

small village of Epulu, where there was an animal collection centre run by Jean de Medina, which seems to have supplied Jezierski with his chimpanzees.¹³⁷ Chimps from Epulu were later supplied to Lindi camp as well.¹³⁸ Later in 1957, Jezierski was forced to terminate his polio vaccine research, and to leave his job with INEAC – for reasons unknown.

What all this underlines is that by 1957, whether it was reported in the medical literature or not, oral polio vaccines such as CHAT were being amplified in locally-prepared cell cultures, derived from locally-available primates. This was not done on an occasional basis, but routinely. This was the normal way in which OPVs were prepared for trials around the world.

Local amplification of the vaccine meant that much smaller quantities of liquid seed virus or vaccine needed to be transported on those long plane journeys, making temperature control that much easier. All that was needed was to prepare a half-litre flask or a 100c.c. bottle of vaccine, pack it around with ice, place it in an insulated box, and then have it taken to the airport.

h) Primate availability in the Congo.

In Stanleyville, between 1956 and 1958, as has already been demonstrated, the available primate was quite clearly the “chimpanzee”, though this term appears to have been used to describe both the common chimp (*Pan troglodytes*) and the pygmy chimp/ bonobo (*Pan paniscus*). The various reports of harvesting tissues and blood at Lindi make it clear that these species were locally cheap and “available”. Furthermore, since safety testing was being performed in these species, it was both logical and consistent to also use them for local vaccine production.

But what if Jervis was the one to amplify vaccines for the Ruzizi trials? What primate cells might he have used? It is reported that the mother-solution of CHAT 10A-11 was stored in the freezer at Usumbura (nowadays Bujumbura in Burundi) in early 1958.¹³⁹ I have already published an eye-witness account from Juma Jamnabas, a former microscopist at the Bujumbura medical laboratory, of chimps and other primates being held in cages behind the lab in the period up to 1957 or 1958, and their kidneys being removed one at a time.¹⁴⁰ This has been strenuously denied by Dr Plotkin in his “Postscript”, who quotes three Belgians who used to work at the lab at that time, all of whom deny that any primates were ever present there. I too have looked into this further, and can add two further denials to those mentioned by Dr Plotkin, both of which come from other Belgian health workers based at the Bujumbura lab. However, I also have two further “positive sightings”, the more compelling of which is from a Belgian who used to visit the animals regularly during the period up to and including 1958, when he was an eight-year-old boy living in Usumbura. He recalls there being monkeys and at least one chimpanzee, and believes that the primates were being used for virus research.¹⁴¹

Although this particular debate is not settled either way, it seems to me that it is far easier to explain the “negative sightings” than the positive sightings. To my mind, these three positive sightings call into question the testimonies of the various Belgian doctors and health workers who deny that primates were ever held at the Bujumbura lab.

In addition, there was from 1956 onwards a small medical laboratory which focussed mainly on malarial and bilharzial studies, at the *Mission Medicale de Ruzizi* (MMR), sited just outside the village of Kabunambo, which served as the health headquarters for the Congolese side of the Ruzizi Valley. This was where Jervis and Flack were officially based during their first four weeks in the Ruzizi Valley. The aforementioned Juma recalled driving here with one of the Belgian doctors, and says that both chimps and other monkeys were caged here as well. Again, there is partial confirmation, in that three other Africans recall monkeys (of unspecified species) being held here. Others, however, recall no monkeys at all. As with the Bujumbura lab, we have contradictory evidence from different quarters, but once again it is difficult to explain away the positive sightings from different sources. My personal belief is that monkeys and chimps were probably held in cages at both Usumbura and at Kabunambo, but only for a brief and finite period – which could well have been around the time of the polio vaccinations.

In any case, although these recollections from Usumbura and Kabunambo are intriguing, they are not crucial to what happened at the Ruzizi Valley vaccinations. If Dr Jervis (rather than Osterrieth) was indeed the one to amplify the vaccines used in Ruzizi, then the likeliest place for him to have done so was at the lab in Bukavu, where it seems he spent much of his time during his African visit. In 2001 I visited Bukavu for the first time, and made repeated attempts to locate the archives of the medical laboratory. I eventually gained access to the director's room in that building, which had been locked and nailed shut, and where, sadly, all that remained were some papers stuffed haphazardly into a filing cabinet.¹⁴² However, in another room I located the blueprints of the lab, and for the large *animalier* which had been built alongside it, many of the brick walls of which are still standing today. The plans showed that there was a section in the animal house devoted to “monkeys”, and the presence of both monkeys and chimpanzees has been confirmed by two ex-workers at the lab.

What this boils down to is that if Jervis amplified the vaccines for Ruzizi, he would have had no shortage of available material. He could, for instance, have obtained chimp kidneys and sera from Osterrieth in Stanleyville, or he could have used chimpanzees or other primates from Bukavu (or possibly from Usumbura or Kabunambo). It is my belief that if Jervis used local primates to produce primary tissue culture to amplify the vaccine, he most likely would have used chimpanzees, for by that stage this was the African primate species which was best known and characterised for the Koprowski collaborators.

i) Commercial concerns.

The fact that local vaccine amplification was not spelt out in contemporary articles by Koprowski and his collaborators would seem to have largely been prompted by commercial concerns, and the fear that competitors might misappropriate one's carefully-attenuated strains – for instance by obtaining a phial of vaccine, or even a stool from a vaccinee.

As Hilary Koprowski once put it to me about his live vaccine strains: “There was no proprietor. As far as I know there was no patent.” In 1960, Albert Sabin (who had distributed his strains quite widely, to places such as the USSR, Singapore and South

Africa), wrote to the WHO, stating: “I would like to say that nobody who has received [vaccine strains] from me is authorised to give them to anyone else without my permission.”¹⁴³ Although the basic passage histories of the Sabin strains had been published in the fifties, it was only in the mid-seventies, long after they had been adopted on a global basis, that Sabin and a colleague wrote a paper detailing the full histories of the strains.¹⁴⁴ This paper described the OPVs which had been prepared in different labs and different countries around the world in terms such as “SO+3” or “SO+4”, to indicate how many further passages had taken place from the “Sabin original” seed strains.

But what is apparent is that the 1950s race to develop effective oral polio vaccines was not only a heroic attempt to control, and even eradicate, a much-feared disease. It was also quite clearly a commercial race, which is why pharmaceutical companies like Lederle (part of American Cyanamid) were involved, and why scientists like Pierre L epine were trying to patent their polio vaccines in the U.S.¹⁴⁵ Not unreasonably, the scientists who had spent years developing their OPV strains wanted to try to protect their considerable investments.

This, I believe, is one of the reasons why no overt reference was made in Koprowski’s papers published in the late 1950s to the onward passage of the vaccine virus in locally prepared tissue culture. From a commercial perspective, he didn’t want to draw attention to the fact that a single phial of vaccine would have been enough to produce enough batches of fresh vaccine to immunise millions.

However, by the mid-sixties, it was no longer necessary to try to keep this a secret, which is why articles about local production of CHAT begin appearing in 1964. By the time I interviewed Koprowski in 1993 there was even less need for secrecy, which is why he was quite open about the fact that local passage of his vaccines had occurred in other labs. Unfortunately, I wasn’t quick enough to pick up on the importance of what he was telling me.

But was this practice of local passage also safe? Until the sensational announcement about the discovery of SV40 in rhesus and cynomolgus cultures in 1960,¹⁴⁶ none of the other 39 adventitious viruses which had been found in monkey kidney tissue culture appeared to be pathogenic for humans. The more far-seeing of virologists (like Sweden’s Sven Gard) expressed concerns about several safety aspects of live vaccines like OPV,¹⁴⁷ but most of those developing live polio vaccines continued working on the basis that the risk was worth it. The question of the safety of the vaccine substrate was all but ignored.

The only time that Hilary Koprowski formally mentioned the question of vaccine substrate during the 1950s, he made it clear that he considered it an issue of little importance. This is what he wrote in a paper published in 1956, just as Lindi camp was opening: “The source of material used for virus cultivation cannot be disregarded altogether. It should be represented by tissue that is least apt to harbor human pathogens – although the dilution factor which can be applied to a poliomyelitis virus suspension may be beneficial for the elimination of other ‘passenger’ viruses.”¹⁴⁸

This paper by Koprowski was originally delivered as a speech at a symposium on “Newer knowledge of viral and rickettsial diseases” held in November 1955, which

focussed on the problems and benefits of different tissue cultures. A remarkably prescient paper delivered at that conference by John Enders' former collaborator, Tom Weller, examined the potential pitfalls of using tissue culture as they "might affect the operations of a hypothetical team of virologists...in Uganda".¹⁴⁹ He pointed out that as an alternative to bringing in tissues from abroad, "the [Uganda] group might equally rely on tissues, either of monkey or human origin, locally available". Weller emphasised the problem of hidden contaminating viruses which might be lurking in the monkey tissues, and which might cause visible cytopathogenic effect (CPE) in tissue culture only after a lengthy period, and he concluded that although such problems could be minimised, they "cannot be entirely circumvented".¹⁵⁰

One wonders what Koprowski thought of Weller's speech. His own approach to such potential problems with substrate seemed to be one of circumvention rather than cure, for this was one of three papers he wrote in 1956 and 1957 in which he claimed to be growing his polio strains in chick embryo tissue culture, when in reality he was already using some variety of primate kidney tissue culture. The reason for Koprowski's misreporting his polio vaccine substrate is not known.

j) The true purpose of Lindi camp.

In recent years, Stanley Plotkin and Hilary Koprowski have frequently repeated that the original purpose of Lindi camp was to test the safety and immunogenicity of Koprowski's polio vaccines in chimpanzees. No persuasive evidence has ever been presented to support this claim, and it now appears an inadequate explanation for the establishment of such a complicated operation. Back in 1994, Stanley Plotkin admitted to me that "I don't think a lot of real importance came out of those studies".¹⁵¹ And indeed, from the account provided by Koprowski, it would appear – anachronistically – that CHAT was fed to humans in Africa before it was tested on chimps.

