CHAT Oral Polio Vaccine Was Not the Source of Human Immunodeficiency Virus Type 1 Group M for Humans

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A book published in 1999 hypothesized that the scientists who worked with the CHAT type 1 attenuated poliomyelitis strain, tested in the former Belgian Congo in the late 1950s, had covertly prepared the vaccine in chimpanzee kidney cells contaminated with a simian immunodeficiency virus, which evolved into human immunodeficiency virus type 1 group M. This article summarizes the results of the investigation conducted by the author to determine the legitimacy of the accusation. Testimony by eyewitnesses, historical documents of the time, epidemiological analysis, and analysis of ancillary phylogenetic, virological, and polymerase chain reaction data all indicate that this hypothesis is false.

Poliomyelitis is rapidly disappearing from the world, thanks in large part to the widespread use of the oral polio vaccine (OPV) strains developed by Albert Sabin. The precursors of the Sabin strains were those developed by Hilary Koprowski, first at the Lederle Laboratories and then at the Wistar Institute in Philadelphia. The results of the first administration of OPV to humans were published by Koprowski et al. in 1952 [1] and concerned the TN strain, later identified as type 2 poliovirus. A type 1 strain, called SM, was reported in 1954 [2], and its descendant, a virus called “CHAT” was reported in 1957 [3].

The latter strain concerns us here, for in 1999, a British journalist published a book called The River: A Journal to the Source of HIV and AIDS [4], which proposed the hypothesis that CHAT had been produced in cells from chimpanzees that were contaminated with the simian precursor of HIV type 1 (HIV-1) group M, the major agent of the AIDS epidemic. This article examines that hypothesis in detail.

For a proper understanding of the events recalled in this article, the state of polio vaccine development in the late 1950s is germane. In the late 1950s, obtaining cultures of kidney cells from rhesus monkeys was routine, and commercial laboratories were selling trypsinized kidneys or monolayer cultures in glass bottles. The plaque technique of Dulbecco and Vogt had also come into general use, permitting for the first time cloning (in the old sense) of genetically distinct virus populations. (Therefore, whereas both the early TN type 2 and SM type 1 attenuated strains of Koprowski had been attenuated in rodents, the SM strain was then further attenuated by alternate passages in chick embryo and cultures of monkey kidney cells.)

However, the SM strain appeared to be too neurovirulent [5, 6], and a strain called SM-N90 was passed 4 times serially in humans by oral administration of filtered fecal virus, isolated after replication in the intestine. After the fourth human passage, the virus was plaque 4 times (“plaque purification”) in cultures of monkey kidney cells, and the resultant strain was renamed CHAT, after the name of the baby in whom the last human passage had been made.

The plaque passage history was shown in detail in Koprowski’s article (figure 1) [3]. The sequence of plaque passage was plaque 9 to plaque 13 to plaque 20 to plaque 36. The most extensively tested virus was derived from plaque 20, from which 7 plaque derivatives were tested for neurovirulence in monkeys. Plaque 20 was also tested in chimpanzees.

Therefore, the situation at the end of the 1950s was that attenuation was accomplished by an empiric process of passage in animals or cultures of kidney cells, hoping that the “un-
natural" conditions would select virus strains with the desired qualities. Attenuation was unpredictable and reversion to virulence was feared. However, by early 1957, Koprowski was convinced that a vaccine trial on a large scale was justified. Meanwhile, Koprowski had met a Belgian scientist named Ghislain Courtois, who held a senior public health laboratory position in the country then called the Belgian Congo. Because polio was endemic in the Congo in both Africans and Europeans [7, 8], permission for human vaccination in this region was readily obtained from the Belgian authorities.

Mass vaccination campaigns were undertaken in the Congo, starting in February 1957 in various villages in northeastern Congo where polio infections were occurring, and leading up to a major campaign in the Ruzizi Valley that was conducted February–April 1958 [9]. At the time, Sabin was preparing for mass vaccination campaigns in what was then the Soviet Union.

In 1992, a journalist wrote a story in a popular magazine suggesting that CHAT had been made in cultures of African green monkey kidney cells [10]. Because African green monkeys are known to carry simian immunodeficiency virus (SIV), contamination of the cultures was postulated to be the source of HIV-1. However, SIVagm is too distant from HIV-1 to have been its origin. The publication of The River in 1999 raised renewed interest in the poliovirus theory because the chimpanzee virus, SIVcpz, is genetically close to HIV-1.

The claims made in The River are based on 2 assertions: first, that the CHAT vaccine was prepared in kidneys obtained from SIV-infected chimpanzees from a colony established in the Belgian Congo; and second, that there was a coincidence in the locations of sites where vaccine was administered and sites where early cases of AIDS were identified. The controversy raised by The River culminated in a meeting held in London on 11–12 September 2000 by the Royal Society to consider the origin of HIV-1 [11]. Many of the protagonists of the controversy were present at that meeting, and this article is a detailed version of the paper I gave at that conference in which both of the major assertions of The River are refuted [12]. (At the Royal Society meeting, the author of The River added new charges to those in the book, now saying that the kidneys of chimpanzees were excised in Bujumbura [Burundi] and sent to 2 veterinary laboratories in Rwanda and the Belgian Congo, where cultures were performed to provide cell substrate for manufacture of CHAT virus. Later investigations by myself and by my colleagues are detailed in an article to be published with the conference proceedings [11]. These investigations showed no evidence of the presence of chimpanzees in Bujumbura and
no evidence for removal of kidneys from chimps at that site; and evidence was found that contradicted the assertion of vaccine manufacture at veterinary laboratories. Therefore, the new wild allegations are also false.)

**PRODUCTION OF THE CHAT TYPE 1 ATTENUATED STRAIN**

As recounted above, there is no mystery about either the name or the origin of the CHAT strain, which is called the source of the HIV-1 group M epidemic by *The River*. Figure 2 illustrates the history of CHAT; I drew this up in 1958 or early 1959. Figure 2 is illuminating for several reasons, as follows:

1. The derivation of the name of the strain is stated. “CHAT” comes from “Charlton,” the name of an infant who was given the SM-N90 predecessor of CHAT at an institution for retarded children in Sonoma, California, in 1956 [5]. That the name of the strain CHAT was so derived was common knowledge around the laboratory in 1957. The name does not stand for “chimp-attenuated,” as suggested in *The River*.

2. All attenuating passages are indicated as having been made in MK, which stands for monkey kidney. These were cells of rhesus and cynomolgus origin received as suspended cells or monolayers commercially prepared by Microbiological Associates. I would not have referred to chimpanzee kidney as MK, considering that the chimpanzee is an ape, not a monkey.

3. Pool 10A-11 is a mixture of pools 10 and 11, evidently combined to provide sufficient volume for the Congo vaccination.

4. No seed system was used. Rather, each pool served as the seed virus for a subsequent pool. Today, to avoid possible changes in the properties of the virus by passage of cell cultures, one would create a seed virus from which all subsequent pools would be prepared. In those days, the system was to passage the virus and to check each passage for neurovirulence in primates. Therefore, when evaluating the PCR results given later, it should be understood that there was no seed virus to test, only the pools themselves.

5. However, when Wyeth, the commercial vaccine manufacturer, became involved in production of CHAT, they did produce a seed pool, which served to generate a vaccine pool called Wyeth 2-4B-5.

**CELL SUBSTRATE FOR CHAT**

*The River* claims that CHAT was produced in cultures of chimpanzee kidney cells, rather than cultures of macaque kidney cells, and that this occurred in Philadelphia, in Belgium, or in the Congo itself.

With regard to Philadelphia, the reason for using chimpanzee cells advanced by the book is that there were difficulties in obtaining rhesus cells during 1957–1960 because of exportation bans by India. However, Dr. Joe Held, a retired veterinary primatologist who was active at that time, comments as follows in a letter to me dated 20 March 2000:

1. No important interruption in the supply of rhesus monkeys occurred between 1957 and 1960.
2. I do not have readily at hand data regarding the actual numbers imported between 1957 and 1960. In 1955, the first year in which polio vaccine was licensed in the U.S., over 200,000 were imported. Following that, the numbers began to decrease incrementally each year, down to a level of about 14,000 per year in the mid-1970s.
3. Both cynomolgus and rhesus macaques were included in those imports.
4. I never heard of polio vaccine being made in chimpanzee cells.

