THE ROYAL SOCIETY

doi 10.1098/rstb.2001.0862

Polio vaccine and retroviruses

John Beale[1*] **and Florian Horaud**[2†]

[1]*The Priest's House, Sissinghurst Castle, Cranbrook, Kent TN17 2AB, UK*
[2]*Institut Pasteur, 25–28 rue du docteur Roux, 75724 Paris Cedex 15, France*

In this paper we consider the main steps in the process of manufacture of oral polio vaccine and assess the probable clearance factor for HIV retrovirus at each step. We conclude that the processes employed would have eliminated retrovirus contamination for all practical purposes.

Keywords: HIV; oral polio vaccine; clearance factors; extraneous agents; manufacture

We examined the probability that simian retroviruses could have contaminated oral polio vaccine (OPV), because we were two of a dwindling band of virologists involved in OPV manufacture in the 1950s. One of us (F.H.) attended the first course on tissue culture methods in virology at the Pasteur Institute under Pierre Lépine and was involved in the manufacture and control of both injectable polio vaccine (IPV) and OPV in Bucharest and Paris. The other (J.B.) worked under Andrew Rhodes at the Hospital for Sick Children in Toronto from 1954 to 1956 and had contact with the group at the Connaught Laboratories pioneering the development of polio vaccines. J.B. was later involved in making first IPV and then OPV for Glaxo Laboratories from 1958. F.H.'s review (Horaud 2000) of Hooper's (1999) *The river* provided the basis for this presentation.

The interest in the possible contamination of OPV with retroviruses stems from Hooper's hypothesis (Hooper 1999) about the origin of acquired immune deficiency syndrome, which he has already powerfully argued. This hypothesis requires that chimpanzee kidney cells carrying the simian immunodeficiency retrovirus SIVcpz were used by Koprowski to make some batches at least of OPV used experimentally in the old Belgian Congo. It also requires that even if contaminated cultures were used that any retrovirus present would survive the process of preparation of the vaccine. This possibility has to be examined seriously because there is a long history of the contamination of vaccines by extraneous agents, well documented by Wilson (1967) in his book *The hazards of immunization*.

The worst incident was during the Second World War, when yellow fever vaccine was contaminated by hepatitis B virus present in the human serum used to stabilize the virus during freeze-drying. There were 28 585 cases with 62 deaths among the 2–2.5 million US army personnel vaccinated in the first six months of 1942. Closer to home, the simian virus SV40 contaminated many of the early batches of both IPV and OPV. J.B. remembers the shock when this new agent was first described and looked apprehensively, to see whether our vaccine was contaminated. I was lucky, because for IPV, Glaxo still used the Maitland style of culture, which fortuitously is an inefficient way of growing SV40 virus. It is ironic that Dr Koprowski was the first to tackle the problem of simian virus contamination, by sponsoring the work of Hayflick & Moorhead (1961) on human diploid cells, and when they came available using them for vaccine production.

Before considering the question of the likely survival of SIVcpz through the production process for OPV, I must on F.H.'s behalf address the question of Lépine's advocacy of OPV as a booster dose after a primary course of IPV. Hooper in his book suggests that Pierre Lépine may have used living attenuated polio vaccine grown in cells of baboons (*Papio papio*) or sooty mangabeys, which harbour the precursor of human immunodeficiency virus type 2 (HIV-2), or even in chimpanzee kidney cells, such vaccine being given subcutaneously as a booster dose to a primary course of immunization with killed polio vaccine. F.H. worked closely with Lépine on polio vaccine, and was in an authoritative position to know about what went on at the Pasteur Institute in Paris where he attended the first tissue culture for virology course in 1956 and later from 1958 worked on the testing of IPV and later OPV. Although Lépine often considered the possibility of using a living vaccine given orally to boost immunity provided by IPV, and went so far as to test live virus in chimpanzees, he never developed nor produced any OPV himself. In the early 1960s he imported Sabin vaccine and did some trials in Africa with vaccine given orally, to humans. Hooper in his book acknowledges (Hooper 1999, p. 297) that his 'writing style does not make it easy to divine his exact meaning'. F.H. believed Hooper failed to grasp his meaning. Both (Hooper and F.H.) state that there was no published record of Lépine using attenuated vaccine in human trials apart from the work he did with Sabin's strains.

He (Lépine) was very conservative, relying on IPV much longer than many of his contemporaries. The IPV he made at the Pasteur Institute differed from standard Salk vaccine in several respects: (i) the monkey species used was *P. papio*; (ii) the poliovirus type 1 strain used was #13.42, an attenuated strain of low neurovirulence, instead of the Mahoney virulent strain advocated by

* Author for correspondence (j.beale@dircon.co.uk).
† Deceased.