It is documented that the first open trials of CHAT began in Stanleyville in February 1957,¹⁵² and the fact that CHAT vaccination was occurring at the time of Koprowski and Norton's visit to that city at the start of that month is proved by a contemporary photo which was published in *Time*, which shows Koprowski and Osterreith at Lindi camp, watching an African woman down vaccine from a tablespoon.¹⁵³ Since Koprowski stayed in Stanleyville for less than a week, which is not long enough for results to be obtained from vaccination and challenge or from intraspinal safety testing, it follows that (as with his first vaccine, TN, in 1950)¹⁵⁴ human subjects were actually fed the vaccine *before* its safety and immunogenicity had been assessed in chimpanzees. This suggests that testing the safety and immunogenicity of CHAT and Fox were not the *raison d'être* of Lindi camp.

All of which begs the question. What did the CHAT-related "experiments in chimpanzees", which had to be conducted at Lindi camp, actually comprise? Or, to put it another way: why would Koprowski go to such expense and trouble to set up a chimpanzee camp in the middle of the rain forest if the vaccines which it was allegedly vital to test there had already been publicly declared fit for human use, and were already being tried out in human "volunteers"?

What the scant historical records reveal is that polio-related experiments were carried out on a total of 83 Lindi chimps up to February 1958 (this representing 20% of the 416 chimps “used” in those twenty months). This work might have required the sacrifice of 48 of the 83 animals. Furthermore, it is apparent that most of the work (just as Plotkin hinted) failed to produce any significant scientific information. This was not least because chimps are a far less accurate barometer of vaccine safety than the lower monkeys – a fact which Koprowski already knew before he started.¹⁵⁵

However, the local press reported that while they were in Stanleyville Dr Koprowski, assisted by Tom Norton, “initiated Dr Courtois, as well as his assistants, doctors Ninane and Osterrieth, into his methods of work”.¹⁵⁶

So what was the work into which they were initiated? Was it intraspinal inoculation? This work is highly skilled, and it seems possible that most, if not all, was carried out by Koprowski’s experienced lab man, Tom Norton, during his six week stay in February and March 1957. As for vaccination and challenge (using whichever strains), this required little in the way of training.

I believe it likely that the principal technique which Tom Norton was teaching the three Belgians in February and March 1957 was how to make tissue cultures from chimp kidney cells and sera, in order to amplify the vaccines. At this stage, in early 1957, the brand new medical lab had not yet been opened, but the old brick-built laboratory was fully operational, and certainly Maitland-type cell cultures could have been prepared there.¹⁵⁷ It may also be that the early attempts at tuition were not completely successful, because when Ninane departed on his triannual leave in late March 1957, and Osterrieth in July 1957,¹⁵⁸ they both received additional training in tissue culture preparation: Ninane at Lise Thiry’s lab in Brussels, where he apparently spent about “ten to twelve weeks”; Osterrieth at the Wistar Institute in Philadelphia. During those periods, CHAT vaccine was present in both labs.¹⁵⁹

However, is it possible that tissue culture preparation may have started even earlier? The 1956 annual report of the medical laboratory has just this to say about Lindi camp: “Poliomyelitis. In collaboration with Doctor H. Koprowski, Vice-President of the New York Academy of Sciences, trials are in progress at the camp of experimentation at Lindi. More than 60 chimpanzees (meant for the study of an oral attenuated virus-vaccine) have already been used in the first trials.”¹⁶⁰ Since CHAT was only brought out to Stanleyville in February 1957, it would seem that these “first trials” in 1956 must have involved a different vaccine. Now, we know that pool 14 of Koprowski’s previous Type 1 vaccine, SM N-90, was present at Lindi, and was still being used in research as late as August 1957. SM N-90 (pool 14) would have been in existence by 1955 or (at latest) early 1956, so this Lederle-made Type 1 vaccine was almost certainly the one utilised for the 1956 trials, with 60 chimps being “used”, perhaps as a dry run for the CHAT vaccine that would arrive with Koprowski the following year.¹⁶¹

Whether chimp tissues and sera were used to amplify SM N-90 pool 14 back in 1956 is, of course, not known. However, one thing is certain. The camp at Lindi was not a small, or easy, or inexpensive operation to set up in the middle of the Congolese rain forest – and yet the scientific benefits which could be gained from its *alleged*

programme of testing the safety and efficacy of OPVs were relatively small. Clearly something is wrong here.

The true purpose of the chimp colony has never been revealed, but certain clues [see below] begin to suggest that the real *raison d'être* for Lindi may have been to test the safety of Koprowski's vaccine *substrates*, rather than that of his vaccines. The latter had, in any case, already been tested quite extensively on monkeys and humans (eg the infants born at Clinton State Farms, New Jersey) in the U.S.

There is also another possibility. Given the very detailed accounts by Joseph, the camp nurse, and by "Antoine", of chimpanzee organs being put into canisters (either in formalin or in a watery liquid, which sounds like Hanks' solution), and then packed off to the USA (and possibly Belgium), accounts that were in large part confirmed by the Stanleyville vet, Louis Bugyaki, it seems that there were possibly other research programmes which linked in to Lindi camp, from the time of its June 1956 opening. If this hypothesis is correct, then the polio research conducted at Stanleyville may not have been the sole reason for the establishment of Lindi. According to this scenario, it may be that chimp kidneys were used for local vaccine production mainly because they were available, as a by-product of the sacrifice of hundreds of chimps, and scores of bonobos for other purposes.

k) The possible use of other tissue cultures.

Since 1994, a number of investigators (such as Billi Goldberg, Blaine Elswood and Raphael Stricker) have proposed that the polio vaccine used in the Congo might have contained a simian immunodeficiency virus (such as SIVcpz) which became human-adapted after being further passaged either in human diploid cell strains (such as the varieties from WI-1 to WI-38, which were developed at the Wistar by Leonard Hayflick),¹⁶² or else in human cell lines (such as WISH, also developed at the Wistar, and HeLa).¹⁶³ The use of human cells, they proposed, might not only have helped an SIV or SIVs to adapt to humans, but might also have allowed different SIVs to recombine *in vitro*, before crossing to humans *in vivo*.¹⁶⁴

For many years I have rejected this idea, mainly because the timings did not seem to fit. Firstly, there was evidence that WISH was not produced until the end of 1958, and did not transform into a cell line until the last day of that year. Secondly, it appeared that Hayflick's human diploid cell strain series, WI-1 to WI-38, did not begin to be produced until 1959, which was the year when Professor Sven Gard was on sabbatical at the Wistar, and when he appeared to have arranged a supply of the embryos on which Hayflick's work was based, obtained from abortions conducted in Sweden. If correct, this meant that amplification of polio vaccines in such substrates could not have started until 1959 or 1960 – which appeared to be too late to have sparked the Group M outbreak.¹⁶⁵

There was also another reason, for it appeared that HeLa, by itself, was unable to grow HIV or SIV, but only in the form of a genetically engineered cell line like HeLa T4+.¹⁶⁶ Such a modified cell line, I was informed, could not have existed before the 1980s.

Furthermore, although HeLa grows poliovirus to a high titre, it was widely accepted (even in the fifties) that this cell line could only be used for research and diagnostic purposes, because of the potential risks of preparing a human vaccine in a culture derived from a particularly vigorous human tumour. For instance in 1954 the Armed Forces Epidemiological Board, the members of which were not renowned for being especially faint-hearted, ruled out HeLa for the production of an adenovirus vaccine for soldiers.¹⁶⁷ So although HeLa had been present in the Congo from 1954 onwards, there was no evidence to suggest that anyone had actually used it as a polio vaccine substrate.

However, in the course of writing the present paper, I was reminded of certain important details, such as Ninane's claim that he was trying to make human cell cultures in Stanleyville, and the fact that Fritz Deinhardt had extensive experience of working with this type of culture. And so I decided to look once again into the possibility that human cells might have been used for polio vaccine production in the Congo.

Whilst I would wish to emphasise that I still differ from the "Goldberg/Elswood school" on several issues, I do now believe that their long-standing hypothesis (that the additional preparation of CHAT vaccine in human cells contributed to the birth of AIDS) may perhaps have merit.

Gaston Ninane told me several times that he had been working with human cells in his lab – but which cells might these have been? Firstly, because he spoke in terms of "trying to make cultures" from human cells, it appears that he was not talking about HeLa, a line which grows continuously in culture, and which is notoriously robust. So what other possibilities are there?

At Lise Thiry's lab in Brussels, where Dr Ninane trained in the summer of 1957, they had been growing CHAT and Fox in different batches of "monkey kidney", and in several different human cells. These included human cell lines (such as Eagle's KB cells, Chang's liver cells, and T1 kidney cells) and human amnion cell lines (including two, "N" and "LoFi", which were reported as being susceptible to poliovirus). They had also grown CHAT and Fox in other cultures too, including SCH, a cynomolgus heart cell line obtained from Jonas Salk, and even a line of "transformed embryo rabbit kidney", ERK-1, which grew poliovirus to a surprisingly high titre.¹⁶⁸

Interestingly, it seems that others who were directly involved in this story were also doing research along parallel lines. Fritz Deinhardt and the Henles were working with several different human amnion cell lines in their virus lab at CHOP in Philadelphia, and by 1955 or early 1956 these included intestine 407, liver 407, MAF-E, and another, "Lung TO", which they subsequently supplied to Lise Thiry's lab.¹⁶⁹ By February 1958, they were reporting work on three other human amnion cell lines (Line T, 103 and 185) which they had apparently obtained from Leonard Hayflick.¹⁷⁰

Meanwhile, at the Wistar itself, between 1953 and 1957, a virology research unit was set up under Geoffrey Rake and William McLimans, which experimented with several different types of tissue culture, and engaged in the semi-industrial production (in 5-litre spinner cultures, and 20-litre stainless steel fermentors) of viruses such as the

polioviruses in substrates such as HeLa, Chang's conjunctival cells, FL (a human amnion cell line), and the embryonic rabbit kidney cell line, ERK-1.