I was in the Koprowski laboratory from August 1957 through June 1961, and never received, worked with, or saw others working with cells from chimpanzees. Macaque cells in suspension or in monolayer were regularly received from Microbiological Associates, a commercial laboratory. We may on occasion have received cells from Flow Laboratories, another commercial source. The testimony of other people in the laboratory during the entire period that vaccine was made at Wistar is also available. Most important, Barbara Cohen, the technician who opened Koprowski’s polio laboratory at Wistar, who was in charge of that laboratory during the entire period, and who made all of the vaccine produced at Wistar, completely denies the existence of any chimpanzee cells in the Wistar lab.
In the following notarized statement dated 15 November 1999, she writes:

1. I came to work at Wistar in June 1957 as chief technician in the laboratory of Hilary Koprowski. I worked there until June 1961.

2. At no time did I ever receive or work on chimpanzee kidneys, nor to my knowledge, cells derived from chimpanzees. I never made, nor know of anyone in the lab who made, polio vaccine in chimpanzee cells. However, I did receive serum and stools from those animals to test for poliovirus and antibodies.

3. The cells used to produce the CHAT and other polio vaccines were labeled “rhesus monkey kidney” and were obtained from a commercial supplier, I believe.

In addition, I have located all the people still alive who were in the Koprowski polio laboratory from 1957 through 1960. Table 1 summarizes their jobs at the time. These workers all deny that chimpanzee kidneys or cells were present in the laboratory. In addition, 2 people involved in the manufacture of CHAT at Wyeth Laboratories, Dr. Alan Bernstein and Dr. Howard Tint (personal communications) both deny that chimpanzee cells were ever used at Wyeth.

A digression is necessary here. Dr. Fritz Deinhardt came to the Congo in late 1957 and early 1958 to attempt infection of chimpanzees with human hepatitis viruses. In the search for a kidney cell culture system for growing hepatitis virus, he had 6 kidneys from chimpanzees sent to his laboratory at the Children’s Hospital of Philadelphia for preparation of kidney cell cultures [13]. Although Dr. Joseph Stokes Jr., chief pediatrician at the Children’s Hospital of Philadelphia, was associated with the vaccine trials in New Jersey, the hepatitis work was carried out exclusively at the Children’s Hospital of Philadelphia research laboratory headed by Werner Henle, which was then located in another part of Philadelphia. There is absolutely no evidence for the assertion that chimpanzee kidneys also found their way to Wistar.

We and many other researchers in the late 1950s referred to animals whose kidneys were used in cultures of tissues as “monkeys” or “macaques.” Further specification of these donor animals was not necessary because no other animals were used for vaccine preparation. If one examines 2 references extensively cited in The River, the New York Academy of Sciences Symposium of 1957 and the Pan American Health Organization conference of 1959, no paper gives the species of the monkey cellular substrate for virus passage! Only in the discussion of the Pan American Health Organization conference are rhesus cells mentioned—and then only once and in passing. However, in a paper published by Koprowski [14] in 1961, he writes:

The material used for growing poliovirus in tissue culture consists of living cells obtained from the freshly harvested kidneys from monkeys brought to the U.S. either from India or from the Philippines…. However, it would be more difficult to justify scientifically a stand that nothing should be done in the immediate future about the host cells in which polioviruses are grown. Not only has the existence in monkey tissue of the dreaded [herpes] B virus (which is definitely pathogenic for man) been known for some time, but it is clear that tissues obtained from the next batch of killed monkeys may contain more “virus surprises.” Monkey tissue can be supplanted as the host system for growth of polioviruses.

Also in 1961, in an article about the intratypic serodifferentiation of polioviruses, we tested 5 lots of the CHAT virus. The Material and Methods section says that “Primary cultures of monkey kidney cells were used in all the work described here, except in the case of a single pool of CHAT virus which was prepared in a culture of human diploid cells as described elsewhere” [15]. In table 2, the pools that were produced outside of Wistar (namely, in Belgium and Wyeth Laboratories, as I will show later) are specified, and in addition, the pool that was produced in human diploid cells was specified as an exceptional cell substrate. In the corresponding text, it says “R_{90} values for 7 pools of CHAT virus (including 2 prepared in this laboratory, 3 in other laboratories, and 1 made in a human cell strain rather than in monkey kidney cells) were determined using anti-CHAT serum” [15].

In 1960, I spoke at a meeting in Wiesbaden, Germany, shortly after the discovery of SV40 in the kidney cells of rhesus monkeys. The published paper based on this presentation [16] refers to that discovery and recounts the medical follow-up of infants

<table>
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<tr>
<th>Site, researcher</th>
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<tbody>
<tr>
<td>Wistar Institute (Philadelphia)</td>
<td></td>
</tr>
<tr>
<td>H. Koprowski, laboratory director</td>
<td></td>
</tr>
<tr>
<td>S. Plotkin, J. Pagano, and R. Carp, postdoctor fellows</td>
<td></td>
</tr>
<tr>
<td>G. Theiss, predoctoral fellow</td>
<td></td>
</tr>
<tr>
<td>B. Cohen, A. Kamrin, and S. Richardson, technicians</td>
<td></td>
</tr>
<tr>
<td>Rega Institute (Leuven, Belgium) and Recherches et</td>
<td></td>
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<tr>
<td>Industries Therapeutiques (Rixensart, Belgium)</td>
<td></td>
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<tr>
<td>A. Prinzie, chief scientist</td>
<td></td>
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<tr>
<td>M. Lamy, C. Huygelen, J. Peetermans, and P. Kolosi,</td>
<td></td>
</tr>
<tr>
<td>scientists</td>
<td></td>
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<tr>
<td>J. Costermans, technician</td>
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<td>Provincial Medical Laboratory (Stanleyville, Belgian</td>
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<td>Congo)</td>
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<td>P. Osterreith, laboratory director</td>
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<td>G. Ninane, pathologist</td>
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<td>P. Dherte, pharmacist</td>
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*This laboratory did not produce CHAT poliovirus vaccine, but it was accused of doing so.*
Table 2. Lots of CHAT poliovirus vaccine that were certainly or possibly used in the Belgian Congo.

<table>
<thead>
<tr>
<th>Lot</th>
<th>Manufacturer</th>
<th>Date</th>
<th>Where used</th>
</tr>
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<tbody>
<tr>
<td>8 or 9</td>
<td>Wistar Institute</td>
<td>1957</td>
<td>New Jersey, northeast Congo</td>
</tr>
<tr>
<td>10A-11</td>
<td>Wistar Institute</td>
<td>Early 1958</td>
<td>New Jersey, Ruzizi Valley, Sweden, Switzerland</td>
</tr>
<tr>
<td>13</td>
<td>Wistar Institute</td>
<td>Late 1958</td>
<td>New Jersey, Léopoldville, Poland</td>
</tr>
<tr>
<td>2-4B-5</td>
<td>Wyeth Laboratories</td>
<td>Early 1959</td>
<td>Congo?</td>
</tr>
<tr>
<td>DS101</td>
<td>RIT</td>
<td>Late 1959</td>
<td>Rwanda-Urundi, Congo?</td>
</tr>
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</table>

**NOTE.** RIT, Recherches et Industries Therapeutiques.

\[ ^{a} \text{Wistar Institute, Philadelphia, PA; Wyeth Laboratories, Marietta, PA; Recherches et Industries Therapeutiques, Rixensart, Belgium.} \]

vaccinated with Koprowski OPV strains to determine if any had developed cancer:

Recently a vacuolating simian virus has been demonstrated by Sweet and Hilleman in almost all Rhesus monkey kidney tissue cultures. Whether they are infectious for humans is a matter of dispute. Our own studies of antibodies to the vacuolating agent in the sera of live-virus vaccines are just getting underway. We have, however, already determined whether the newborn infants we have vaccinated since 1955 have suffered an unusual incidence of serious illness in the intervening years. Approximately 200 children have been investigated, 1–3½ years after oral ingestion of live-virus vaccines which presumably contained the vacuolating agent. Only one death has occurred in this group of children (due to accidental trauma) and there have been no other cases of serious illness.

