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Review

Oral polio vaccine: fact versus fiction

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Abstract

The author of this article has now become the principal target of the accusation that HIV-1 was introduced into man through the wildcat production of an oral polio vaccine in chimpanzee cell culture more than 40 years ago in the Belgian Congo. The putative evidence presented in support of this accusation rests essentially on testimonials by local witnesses. Such an accusation is strongly rejected on basis of a factual review of the author's career and his publications, as well as his total incapability to have managed such a feat at the supposed time.

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1. Introduction

It is well known that infections can occur after medical interventions because of a lack of sterility; to imagine that such could be the case with vaccinations may therefore appear to be a conceivable hypothesis which then must be verified on basis of facts. As many vaccines are produced in living cells, any adventitious infectious agent present in those cells may represent a potential danger. Well aware of this risk, vaccine manufacturers test their products with all available tests, but evidently they cannot check against the presence of the unknown. This is what Mr. Edward Hooper says, but he goes one step further by affirming that a contaminating agent was present and that an accident did occur [1]. After having suggested several prior hypotheses and having to eliminate these on basis of factual evidence, Hooper now says that HIV was introduced into humans in Kisangani, Democratic Republic of the Congo, (formerly Stanleyville, Belgian Congo) because oral polio vaccine (OPV) was produced there in SIV-infected chimpanzee kidney cells [2].

As Mr. Hooper, acting like a prosecutor, goes on and on with his theory about this alleged origin of HIV, [3] and as I, his designated defendant, am the target of his wild accusations, I feel obliged to provide precise additional information on what actually happened in the Laboratoire Médical de Stanleyville (LMS) during the 1950s, beyond any already given [4,5].

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2. The old laboratory at Stanleyville

When I arrived in 1956, the LMS was located in a very old building that was extended on several sides by small additions covered by prolongations of a corrugated iron roof. It was a miserable place, extremely hot and overcrowded with men and material. For example, the cleaning and sterilization of glassware and culture media were carried out under a simple roof with no walls. It was miraculous that one could manage to perform bacterial cultures and analysis in such surroundings.

Dr. Ghislain Courtois, director of the LMS, had a small office full of documents, with a microscope that he used to examine liver biopsies for Councilman bodies in search for cases of yellow fever. By identification of Councilman bodies, Dr. Courtois had shown for many years that yellow fever was present in the Belgian Congo. When declared positive by Dr. Courtois or his associate, Dr. Gaston Ninane, the sections would always be sent to outside experts for confirmation¹. Dr. Ninane was in charge of anatomic pathology and had a small lab in the center of the building.

However, as the presence of Councilman bodies is not as direct a proof of yellow fever as virus isolation. Dr. Courtois therefore longed for the availability of an isolation technique

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¹ Dr. Levaditi (Institut Pasteur de Paris), Dr. Bearcroft (Virus research Institute Lagos), Dr. Thys (Institut Princesse Artrid Léopoldville), Dr. Smithburn (South African Institute for Medical Research), Dr. Gast-Galves (Institut Carlos Finlay Bogota), Dr. Camain (Institut Pasteur de Dakar).

at the LMS. It was in this context that he asked me to become a virologist, as detailed below.

The bacteriological unit was somewhat larger, but too crowded to properly accommodate Mr. Pierre Doupagne, a bacteriologist, Mr. David Bisuta, a Congolese nurse, and myself. Nevertheless, during my first stay in Stanleyville (24 July 1956 to 19 July 1957) it was there that I was trained in bacteriology. At that point, I was scheduled to go on leave, and although I wanted additional bacteriological training, Dr. Courtois insisted on obtaining for me a WHO Fellowship in Virology so that I could help with vellow fever diagnosis. Accordingly, I was sent to the CDC laboratory in Montgomery, Alabama, USA, to take a course in laboratory diagnosis of viral and rickettsial diseases. After the course, I spent another 6 weeks at CDC, and then went to the Wistar Institute in Philadelphia. There, I spent a month observing tissue culture and virology techniques, although I did not myself do any work at the bench, but rather observed and asked questions. During that period, I saw Dr. Koprowski once or twice for casual conversations.