Leonard Hayflick, who worked at the Wistar for most of the fifties, told me that this period, just after John Enders proved that you could grow poliovirus in human cells (and ones which, in contrast to the previous accepted wisdom, did not derive from nervous tissue), was "the Golden Age of Virology".¹⁷¹ Between 1952 and 1956, Hayflick did his doctoral dissertation at the Wistar on the growth of mycoplasmas in tissue culture, and then he did two years of post-doctoral work, again concentrating on cell culture, at Charles Pomerat's lab in Galveston, Texas. (Pomerat, known to his friends as "Pom-Pom", had headed the U.S. Navy's Subtropical Marine Laboratory at Woods Hole during the second world war.) Hayflick returned to the Wistar at the end of 1957, after Koprowski had taken over as director, "to organise a cell culture lab and provide cells of different types to the investigators".¹⁷²

There was in fact a small coterie of virologists (including Koprowski, McLimans, Rake, Pomerat, the Henles, Deinhardt and Thiry) who were at the forefront of research into human cell cultures in the second half of the fifties, and there were close links between different members of the group, which extended to the young scientists (like Hayflick, Osterrieth and Ninane) whom they trained. Pomerat oversaw Hayflick's post-doctoral work, and Hayflick then moved back to the Wistar (together with two others from Galveston, Moorhead and Fernandes). Hayflick thanked Thiry for her help at the end of his WISH article, and he provided human amnion cell lines to the Henles and Deinhardt. They, in turn, provided one of their cell lines to Thiry. Thiry trained Ninane. The Wistar trained Osterrieth, and while in Philadelphia Osterrieth became close friends with Deinhardt.

The one figure linking all these people together was Hilary Koprowski.

One particular preoccupation of these scientists during the "golden age of virology" was the question of which cells would grow which viruses, and – in particular – which cells would grow the benchmark viruses of the era, the polioviruses. In short, there was a lot of experimentation with different substrates, which tended to be reported separately from what some apparently viewed as the pure virus research, on subjects such as attenuation.

And there was one particular substrate that looked especially promising in 1957 and 1958. At that key polio conference in Geneva in July 1957, the one which was attended by Koprowski, McLimans, Thiry and Ninane, and the one at which the WHO Expert Committee gave the go-ahead for open trials of OPV "in the face of epidemics", William McLimans from the Wistar spent ten minutes or more singing the praises of the embryonic rabbit cell line, ERK-1. He reported that in the Wistar labs all three types of poliovirus grew to very high titres in ERK-1, with 7.5 log doses or better being obtained within 40 hours. He stressed that, because of the fear of malignant agents, stable cell lines of human or monkey origin should not be used for producing polio vaccines – at least not IPVs, which were inoculated into the bloodstream. However, he continued, the use of a stable cell line from a species far removed from man (such as the rabbit) would "obliterate the fear...of malignancy". He ended up by posing a question: "can we take the monkey business out of polio vaccine production?"¹⁷³

In fact, in several respects ERK-1 seemed a miracle – the first non-primate cell line to be discovered that grew poliovirus, and – furthermore, which did so to titres that were just as high as those of HeLa.¹⁷⁴

Should anyone have seen the warning signs? Although misgivings were officially expressed in the early sixties,¹⁷⁵ it was not until 1966 that a geneticist, Stan Gartler, pointed out that many of these cell lines appeared to be not just similar, but the same – and they all looked like HeLa. Apparently the cell biologists in the audience received the news with extreme hostility.¹⁷⁶ So it was not until 1973 that the painstaking work of Walter Nelson-Rees *proved* that many of the world’s commonly-used cell lines had in fact been taken over by HeLa cells. The HeLa cell line was so vigorous that if someone opened a stoppered flask of it, or pipetted a sample, a little carelessly, and sent a few micro-droplets drifting into the air to land on another cell culture, the latter would be outgrown and replaced within days. Suddenly it became apparent why, back in the fifties especially, so many normal cells (like human amnion cells) had suddenly miraculously “transformed” into cell lines. In reality, their nests had been taken over by HeLa cuckoos.

Among the cells which Nelson-Rees “outed” as HeLa cultures in his two famous articles in *Science*, in 1976 and 1981,¹⁷⁷ were most of those really productive cell lines of the fifties.

These included seven of those mentioned above: KB, T-1, FL, Chang’s liver cells,¹⁷⁸ Salk’s cynomolgus heart, Henle’s intestine 407, and Hayflick’s WISH.¹⁷⁹ All seven cultures had been present in the labs of Koprowski, the Henles and Fritz Deinhardt, and Lise Thiry, in the period 1955-1960, and all seven had been colonised by HeLa.

The Nelson-Rees announcement was one to which many members of the medical establishment did not respond either wisely or graciously, and shortly afterwards, he was rewarded with the sack. Several of his detractors seemed determined to ignore the bad news, and to go on as if nothing had changed. For instance, when Jonas Salk rather courageously admitted, at the Lake Placid conference in 1978, that some of the injectable polio vaccine he had produced in the fifties must have been mistakenly prepared in a culture of HeLa, the organisers apparently persuaded him to omit this detail from the published proceedings.¹⁸⁰ Even today, many cell lines are wrongly described, and, as a contemporary article expresses it: “Chaos reigns and fraud – unwitting or deliberate – is condoned”.¹⁸¹

Nowadays it is known that polioviruses grow only in primate cells, so whatever the ERK-1 cell line was, it was certainly not embryonic rabbit kidney. Walter Nelson-Rees never reported in the literature on this particular cell line, but written on one of the copies of his own 1981 paper is a contemporary note, in his hand, which indicates that “ERK-1 (rabbit) of Westbrook, 1957, was also shown to be HeLa.”¹⁸²

It is clear that even the 1981 list from Nelson-Rees was not exhaustive, for he was not invited to test every cell culture that was then in use. An example of another possible omission is Chang’s conjunctival cell line. However, Nelson-Rees has recently sent me a page of detailed documentation on this subject, which concludes: “I am 99.99% certain that Chang’s conjunctiva [cells], like his liver [cells], are HeLa”.¹⁸³ Nowadays,

he says that many of the other cell lines of the era are also likely to have been HeLa, from the moment that they miraculously “transformed” from normal cells. And of course, it is precisely because they had transformed and become more robust that virologists valued the presence of such cells in their laboratories.

It is worth reiterating that four of the five major cell lines being researched at the Wistar in the mid-fifties (HeLa, FL, Chang’s conjunctival cells and ERK-1) were actually different forms of HeLa.

The repeated failures by doctors Koprowski, Plotkin, Ninane, Osterrieth and others to give a proper account of what they were doing in the Congo, the increasingly unbelievable retrospective denials that polio vaccines were, or could have been, prepared there (denials which are quoted extensively by Dr Plotkin, as if he and Dr Koprowski were unaware that local vaccine amplification was taking place), the ongoing attempts to persuade witnesses around the world to modify their stories – all these things lead one to suspect that it may be that more than just chimpanzee cells were being field-tested in the Belgian Congo in the late fifties.

Meanwhile, there is some rather significant evidence “from the horse’s mouth”. In their various polio articles in the mainstream medical literature in the years up to and including 1960, Doctor Koprowski and his various collaborators hardly, if ever, mentioned the possibility of using human cells for making oral polio vaccines. However, in a lecture which he delivered in Kenya and South Africa in July and August 1955, and which was later reprinted in the *South African Medical Journal*, Koprowski presented two tables which encapsulated “the author’s views on the acceptability of attenuated viruses as vaccines”. One of these tables (sub-titled “Personal Credo of the Author”) listed various potential OPV tissue cultures, and made it clear that both “monkey epithelial” and “human epithelial” cultures would be considered acceptable as substrates.¹⁸⁴

The next reference by Koprowski to the use of human cells as OPV substrates that I have been able to find came five years later, in November 1960, when he and Plotkin co-wrote a letter to the WHO, entitled “Notes on acceptance criteria and requirements for live poliovirus vaccines”.¹⁸⁵

In a section entitled “Viruses other than polio”, they analysed the new-found (post-SV40) fear of adventitious simian viruses which might cause human cancers. “Any tissue that is obtained from a normal animal”, they wrote, “may be parasitized by viruses probably harmless to the host most of the time. When such an organ is removed from the host and the cells allowed to multiply outside the control of the whole organism, as, for instance, in tissue culture, the virus ‘infected’ cells seem to multiply...and the virus which parasitized them is released.” Their response to this, however, was a pragmatic one, for they suggest that “vaccine be allowed to contain these agents if the titre is so low that each human subject will receive only a small dose of these [adventitious simian] viruses.”¹⁸⁶

Then, in a section titled “The phantom of cancer virus”, Koprowski and Plotkin begin to get more controversial. “One may consider that the chance or the presence of a cancer virus in a cell-free preparation obtained from monkey kidney tissue culture is

as small or as great as in a cell-free product obtained from cultures of HeLa cells, even though the latter originated from a malignant tumour of man.”

And then comes the punch-line. “Even if HeLa cultures”, they write, “will not be considered as suitable menstrea for growth of poliovirus strains, there are at present in existence several tissue culture lines which have been originally isolated from normal human embryo, and which grow only for a limited number of passages (30-40) *in vitro*. These culture lines which are susceptible to poliovirus growth have morphological and chromosomal characteristics of a *normal human cell*.”

This is clearly one of the first public references to Hayflick’s human diploid cell strains (WI-1 to WI-38), which would later feature in an article by Hayflick that was published in 1961 (but which was apparently first submitted, and rejected, in 1960).¹⁸⁷ At around the same time, Hayflick had also been examining the potential of another culture, WISH (Wistar Institute Susan Hayflick), based on amniotic cells obtained at the birth of his own daughter in November 1958, which transformed into a cell line on the last day of that year.¹⁸⁸ Like the WI-1 to WI-38 series, WISH was good at growing Koprowski’s polio vaccines, CHAT and Fox. Walter Nelson-Rees later identified WISH as yet another front for HeLa, although Leonard Hayflick has apparently never accepted this.¹⁸⁹

The question that has to be asked is: given Koprowski’s ambivalent position, between 1955 and 1960, about the use of human cells for making OPVs, and the fact that human cell substrates of various types were being prepared at the Wistar Institute between those same years, 1955-1960, just what is the possibility that Koprowski and/or his collaborators might have tried out vaccines prepared in human cells on “volunteers” in central Africa during this period? This category would include vaccines prepared in human amnion cells, or HeLa cultures, or human cell lines that were really HeLa, or other cell lines (like ERK-1) that were really HeLa, or Hayflick’s new “semi-stable human cells” (such as the diploid strains, the WI-1 to WI-38 series).