Table 1. *Estimated clearance factors (log ID_{50}) for SIV during manufacture of OPV*

process step	clearance	evidence
washing kidney pieces	> -1	F.H.'s and J.B.'s experience
trypsin (0.25%) 1.5 h	-5	Garrett *et al.* (1993)
incubation 37 °C, seven days, and 34 °C, two days	> -1	McDougal *et al.* (1985)
washing before poliovirus inoculation	> -1	F.H.'s and J.B.'s experience
freezing and thawing at −20 °C four times	-2	F.H.'s experience
Seitz filtration	-3	F.H.'s and J.B.'s experience
total clearance	-13 (i.e. 10^{13}-fold)	—

Salk; and (iii) influenced by the Cutter disaster he used two virus-inactivating agents, formaldehyde and beta propriolactone. F.H. was certain he (Lépine) did not use any attenuated vaccine other than Sabin strains and also never gave an OPV subcutaneously.

Lépine was thus very conscious of the risks of vaccination and cautious in dealing with them.

The question as to whether chimpanzee kidneys were used for vaccine production by Koprowski and his colleagues has been considered by others already. I have only one contribution to this part of the debate and that is to calculate how many doses of vaccine could be made from a pair of kidneys. From our experience you could expect in those days to get between 1 and 5 litres of virus harvest from a pair of kidneys and the harvest titre would be some 40 million infectious virus particles ml^{-1}. Since in the Congo trials of monovalent vaccine they used the relative low dose of 100–200 000, so that there was at least a 200-fold dilution to make the vaccine judged by the titres reported by Plotkin *et al.* (this issue) for various pools of CHAT and a pair of kidneys would produce about 100 000 doses of OPV, and maybe 200 000 doses. Thus less than a dozen chimpanzee kidneys would be needed to provide the vaccine used in the Congo, much less than the 80 postulated by Hooper; but likewise there would be no shortage of rhesus or cynomolgus monkeys either.

We have to assume for today's discussion that chimpanzee kidneys infected with SIVcpz were used to make OPV; although it seems unlikely given the contemporary evidence. The question is, therefore, is it probable that the contaminating virus would survive the steps involved in OPV production? Here I consider six steps, as shown in table 1.

First the kidneys were dissected to yield cortical tissue, cut into 1 mm^3 pieces and washed at least three times in 0.25% trypsin in medium 199. I have estimated that this procedure would reduce the titre of virus at least tenfold; although by dilution the figure should be nearer 1000-fold. The figure is reduced because of the inefficiency of washing clumps of tissue. Certainly the amount of extracellular virus should be substantially reduced. Cell-associated virus in macrophages, a small amount in total, would, however, remain.

The second step was trypsinization. By the time J.B. and colleagues started to work on polioviruses all progressive laboratories were using trypsinized monolayer cultures for all experimental and assay work with polioviruses, although we at Glaxo were still using the Maitland culture technique for IPV production. It is overwhelmingly likely that such monolayer cultures would have been used at the Wistar Institute, for work on an experimental OPV. J.B. has found a paper from Glaxo that we submitted to *Virology* in November 1959, although it was not published until 1960 (Bishop *et al.* 1960) detailing the results of more than two years' experience with trypsinized monkey kidney cells. Thus a revised improved procedure was in use on a routine basis in our laboratories by 1957. Based on the careful work of Garrett *et al.* (1993) it seems that at least a 100 000-fold reduction of titre would be produced by exposure to 0.25% trypsin solution at 24 °C for 1.5 h (at Glaxo we used 0.25% trypsin at 37 °C for at least 1 h).

Hooper considers Garrett's results a near fatal blow to his hypothesis, but raises three objections:

(i) Koprowski may not have used trypsinized monolayers; of course, he may not have done but it is in my view improbable, since he had a laboratory at the cutting edge of the technology. Plotkin *et al.* (this issue) have provided clear evidence that such trypsinized cell monolayers were used. In their fig. 1 they refer repeatedly to 'MK' plaques. This plaque technique for selecting pure strains of virus requires the use of trypsinized monolayer cell cultures. Their second piece of unwitting evidence is in their fig. 2, which records the titres of various pools of the SM CHAT plaque line. Most of the titres are in the range 7.0–8.2 ID_{50}, figures rarely if ever reached in Maitland type cultures.

(ii) He may have used less trypsin for a shorter time. Again, he may have done but it seems more likely he used the techniques then generally available in the USA, for example by Bodian (1956).

(iii) Macrophages or leucocytes present in the cultures may be infected with SIV and escape inactivation by trypsin, only later to emerge and contaminate the vaccine. We may have missed something but neither of us can recollect seeing the numerous macrophages in such cultures reported by Hooper, to be seen by Robin Weiss for example (Weiss, this issue). However that may be, the results of Garrett's experiments with monolayer cultures from kidneys of SIV-infected monkeys, or cultures infected *in vitro*, do not support the view that SIV can survive the trypsinization process whether extra- or intracellular. Neither type of experiment produced any SIV, as assessed by a sensitive polymerase chain reaction technique for detection of minute amounts of virus. Moreover, after preparation of the monolayers the cultures have

at least one medium change and are washed before infection with poliovirus. These procedures would remove more macrophages from the monolayers. Moreover, the freezing and thawing and filtration steps would remove any remaining cells and cell debris.