Hooper reports that Dr. Courtois had the knowledge to carry out tissue culture on the basis of notes he took at the Oswaldo Cruz Institute, and that he did produce tissue culture in the old LMS as early as 1956. That Dr. Courtois made tissue culture in the old LMS must be discarded as fantasy for many reasons: the lab was an awfully overcrowded place, hot and dirty, where it would be hard to try to carry out tissue culture. Moreover, to my knowledge, Dr. Courtois had no practical experience at all in tissue culture; he mainly administered the lab, took care of relations with other services, examined liver slides for evidence of yellow fever, and inoculated mice with the help of Madame Paula Liègois and Mr. Victor Amunga. That he carried out tissue culture from chimpanzees is pure fantasy: extending this fantasy to Dr. Ninane's laboratory is delirium.

In addition, I strongly doubt that Courtois carried out any research on chimpanzees before the opening of the Lindi camp (see below).

3. The Lindi Camp

When I joined the laboratory in 1956, the Lindi Camp for chimpanzees was already established in the forest on the far side of the Lindi River. I do not know exactly when it was opened. However, whereas it was not a secret place, it was restricted to visitors as a precaution against spread of infectious agents. Because of these precautions, few people knew exactly what was going on there, and many rumors could circulate owing to ignorance among lay people of microbiology and epidemiology laboratories. One could afford no risk of chimpanzees infecting humans or of humans infecting chimpanzees.

Mr. R. Daenens was in charge of the camp, and he was helped by a Congolese named Joseph, I believe. The chimpanzees were collected by a French hunter, Mr. Rolley. Only chimpanzees out of the wild could be used, with no history of contact with the human population. Chimpanzees that had lived captive in a native village were thus usually not accepted.

The chimpanzees were housed in cages built of wooden planks and wire mesh. Each harbored two chimpanzees, but the animals were separated by a wooden wall and there were separate entrances so that each chimp was in fact alone, which was necessary for handling, although very young ones were kept together. Bonobos were sometimes in one side of a divided unit with a common chimp occupying the other side.

Lindi Camp was established to test polio vaccines, but as the chimpanzees were available, other experiments were carried out to obtain a full return on investment. Chimpanzees were not killed for tissue culture purposes, but to check the results of experiments involving poliomyelitis, hepatitis, and other pathogens. Many chimpanzees died of illnesses or malnutrition, but I have no exact data regarding specific causes of death. It is inconceivable that serum or tissues from dead animals or from animals sacrificed after experiments could be used for tissue culture purposes.

Mr. Hooper has greatly exaggerated my role at Lindi Camp. If Antoine and Joseph say I was the one who did most of the autopsies, they are wrong; that was the job of Dr. Ninane. I might have helped from time to time, when necessary, but not more. I did not perform autopsies even if I happened to be present by chance when Dr. Ninane was performing one. However, based on my observations of those autopsies that I did attend, organs were removed with aseptic precautions as they should be, particularly if they had to be checked for bacterial infection or sent to Dr. F. Deinhardt at Philadelphia Children's Hospital for work on his hepatitis project. Blood was also taken for serology, but in limited quantity (laymen frequently overestimate the quantity of blood taken). Post-mortem blood or tissues are not useful, as bacteria from the gut quickly disseminate and sterility is no longer possible. Because of tropical conditions, it was good practice to store all biological samples in the cold. We had green metal canisters that contained Dewar flasks.

I did use sera from chimpanzees for serological surveys. On a few occasions, I obtained these sera myself, but certainly not every Saturday, as suggested, on a regular basis. In fact, I went to Lindi Camp mostly to check cages containing baby mice that had to be renewed often. These cages were positioned here and there around the camp as bait for mosquito bites, as I was hunting for arboviruses.