Two points need to be appended here. Firstly, there is substantial evidence that other hitherto untested, and potentially unsafe medical, pharmaceutical and chemical preparations were administered experimentally to populations in central Africa during this period, especially during the late fifties.¹⁹⁰ Some of these trials were fully reported in official documents, but for others one has to read between the lines, or follow up on stray references and other, similar clues. Secondly, some of these trials took place without even a nod towards what is nowadays referred to as “informed consent” – and it is clear that as independence approached, the time for conducting such trials on convenient “volunteers” in Africa was growing short.¹⁹¹

I wrote earlier that I have long been sceptical that polio vaccines made in human cells could have been tested in Africa, and could have been connected to the birth of AIDS. However (and not for the first time), Bill Hamilton was more far-seeing than I was. In a hand-written note he sent me about a draft of one of Blaine Elswood’s early papers on this subject, he commented as follows: “I haven’t had time to read his piece and size up his references with full care, but at the moment I don’t see any major snags with his ideas, and don’t see them as exclusive to most of yours about the Congo events. K. [Koprowski] may have been experimenting with several culture techniques,

and [viruses in] kidneys brought back from the Congo could have infected HeLa lines in his labs. Maybe these lines were used in the Congo, maybe Pan paniscus kidney cultures...I had forgotten (if I knew) that HeLa was long known as an excellent medium for poliovirus, but now reminded, I can see that for a man as determined and uncaringful as K., any cell lines that reared the virus well would have been tempting ...¹⁹²

There appears to have been a considerable degree of interest in the African CHAT trials by scientists who were at the forefront of research into human cell cultures. Apart from the frequent visits by members of the Koprowski group at the Wistar, there was Fritz Deinhardt's three month visit to the Congo in early 1958. The fact that Deinhardt is reported to have been present at some of the CHAT trials (apparently those in Stanleyville itself)¹⁹³ suggests that his visit may not have been prompted solely by the hepatitis research, as does the fact that for some reason his bosses, the Henles, were apparently unenthusiastic about his visiting Stanleyville.¹⁹⁴ Also interesting is the visit by several Belgian virologists (such as Lise Thiry, Piet De Somer and E. Nihoul) to the Stanleyville lab for the virus symposium in September/October 1957, a visit which some of them combined with longer stays in the country. De Somer, who later (in 1959, it seems) produced CHAT vaccine for use in Burundi at the Belgian pharmaceutical house, RIT,¹⁹⁵ stayed on in the Congo for two more months, and is said to have become a keen supporter of OPV during that visit.

Then there are other intriguing links. Lise Thiry met Agnes Flack at Brussels airport on her way out to the Ruzizi trials, and later spent two days with her when she passed through Brussels on her way back home, which suggests that she may have had some particular interest in the progress of those trials, or in certain sections thereof.¹⁹⁶ Again, there is the fact the CDC was apparently interested, shortly after independence in the Congo, in testing pre-vaccination and post-vaccination blood samples from one of the final CHAT trials, that at Coquilhatville.¹⁹⁷ Even though this mooted collaboration apparently never materialised, is it possible that there was something especially interesting about that particular trial?

But let us leave conjecture, and return to documented facts. Precisely which tissue cultures were available, or being used, in Belgian colonial laboratories during the 1950s? I can find no records mentioning ERK-1 cells, although this is not to say they were not present. What is documented is as follows. The first lab to make tissue culture in the Congo was the virology lab that opened in 1954 as part of the *Laboratoire Médical d'Elisabethville*, in the heart of the Copper Belt, in Katanga province in the south of the Congo. This lab used HeLa cells from late 1954 onwards,¹⁹⁸ and human amnion cells from an unspecified date between 1954 and 1957. (The virology lab was conveniently situated near to a maternity department.)¹⁹⁹ With regard to the capital, Leopoldville, Dr Michel Vandeputte told me that he joined the central laboratory there in July or August 1956, soon after which he was asked to set up a virus lab. He wrote to Stefan Pattyn, who then headed the virus lab in Elisabethville, and they exchanged sera, viral strains and tissue cultures, namely HeLa cells and amniotic cells, which, Vandeputte told me, "we used more or less for experimental purposes". The Leopoldville virology lab opened in October 1957, and subsequent papers show that Dr Vandeputte was using both HeLa and human amnion cells by that month at latest.²⁰⁰ Vandeputte told me that the Leopoldville lab had mice,

rats, guinea pigs, rabbits and monkeys (which in this case would appear to mean “primates of unknown species”, which may or may not have included chimpanzees).

Two other large and impressive purpose-built medical laboratories also opened in the Belgian Congo in 1957, at Stanleyville and Bukavu. Both had virology sections, and large animal houses, and we know that both either had (or had ready access to) chimpanzees. We do not know which tissue cultures were available at Bukavu, but (as explained above) it appears that Maitland-type tissue cultures based on chimpanzee cells and sera were present at the Stanleyville virology lab by February 1958 at the latest. However, the fact that there was a mass sacrifice programme of the Lindi chimps, beginning in the second half of 1956, and that Courtois was doing research with chimps in Stanleyville even before Lindi camp opened, suggests that these types of cultures may have been prepared at the *old* medical laboratory from as early as 1955. According to Dr Osterrieth, it was not until “several months” after February 1958 that he began attempting to make tissue cultures using trypsin, and he only succeeded in making a few trypsinised cultures during 1958, these being from baboons. He states that he first obtained HeLa cells in 1959. Gaston Ninane, meanwhile, spoke to me about trying to make human cell cultures during 1957, and it seems likely that, like Pattyn in Elisabethville, he could have been using amniotic cells obtained from the local maternity ward.

If we collate the documentary and testimonial evidence, then HeLa cells were available in the Belgian Congo from 1954 onwards, human amnion cells (and perhaps cell lines) from some time between 1954 and 1957, chimpanzee cultures from some time between 1955 and February 1958, and trypsinised monkey kidney tissue cultures (for instance from baboons) from around the middle of 1958. The relevant articles refer only to “amniotic cells” and “human amnion cell tissue culture”; no attempt is made to clarify which of these, if any, had “transformed” into human amnion cell *lines*. However, it is worth repeating that in many other labs where human amnion cells and HeLa were both present during the 50s, it was only a matter of weeks, or months, before the amnion cells were overtaken and colonised. One article from Gertrude Henle and Fritz Deinhardt describes five such “transformations” in the CHOP virology lab in the space of a few months in 1955-6.²⁰¹

It is likely that different variants of HeLa (*both recognised and unrecognised*) were present in Congolese laboratories from an early stage. Whether or not any of these cells were ever used as a polio vaccine substrate is not known. However, it certainly seems possible that the very availability of these cells, especially those which were officially “not HeLa cells”, might have led to their being assessed in one or more of the vaccine field-trials.

1) The potential significance of HeLa contamination.

The fact that chimpanzee cells (which may well have been SIV-contaminated) appear to have been used to make human vaccine in Stanleyville in the period up to April 1958 is, in itself, alarming. But if such vaccines, in turn, were subsequently contaminated with, or passaged in, or diluted by, HeLa cells, then this could potentially have been far more serious in terms of the impact on human health.

Although much work has been done on the natural history of HIV in the human body (and of SIV in the bodies of primates), many details, such as the precise mode of viral entry, remain uncertain. What is clear, however, is that both lymphocytes and macrophages are prime target cells for HIV and SIV. However, there is a difference, for SIV-infected lymphocytes have one furious burst of virus production *in vitro*, and then die off. By contrast, SIV-infected macrophages continue throughout the life of a culture to spew SIV out into the supernatant.²⁰² Macrophages do not die off and, as Robin Weiss has observed, they make up approximately 1% of all epithelial cell cultures.²⁰³ They are sometimes referred to as cellular vacuum cleaners.

So let us imagine a chimp cell culture of which one part in a hundred is made up of SIV-contaminated macrophages, which is then combined with cells that are described as KB, or FL, or ERK-1, but which are in fact HeLa. Is it conceivable that SIVcpz could grow in HeLa? When I first asked this question of microbiologists and virologists back in the late nineties, most of them said that this could only happen in a genetically engineered cell line, such as HeLaT4+, which was not created until the 1980s. But is that really the only way?

What follows in the next two paragraphs is sheer speculation, for I can find nothing in the literature about whether or not SIVcpz will grow in a HeLa culture – or, indeed, about what impact HeLa might have on the chimp virus, if it did support its growth.

Some microbiologists I have spoken with believe that because macrophages are fusogenic, the act of transferring chimp cells containing macrophages into HeLa, that most turbocharged of tissue cultures, could in itself generate a hybrid cell line which would combine chimp CD4 cells with the immortality of HeLa. They say that such a hybrid cell line would, from that point on, represent an excellent substrate for growing SIVcpz (for instance if SIVcpz-contaminated tissue culture was introduced into the HeLa/chimp hybrid). Furthermore, they say, the necessary process might be even simpler, and require just a single step – that of putting primate cells which contained already SIV-infected macrophages into HeLa. However, in this instance the outcome is less readily forecastable, in that the hybrid cells might spew out SIV, or might be killed off by the SIV.

If such a hybrid HeLa/chimp cell line was accidentally created in the course of the CHAT vaccine research that was taking place in the Congo during the fifties, then the potential implications could have been “a recipe for disaster”, in the words of one respected microbiologist. Such a cell line could introduce a crucial amplification step to the basic OPV theory, by allowing the mooted chimp SIV contamination of CHAT to become human-adapted. Furthermore, if two or more SIVs were present in a HeLa/chimp hybrid culture, they would be likely to recombine even more rapidly than in a chimp cell substrate. [For the potential implications of such recombinations, see “Dating the epidemic”, below.]

I must repeat that such analysis clearly remains in the realm of the hypothetical – at least until such time as someone stages an appropriate *in vitro* experiment.

