculate" to parenteral administration of a vaccine in order to produce immunity. In the light of your letter, and with your permission, I shall in future follow your interpretation of these words.

I thank you for sending your copy of the history to me and I realise I should have asked you at the beginning about this matter. However, I must admit that reading the relevant literature has given me a greater understanding of the enormous amount of work you have done (plus a certain amount of personal satisfaction in producing a fairly accurate record from the references).

There is only one detail about which I should be grateful if at some future date you would kindly explain. I have called your original type 1 virus "LS-c,2ab/KP2" based on table 8 in your paper in J.Amer.Med.Ass.(1957),164, page 1219, assuming that the 25-litre lot prepared by Merck, Sharp and Dohme Research Laboratories was produced in one passage from plaque line "11" (pH 6.8), kidney passage level "2" with a volume of 100 ml etc. (as described in the upper part of page 2 of my memorandum). Is the passage level of KP2 given in your article a misprint so that in fact your original type 1 virus is Ls-c,2ab/KP3 of 10/10/56 possessing the properties I described for LS-c,2ab/KP2?

With kind regards,

Yours sincerely,

L.R. Boulger
The **Mahoney** virus was isolated in 1941 by Drs. Francis 4- Mack from the pooled faeces of 3 healthy children in Cleveland.

Drs. Li and Schaeffer received the strain from Dr. Salk after it had undergone 14 monkey in-vivo and 2 monkey in-vitro testicular tissue culture passages. Li and Schaeffer (1954) subjected this strain, Monk 14 T2 (Mahoney strain), to a further 9 similar in-vitro passages. From Monk 14 T11, they established 4 separate virus lines by further passages in monkey testicular tissue and kidney cell cultures, by passages in the central nervous systems of white mice usually by the intraspinal route of injection, and by alternate passages in the skin of rhesus or cynomolgous monkeys and tissue cultures. For the intradermal injections, ten 0.1 ml amounts of undiluted culture fluid were introduced adjacent to each other into the shaved skin of the abdomen. The 4 passaged strains were designated LS, LS-a, LS-b and LS-c and as they continued to grow in cell-culture, they were differentiated by their host reactions. LS and LS-a reacted similarly in that they were mouse and monkey spinal cord variants. LS-b acted as a mouse cerebral strain, but LS-c was a non-neurotropic strain for mice and monkeys by either route. It was derived from the 33rd consecutive in-vitro passage of Monk 14 T2 (the first 15 in testicular tissue and the subsequent 18 in kidney cells), then by alternate passage in monkey skin (the first 2 were in-vivo rhesus passages and the later ones were performed in cynomolgous monkeys) and monkey kidney cell cultures. The LS-c strain is Monk 14/MS 10 T 43 level.

The LS-c strain underwent 5 passages in cynomolgous monkey kidney cell cultures, including 3 terminal dilution passages, prior to submission to a series of 3 consecutive single plaque passages (Sabin 1956). The progeny of 10 selected individual plaques were tested for neurovirulence in cynomolgous monkeys injected intraspinally.
with $10^6$, $10^5$ and $10^4$ tissue culture infective virus particles, and the $L_{S-C_2}, 2ab$ strain was selected because it possessed the optimum properties. The original type 1 virus ($SO$) was prepared by 2 further passages in cynomolgous monkey kidney cell cultures and designated $L_{S-C_2}, 2ab/KP_2$. Its volume was 100 ml, the pH was 8.2, the titre was $7.9 \log_{10}$ TCID$_{50}$ per ml and only one cynomolgous monkey out of 5 receiving undiluted material intraspinally exhibited slight paralysis. The two groups of animals injected with suspension diluted 1/10 to 1/100 showed no paralysis. At the end of 1956 Merck, Sharp and Dohme Research Laboratories prepared a lot of 25 litres by one passage of the original virus in rhesus monkey kidney cell cultures. This material was designated $L_{S-C_2}, 2ab/KP_3$ (NSD, SOM or SO+1), and aliquots were used for the world-wide field trials before it was licensed as the Sabin original vaccine, and as the Sabin seed virus for the production of vaccine.

**Type 2 $P712, Ch, 2ab/KP_2$ (Sabin Original Virus = $SO$)**

The original $P712$ virus was a natural occurring strain of poliovirus possessing low neurovirulence for cynomolgous monkeys by the intraspininal route (Sabin 1956). It was isolated from the stools of a healthy child in Louisiana and sent to Dr. Sabin by Drs. Fox and Gelfand of New Orleans. It was passaged 4 times in cynomolgous monkey kidney cell cultures, 3 of which were terminal dilution ones. The progeny from a number of plaques were obtained, and 9 were submitted to 3 consecutive plaque passages (Sabin 1957). The purified plaque progeny with the least neurovirulence for cynomolgous monkeys injected intraspinally as with type 1 progeny was fed to chimpanzees and the excreted strain possessing the least residual neurovirulence ($P712, Ch$) was further purified by 3 consecutive passages from single plaques, and the strain designated $P712, Ch, 2ab$ selected as the vaccine virus. The original type 2 virus ($SO$ was prepared by 2 further passages in cynomolgous monkey kidney cell cultures and named $P712, Ch, 2ab/KP_2$. Its volume was 100 ml, the pH was 8.2, the titre was $7.3 \log_{10}$ TCID$_{50}$ per ml and none of the 3 groups of 5
cynomolgous monkeys each injected with 0.1 ml amounts of undiluted virus suspension, and suspension diluted tenfold and hundredfold showed any degree of paralysis. As with the type 1 attenuated poliovirus Merck, Sharp & Dohme Research Laboratories made a 23 litre lot by one passage of the original type 2 virus in rhesus cultures. This is the P712,Ch,2ab/KP3 (MSD, SOM or SO+1) and aliquots were used for the field trials before it was licensed as the Sabin original vaccine, and as the Sabin seed virus