4. The new laboratory

A new and larger laboratory was absolutely necessary: it was built by the Belgian Congo authorities and opened in late 1957, while I was still absent. I returned to the LMS on

23rd February 1958, as stated in the 1958 Annual Report $[6]^2$.

My family and I arrived in Stanleyville several days earlier, and as customary, stayed in a hotel until we were assigned a house. I officially began to work on the date stated in the report. Hooper quotes Jacques to the effect that he began to work on 12th February. If so, his assignment was to the LMS and not specifically to me. The precise memory recall of Jacques after over 40 years is amazing and puzzling, but he pretends also that I was already making vaccine when the virus lab was not even yet created. It may be he confuses polio vaccine with different types of bacteriological vaccines that I and others made in the old LMS, such as auto-vaccines against staphylococcus, vaccines against enteric bacteria, etc. In any case, how could he know what vaccines I was making?

Besides, there were additional factors that prevented me even from doing routine virology: first, on my return, Prof. M. Welsch from the University of Liège, on visit to the LMS, consumed most of my time for 2 months as we studied the transformation of gram negative rods to protoplasts [7]. I also carried out a serological study of Lymphogranuloma venereum [8]. Furthermore, Dr. Courtois was absent from 27 March to 21 October 1958, with the consequence that I had to take care of additional routine duties.

Thereafter, I began to install the virology and tissue culture laboratory, starting roughly in May 1958. Obtaining the glassware, building the hood, installing the UV lights took time, and no work was possible until later in the year, not even the Maitland type cultures postulated by Hooper. The idea that I was making poliovaccine early (or late) in 1958 is quite impossible under the conditions that existed at that time, so this allegation is totally untrue.

I had brought back to Stanleyville various viruses of the arbovirus group that could be passed in baby mice, and therefore these could be used for in vivo neutralization tests. Using this technique, I tested a large number of sera against a battery of viruses, involving the inoculation of large numbers of mice and observation of those mice for at least 2 weeks. The results of those experiments were published [8,9]. Additional work on arboviruses is mentioned below.

5. My responsibilities at the LMS

As mentioned above, my initial duties in Stanleyville were bacteriological. I published with Pierre Doupagne a paper on the incidence of different bacteria in cultures of human urine [10]. I had noticed that there was a large variety of Enterobacteriaceae in Stanleyville, including new salmonellae such as *S. kisangani*, *S. simimi* and *S. stanleyville*. Klebsiellae were not well identified, and were therefore worth studying. These bacteria are common in human samples where

they are often present with other enteric bacteria. We decided to collect klebsiellae and to type them biochemically.

Hooper implies that our Klebsiellae isolates were the result of concurrent HIV infection. Although Klebsiellae may cause opportunistic infections, HIV infection is far from the only predisposing cause. Their presence in chimpanzees can be explained by the stress of captivity, poor nutrition, or coinfection with other agents. Some of the chimps died with pneumonia, from which Klebsiellae were cultured, but they were not established as the cause of death. Furthermore, the human isolates had nothing to do with an 'epidemic'. They were selected because they were Klebsiellae isolates obtained in the hospital and we were interested in the study of different biotypes involved from humans and from the chimpanzees [10].

To return to my arbovirus duties, Dr. Courtois and I asked the doctors in rural hospitals to alert us to cases that might be caused by yellow fever or other viruses. In fact, one such case caused me to spend some time, from 11 November to 15 December 1958, in Doruma, Belgian Congo. Various strains of yellow fever and Chikungunya viruses were isolated in mice from this and other outbreaks, and identified with the help of Dr. Haddow from Entebbe, Uganda. Several publications resulted, [11–16] as well as my doctoral thesis later completed in Belgium [9]. The arbovirus work consumes 6.5 of 10 pages in the 1958 report [6] of the LMS and three of five pages in the 1959 report [17]. To argue, as Mr. Hooper does, that my real task was to prepare vaccine, is to fly in the face of all documented facts.