The basic OPV theory, involving a contaminated chimpanzee tissue culture used to make the polio vaccines fed in the Congo, stands up on its own. However, if HeLa contamination of those cultures also occurred, this just might represent the “extra

ingredient”, the amplification step, that could help to explain why this particular zoonotic transfer was some orders of magnitude more serious, in terms of human disease, than those other SIV transfers which resulted in the human outbreaks of HIV-2, and HIV-1 Groups O and N. These latter three outbreaks may have resulted from contact with bushmeat, or from iatrogenic (possibly polio vaccine-related) episodes [see below] in west Africa and west central Africa, but they have probably resulted in fewer than 20,000 fatalities in total.²⁰⁴ By comparison, by 2002 the Group M-related AIDS pandemic seems to be some three orders of magnitude greater, having caused an estimated 20 million deaths.

At this point, two historical details which may be relevant to the hypothetical CHAT/HeLa scenario need to be mentioned. Firstly, an article from a Leopoldville newspaper in August 1958, published a week before the start of the vaccinations of all the children aged five and under in the capital, reported that the new polio vaccine of Dr Koprowski “has been prepared at Elisabethville by the Wistar Institute, and is controlled from the point of view of efficacy and safety by the Stanleyville laboratory”.²⁰⁵ To date, nobody has been able to explain what this sentence actually means. **[Figure 5]**

The opening paragraph of this article reveals that the new polio vaccine in question had previously been “perfected [*mis au point*] by Koprowski at Stanleyville, in collaboration with the medical services of the Congo”. The phrase *mis au point* also embraces the idea of something which is being “fine-tuned”, or to which someone is “putting the finishing touches”. The concept fits nicely with that of amplification in a new substrate.

This link between Elisabethville and the CHAT vaccine that had been perfected in Stanleyville is intriguing. Dr Stefan Pattyn, who headed the virus lab at Elisabethville in the fifties, has since stated to Stanley Plotkin that “certainly poliovaccine was never produced” in Elisabethville between 1955 and 1960.²⁰⁶ This, as Plotkin points out, is a clear denial, but it leaves unexplained why such a specific statement should have appeared in the local press in 1958, in an article which was apparently based on an interview with one of the Belgian doctors involved, and which was, in all other respects, extremely well-informed. It is worth noting that Pattyn appears to have been familiar with the work of Koprowski, Plotkin, Hayflick and Gelfand. An article he wrote in the early sixties on “Anti-poliomyelitic vaccination in tropical countries” focuses on the Koprowski strains, and on attempts that Koprowski and Hayflick had made to get away from the potential dangers of contaminated primate tissue cultures by adopting “a technique for culturing fibroblasts from human embryos”.²⁰⁷

Let us suppose for one moment that Dr Pattyn was not fully informed about all that happened in Elisabethville in the second half of the fifties, and that one of the many labs in that city *was* producing the Koprowski vaccines as reported in the article.²⁰⁸ In that case, which locally-available substrate would have been used?

During my various interviews with Belgian doctors who used to work in the Congo, the fact that the Elisabethville lab had used human cells rather than monkey cells for its virus research was mentioned quite often. This conclusion is supported by a paper which the two leading scientists from the Elisabethville lab, the director (Jean Delville) and the head of virology (Stefan Pattyn) delivered to the Stanleyville virus

symposium in September 1957, which explained that in their virus research in Elisabethville they had worked since 1954 with HeLa cells, which had considerable advantages over “monkey kidney cell cultures”.²⁰⁹ This account is expanded in a review by Pattyn of his own enterovirus research in the Congo, which was published in 1962. Here, Pattyn explains that he decided to concentrate on poliomyelitis research after he witnessed a polio epidemic in the savanna region of Upper Katanga. He continues: “It was my good fortune to work in a well-equipped laboratory where a centre for tissue culture was established. The HeLa cell line was used as a source of continuously proliferating cells, whereas human amnion cells were used as a source of freshly trypsinised cells, with broader susceptibility to polioviruses. Amnion cell tissue cultures could be produced on a large scale as our laboratory was near the maternity department where an average of 24 deliveries were carried out in 24 hours.”²¹⁰

The descriptions of the research makes mention only of virus isolation and diagnostic work, but if CHAT was indeed prepared by Wistar Institute scientists in that city in 1958, is it not possible, indeed likely, that they would also have used one of these human cultures as a vaccine substrate?

Pattyn’s various articles make it clear that he was actively engaged in polio research in the Congo throughout the fifties. For instance, it was he who coordinated the polio antibody studies in Leopoldville, Elisabethville and Bukavu – and who may have done the same in Astrida (now Butare, Rwanda). If the newspaper article is correct, then many will feel that it is Pattyn’s virology lab, where they used only human cells, that is the likeliest venue for CHAT vaccine (which had previously been perfected in Stanleyville) to be amplified for the Leopoldville trials.

It is my impression that Dr Pattyn may know more than he says about the various polio vaccines that were prepared and tested in the Belgian Congo. After all, it was he, in the mid-nineties, who once advised me that if I wanted to know more about the chimp research at Lindi, I ought to “ask Osterrieth. He was implicated in this whole thing.”²¹¹ Having said that, however, he declined to elaborate further. When I interviewed Dr Pattyn again in 2000, I was surprised by the change in this previously helpful man. On this occasion, he was hostile and defensive, and referred to me with heavy sarcasm as “the man who wants to be famous”. He had not read *The River*, but told me he thought that the OPV theory was “foolish”. When asked why, he said because there was a “lot of evidence” that AIDS had existed before 1959, but was unable to support this claim.

The second historically relevant point is that in July or August 1958, Dr Henry Gelfand, an American epidemiologist who was based at Tulane University in New Orleans, hand-carried the latest Koprowski strain (CHAT pool 13) from Brussels²¹² to Leopoldville. He also visited Stanleyville, Bukavu and Elisabethville, in order, as he put it, “to acquaint regional authorities about the vaccine, and its proposed use”. However, the precise purpose of his journey outside the capital has recently become a bone of contention, for Dr Gelfand’s accounts of these events (which in 1996 included his declaration that he “must have carried” polio vaccine to labs in the three other cities) have changed as the years have passed. In a letter which he wrote to Stanley Plotkin in 2000, Dr Gelfand claimed that it was “extremely unlikely” that he had

taken polio vaccine to the other three cities. He also claimed that this is what he had told me, which is untrue.²¹³

In a letter which he wrote me in 1996, Dr Gelfand closed as follows: “P.S. I forgot to mention that I went to B.C. [Belgian Congo] only as a consultant to Koprowski and the Wistar Institute”.²¹⁴

This postscript is interesting in the light of the 1958 newspaper report which records that the new Koprowski polio vaccine, which had previously been perfected in Stanleyville, “has been prepared at Elisabethville by the Wistar Institute”.

Let us suppose for a moment that, despite what Dr Pattyn says, a visiting scientist or consultant representing the Wistar Institute did prepare the CHAT vaccine that was used in the Leopoldville trials at one of the several Elisabethville labs. The scientist in question could possibly have been Dr Gelfand (who, despite officially being an epidemiologist, was also an acknowledged expert on polioviruses).²¹⁵ Or that scientist could have been someone else representing the Wistar, someone who had visited Elisabethville in the preceding weeks or months.

Because of Dr Gelfand’s ambivalent answers about what he actually did with the CHAT pool 13 vaccine, we are left to speculate about how the vaccine used in Leo might have been prepared in Elisabethville. Did Dr Gelfand, despite his recent protestations to the contrary, carry a bottle of pool 13 to Elisabethville, and amplify it there – perhaps in the virus lab which was the oldest, and almost certainly the best-equipped, in the Congo? Or had another doctor already brought some locally-prepared CHAT from Stanleyville to Elisabethville, in order to amplify it further? Or did Gelfand (and/or another Wistar representative) amplify both pools in Elisabethville – the newly-arrived pool 13, presumably made at the Wistar, and the older pool 10A-11, which had already been amplified in Stanleyville? Both the second and the third scenarios would theoretically allow a batch of CHAT vaccine made in chimp cells to be further passaged in human cells, which might (for instance) have been human amnion cells that had been overtaken by HeLa.

Henry Gelfand’s clarification of his official status in the letter he wrote me is also intriguing for another reason. If he was in the Congo “only as a consultant to Koprowski and the Wistar Institute”, then he was clearly representing neither Tulane University (which is the home of tropical disease research in the US), nor the CDC, where he moved soon after the Leopoldville trials to take over the enterovirus unit. This is further underlined in the paper which Gelfand, Plotkin, Koprowski, Courtois and two other Belgian doctors subsequently wrote about the Leopoldville trials, for a note on the title page states that: “the participation of Dr Gelfand does not necessarily imply endorsement of the studies by the Public Health Service.”²¹⁶

Another note on this paper makes a similar claim about Stanley Plotkin’s participation. And the next paper in the series, on which Plotkin was lead author, claims that “This work was done when Dr Plotkin visited Leopoldville in May 1959 while on leave from the Public Health Service, and the opinions expressed are those of the authors only.”²¹⁷ It is for the reader to decide whether these are *pro forma* disclaimers, or whether one is meant to take them seriously.

m) Sources of funding.

Some have questioned why it was that so much money was poured into medical research in the Belgian Congo in and around 1957, when it was already clear that independence was approaching fast. Large and impressive new medical laboratories were erected at Stanleyville, Bukavu, and Bunya, all of which featured virology departments. Furthermore, a dedicated virus lab was opened in Leopoldville. Since the colony was officially self-financing, and was famously short of cash, this has suggested to some observers that foreign funds might have been involved.