The third step in vaccine production was the incubation at 37 °C for seven days followed by three to four days at 34 °C. Based on McDougal *et al.* (1985) this would reduce the titre of virus at least tenfold. Resnick *et al.* (1986) suggest a higher clearance, probably a 1000-fold.

Fourth was another washing step. Before the cultures were infected with poliovirus one week after being set up, they were thoroughly washed to remove the serum added to the growth medium. This was accomplished by washing the culture bottles with 100 ml of saline twice. This should make at least a 100-fold reduction in the titre of any contaminant. In order to be conservative we have claimed a tenfold reduction.

The fifth step was the repeated cycles of freezing and thawing, in those days to −20 °C at least three times; first after harvesting the virus, second at the time of filtration and finally just before the virus was diluted to make the final preparation for administration to vaccinees. According to F.H.'s experience these freezing and thawing cycles would lead to a 100-fold reduction in titre. No one now uses −20 °C storage because of its deleterious effect on viruses, and although J.B. has not been able to find data to substantiate F.H.'s opinion, he thinks it is a valid estimate.

The sixth and final step, which might have reduced the titre of any contaminating retrovirus, was filtration to render the product sterile. In those days, this was achieved by filtration through Seitz filters made of cellulose and asbestos. These filters were notorious for taking out viruses by adsorption to the fabric of the filter. Polioviruses could be filtered with little loss of titre by first satisfying the filter with gelatin solution, but enveloped viruses, for example influenza viruses, were associated with such high losses as to make the procedure impracticable as a step in vaccine production. We estimated a loss of at least 1000-fold.

In summary, there was therefore an approximate 10–100 UK billion-fold (million×million, i.e. 10^{12}) reduction of titre of any contaminating retrovirus that might have been present. It is not surprising therefore that Garrett *et al.* (1993) found no SIV present in early batches of OPV, even those produced before it was required that monkeys used for vaccine production lacked antibodies to the appropriate SIV.

The next question is how much SIVcpz would be present in chimpanzee kidney cells. As mentioned already, the overwhelming weight of the evidence makes it unlikely that such cells were used. Even if they were, the accepted level of contamination is 2% so that since only a very few chimpanzee kidneys are required for 100 000 doses of monovalent vaccine, the chances of using kidneys from an infected monkey are low. Hooper (1999) has pointed out, however, that cross-infection could easily occur, so we should assume infected kidneys might have been used. It seems that the level of contamination in kidney tissue would be relatively low. However, Platak *et al.* (1993) estimated that the level of HIV infection in humans may be as high as 10 000 50% infectious doses (ID_{50}) in plasma. Although this seems a very high infectivity for kidney tissue, our summary shows a very large safety factor in the clearance data presented here. Thus, in conclusion, although it may be more by good fortune than deliberate intent, it seems impossible that any SIVcpz could have contaminated Koprowski's experimantal OPV CHAT used in the old Belgian Congo; even in the very unlikely event that chimpanzee kidney cells were used as the substrate.

REFERENCES

Bishop, L. W. J., Smith, M. K. & Beale, A. J. 1960 An apparatus for producing trypsinized monkey kidney cell suspensions. *Virology* **10**, 280–283.

Bodian, D. 1956 Simplified version of dispersion of monkey kidney cells with trypsin. *Virology* **4**, 575–576.

Garrett, J., Durham, A. & Wood, D. J. 1993 Retroviruses and polio vaccines. *The Lancet* **342**, 932–933.

Hayflick, L. & Moorhead, P. S. 1961 The serial cultivation of human diploid cell strains. *Exp. Cell Res.* **25**, 585–621.

Hooper, E. 1999 *The river: a journey to the source of HIV and AIDS*. Boston, MA: Little, Brown & Co.

Horaud, F. 2000 La pandemie du sida a-t-elle une origene iatrogene? *Virologie* **2**, 87–93.

McDougal, J. S., Martin, L. S., Cort, S. P., Mozen, M., Heldebrant, C. M. & Vatt, B. L. 1985 Thermal inactivation of the acquired immune deficiency syndrome virus, human T lymphotropic virus III/lymphopathy-associated virus, with special reference to antihemophilic factor. *J. Clin. Invest.* **76**, 875–877.

Platak, M., Saag, G. M., Yang, S. L., Clark, S. J., Kappes, J. C., Luk, K. C., Hahn, B. H., Shaw, G. M. & Lifson, J. D. 1993 High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science* **259** 1749–1753.

Resnick, L., Veren, K., Salahuddin, S. Z., Tondreau, S. & Markham, P. D. 1986 *J. Am. Med Ass.* **255**, 1887–1891.

Wilson, G. 1967 *The hazards of immunization*. London: Athlone.