Then 2 methods for elimination of SV40 from the vaccine–ion exchange chromatography or passage in human diploid cells were also discussed: “Despite the probable harmlessness of these adventitious agents, 2 lines of research are being pursued at the Wistar Institute in an effort to obtain live-virus vaccines containing only poliovirus. First is an attempt to purify live-virus vaccine by passage through a cellulose ion exchange resin. In this technique, virus prepared in Rhesus monkey kidney is dialyzed and put on a diethyl aminoethyl cellulose (DEAE) column in 0.01 M tris buffer” [16]. Can this be interpreted in any other way but that we had been using kidney cells from rhesus monkeys to make the vaccines?

In 1959 I wrote about “the monkey kidney–adapted CHAT strain” [17], and in 1965, with regard to the use of OPV in newborns, I stated that “CHAT, type I attenuated poliovirus prepared in primary rhesus monkey kidney tissue culture, was used” [18]. In another paper written with Leonard Hayflick on the adaptation of CHAT to human diploid cell strains [19], one finds the statement, “when the CHAT strain of poliovirus, prepared in monkey kidney tissue culture (CHAT-MKTC) was inoculated directly into human diploid cells, titers were usually 1 to 2 \( \log_{10} \) TCID\text{\textsubscript{50}} lower.”

In 1962, in an unpublished document submitted to the World Health Organization, Tom Norton (personal communication) wrote, “The ID\text{\textsubscript{50}} [in humans] of the CHAT strain prepared in human diploid cells appeared to be about \( 10^{4.5} \) TCID\text{\textsubscript{50}}, which is similar to the ID\text{\textsubscript{50}} as previously determined with vaccine prepared in monkey kidney tissue culture. The monkey kidney vaccine has been fed to approximately 10 million children.” Clearly, he was contrasting human cell–grown virus with what came before.

Finally, the only surviving protocol for making a pool of CHAT, dating from 1960, states that its substrate is primary monkey kidney [20]. Therefore, there is no evidence for the assertion that cells from chimpanzees were imported to Wistar and no evidence that vaccine made in Philadelphia was prepared in any other cells than those derived from cultures of kidney cells from macaque monkeys.

**BELGIUM**

A second site where The River says chimpanzee cells were used to make OPV was in Belgium. Early in the development of the vaccine, Koprowski was contacted by Pieter De Somer, a Belgian virologist at the University of Leuven, who in 1953 founded an institute for virology called the Rega Institute. In 1957, with others, he founded a commercial vaccine laboratory as part of a pharmaceutical company called Recherches et Industries Therapeutiques (RIT), the precursor to SmithKline. This laboratory first made a Salk-type vaccine, and then in 1960, it went into the production of Sabin strain OPV.

Three questions are raised in The River: (1) was CHAT strain OPV ever made at the Rega or at RIT; (2) were chimpanzee cells used to make the vaccine; and (3) was Belgian-made vaccine used in the Congo?

Fortunately, most of the witnesses in these matters are still alive, although De Somer is not. With regard to the first question, the manufacture of CHAT, it is possible that small amounts of CHAT were produced at the Rega for research purposes, but it is extremely unlikely that Rega would have
undertaken vaccine production, in view of the small size of the unit and its dedication to research. Such would have been feasible only from 1957 on at RIT, the commercial laboratory. Indeed, in early 1959, I tested CHAT vaccine produced in Belgium and administered it to babies at a women’s prison called Clinton Farms, in Clinton, New Jersey, in April 1959.

Were chimpanzee cells used to make the vaccine? The testimony of 6 people is relevant: Abel Prinzie, Julian Peetermans, Josette Costermans, Monique Lamy, Paul Kolosi, and Constant Huygelen, whose functions are listed in table 1. In a signed statement dated 9 December 1999, Prinzie states:

> We never (and I absolutely underline never) used chimpanzee tissues or cells; we only used kidney tissue cultures from Macacus rhesus, Macacus cynomolgus, and later, Cerco pithecus (AGM).

As far as CHAT strain production is concerned, we may have produced a small pool of virus at the Rega around 1958, just as we reproduced many other polio-virus strains for laboratory purpose. I emphatically deny Hooper’s annotation on p. 789 (The River) that I said it was “intended for the Ruzizi valley vaccination.” I resent such a false quotation where a mere hint made by the interviewer during a conversation later in the book is presented as a solid fact attributed to the interviewee.

Costermans, in a signed statement dated 18 February 2000, states: “I was technician at the Rega Institute (Leuven) from 1956 to 1974. During that time I was in charge of tissue cultures and serological testing in the laboratory. I can state categorically that during my stay at the Rega Institute, there was never was a chimpanzee in the animal house and we never prepared tissue cultures from chimpanzee organs or tissue.” In a signed statement dated 27 December 1999, Lamy states:

> When I assured with Professor P. De Somer (1956–60) the development and production of antipolio vaccines, I can certify the following:

> We never used chimpanzee cells, neither for the adaptation of strains nor for the production of vaccines, whether it be for the Salk type vaccine or the Sabin live-type vaccine (author translation).

In a signed statement dated 8 December 1999, Huygelen states, “I had quite a few conversations with Julien Peetermans and Monique Lamy, who had also been directly involved in the production of the Koprowski virus and also in the testing of that virus, both CHAT and Fox. Never was the use of chimpanzees mentioned, and I am sure that if these apes or their tissues had been used at RIT, I would have heard about it.”

As summarized in table 1, the Belgian workers uniformly deny the allegation that chimpanzees, kidneys from chimpanzees, or cells from chimpanzees were ever introduced into Rega or RIT. Their testimony confirms that it would have been inconceivable for a commercial vaccine company to produce a human vaccine in a completely unknown and untested substrate.

With regard to the shipment of RIT vaccine for use in the Belgian Congo, because CHAT vaccine produced in Belgium was administered to babies in New Jersey for the first time in April 1959, it could not have been used before mid-1959 because no results would have been available before that time. Moreover, an unpublished document from 1959 (a protocol for vaccination in Rwanda-Urundi) relative to the completion of the vaccination campaign of Rwanda-Urundi mentions the use of CHAT lot 101. Therefore, Belgian vaccine could only have been used in late 1959 or 1960, too late to have been involved in the implantation of HIV. In any case, the essential point is that even if Belgian vaccine was used in the Congo, it was not made in cells from chimpanzees.

**STANLEYVILLE**

Now we come at last to the Congo itself. *The River* hypothesizes that CHAT vaccine was produced in cells from chimpanzees at the Provincial Laboratory of Stanleyville (now known as Kisangani), in the Belgian Congo (now northeastern Zaire) on the Congo River, early in February–March 1958, to allow for the completion of vaccination in Burundi by Dr. Gaston Ninane. The source of the cells is alleged to have been from autopsies practiced on chimpanzees previously involved in studies of neurovirulence and efficacy of attenuated polio strains (see section below, “Chimpanzee Camp at Lindi”). The book argues that CHAT vaccine was in short supply, kidneys from chimpanzees were available locally, and Dr. Paul Oster-rieth whipped up a batch of CHAT vaccine in his virology laboratory at Stanleyville.

Vaccine was indeed in short supply in March 1958, as confirmed by Koprowski in a letter dated 4 March 1958 to George Jervis, who was then in the Congo. But in a postscript to the same letter, writing in response to a telegram from Ninane, Koprowski promised to send more vaccine by the end of March 1958: “P.S. Since I have written this letter, a telegram from Ninane arrived requesting 10,000 capsules of Type 1 and Type 111 and as much liquid vaccine as possible. I am sending him 5000 capsules of each and small amounts of liquid vaccine. I have advised him to request from you more of liquid Type 1 which will be sent to Usumbura end of March.” He makes no reference to vaccine from Belgium or to local production in the Congo.