Whether or not the United States Public Health Service (PHS) endorsed Koprowski's research programme and vaccine trials in the Belgian Congo, what is certain is that that Service was supporting and at least partially bank-rolling the venture.²¹⁸ In *The River*, I detail several documented links. There was a PHS research grant [E-1799] which was cited in all the papers about Koprowski's polio research in Africa; an internal paper by Ghislain Courtois which clearly stated that the PHS was supporting the research, and was paying for Koprowski and members of his staff to visit Stanleyville annually for the next five years;²¹⁹ a published comment by Agnes Flack indicating that the Congo vaccinations, including the Ruzizi trial, represented a joint undertaking of the Belgian and American public health services; and the fact that Stanley Plotkin, who did much of the organisation and preparation not only for sections of the Congo trials, but also for those in Poland, Croatia, and the US, was apparently one of a team of "top epidemiologists" who were working for "the USPHS field post that is located in the Wistar".²²⁰ Indeed, despite the claim that Plotkin was on leave from the PHS while he was working in the Belgian Congo, a letter that he wrote to Fritz Deinhardt from the Congo in May, 1959 was typed on Public Health Service headed notepaper.²²¹ There are other examples as well, but these make the point.

And then there is the role of Karl Friedrich Meyer.

Here, a little additional history is needed. There were plans for a second stage of the Lindi project, which would have involved the United States and Belgium establishing a chimpanzee colony dedicated to medical research in the Congo, one which was intended to survive beyond independence. The venture had the backing of the Belgian king, and of the US Public Health Service.²²² Karl Meyer, the long-time director of the George Williams Hooper Foundation (an institute of medical research in San Francisco, financed by a bequest from a leading industrialist) led a team of four American scientists to the Congo in May 1960. They discussed possible locations for the new chimp camp, with an island near Lindi and the IRSAC²²³ establishment near Bukavu being the leading candidates. Koprowski was meant to be a member of the group, but finally did not attend; the Belgian representatives were Ghislain Courtois and A. Lafontaine, and there were two chimp specialists from IRSAC. In the end plans for the research centre fell through because of the collapse of the political situation so soon after independence.

However, there is another interesting clue from Lindi itself. In 1999, the local villagers unearthed what appeared to be a foundation stone at the camp, which was inscribed on all four sides. There was 1956 (the year of opening), N (for North), IGCL (unknown), and KF/003. Initially I thought that the latter might be a burst of egoism –

Koprowski Foundation Number 3, or something of that sort. Only much later on did I remember that Karl Friedrich Meyer of the George Williams Hooper Foundation was universally known as “KF” by his contemporaries.²²⁴ The nickname is even referred to in his obituaries.

In his Alvarenga Prize Lecture in 1959, Hilary Koprowski recounts the history of his ten year association with polio vaccines, and identifies a key moment. In January 1952, he had what he calls a “fateful meeting” in New York with K.F. Meyer, and with Joseph Smadel, whom he identifies as an associate director of the US Public Health Service. Apparently Koprowski sought advice from these two great men, and Smadel suggested that he and Meyer should establish a cooperative study. “This led to prolonged and fruitful collaboration” which lasted several years, explains Koprowski, and “the results...of the investigations were very gratifying”. In 1952, and again in 1955, Meyer helped Koprowski set up trials of his OPVs in Sonoma, a facility for the developmentally disabled just north of San Francisco. Thus the setting up of Lindi camp in 1956 appears to have been the third aspect of the Meyer/Koprowski collaboration, KF/003.

But there may be a little more to that “fateful meeting” than meets the eye. In 1952, Jo Smadel was not yet with the PHS. In those days, he was head of the department of viral and rickettsial diseases at Walter Reed Army Medical Service Graduate School (later known as the Armed Forces Institute of Pathology) in Washington DC, and a renowned (and sometimes feared) organiser and power-broker in the worlds of medicine and military medicine. He played a key role in several commissions of the Armed Forces Epidemiological Board (AFEB), and was one of the scientists who helped coordinate America’s biological warfare programme. In the latter role, he helped to organise investigations into the potential dangers from, and uses of, different pathogens, notably rickettsial diseases and arboviruses (arthropod-borne viruses).

Karl Meyer meanwhile, was a hero of public health, having developed several different human vaccines, as well as a technique for eliminating botulism which opened the way for the canning of food. Some of the diseases which these vaccines protected against (such as psittacosis, pneumonic plague and brucellosis) were rather rare in the United States, but they were described as “diseases of military importance”, in that they were viewed as constituting a potential threat to the armed forces of the United States. Much of Karl Meyer’s work, therefore, involved developing vaccines for the troops, vaccines which would protect them against new or little-known diseases when travelling into tropical areas, or alternatively when they were exposed (from whichever quarter) to biowarfare agents. There was, in short, an ongoing collaboration between Smadel and Meyer: the military medic, and the civilian researcher who developed vaccines against some of the same diseases.

A good example of this type of collaboration relates to 1954, and gives some sense of the eminent American scientists who were at least knowledgeable about (if not indirectly involved with) biowarfare (BW) research during the Cold War years. In that year a Dr Devignat, who was director of the “Ecole A.M.I.”²²⁵ at Elisabethville in the Congo, wrote to Joshua Lederberg, offering to dispatch three strains of pneumonic plague – one highly virulent, one which had been attenuated in the lab and which could perhaps serve as a vaccine, and one intermediary strain, which possibly showed

evidence of recombination. Lederberg immediately contacted Dr Ellis Englesberg, one of Karl Meyer's plague experts at the George Williams Hooper Foundation, and Dr Werner Braun, a significant figure at what was then called Camp Detrick, and which later became Fort Detrick [see below]. Professor Lederberg wrote back to Dr Devignat to say that Englesberg was otherwise engaged, but that Dr Braun (not to be confused with rocket scientist Wernher Von Braun) "would be interested in the question of genetic recombination in this species", and would like to collaborate.²²⁶

So, doctors Smadel and Meyer were the men from whom Koprowski sought counsel in 1952, and with whom he established a long and fruitful collaboration. And those two initials, KF, sat quietly on the foundation stone beneath Camp Lindi, the experimentation centre of the *Mission Courtois/Koprowski*, from 1956 until they were uncovered again, forty-three years later.

n) Events at the Wistar Institute.

At this point, we need to turn back a few years, and examine what was happening at the Wistar Institute before Hilary Koprowski was appointed director in 1957. The Institute had officially been without a director for the previous nineteen years.²²⁷ However, it had not been asleep.

In 1952, it came under the wing of the new vice-president for medical affairs of the University of Pennsylvania, Professor Norman Topping. An ex-navy man, and an expert on rickettsial diseases, Topping came from the milieu of military medicine which had taken control of American public health during the years of the second world war, and which had, among other things, responded to the threat posed by the Japanese biological warfare programme. Topping had also helped oversee the process whereby crucial fields such as viral and rickettsial research were officially returned to the civilian fold after the end of that war. Before arriving in Philadelphia, Topping had been the first associate director of the National Institutes of Health, serving from 1948 to 1952. Like Smadel and Meyer, he was a man of substantial influence and power, and one who tended to dispense it quietly, behind the scenes.

Topping knew Lederle Laboratories well, for under Herald Cox, the viral and rickettsial division had prepared vaccines against several of the rickettsial diseases which were felt to pose threats to American troops. These included Topping's own speciality, Rocky Mountain Spotted Fever. And so it was that Topping got to know Cox's deputy, Hilary Koprowski, who had spent the years from 1944 to 1948 conducting research into a wide variety of arboviruses, and had then shifted his attention to two of the great neurotropic viruses: rabies and poliomyelitis. By this stage, Koprowski was probably known as a determined and ambitious man, and also as one who was adept at modifying viruses by growing them in different cell cultures.

In his autobiographical memoir, Topping explains that when he took over the running of medical affairs at the University of Pennsylvania in Philadelphia in 1952, his first job was to reinvigorate the Wistar Institute, which, like the G.W. Hooper Foundation at UCSF, was an independently-funded biomedical research organisation situated at the heart of the campus. In order to do this, he recruited Hilary Koprowski. According to the account given by Dr Topping, it seems that Koprowski must have been brought on board between 1952 and 1954.²²⁸

This may seem strange to those who know that Koprowski was not formally appointed director of the Wistar until May 1957. However, it appears that although he continued to work at Lederle in the intervening years, he may have been wearing two hats. There are at least four separate instances in which witnesses have apparently seen Koprowski at the Wistar, or have had contact with him via the Wistar, in the years before 1957. One example involves Joshua Lederberg, who Koprowski tried to sign up in October 1956, presumably once he had been given the green light to recruit his own team.²²⁹ But there are others from long before that. The doctor who was in day-to-day charge of Koprowski's second polio trial at Sonoma, between April and July 1955, has repeatedly stated that his cheques were paid not by Lederle, but by the Wistar. And Dr Andrew Hunt, who played a similar role at the Koprowski vaccine trials in Clinton, New Jersey, from October 1955 onwards, recalls that Koprowski's links with the University of Pennsylvania (and in particular with Joseph Stokes, who also worked at CHOP) began in 1953 or 1954. He also, strangely, recalls a meeting with Koprowski in 1955 which took place at a business school adjoining the Wistar.

It may well be that Koprowski played only a behind-the-scenes role at the Wistar Institute in the years before 1957. But there are indications that, whether organised by Topping, Koprowski or both, substantial funding started rolling in during this period. The Institute was now publishing the proceedings of the quasi-annual symposia held by the Division of Biology and Medicine of the Atomic Energy Commission (headed by Shields Warren), and by the Biology Division of the Oak Ridge National Laboratory. Several of these AEC meetings dealt with cutting-edge subjects such as "The effects of radiation and other deleterious agents on embryonic development", and "Genetic recombination", and some were co-sponsored by the Biology Council of the National Academy of Sciences-National Research Council. In addition, there are indications that the AEC may have provided further funding, perhaps *sub rosa*, to the Wistar during the fifties. It is worth noting that before she became medical director of Clinton prison in 1953, Agnes Flack had apparently worked for eight years at Union Carbide, the main contractor at Oak Ridge, where she was engaged in "medical research at the...atomic center".²³⁰

Soon after he brought Koprowski on board, Norman Topping recruited two other luminaries for the Institute. "Each was recruited without a committee", writes Topping; "I've never believed in committees." The first was Geoffrey Rake, a famous microbiologist from the Rockefeller Institute and Squibb Institute, and the second William McLimans, who had helped build up the virus and rickettsial lab at the Navy Research Institute in Bethesda.