Although the documentary evidence is all negative, I want to respond specifically to Hooper's allegations regarding chimpanzee cell culture. I repeat that the laboratory was not an ideal location for cell culture. We had one freezer, which often failed, a centrifuge for serum separation, and our hood was simply a box with a UV light. Hooper tries to get around the evident limitations of our training and environment by postulating the use of Maitland cultures to cultivate polio. This assumption is linked to the samples prepared for Dr. Deinhardt. However, one can not assimilate suspensions of minced kidneys in Hanks' balanced salt solution and serum for dispatch overseas at low temperatures, with Maitland cultures that require medium changes, agitation, oxygenation at 37 °C to enable cell replication. In any case, I never tried Maitland type cultures.

As for trypsinized cell culture, as I have shown above, this type of work could not have been done before May 1958, and probably even later. The 1958 LMS report clearly states that only baboon tissue cultures were made. Baboons were not very numerous around Stanleyville but nevertheless were easy to buy at cheap prices. Paradoxically, in 1958, travel was easier than today, with intense and rapid truck traffic between Stanleyville and the towns of Kivu such as Goma. All sorts of supplies and edibles were brought in this way, including monkeys and other "bushmeat." From baboon kidneys, as stated in the LMS report, 200 tubes and 16 flasks of cells were obtained and used for diagnostic

 $^{^{2}}$ In an unpublished paper [5], I said February 28 having mistaken a 3 for an 8.

purposes. Had we been able to make polio vaccine, one can imagine how we would have been proud and boasted about it in the annual report!

Chimpanzee kidneys were not used because they originated from diseased or experimental animals, because they were from a relatively expensive and rare species, because from our work we knew that chimpanzees could acquire human infections, and because they were not a well-characterized substrate for virus isolation (or for that matter, vaccine production).

The idea that we might have used chimpanzee sera for cell culture is also false. Calf serum could easily be obtained from various commercial sources in Europe, or from African cattle (zebu near Stanleyville) and European cattle (in Kivu). However, we did collect chimpanzee blood for serological surveys, for experimental purposes such as Deinhardt's hepatitis experiments, and for studies of blood groups by Prof. Moureau.

With regard to cell culture work by other persons, it is my recollection that the type of cell culture prepared by Dr. Ninane was carried out infrequently by fixing small pieces of tissue on plasma clots, using the technique of Prof. Chevremont. However, those attempts failed repeatedly. Dr. Deinhardt had the expertise to do cell culture, but he was working on his own experiments, and had neither the time, the necessity or the possibility of carrying out cell culture, and even Hooper does not accuse him of making polio vaccine. Dr. Deinhardt did try cell culture from minced kidneys in survival medium we sent him, in Philadelphia at the Children's Hospital but that was after his return to the United States.³

6. Vaccination with Koprowski OPV

While I was at Stanleyville the source of CHAT virus and other attenuated OPV strains was from the Wistar Institute, sent as frozen concentrated stock. The transport from Philadelphia to Stanleyville could be achieved quickly under refrigerated conditions. Sabena, the former Belgian airline, was very cooperative, and the dry ice was replenished in transit with the help of colleagues in Brussels. My memory is that such a trip took a maximum of 48 h.

Hooper insists that the vaccine was "amplified" locally in Stanleyville. I have already explained why this was not feasible, but in addition there was no need to do so. However, dilution of the concentrated stock was necessary before administration, and I probably did do some of that work, to help Dr. Courtois and Dr. Ninane. In addition, once or twice I helped with the vaccination itself, again to relieve others.

Although as noted above, I was away between July 1957 and February 1958, and none of the other personnel at the

LMS—Dr. Courtois, Dr. Ninane, and Dr. Doupagne—were capable of producing or "amplifying" vaccine.

Hooper quotes a Léopoldville (now Kinshasa) newspaper to the effect that vaccine was prepared in Elisabethville and controlled in Stanleyville [2] (p. 50) Aside from the likely exaggeration by a provincial reporter, and aside from the fact that there is no evidence that vaccine was ever prepared in Elisabethville [18,19] both "prepared" and "controlled" are vague words. "Prepared" could mean made ready for use and "controlled" could mean checked for sterility, etc., but it does not mean "produced" or "amplified".