With regard to the possibility that CHAT vaccine was produced in Stanleyville, Dr. Gaston Ninane is quoted in *The River* to the effect that he tried but failed to cultivate cells from chimpanzees at the Stanleyville lab. Dr. Ninane himself violently denied this allegation in a signed statement, dated 22 February 2000:
I never tried to make cell cultures in Stanleyville. The only time I made such attempts was at the University of Liège…. Consequently, I categorically deny having tried to make tissue cultures from chimpanzees. The statements that are attributed to me on this subject are false and are lies (author translation).

The only other person who could have prepared cultures of kidney cells was Dr. Paul Osterrieth, director of the virology laboratory. In his written statement, dated 28 February 2000, he denies that any vaccine was or could have been produced by him:

I was absent from Stanleyville between October 1957 and January 1958, at which time I attended a course on cell culture and serology at CDC and Wistar. While I was at Wistar I never saw or heard of the use of chimpanzee tissue or cell culture…. At my return from the U.S.A., I attempted to set up a cell culture laboratory in Stanleyville. It was difficult to do so because of the lack of the adequate equipment and material. As I recall, several months passed before I was able to succeed in the cultivation of HeLa cells and of kidney cell cultures from baboons. Aside from the limited success with baboon kidney cell culture I also tried to start cell cultures from the kidney of other species of small monkeys. Trypsin was uniformly used to disperse the cells from tissue…. However, at no time did I ever attempt to make cell culture from chimpanzee tissues. In addition, I wish to state categorically that no poliovaccine was ever produced or could have been produced in Stanleyville, since the facilities were totally inadequate for a production or control of poliovaccine.

It should be noted that Dr. Osterrieth had just come back from the United States in February 1958 and would have needed several months to set up a tissue culture laboratory, even if he wanted to produce polio vaccine.

In addition, Paulette Dherte, the pharmacist mentioned who served as assistant to Dr. Osterrieth, confirmed to me that the idea of vaccine production in Stanleyville was ridiculous (personal communication, 2000).

In addition to these testimonials, there is documentary evidence from the annual reports of the Stanleyville laboratory. The annual reports for 1956 [21] and 1957 [22] say nothing about tissue culture. The crucial 1958 report [23] says the following: “Tissue culture: was done exclusively on cynocephalus [baboon] kidney. 200 tubes and 10 bottles were made. Of the tubes, 36 served for 9 negative analyses. The rest of the tubes and the bottles served to prepare adenovirus antigen for complement fixation.” 1958 is the same year when The River says that CHAT vaccine was prepared at the Stanleyville laboratory in cells from chimpanzees. In the 1959 Stanleyville laboratory report [24], the report of tissue culture is limited to isolation of enteroviruses in HeLa cells or in baby mice. No culture from monkey or ape kidneys is mentioned.

However, The River presents another fallback hypothesis; namely, that autopsies were performed on chimpanzees at the Stanleyville laboratory and could have contaminated CHAT vaccine produced elsewhere. The stimulus for this hypothesis is a passage in the 1959 report which mentions that 250,000 doses of polio vaccine were put into final form at Stanleyville and sent to Usumbura. Putting into final form (the French word conditionné) could mean dilution, transfer to final containers, or both.

Could the vaccine have been contaminated by tissues from chimpanzees? Both Dr. Osterrieth and Dr. Ninane deny this possibility. In a signed statement dated 28 February 2000, Dr. Osterrieth remembers:

When I was not in my laboratory, the room was locked for fear of contamination of tissue culture and nobody else had access to the virological laboratory. Dead chimpanzees were autopsied in Stanleyville, but I wish to state that such autopsies of chimpanzees were never done in the laboratories within the main building. Moreover, I have no knowledge of poliovaccine being diluted or distributed into smaller flasks at the Stanleyville laboratories, and in any case it was never done in my laboratory…. I have no knowledge of a “conditioning” of vaccine which might have been done in Stanleyville. I never tried to dilute the polio vaccine that was received.

In a signed statement dated 22 February 2000, Dr. Ninane adds, “I never tried to dilute the polio vaccine that was received.” Furthermore, he adds, “No chimpanzee autopsy was performed in the same building as the laboratories” (author translation).

In any case, as the conditioning was done in 1959, it was too late to account for the positive serum from Léopoldville in 1959 or the launch of HIV in humans postulated by the book in 1958. The vaccine conditionné was probably the Belgian vaccine, because the same document that mentions the RIT vaccine says it will be sent to Stanleyville for dilution (unpublished protocol for vaccination in Rwanda-Urundi, 1959).

Thus the evidence presented by the scientists who were in the Stanleyville laboratory during the crucial period of 1957–1958 and the documentary evidence preclude the notion that vaccine was produced there or that chimpanzee cells were introduced into the vaccine.

There is another inference to be gleaned from the annual reports of the Stanleyville laboratory. The mere final preparation of a vaccine produced elsewhere was sufficiently daunting to be mentioned proudly, and yet we are asked to believe that the local production of a polio vaccine for human use would have gone unmentioned. Indeed, there appears to be no motive
in the late 1950s for the people concerned to have hidden the use of chimpanzee cells.

WHICH CHAT LOTS WERE USED IN THE CONGO?

Table 2 lists the lots of CHAT vaccine that were definitely or possibly used in the Congo. Pools 10A-11 and 13 and pools 8 or 9 were administered in Stanleyville and some other towns in northeastern Congo during 1957. Pool 10A-11 was used in the campaign conducted in the Ruzizi Valley, in 200 children in Sweden [25, 26], in Switzerland [27, 28], in infants at Clinton Farms, New Jersey [29], and probably in the Moorestown family trial conducted in Moorestown, New Jersey [28]. Pool 13 was used in Léopoldville [30], in several thousand children in Poland [31, 32], and in infants at Clinton Farms [29]. There is no evidence that any of the vaccinations led to infection with HIV. It is of interest to note that in one of the Léopoldville papers, it says, "exactly the same lot as used in Poland" [33].

The Wyeth pool (2-4B-5) was available by January 1959 (H. Koprowski, letter dated 4 December, 1958) and may have been used in the Congo, but there is no certainty about this. RIT Lot 101 was used in Rwanda-Urundi late in the vaccination campaign (1959–1960). There is no objective evidence that pools that bore the same numbers were produced locally in the Belgian Congo.

PCR STUDIES

PCR was performed for the detection of HIV-SIV on pool 10A-11 both by Dr. Jan Albert at the Karolinska Institute in Stockholm and by Dr. N. Almond at the National Institute of Biologial Standards and Controls in the United Kingdom. The results were negative (J. Albert and N. Almond, personal communications). In addition, the UK lab tested for the DNA of the cell substrate. The result was positive for macaque DNA but negative for chimpanzee DNA. More recently, the Wistar Institute organized blinded tests in 3 laboratories of 7 other pools of CHAT, including pool 13, which was used in the Congo. All were negative for HIV-SIV, and all lots gave PCR evidence of cellular DNA from macaque monkeys, but not of cellular DNA from chimpanzees [34]. Therefore, the PCR data are contrary to the OPV-HIV hypothesis.

ADAPTATION OF CHAT TO HUMAN DIPLOID CELLS

Aside from the chimpanzee SIV hypothesis of OPV contamination, it has been suggested that the CHAT virus was used in Africa had been passaged through human diploid cells, leading to what is termed “fast forward evolution” [35]. In fact, CHAT was adapted to human diploid cells as soon as the work of Hayflick and Moorhead [36] made them available, to avoid extraneous viruses present in primary kidney cells from rhesus monkeys. However, the work did not start until 1960 [19], and my records show that adaptation was still incomplete in September 1960, but in any case, no vaccine made in human diploid cells was ever used in Africa.