In 1953 or 1954, Rake and McLimans started assembling a team to concentrate on the semi-industrial production of viruses and rickettsia, most notably the polioviruses, at the Wistar. The young Leonard Hayflick was probably a member of this team. They grew the viruses in sealed containers of between five and twenty litres capacity, and the work was conducted as part of a programme called "Microbiology in Medicine", set up jointly between the Wistar Institute and the University of Pennsylvania's School of Veterinary Medicine, which had just opened an annexe at the New Bolton Center. (This is the same NBC where, two decades later, researchers would report inducing leukemia and *Pneumocystis carinii* pneumonia, PCP, in two chimpanzees by feeding them milk from cows infected with a retrovirus, Bovine Leukemia Virus.)²³¹

Almost all of the Microbiology in Medicine studies of mass production of viruses in different substrates were funded by the US Army Chemical Corps at Fort Detrick, Frederick, Maryland. Since the Second World War, Fort Detrick had been America's major centre of biological warfare research, both defensive and offensive.

One such Fort Detrick/Wistar "Microbiology in Medicine" collaboration was a study of anthrax in industrial settings, of which Dr Philip Brachman was one of the co-authors.²³² As an apparent follow-up to this study, in May 1957, just as Dr Koprowski formally took over as director, a Fort Detrick anthrax vaccine was tried out at one of the industrial plants in question: a wool mill in Manchester, New Hampshire. Three months later, there was a sudden outbreak of inhalation anthrax at the mill, the first and only such epidemic outbreak in the USA in the twentieth century – although nowadays, sadly, we are more familiar with such events. Four of the mill workers died, and a two-man team from the PHS's Epidemic Intelligence Service field post at the Wistar was sent in to investigate. Its members were Philip Brachman and the young Stanley Plotkin.²³³

There appeared to be two remarkable coincidences. The first was that an unprecedented outbreak of inhalation anthrax had followed so soon after a vaccination against that very disease. The second was that a new commercial detergent of a type which was known to increase the virulence of inhaled anthrax spores by an order of magnitude²³⁴ had been introduced to certain departments at the mill (to replace the soap and soda ash which had previously been used to clean the goat hair) on the same morning that the first patient fell sick. Despite this, the Wistar investigators concluded that there was no provable link between the two events (because the first patient had not worked in one of those departments where the detergent was being used), and then declared the anthrax vaccine a success, with an "effectiveness of...92.5 per cent".²³⁵ (Others have since disputed their analysis.)²³⁶

A few months later, in June 1958, doctors Brachman and Plotkin attended a meeting of a medical advisory committee at the Fort Detrick Biological Warfare Laboratories (BWL), and Brachman reported in detail about the anthrax vaccine. There were 56 scientists present, of whom only seven appear to have been civilians.²³⁷ Certain information relevant to the Manchester tragedy appears to have been withheld from the minutes of the meeting, such as (it would seem) certain details concerning sampling by Fort Detrick scientists which took place at the mill six months after the outbreak, in February 1958. During the discussion that followed, it was revealed that the unusually virulent pathogen which had caused the Manchester anthrax outbreak was "about as virulent" as the highly virulent anthrax strain then being used at the Fort Detrick BWL. (This whole episode strikes an eerie historical echo, in the light of the recent revelations that the anthrax sent though the US mails in 2001, with such devastating consequences for several persons, apparently had an "identical" genetic sequence to a strain that was developed at a laboratory at Ames, Iowa, and weaponised at Fort Detrick.)²³⁸

Immediately after this discussion of the Manchester incident, the assembled doctors (with Plotkin, at least, still in attendance) began discussing the stability, mass production, stockpiling and aerosolisation of "Agent N". This is the military term for weaponised anthrax. Some might feel that the presence of the two Wistar doctors at

such a meeting at the Biological Warfare Laboratories raises issues about potential conflicts of interest.

Virtually the same anthrax vaccine is still in use in the US today. Apparently even now, in semi-retirement, Philip Brachman and Stanley Plotkin continue to be regular participants at committee meetings at which the safety, efficacy and production of the U.S. anthrax vaccine are discussed and assessed.

This gives some idea of the type of work which the Wistar Institute was undertaking in the 1950s, both during the early years when Koprowski was only informally involved with that institute, and after May 1957, when he officially took over as director.

But let us return to the other Microbiology in Medicine studies published by Wistar scientists. The first study, involving poliovirus production in HeLa cells and published in 1956, was funded by the National Foundation for Infantile Paralysis.²³⁹ But the later studies, which included investigations of virus growth in L cells (a cell line derived from a mouse), FL (human amnion cells), Chang's conjunctival cells (a human cell line), and ERK-1 cells (the cell line derived from rabbit embryos), were funded by Fort Detrick.²⁴⁰

It is not certainly known why Fort Detrick was involved with such investigations, but one of the final Microbiology in Medicine papers explains that this research "permits one to contemplate the production of viral vaccines, hormones and other physiological agents by methods analogous to techniques employed in microbiological fermentations".²⁴¹

It has been suggested that the Fort Detrick and Wistar scientists may have been interested in the potential for the rapid production of vaccines against specific diseases during a time of national emergency, as, for instance, a BW attack. But of course the viruses mass-produced as "discrete units" (in other words, in sealed containers) did not necessarily have to be attenuated ones. Among the other viruses being studied were human adenovirus 4 (in FL cells), herpes simplex virus, and Venezuelan equine encephalitis virus (VEEV) – the latter two being grown in L cells, the mouse cell line.

The presence of VEEV on this list is significant, because this is not a virus which one would normally expect to encounter outside the laboratory, or unless one was wandering through central or south America. It is, however, considered "a disease of importance to the military". The history of scientific research into VEEV is rather interesting. This was the virus with which Koprowski first made his name in 1943, while he was working at a Rockefeller Foundation-funded lab in Rio de Janeiro, Brazil. While engaged on a contact experiment with VEEV-infected mice, he discovered in the most vivid fashion that the virus was capable of infecting humans. In the course of the experiment, he and several other workers in the lab became infected, and were incapacitated for several days with blinding headaches and fevers.²⁴² By the fifties, the U.S. army had prepared VEEV strains of varying strengths, and developed a vaccine against the virus, which promptly became one of the favoured weapons in America's biological warfare arsenal.²⁴³

The first Microbiology in Medicine study, involving the production of poliovirus in HeLa cells, was based on MEF-1, a strain which several virologists (including Koprowski and colleagues at Lederle) had modified to create their Type 2 polio vaccines. Of course, the use of HeLa cells for human vaccine production was already acknowledged to represent an unacceptable risk. But presumably nobody realised the extent to which HeLa had already taken over the world's virus labs. According to Walter Nelson-Rees, ERK-1, FL, and Chang's conjunctival cells had almost certainly been taken over by HeLa by this stage. Even though there are some indications that, as early as 1956, some scientists were beginning to twig that HeLa contamination of other cell lines might be occurring,²⁴⁴ there is no reason to believe that anyone at the Wistar had been entertaining such suspicions.

In July 1957, just after Koprowski's formal taking over as Wistar director, he and William McLimans attended the Fourth International Poliomyelitis Conference in Geneva. Here, Koprowski made his first public announcement about his new polio vaccines, CHAT and Fox.²⁴⁵

Some of his listeners may have been surprised that Koprowski was speaking about vaccines which must have been largely developed at Lederle, where he had been working until ten weeks previously, and that Lederle had apparently accepted the departure of those vaccines without comment – or at least without legal intervention. However, the *sub rosa* relationship he enjoyed with figures such as Topping and Smadel might explain a great deal. One source from Lederle has told me that Koprowski acted as a law unto himself during his final two years there, taking virus strains from the lab without permission, and engaging in “under-the-table dealings”.²⁴⁶

Despite this, it seems possible that Koprowski's departure may not have come as a complete surprise to the Lederle management. The suggestion that Lederle may have retained an interest in Koprowski's polio vaccine research even after May 1957 is also supported by further clues, such as the fact that it was apparently a Lederle car which took George Jervis to Idlewild airport when he set off for the Ruzizi Valley trials in February 1958.²⁴⁷ This is despite the fact that Dr Jervis worked at the Letchworth Village facility for developmentally disabled kids, and had no formal links with Lederle.

There is no doubt that Lederle was where the CHAT and Fox vaccine strains were developed,²⁴⁸ and this is where Koprowski had been misreporting the substrate he had been using to grow his polio vaccines during 1956 and 1957.²⁴⁹ Meanwhile, however, he had a clandestine relationship with the Wistar, where research work was clearly focussing on the different tissue cultures and cell lines in which vaccines could be grown.

In other words, *the vaccine research on CHAT and Fox was done at Lederle, but it seems that the vaccine substrate research may have been carried out at the Wistar.*

But back to the Geneva conference. On the afternoon of the day that Hilary Koprowski made his first announcement about CHAT and Fox, William McLimans got up to sing the praises of ERK-1, the embryonic rabbit kidney cell line, and made his comment about taking the monkey business out of vaccine production.

He may have been a little over-eager. In the paper which described the original development of ERK-1, written by J.C.N. Westwood from the Microbiological Research Establishment at Porton Down (Britain's equivalent of Fort Detrick) and published in early 1957, the author emphasised caution.²⁵⁰ "In our experience the transformation [of ERK-1 and other cell lines] has occurred with sufficient frequency to suggest that it may be expected in a large proportion of cell lines derived from normal tissues and, in view of the growing importance of serial subcultivation, it is imperative that a fuller understanding of its true nature be obtained", he wrote. Westwood went on to note the similarity of ERK-1 (and five other cell lines developed at Porton) to HeLa, and began the discussion section as follows: "*The possibility of using cancer cells for the production of virus vaccines for human use raises issues as much of a political as of a medical nature, and the political issues are less susceptible to the results of experimental investigation...*" [My italics]

However, in the very next article in the same journal, two scientists from the Bacteriology department at University College Hospital Medical School in London describe the ease with which ERK-1 can produce polioviruses.²⁵¹ Again they emphasised that further investigations were needed before virus grown in ERK-1 could be used for immunising humans, but now at Geneva, just a few months later, McLimans from the Wistar was actively promoting the making of human polio vaccines in this substrate.