**Type 3. Leon 12a,b/KP3 (Sabin Original Virus = SO)**

The Leon virus was obtained from the brain-stem and spinal cord of a boy, aged 11, who died of bulbo-spinal poliomyelitis in Los Angeles in 1937. It was isolated by Drs. Kassel and Stimpert in rhesus monkeys and maintained in the same species by the intracerebral route for 20 subsequent passages (1951). It underwent 8 further passages in rhesus monkey testicular tissue culture before the strain was sent by Dr. Melnick to Dr. Sabin (Sabin et al 1954). After 3 passages in cynomolgous monkey kidney cell cultures, the virus produced prostrating paralysis in 4 to 6 days in each of 4 intracerebrally injected cynomolgous monkeys. After 30 rapid passages, approximately 24 hour intervals, using large inocula ($10^5$ to $10^6$ TCID$_{50}$) in cynomolgous kidney cultures. These were succeeded by 3 terminal dilution passages, followed by one passage using a large inoculum of the progeny of the 3rd terminal dilution. This strain, Leon KP34, exhibited a marked reduction in its neurovirulence in that none of the 28 cynomolgous monkeys injected intracerebrally with 7.2 log$_{10}$TCID$_{50}$ per ml developed either paralysis or histological poliomyelitis. The progeny from 9 selected plaques, after purification by 3 consecutive plaque passages, were subjected to the neurovirulence test in 3 groups of cynomolgous monkeys injected intraspinally with 6.0, 5.0 and 4.0 log$_{10}$TCID$_{50}$ of virus. The progeny designated as 12a,b showed the least neurovirulence and was selected for the production of vaccine (Sabin 1956). This strain was passaged 3 times in cynomolgous monkey kidney cell cultures to give the original type 3 virus (SO)
named Leon 12a\(_{1-2}\)/KP\(_3\) (Sabin 1957). The volume was 10 ml, the pH was 6.8, the titre was 6.5 log\(_{10}\) TCID\(_{50}\) per ml, and 3 groups of 5 cynomolgous monkeys were each injected intraspinally with 0.1 ml amounts of undiluted virus suspension as well as 10\(^{-1}\) and 10\(^{-2}\) dilutions. The monkeys receiving the undiluted material and those injected with suspensions diluted one hundredfold remained symptomless, whereas one of the 5 animals which had the 10\(^{-1}\) dilution showed minimal clinical signs and focal histological poliomyelitis adjacent to the site of injection in the lumbar cord. Merck, Sharp and Dohme prepared a lot of 25 litres by one passage (as with types 1 and 2) using the original type 3 virus. This is the Leon 12a\(_{1-2}\)/KP\(_4\) (MSD, SOM or SO+1) and was used in the field trials before being licensed as the Sabin original vaccine and as the Sabin seed virus.

The three types of the large lots produced by Merck, Sharp and Dohme in rhesus monkey kidney cell cultures contained SV40 (WHO 1969).

**Alternative Leon 12a\(_{1-2}\) Vaccine Strains**

Dr. Sabin supplied Lederle Laboratories with his original type 3 virus (SO) designated Leon 12a\(_{1-2}\)/KP\(_3\) (Sabin 1969). As mentioned, this strain was prepared in cynomolgous monkey kidney cell cultures and was free of SV 40 virus. Lederle prepared their seed lot by one passage in monkey cell cultures and this was used to make a larger lot (No. 3-393). This preparation was approved by the Division of Biologics Standards, United States Public Health. Lederle lot No. 3-393 represents SO + (Led 1+1) i.e. SO+2 (WHO 1969) and aliquots have been used by some manufacturers for the production of working seed i.e. SO + (Led 1 + 1) + 1 so that their vaccines would represent SO + 4. Dr. Sabin also gave his original type 3 virus to other manufacturers, of whom 3 prepared working seed in one passage i.e. SO + Man = SO + 2 so that their vaccines represent 2nd cell culture passage level or SO + 2. Of the remaining 2 producers, one made a further cell culture passage before preparing the working seed, i.e. SO + Man + 1 = SO + 2 meaning their vaccines are 3rd passage level or SO + 3. The other manufacturer, who
also received Dr. Sabin's original type 1 and 2 virus, prepared their working seeds by 3 consecutive cell culture passages, i.e. SO + Man + 2 or SO + 3 so that their vaccines are 4th passage level or SO + 4. The majority of the manufacturers receiving the Sabin seed virus (SOM or SO + 1) types 1, 2 and 3 made their working seeds by one passage to free SOM from SV40. Hence their vaccines are 3rd passage level or SO + 3 (WHO 1969). One producer (Chumakov et al 1964) freed SOM + 2 from SV40 by 3 heat treatments at 34°C in the presence 1M MgCl₂ after which six plaques were selected and grown on vervet monkey cell cultures and pooled to form the working seed (SOM + 5 or SO + 6) so that the vaccine was SOM + 6 i.e. SO + 7. Finally, another manufacturer (Stones et al 1964) grew SOM type 3 in vervet monkey cells in the presence of SV40 antiserum. This material was then subjected to phenol extraction and plaque purification to form an RNA working seed equivalent to SOM + 4 and RNA vaccines to SOM + 5 i.e. SO + 6.

[Signature]
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REFERENCES


Immunologic Classification of Poliomyelitis Viruses (1951) Am. J. Hyg. 54, 191.


