Dear Dr. Sabin,

I should like to refer to previous correspondence regarding the history of your poliovirus vaccine strains.

Since the compilation of the memorandum, many people, usually visiting workers, have shown great interest in the document. In fact, I have been asked many times to publish it, and as a result I have discussed the matter with Dr. Perkins. He agrees that although the information is available in various journals, the presentation of all the facts as one document would be extremely useful. Furthermore, as editor of the new 'Journal of Biological Standardisation' he feels that the document would be considered very sympathetically for inclusion in the second number (April 1973).

I realise that for publication a reappraisal, etc. of the memorandum would be useful, but before I take any action I should like to ask if you do agree to publication and would you do me the honour of being co-writer.

I enclose a copy of the document.

I hope very much you are keeping well.

With kind regards,

Yours sincerely,

L.R. Boulger

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HISTORY OF SABIN ATTENUATED POLIOVIRUS ORAL
LIVE VACCINE STRAINS

Type 1 LSc, 2ab/KP2 (Sabin Original Virus = SO)

The Mahoney virus was isolated in 1941 by Drs. Francis and 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, Monkl4 T2 (Mahoney strain), to a further 9 similar in-vitro passages. From Monkl4 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 cynomolgus 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 Monkl4 T2 (the first 15 in testicular tissue and the subsequent 18 in kidney cells), then by alternate passage in monkey skin (the first 2 of these were in-vivo rhesus passages and the later ones were performed in cynomolgus monkeys) and monkey kidney cell cultures. The LS-c strain is Monkl4/9510 T43 level.

The LS-c strain underwent 5 passages in cynomolgus 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 cynomolgus monkeys inoculated intraspinally with $10^6$, $10^5$ and $10^4$ tissue culture infective virus doses, and the LS-c,2ab strain was selected because it possessed the optimum properties. The original type 1 virus (S0) was prepared by 2 further passages in cynomolgus monkey kidney cell cultures and designated LS-c,2ab/KP$_2$ of 10.10.56 (Sabin 1971). Its volume was 100 ml, the pH was 8.2, the titre was 7.9 log$_{10}$ TCID$_{50}$ per ml and only one cynomolgus 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 LS-c,2ab/KP$_3$ (M3D, S01 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 = S0)

The original P712 virus was a naturally occurring strain of poliovirus possessing low neurovirulence for cynomolgus monkeys by the intraspinal route (Sabin 1956). It was isolated by Dr. Sabin from the stools of one of a number of healthy children in Louisiana sent to him by Drs. Fox and Gelfand of New Orleans. Because of its low initial neurovirulence for monkeys it was passaged 4 times in cynomolgus monkey kidney cell cultures, 3 of which were terminal dilution ones. The progeny from a number of plaques was obtained, and 9 were submitted to 3 consecutive plaque passages (Sabin 1957). The purified plaque progeny with the least neurovirulence for cynomolgus 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 cynomolgus monkey kidney cell cultures and named P712,Ch,2ab/KP2 of 10/10/56 (Sabin 1971). Its volume was 100 ml, the pH was 8.2, the titre was 7.3 log<sub>10</sub> TCID<sub>50</sub> per ml and none of the 3 groups of 5 cynomolgus monkeys each inoculated 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,SO: 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 an 11-year old boy, who had died of bulbospinal poliomyelitis in Los Angeles in 1937. It was isolated by Drs. Kessel and Stimpert in rhesus monkeys and maintained in the same species by the intracerebral route for 30 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 cynomolgus monkey kidney cell cultures the virus produced prostrating paralysis within 4 to 5 days in each of 4 intracerebrally inoculated cynomolgus monkeys. Thirty rapid passages at approximately 24-hour intervals, using large inocula (10<sup>5</sup> to 10<sup>6</sup> TCID<sub>50</sub>) were carried out in cynomolgus 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 KP3<sup>4</sup>, exhibited a marked reduction in its neurovirulence in that none of the 28 cynomolgus monkeys inoculated intracerebrally with 7.2 log<sub>10</sub> TCID<sub>50</sub> per ml developed either clinical or histological poliomyelitis. The progeny from 9 selected plaques, after
purification by 3 consecutive plaque passages, was subjected to the neurovirulence test in 3 groups of cynomolgus monkeys inoculated intraspinally with 6.0, 5.0 and 4.0 log_{10} TCID_{50} of virus. The progeny designated as 12a_{1,2} showed the least neurovirulence and was selected for the production of vaccine (Sabin 1956). This strain was passaged 3 times in cynomolgus monkey kidney cell cultures to give the original type 3 virus (SO) named Leon 12a_{1,2}/KP_{3} of 10/10/56 (Sabin 1957 and 1971). 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 cynomolgus monkeys were each inoculated 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 inoculated with suspension diluted one hundredfold remained symptomless, whereas one of the 5 animals which had the tenfold 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 (SO). This Lot is Leon 12a_{1,2}/KP_{3} (MED, SO 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 cynomolgus monkey kidney cell cultures and was free of SV40 virus. Lederle prepared their seed Lot (No.453-25) by one passage of Sabin's original virus previously mixed with SV40 antiserum in cercopithecus monkey kidney 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 represented 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 + Han = SO + 1 so that these vaccines represented 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 + Han + 1 = SO + 2 meaning their vaccines were 3rd passage level or SO + 3. The other manufacturer, who also received Dr. Sabin's original types 1 and 2 virus, prepared their working seeds by 3 consecutive cell culture passages, i.e. SO + Han + 2 or SO + 3 so that their vaccines were 4th passage level or SO + 4. The majority of the manufacturers receiving the Sabin seed virus (SO4 or SO + 1) types 1, 2 and 3 made their working seeds by one passage to free SO1 from SV40. Hence their vaccines were 3rd passage level or SO + 3 (WFC 1968). One producer (Chumakov et al 1964) freed SO1 + 2 from SV40 by 3 heat treatments at 34°C in the presence of 1M MgCl₂, after which six plaques were selected and grown on vervet monkey cell cultures and pooled to form the working seed (SO1 + 4 or SO + 5) so that the vaccine was SO1 + 5 i.e. SO + 6. Finally, another manufacturer (Stones et al 1964) grew SO1 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 PNA working seed equivalent to SO1 + 4 and PNA vaccines to SO1 + 5 i.e., SO + 6.

L.R. Boulger
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