CHIMPANZEE CAMP AT LINDI

The centerpiece of The River’s detective story is that a camp for chimpanzees was organized by Koprowski and a Belgian scientist named Ghislain Courtois at a place called Lindi, near Stanleyville. The exact number of chimpanzees that passed through this camp is unknown but may have exceeded 400. However, the purpose of the camp was not at all mysterious and is stated clearly in an article published by Courtois [37]:

The proposal to establish this monkey house came from H. Koprowski, who wanted to experiment with, in a sufficient number of chimpanzees, the attenuated strains of poliovirus that he was developing with a view toward their use as vaccine. The first trials on lower monkeys had shown the quasiabsence of neurovirulence.... The goal of the experiments performed in chimpanzees consisted of vaccinating by the oral route a sufficient number of these animals, checking the immune changes thus provoked, then lastly infecting them by the oral route with a paralytogenic strain of polio to verify the protective power conferred. It was also a question of checking by intraspinal injection the neurovirulence of the strains used, as well as the possible changes that could have occurred in their pathogenicity after passage through the digestive tract of vaccinated infants (author translation).

The 1958 report of the Stanleyville lab [23] states, “The experiments performed on chimpanzees at Camp Lindi consisted principally at the beginning of the year to finish the polio experiments (infection trials, test of efficacy, test of intraspinal innocuity, etc.). Other experiments performed on new animals as well as those already used for polio had to do with trials with the virus of Infectious Hepatitis.”

The 1959 report [24] mentions additional polio experiments, in addition to measles and canine distemper virus inoculations in 32 animals, plus isolated experiments on allergic encephalitis, atherosclerosis, and diabetes. Studies of cancer induction in chimps are also discussed for the future.

With respect to the hepatitis experiments mentioned above, we have the unpublished notebook of Dr. Fritz Deinhardt. From this notebook, we learn that 54 chimpanzees were used in the hepatitis experiments. Their approximate ages, as recorded in the book for 52 of them, show that with 2 exceptions, all animals were aged <7 years. One of the 2 older chimps was acquired from a zoo, but it is not known at what age it came...
to the zoo, so only 1 of 52 animals was likely to have been sexually active. That animal was aged 7–10 years, as determined on the basis of his weight of 26 kg [38, 39]. The reason that the chimps were young (aside from the fact that they are easier to catch) is stated by Courtois in another article: “Apes weighing more than 20 to 25 kg must be considered as unmanageable and very dangerous” [40].

Deinhardt’s notebook also contributes other important pieces of information. The hepatitis experiments began in January 1958 and ended in May 1959. During that 17-month period, 187 biopsies of liver specimens were performed under anesthesia. The anesthetic used was called Nesdonal, which is the brand name of thiopentone sodium, a barbiturate much used for anesthesia at the time. Undoubtedly these numerous episodes of anesthesia for biopsy of liver specimens account for the description of anesthesia mentioned by an African attendant at Camp Lindi [4].

As far as the issue of kidney removal is concerned, we know that it was practiced 6 times for Deinhardt’s experiments. However, the author of The River claims that additional kidneys were sent to Wistar and to Belgium for polio vaccine manufacture. His evidence for this is remarks allegedly made to him by 2 veterinarians who were in Stanleyville, L. Bugyaki and J. Mortelmans. However, in a letter to Abel Prinzie dated 4 February 2000, Dr. Bugyaki writes:

In the course of my stay in Africa (1949–1959), I heard about the occasional dispatch of chimpanzees from Leopoldville to Belgium, and that already from 1949. Although I don’t know towards what destination and for what use, it was certainly not for laboratory use. I have no knowledge of dispatch of chimpanzees to the University of Louvain, nor of the dispatch of chimpanzee kidneys from Lindi camp to Belgium or to other countries.

Dr. Mortelmans, who in any case was not present in Stanleyville after 1956, denies any firsthand knowledge of chimpanzee organs sent to Philadelphia or Belgium. As a primatologist, he only evoked the possibility of using primate tissues to grow polioviruses. In a signed statement dated 20 April 2000, he notes:

On p. 573 [of The River] I am alleged to have said that chimp cell culture could have been used for manufacture of polio vaccine. In fact I have no personal knowledge of such ever having been done, and this was stated as a hypothetical possibility. Indeed, there are very good reasons not to use the chimp for such purpose. Chimpanzees are difficult to catch, difficult to keep, and expensive to maintain. Moreover, around Stanleyville, baboons, Cercopithecus monkeys and colobus monkeys were plentiful and easily available as a preferable source for tissue culture. Similarly, on p. 572, it is alleged that I supported the hypothesis that chimp kidneys could have been sent to Philadelphia or Belgium. In fact, it only said that such might well have been possible, but I have no personal knowledge that it has ever been done.

And what about the people who would have performed the autopsies on the animals and prepared the kidneys for dispatch elsewhere? In his signed statement, Dr. Ninane writes, “I firmly and categorically declare that I never sent chimpanzee kidneys nor any other organs of these animals towards other countries. I only sent microscopic slides for verification by other laboratories.” In his signed statement, Dr. Osterrieth acknowledges that he may have helped Fritz Deinhardt send minced kidneys to Philadelphia Children’s Hospital, but he adds, “I also want to state very clearly that I never sent chimpanzee kidneys to the Wistar Institute, Philadelphia.”

Dr. Henry Gelfand, the noted retired epidemiologist from Tulane who visited Camp Lindi in 1958, comments in a letter to me, “I remember Lindi as rather primitive, housing about a dozen chimps, and engaged in studies of polio pathogenesis.”

In The River, there is repeated speculation about the number of chimps that may have been at Camp Lindi and what happened to them, implying without specific data that some must have been designated as donors of kidneys. The mortality rate of the chimps, according to witnesses such as Ninane, was very high, perhaps as high as 50%. Even if 400 chimps were captured, this mortality plus experiments on polio, hepatitis, distemper, and other viruses could account for the large number. However, the crucial point is not how many there were but what they were used for, and there is absolutely no documentary evidence that they served as donors of organs for polio vaccine manufacture. Nor is there any evidence for the inference that large quantities of chimpanzee serum samples were sent anywhere, other than specimens sent for serological testing or blood group analysis [41].

A minor correction may be added here. In The River a major point is made concerning a paper on bacteriology written by Osterrieth on isolates of Klebsiella species obtained from chimpanzees and patients hospitalized in Stanleyville. The book states that “Klebsiella pneumoniae is one of the classic opportunistic infections of AIDS” (p. 351). However, Klebsiella species are only the fifth most common bacterial cause of pneumonia in AIDS patients [42]. Moreover, monkeys who die of simian AIDS do not commonly have Klebsiella infections (M. Simon, personal communication).

EPIDEMIOLOGY OF CHAT VACCINATION AND AIDS

Alleged coincidence of vaccination and early AIDS cases in the Congo. The second major assertion in The River is that there is an amazing overall coincidence between sites of CHAT
vaccination and early cases of AIDS. Two coincidences are given particular emphasis to show a relationship between CHAT vaccination and AIDS. One is a Belgian cartographer and his wife, who are said to have been infected in a place called Kikwit, and which cases have been added to the map; and the other is vaccination in a place called Lubudi, which is said to explain the cases of AIDS occurring in Katanga.

Kikwit was a town of about 15,000 inhabitants; I was there in May 1959 to start a study of immunogenicity. By that time, we knew that African adults were almost all immune to polio, and, therefore, vaccination was restricted to children aged <5 years. We vaccinated with CHAT and also took blood specimens for prevaccination titers. Unfortunately, after blood was taken from 160 children, the rumor began to circulate that the blood was being taken for the purposes of witchcraft, and the vaccination center was soon encircled by angry people. Vaccination records from Kikwit in 1959 note that the next day, we were able to vaccinate only 15 children. We were forced to terminate the study—indeed, we had to be evacuated from the center by soldiers.

I was therefore surprised to read in The River (p. 749) that between one-third and one-half of the population had been vaccinated in May and that vaccinators had returned to Kikwit in November to vaccinate 600 more people. The Belgian cartographer was supposedly included in one of those vaccination sessions.