My virus unit in the new LMS had two rooms, one of which contained a centrifuge, while the other was a sterile room for attempts at cell culture. The cell culture room was flooded day and night with UV light and locked. I was the only person to enter, as I needed to master the techniques under primitive conditions, far different from those at CDC! Apparently some local people remember that I stayed alone in the evenings in the sterile room. This was not for reason of secrecy, but merely not to be disturbed during my attempts at cell culture work. Once more, I solemnly state that I never produced polio vaccine of any sort, never attempted to multiply polio virus, and never attempted to culture chimpanzee cells.

7. The memories of elderly Congolese and Belgians

When one examines carefully Mr. Hooper's presentation, it becomes clear that the only "evidence" for his hypothesis is the memories of some elderly people who had indirect and partial knowledge of the work circumstances some 48 years ago. Primarily, he cites Congolese men who worked at the LMS or Lindi Camp. [2] (p. 52) Three are identified by name: "Joseph", the camp nurse; Jacob Kanyama, who worked with me; and Philippe Elebe, who prepared media. The latter two are Hooper's star witnesses for the idea that I made polio vaccine at the LMS.

Mr. Kanyama is quoted as saying that he started work on 12 February 1958, and that I had prepared vaccine even before that date. I have shown above that I was on study leave for 7 months before that date, and that I returned to the lab on 23 February, so how could Mr. Kanyama know what I did before that date? Mr. Kanyama is also quoted as saying that I brought vaccine out of the sterile room each time a new order was received. How could Mr. Kanyama differentiate between an alleged new production and simple dilution of stock? Mr. Elebe is quoted as saying that tissue cultures were made at the LMS. How could he be certain that they were made before late 1958; date at which time I did make baboon cell cultures?

I must also give some information about the Congolese technicians in the era of Belgian administration. The Congolese who came to work with us only received limited training as nurses or nurses' aides, with little scientific background. In the late 1950s, the administration's aim was to

³ Note that in Ref. [4], I mistakenly said that the kidneys were sent to the Wistar Institute, but in fact Dr. Deinhardt worked only at the Children's Hospital.

school progressively the native population. For example, a school for training medical assistants (Ecole pour Assistants Médicaux Indigènes) was founded at that time. Several large laboratories opened in the late fifties, like the new LMS in 1957, in anticipation of independence. These were built with Belgian Congo funds. Sadly, subsequent to independence, internal ethnic divisions, terrorism and disorganization resulted largely in destruction of the scientific infrastructure.

Hooper also quotes Dr. Maurice Kivits, an administrator in Brussels, Dr. Louis Bugyaki, a veterinarian at the Provincial Medical Laboratory (an entirely distinct organization from the LMS), and Dr. Joseph Mortelmans, also a veterinarian, as saying that they *think* chimpanzee tissues were used for culture. Apart from subsequent contradictory statements from the above three persons, [10,20] the fact is that none of them was ever in my laboratory.

8. Summary and conclusions

I want to reiterate that I did not participate in any program of sacrificing chimpanzees in order to obtain organs and/or blood for tissue culture purpose. I did not prepare CHAT or other OPV in cell culture. In particular, I never prepared chimpanzee cell culture.

In addition as confirmed by Joseph Limbaya [21], I never wrote at any time to a former Congolese assistant, as stated by Hooper. [2] (p. 52). Moreover, I have never purposely given misleading answers to anyone about my work in Stanleyville. Mr. Hooper constantly implies that "secrets" have been maintained for over 40 years, and relies on testimony of people with limited education or scientific background and only with second or third hand knowledge, despite documented evidence to the contrary. Scientists are notoriously poor at keeping secrets, and it does not fit at all with scientific culture.

Mr. Hooper's method is to take any word slip or hesitation and to convert it into a mysterious allusion to hidden crimes. This is the way of a prosecutor, not an investigator, and it has more to do with science fiction than fact.

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