The sources given for these statements are Drs. André Lebrun and Michel Vandeputte, plus one of my own papers [43]. Contact with the Belgian physicians resulted in denials from both. Dr. Lebrun writes, "We went there to vaccinate primarily the African children under 5 years of age. The very first day we had a Belgian settler, covered with dust, who came all excited from the bush in his jeep. He told us that trouble was brewing because the natives thought that we were taking blood from the children to practice some kind of magic with that blood. The local population became restless and aggressive. We had to abort the campaign and leave rather precipitously" (A. Lebrun, personal communication). In a signed statement dated 2 February 2000, Dr. Vandeputte writes, "On the first day, we vaccinated the children of the military camp of Kikwit. Blood samples were taken from most of these children for antibody testing. The second day, the vaccination was pursued among the children of the local community but had to be stopped very quickly because of local unrest and protest from the people (blood sampling at the femoral vein was thought to be cause for later sterility). As far as I can remember, we vaccinated during this period no more than a few hundred children."

None of us recollected the vaccination of European adults in May, but what about November? In an article I wrote with several coauthors, there appear the following sentences: "Of particular interest is virus 525, isolated from a 32-year old European woman who developed poliomyelitis one month after coming to a village in the Congo. In this village and in neighbouring villages during the latter part of 1959 there had been several cases of type 1 poliomyelitis. Consequently, on 30 November 1959, CHAT was administered to 374 Europeans of all ages and 253 African children less than 5 years old" [43]. In my files, there is a facsimile transmission that refers to vaccination in a place called Moanda of 374 Europeans and 253 Congolese, in relation to the case of Madame de Jonghe, a Dutch woman who developed polio on 10 January 1960. This corresponds exactly with the episode referred to in my article. Moanda is on the Atlantic Coast, ~730 km (453 mi) from Kikwit, which may be too far even by The River’s generous standards of geographical proximity.

Moreover, I found a letter from Dr. Lebrun dated 6 October 1959 responding to my request for him to go back to Kikwit to continue the study. He quotes a telegram that he had received from the government physician on site, saying “bloods not taken. I assess that it is inopportune at the moment because of public safety.” Therefore, it is unlikely that the Belgian cartographer was vaccinated.

The second putative vaccination site in the Congo emphasized in The River is at Lubudi, which supposedly explains AIDS cases in Elisabethville, ~282 km (175 mi) away. The evidence for vaccination is based on a newspaper article [44]. Lubudi is in Katanga province, where as far as I know, no vaccination was ever done because the health authorities were opposed to the concept of vaccination using live poliovirus. Two of those authorities are still alive: Dr. Jean Delville and Dr. Stephane Pattyn, formerly provincial medical director and laboratory director, respectively. In a signed statement dated 6 January 2000, Delville says “I...declare having no knowledge of polio vaccinations by means of attenuated virus in Katanga during the period 1955–59” (author translation). Pattyn says in a signed statement dated 25 November 1999, “my recollection [is] that before 1960—I left the Congo on 1.6.1960—there had been no poliomyelitis vaccination done in Katanga.”

Communication with the medical department of the Union Minière of Katanga, which owned the mines that formed the main industry of Katanga, showed that during the time of the CHAT trials, they were using the Salk-type vaccine, not OPV [45].

The newspaper article [44] that spoke of the planned vaccination referred to “Kabare-Lubudi.” Kabare is just north of the Ruzizi Valley, and to the west by road is Lubutu, whereas Lubudi is almost 805 km (500 mi) to the south. I suggest that it is more likely that the reporter made a mistake in spelling rather than that the investigators quoted in the article connected 2 places so distant from one another.

Therefore, the 2 early AIDS cases in the Congo that would
be most useful to bolster the CHAT vaccine case are unrelated to sites of vaccination.

With regard to the general idea that vaccination sites and AIDS cases before 1980 are juxtaposed, it is important to note that only 10 of 38 cases listed in The River were confirmed to be HIV infections. However, for the sake of argument, let us accept them all as AIDS cases. The River claims that "of the 28 patients for whom a specific town is cited, 23 come from the Congo, Rwanda, or Burundi—and of these, fully 17 are linked to towns where CHAT was previously fed. The other six are linked to places situated within 175 mi of towns where CHAT is known or believed to have been fed" (p. 743). No statistical justification is cited for the choice of 175 mi (282 km) as an indication of propinquity, rather than some shorter or longer distance.

Just how significant is the relationship? In fact, 13 of the 17 cases from the Belgian Congo that match with vaccination sites came from the metropolis of Léopoldville and 2 from the city of Stanleyville. Therefore, 15 of the 17 matches occurred in the 2 major urban areas.

Moreover, as shown in table 3, the distribution of AIDS cases (if they were AIDS cases) in the Congo shows a strong urban-rural difference. For the 4 urban areas where there were cases, the incidence rate was 3.4 per 100,000 population; for all urban areas in the Congo, the incidence rate was 1.3 per 100,000 population, whereas for rural and suburban areas, the incidence rate was down to 0.4 per 100,000 population [46].

Figure 3 is a map showing the vaccination sites and the AIDS cases listed in The River, but with removal of the Kikwit cases and the Lubudi vaccination site. The map shows that in the Bas Congo (west of Léopoldville) and in Province Oriental (northeast Congo), there is CHAT vaccination and no AIDS, whereas in Katanga (southeast Congo) there is AIDS but no CHAT vaccination.

<table>
<thead>
<tr>
<th>Site</th>
<th>Estimated population, thousands [46]</th>
<th>No. of cases</th>
<th>Incidence per 100,000 population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Léopoldville</td>
<td>273</td>
<td>13</td>
<td>4.8</td>
</tr>
<tr>
<td>Stanleyville</td>
<td>72</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>Bukavu</td>
<td>30</td>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td>Elisabethville</td>
<td>140</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td>515</td>
<td>17</td>
<td>3.4</td>
</tr>
<tr>
<td>All urban</td>
<td>1,261</td>
<td>17</td>
<td>1.3</td>
</tr>
<tr>
<td>Rural + mixed</td>
<td>11,510</td>
<td>5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Vaccination in Burundi

In fact, The River makes a particular case for CHAT having initiated HIV infection during a vaccination campaign in Burundi, along the eastern shore of Lake Tanganyika, from Usumbura to Nyanza Lake: "It is rather the CHAT 10A-11 vaccine used in 1958 for the second (Lake Tanganyika) leg of the Ruzizi Valley trial, vaccine that was fed in places like Usumbura and Rumonge, which coincides most precisely with the early appearance of HIV some years later" (p. 788).

The basis for this idea is seroprevalence data obtained in 1980–1981 by Morvan et al. [47]. They found that the prevalence of HIV antibodies was higher in the city of Usumbura (8.1%) than it was in rural areas (2.8%), which is consistent with an epidemic propagated by sexual activity, but the prevalence in a village named Rumonge was 11.9%. Although that figure was based on only 8 positive serum samples among 67 tested, The River nevertheless makes a great point about this but fails to quote the explanation given in the original paper by the authors, which is as follows: "At Rumonge, where there existed a high frequency of seropositives (11.94%), there was a Burundu-Zairois population comparable to a semi-urban environment with free sexual morals" [47]. Dr. Bernard Carteron,
one of the authors, also told me that there were many Zairean truck drivers, who are known vectors of HIV, in Rumonge.

Other data collected during the 1980s by Van de Perre [48] (letter, P. Van de Perre to H. Koprowski, 25 May 1992) showed urban–rural prevalence of 17.8% versus 1.3% in Rwanda and a rural prevalence of 0.7% in Burundi.

**CLINTON FARMS AND THE AMERICAN AIDS EPIDEMIC**

*The River* also proposes an explanation for the introduction of HIV into the United States—namely, that during early trials of immunization at Clinton Farms, a women’s prison in New Jersey, CHAT vaccine contaminated with SIV was administered to infants. Attention is called to a case of pediatric AIDS reported by Dr. James Oleske [49], a pediatrician from Newark. The child in question was born in 1974 to a 16-year-old drug-addicted mother. The contention is that the mother had been an infant at Clinton Farms and was one of the CHAT vaccinees.

However, Dr. Oleske has ruled out this possibility. In a letter to me dated 11 July 2000, he writes, “I have reviewed a list of names of children born during 1956 to 1958 to mothers incarcerated at what is now known as the Clinton Detention Center, New Jersey. The list was supplied to me under confidentiality by the New Jersey State Department of Health. Based on my patient records and a review of this list, I cannot identify any name on the list of babies that corresponds with the mother of an HIV-infected infant seen by me in the late 1970s and reported in the *Journal of the American Medical Association*.” Therefore, this hypothesis for the origin of HIV infection in America has no facts to support it.

**CORROBORATIVE INFORMATION**

Were there AIDS cases before 1959? *The River* argues that the absence of confirmed HIV infections or AIDS before 1959 is a strong argument in favor of the polio vaccine hypothesis. However, medical care was in general available in the Congo only in towns. We know from analysis of the data that infection was rare before 1970. In that year, the prevalence of HIV infection was only 0.2% among pregnant women in Léopoldville, but it rose to 3% 10 years later [50]. Auvert et al. [51] plotted the epidemic curve for Léopoldville. They back-calculate that the number of infected individuals already exceeded 10 in Léopoldville in 1952 ± 5 years, placing the introduction well before the first OPV vaccination.

Moreover, it is well known that the first serum to test positive for HIV antibodies was obtained early in 1959 by Arno Motulsky in Léopoldville [52–54]. Although the serum came from an adult, only children were vaccinated in Léopoldville. Several explanations of how this could have been a vaccinated individual are offered in *The River*. However, Motulsky also collected 98 specimens from adults in Stanleyville, 116 children in Bukavu in Ruzizi Valley, and 99 children in Nyanza. All of those are places where CHAT vaccination was conducted in 1957–1958. Nyanza is particularly interesting because vaccine was given there during the campaign that was suspected to have been from a contaminated lot (see section above, “Vaccination in Burundi”), yet none of the serum specimens was positive. Although the numbers are small, these are striking negative data.

Whereas the absence of known HIV-positive patients before 1959 cannot prove or disprove an association between infection and CHAT vaccination, one can certainly say that there is no epidemiological resemblance to a common source outbreak. The specimens collected by Motulsky in 1959 yielded only 1 positive result in 500 specimens, and in 1970, only 0.2% (1 in 500) of pregnant women in Léopoldville yielded positive results [50].

The combination of rarity of infection, perhaps confined to individuals in rural areas, plus the lack of medical care in those areas would have allowed isolated illnesses and deaths to pass unnoticed. Moreover, the fact is that AIDS in the Congo went unnoticed for over 20 years (from the early 1960s to 1983), even as it was becoming more prevalent. Even in the United States and Europe, where AIDS cases go back to at least 1974, the disease went unrecognized until 1981. A case of possible AIDS seen in Léopoldville in 1962 [55] is mentioned in *The River*, and an attempt is made to link it to CHAT vaccination in Lisala in 1958. However, the case history related in the book shows that the woman was already symptomatic in 1958. Moreover, a later study [56] showed that HIV seroprevalence was not higher in Lisala than in Bumba, where vaccination had not been done.

Two longtime African observers commented to us on the possibility that AIDS could have gone undetected in Africa for many years. In a letter to me dated 6 March 2000, Dr. Michel Garenne writes:

Is it likely that an unidentified disease could be noticed by colonial physicians before 1959, the date of the first documented HIV/AIDS death in tropical Africa? The answer is clearly yes, and there are many arguments for this statement, assuming that HIV/AIDS was sporadic before that date. First, several very lethal tropical diseases, which most likely existed before, were discovered after 1960, such as Lassa fever (1969) and Ebola (1976). These diseases were identified in towns where colonial hospitals or clinics had been functioning since the mid-1930s.

Second, even very well known lethal diseases, such as measles and whooping cough, were ignored by colonial physicians before 1960. One of the first colonial reports
on medical geography argued that measles did not exist in tropical Africa outside of imported cases along the coast.

In an e-mail to me dated 15 February 2000, Dr. Philippe Van de Perre commented, "In fact, I think that certain cases of AIDS could have passed unperceived for a long time. During my first sojourn in Kigali (Rwanda), of all the cases that I reviewed in the first article published in The Lancet, another diagnosis had initially been erroneously proposed. Even more so in the isolated health centers, it is probable that cases of AIDS have escaped the curiosity of clinicians during several years" (author translation).

It should also be mentioned that extensive urbanization took place in the Congo during the last 40 years. Between 1957 and 1985, the urban population rose from <10% to 40% [8].

SURVIVAL OF HIV-SIV IN KIDNEY CULTURES

Despite the facts presented above which show that kidneys from chimpanzees were not used in the production of polio vaccine, it is pertinent to comment on the possible survival of lentiviruses if contaminated renal tissue cultures had been used.

This subject was carefully examined by Florian Horaud [57], who was involved in polio vaccine research and manufacture since the 1950s. It is important to recognize that SIV infection of chimpanzees is uncommon, occurring in 7% of animals from Gabon or Cameroon, and probably fewer animals from the Congo [58]. This figure is undoubtedly influenced by the fact that chimpanzees are almost always captured young, before they are exposed by means of sexual intercourse. Second, although HIV can be demonstrated in the kidneys of patients with nephropathy [59], in general, AIDS patients do not show replication of virus in their kidneys [60], and renal cells are apparently not highly susceptible to replication of lentiviruses after inoculation in the laboratory [61, 62]. To overcome this objection, The River postulates the persistence of macrophages and lymphocytes in the cultures used to make vaccine. However, in the production of poliovirus, simian kidneys are trypsinized overnight, and the suspended and washed cells are allowed to grow to confluence in monolayer cultures for about a week, with frequent media changes. Then poliovirus is inoculated, after which the cells are further washed. Cytopathic effect eventually kills the cells. The harvest is then passed through a filter, which removes bacteria and much cell debris, including material potentially infected with lentivirus.

The importance of trypsinization must be emphasized because trypsin is a potent destroyer of HIV-SIV. Indeed, several attempts to reproduce the conditions of cell culture used to make polio vaccine, including tests of kidneys derived from infected monkeys, failed to show recovery of SIV [61–64]. Recently, Narayan and associates (personal communication) attempted to recover SIV from the passaged kidney cultures of infected monkeys by coculture with susceptible lymphocytes, but were unable to do so. Therefore, even if an HIV-SIV contaminated tissue had been used in manufacture, the amount of residual virus would have been small or none.

CROSS-INFECTION BETWEEN PRIMATE SPECIES

The accumulated information of recent years has shown that transmission of retroviruses from one primate to another (including humans) is not at all rare. Table 4, although certainly incomplete, summarizes many reports of these cross-infections [58, 65–83]. Of particular interest is the evidence that the simian T cell lymphotropic virus 1 of chimpanzees was transmitted to humans, where it evolved into human T cell lymphotropic virus type 1 (A.-M. Vandamme, personal communication) [68, 70].

Hahn et al. [65] have shown that the chimpanzee SIV strains of known geographic origin closest to HIV-1 have been isolated thus far only from west-central African chimpanzees (formerly called *Pan troglodytes troglodytes*), rather than in chimps found in east Africa, including the Congo (formerly called *Pan troglodytes schweinfurthii*). One more divergent strain was recovered from a chimp of unknown geographic origin caught in the wild. Hahn et al. have proposed that the chimpanzee-human transfer took place in west equatorial Africa, rather than in east Africa [63]. In particular, HIV-1 group N is very close to SIVcpz isolated from west-central African chimpanzees [58]. Because there are 3 groups of HIV-1 viruses—M, N, and O—it appears that 3 separate crossovers are needed to explain the presence of the 3 groups of HIV-1 in humans.

Fortunately, Dr. Courtois left a precise record of the geographic regions where chimps were collected [37]. All of those areas were in the eastern half of the Congo, the closest being >800 km (500 mi) from Léopoldville. Moreover, in the same article, he says, “If the characteristics that differentiate *Pan paniscus* and *Pan troglodytes schweinfurthii* are numerous and evident, we will not take the risk of discussing those much less clear that differentiate *P. troglodytes schweinfurthii* from other *P. troglodytes*, which besides we have never had the occasion to use.” Although the subspecies idea has since been abandoned, the statement shows clearly that Courtois collected chimps from the eastern Congo, and so far, the isolates closest to HIV-1 SIVcpz have not been reported in the area from which he collected. One of the chimps used by Fritz Deinhardt is reported as coming from Coquilhatville (now Mbandaka), but this animal was still alive in May 1959, after the first known HIV infection.

An argument is made in The River that the “cut hunter” theory accepted by most scientists is not provable. The “cut hunter” theory contends that the practice of hunting and butch-
Table 4. Some examples of interprimate lentivirus transmission.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Lentivirus</th>
<th>Transfer from</th>
<th>Transfer to</th>
</tr>
</thead>
<tbody>
<tr>
<td>[58, 65]</td>
<td>SIVcpz ← HIV-1</td>
<td>Chimpanzee</td>
<td>Human</td>
</tr>
<tr>
<td>[66, 67]</td>
<td>SIVsm ← HIV-2</td>
<td>Sooty mangabey</td>
<td>Human</td>
</tr>
<tr>
<td>[68, 69, 70, 71]</td>
<td>STLV ← HTLV&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Chimpanzee</td>
<td>Human</td>
</tr>
<tr>
<td>[68, 72]</td>
<td>STLV ← HTLV&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Baboon</td>
<td>Human</td>
</tr>
<tr>
<td>[68]</td>
<td>STLV ← HTLV&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Cercopithecus</td>
<td>Baboon</td>
</tr>
<tr>
<td>[68]</td>
<td>STLV ← HTLV&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Macaque</td>
<td>Human</td>
</tr>
<tr>
<td>[73, 74]</td>
<td>STLV ← HTLV&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Macaque</td>
<td>Baboons</td>
</tr>
<tr>
<td>[75]</td>
<td>SFV</td>
<td>African green monkey</td>
<td>Human</td>
</tr>
<tr>
<td>[75]</td>
<td>SFV</td>
<td>Baboon</td>
<td>Human</td>
</tr>
<tr>
<td>[76, 77]</td>
<td>STLV&lt;sub&gt;i&lt;/sub&gt; ← HTLV&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Chimps</td>
<td>Human</td>
</tr>
<tr>
<td>[78]</td>
<td>SIV&lt;sub&gt;AGM&lt;/sub&gt;</td>
<td>African green monkey</td>
<td>Rhesus monkey&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[79, 80]</td>
<td>SIV&lt;sub&gt;AGM&lt;/sub&gt;</td>
<td>Vervet monkey</td>
<td>Yellow baboon, Chacma baboon</td>
</tr>
<tr>
<td>[81]</td>
<td>SIV&lt;sub&gt;MAC&lt;/sub&gt;</td>
<td>Macaque monkey</td>
<td>Human&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>[82]</td>
<td>SIV&lt;sub&gt;SM&lt;/sub&gt;</td>
<td>Sooty mangabey</td>
<td>Macaque&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[83]</td>
<td>SIV&lt;sub&gt;AGM&lt;/sub&gt;</td>
<td>Sabaæus monkey</td>
<td>Patas monkey</td>
</tr>
</tbody>
</table>

<sup>a</sup> Complex interspecies transmissions.  
<sup>b</sup> Transfer occurred in captivity.  
<sup>c</sup> Laboratory workers.

Table 5. Recent estimates of the year when HIV type 1 group M virus started to spread from a common ancestor.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Confidence limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>[84]</td>
<td>1931</td>
<td>1915, 1941</td>
</tr>
<tr>
<td>[86]</td>
<td>1940 or earlier</td>
<td>Not given</td>
</tr>
<tr>
<td>[85]</td>
<td>1931</td>
<td>1907, 1955</td>
</tr>
<tr>
<td>[87]</td>
<td>Before 1959&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Not given</td>
</tr>
</tbody>
</table>

<sup>a</sup> On the basis of a 1959 isolate, which was already beyond the node for clades B and D. Therefore, the common ancestor was earlier.

PHYLOGENETICS AND MOLECULAR DATING

A number of attempts to date the origin of HIV-1, or at least the beginning of its starburst radiation from a primitive infection, have been made, and the technology for analysis of virus evolution has greatly improved in recent years. The best known of these analyses was recently published by Bette Korber et al. at the Los Alamos National Laboratory. Their conclusion, which was based on an analysis of the phylogenetic data using a bank of supercomputers, was that HIV-1 started its evolution in humans around 1930 [84]. Other workers have reached similar conclusions [85, 86], as summarized in table 5. Therefore, the majority view of evolutionary virologists is that HIV-1 group M originated well before the use of OPV in the Belgian Congo during 1957–1960.

In any case, the fact that HIV-1 infection was present in 1959, and that the virus from that infection had already evolved beyond the B-D clade junction [87], means that evolution would have had to have been enormously fast between 1957 and 1959 to be compatible with introduction by OPV. Other explanations are the evolution of all or most of the clades of HIV-1 group M in a single chimpanzee (which is unlikely) or their introduction into the OPV through the use in manufacture of multiple infected chimpanzee kidneys, each carrying a different clade. In view of the absence of any evidence that chimpanzee kidneys were used, the lack of known HIV-1 group M isolates in chimpanzees, and the low rate of SIVcpz infection in chimpanzees, the likelihood of such events is small.

REFLECTIONS

After all is said and done, we are left with the same facts that were widely known before publication of The River: namely, that AIDS became apparent as a disease in the same country where CHAT OPV vaccination was conducted, and that chimpanzees were available to those who made the vaccine.

There is some irony in the accusation that Koprowski and I were oblivious to the threat of extraneous agents in primary...
cell culture. To avoid that threat, we were the first to apply the human diploid cell strains developed by Hayflick and Moorhead [88] as substrates for vaccines, first for polio, then later for rabies by Wiktor and Koprowski [89] and for rubella by Plotkin et al. [90].

The River has been praised for its precise detail and wealth of footnotes, but one can be precise without being accurate. The issue in the case under consideration is not whether contamination of vaccine with HIV might have happened, but whether in fact it did happen. To accept a hypothesis, science demands not only association in time but also the absence of contradictory data. By this test, the OPV-AIDS hypothesis fails.

The River contains serious accusations against scientists. Yet the issue goes beyond personal reputations. The River is an attack on vaccination, and it is also the Faust myth brought up to date: the myth of evil scientists who stop at nothing. As attractive as that story may be in today’s antiscientific climate, it is false.

In summary, no evidence supports the idea that chimpanzee cells were actually used to make polio vaccine, and the supposed geographical correlation between vaccination and AIDS is an illusion. Moreover, the ancillary virological and epidemiological data are against the OPV-AIDS hypothesis. The River is a house of cards built on a swamp of conspiracy theory, unsubstantiated insinuations, and character assassination. It is fundamentally meretricious and does not withstand critical analysis.

Acknowledgments

The sole positive aspect of this experience was the spontaneous support of many scientists and other interested parties, both those whom we knew well and others who were strangers before. There is not enough room to name them all. In particular, profuse thanks and heartfelt gratitude go to Abel Prinzie, Dirk Teuwen, and Jan Desmyter. I am equally grateful for the advice and help of Beatrice Hahn, Bette Korber, Steve Wolinsky, Paul Sharp, Vincent Racaniello, James Oleske, and Grace Fries. Finally, I thank John Moore for lending me some of his courage to face defamatory accusations.

This article is dedicated to the memories of Drs. Florian Horaud and Gaston Ninane, two old warriors in the fight against polio, who died just before this article was presented as a paper at the Royal Society meeting.

References

28. Plotkin SA, Koprowski H, Richardson SM, Stokes J. Vaccination of families against poliomyelitis by feeding and by contact spread of living