This seems a good example of the way in which the science from one article may be inoculated into a second, leaving only the sensible caution behind. Sometimes, however, one suspects that both groups of scientists may have been party to the process, as an effective way of moving a debate forward, especially one that occupies politically sensitive areas.

It is not known, of course, if CHAT and Fox were ever grown in ERK-1 (or any other HeLa-contaminated substrate) in the Congo. It is worth noting, however, that the necessary materials for serial propagation of such lines were available, since all the major Congolese labs had rabbits in their *animaliers*, and nearby maternity wards where amniotic cells were in good supply. Given this availability, combined with the background history, it does not seem absurd to propose that Koprowski's vaccines may have been tried out in some of these substrates.

This is the first time in the course of this ten year investigation that I personally have believed that there is substantial evidence to support the scenario that human cells (as well as chimpanzee cells) may have been used to grow some of the polio vaccines that were being field-tested in Belgium's African colonies in the 1956-60 period.

But it must be emphasised that there is a difference in the quality of evidence for the two substrates. Whereas there is documentary evidence that chimpanzee cells were present in Osterrieth's lab in February to April 1958, at the time when we have a convincing first-hand account of his "making polio vaccine", the links between HeLa and CHAT vaccine are more tenuous. They depend on an August 1958 newspaper article which states that CHAT vaccine had been prepared in Elisabethville, which was written at a time when both HeLa and human amnion cells (some of which may have been in the form of HeLa-transformed cell lines) were present in that city, but

when primate cells were apparently not. (Both the principal doctors from the Elisabethville medical lab during the fifties, Jean Delville and Stefan Pattyn, used to stress that they found HeLa and similar stable cell lines far more reliable than simian cultures.)²⁵²

It is important to stress that there is no direct evidence that vaccine made in chimp cells was later amplified in HeLa, or cell lines taken over by HeLa. However, according to Hayflick's detailed accounts, this would represent a precise parallel to what happened when he first tested his human amnion cell line, WISH, and his first human diploid cell strain, WI-1, for susceptibility to polioviruses. In both instances, Hayflick describes taking CHAT and Fox vaccines which had previously been prepared in "monkey kidney tissue culture" and growing them up in the new human substrates.²⁵³ If similar experimentation had taken place in the Congo, then vaccines made in chimp kidney tissue culture would have been grown up in substrates such as WISH and WI-1 (both of which appear to have been developed after August 1958), or in other cell lines like FL, ERK-1 and "Fernandes" (which appear to have been developed in 1956 and 1957). FL and ERK-1 were being investigated by the Rake/MacLimans team at the Wistar by 1956, while "Fernandes" (which was originally called a "cell strain", though it bore close similarities to both HeLa and WISH) was developed by Mario Fernandes, a Portuguese scientist trained in Lisbon who collaborated with Hayflick while he was at Galveston, Texas in 1956-7, and who later, together with his cell line namesake, followed him to the Wistar.²⁵⁴

Given what generally happened when polio vaccine ran out, it does not seem implausible to propose that CHAT vaccine from one lab (such as Stanleyville) might have been sent to another (such as Elisabethville), and then amplified in a different cell substrate. This might have happened occasionally, to take care of a shortfall, or it might have happened several times. But because, as Plotkin has revealed, CHAT vaccine was routinely prepared from previous batches of vaccine (rather than from seed virus), such an event only needed to happen the once, from the perspective of viral contamination, for the entire output of the second lab to be compromised. And because of the nature of the virus, it is unlikely that, back in the fifties, even careful observation of the cultures would have led to the recognition of SIV contamination.

If the scenario of further CHAT passage in human cells has substance, then this would introduce an important new element to the OPV theory. I believe that this historically-supported possibility demands further investigation.

o) The notebooks.

But is it ever going to be possible to know for sure what work was being conducted by Dr Koprowski and his colleagues in Stanleyville and elsewhere? Well, as it happens, there is one way of gathering more information. It appears that Hilary Koprowski's and Tom Norton's lab notebooks from 1950 to 1957 are still held at the facility that used to be Lederle Laboratories, on microfiche. I have a list of the numbered notebooks, and copies of certain pages from them. These reveal, for instance, that Koprowski was already plaquing out the Charlton strain in July 1956, the month after Lindi camp opened. (Charlton was the original name for CHAT, which was based on Charlton plaque 20, which we know was tested intraspinally on five chimps – presumably from Lindi.)

In January, 2000, I wrote to Professor Patrick Gage, who was heading the research and development arm for Lederle's then-owners, and I requested that I should be allowed to view the microfiches in the company of a Lederle scientist. A detailed letter of support (probably the last professional letter he ever wrote) had been prepared by Bill Hamilton, and I enclosed that with mine.

Two months later, Dr Gage wrote back to refuse my request, explaining that the notebooks contained "highly confidential and proprietary information", and that it was the company's policy to "protect against disclosure of confidential proprietary information and the potential loss of valuable intellectual property rights".²⁵⁵ He added that he had "assigned a team of scientists experienced in virology and vaccine development" to review the relevant notebooks, and explained to me that they did "not contain any evidence that Lederle used chimpanzee kidney tissue as a substrate for the development of any oral polio vaccine".

Given what is now known about the making of chimpanzee kidney cultures *in Stanleyville*, that may well be the case. However, the notebooks do make it clear that among the substrates being used in the lab were "Detroit 6" (a human cell line, later revealed to have been taken over by HeLa), together with "human lung" and "human kidney". They also reveal that embryos were arriving in the lab, presumably for making tissue culture.

Later in 2000, I again wrote to Dr Gage, asking him to reconsider. Once again he declined.²⁵⁶ I am unable to see what commercial secrets might require protection fifty years on. Furthermore, I am reliably informed that the review of the microfiches was in fact carried out by a team of lawyers.

I believe that one of the more obvious reasons for my request being refused might be that the notebooks contain information which would lend support to the OPV/AIDS theory of origin. However, if the vaccine strains were developed at Lederle, and the vaccine substrates at the Wistar – as now appears to be the case, then there should be nothing in the notebooks which needs to be concealed.

I am therefore calling publicly on Wyeth, the new owners of Lederle Laboratories, to reverse the previous decision by Dr Gage, and to invite me (together with one of their own scientists, and perhaps one other party, who might serve as a "neutral observer") to view these microfiches, together with the report submitted by the team of lawyers. A continued refusal to grant access to these materials might well lead people to the unfortunate conclusion that Wyeth has something to hide about this period of research.

p) "Weisswash".

One last point: after the Lincei meeting, Dr Weiss told me that he thought it was not fair for me to "accuse" Dr Osterrieth when he was not present. Dr Osterrieth has now had six separate opportunities, and more than eight years, in which to clarify what happened at Stanleyville lab, and at Lindi camp.²⁵⁷ Despite this, Dr Osterrieth's accounts still contain major gaps and contradictions.

I suspect that what Dr Weiss really means is something rather different. When he himself suggests, on an entirely theoretical basis, that local OPV production in chimpanzee cells might have happened in Africa, as he has now done on several occasions, then this, apparently, is acceptable.²⁵⁸ But when I go out to Kisangani (without, I should stress, a “prior agenda”, not least because I didn’t really believe that Weiss was right about local vaccine production), and I return with information indicating that this is precisely what did happen, then Dr Weiss changes his tune.

According to him, I am now being unfair. Why? Because “Dr Osterrieth has categorically stated...that chimp tissues were not used.” What this argument seems to boil down to is that one should not embarrass those of whom Dr Weiss approves. And he certainly does approve of Dr Osterrieth, having referred to his Royal Society speech as both “elegant” and “resonant”.²⁵⁹ (It may well have been both, of course, without being accurate.)

The bottom line, it would appear, for Dr Weiss is that whereas a theoretical and non-specific argument is acceptable, one which spells out a detailed scenario and which “names names” is not.

Whatever Dr Weiss may think or say, this isn’t a witch-hunt. This is, and always has been, an attempt to get to the bottom of a hugely important issue.

Scientists, like those from most other walks of life, tend to shy away from the whiff of scandal – and there is always, of course, a natural tendency for the establishment to try to defend its own. However, there is now substantial evidence (some of which will be presented later in this paper) that several eminent members of this profession have participated in an attempted cover-up.

Indeed, Professor Weiss himself does have something of a reputation for defending establishment views (at least in public) over the years. Back in 1985, when the first story questioning the role of Robert Gallo in the “discovery of the AIDS virus” debacle broke in *New Scientist*, Dr Weiss wrote in with a letter stating that the article “was the nastiest piece of writing I have seen in twenty years of studying retroviruses”. Later, Abraham Karpas commented that the story in question might have revealed a “‘Gallogate’, in spite of a *Weisswash*”.²⁶⁰

This is most certainly not the right time for a *Weisswash*, for it is no longer possible to brush the less comfortable aspects of this debate under the carpet. If these issues are dismissed unfairly and unscientifically by a scientific establishment which has merely gone through the motions of proper investigation, then they will keep returning (not least because there is more of this story which remains to be told). If that does happen, then those who have initiated, or contributed to, the process will bear a heavy moral responsibility.

For many reasons it is imperative, I believe, that what began as an open debate should continue to be so, and should be played out to its natural conclusion, however enormous the potential implications may be in terms of finance, ethics and – indeed – the future conduct of science.

4. The scientific debate: could a chimp-based vaccine have sparked AIDS?

In this section I shall review the latest developments in the scientific debate about how AIDS started, and in the process will respond, one by one, to the various scientific arguments which have been put forward over the last three years, and which, it is claimed, have “seriously weakened”, “disproved”, or “destroyed” the OPV theory.

The first two responses below relate to scientific evidence about whether or not CHAT vaccine batches were made in chimpanzee cells. The remaining responses relate to those scientific arguments which come into play if CHAT, in any shape or form, *did* incorporate chimpanzee cells.

a) The testing of the Wistar vaccines.

In the year 2000, samples of CHAT vaccine which had been released by the Wistar Institute were tested, and found to contain the DNA of macaques from Asia, but not that of chimpanzees. They were also found not to contain either HIV or SIV. The results of such testing, it has been claimed, have prompted the OPV theory to “die its final death”.

In fact, they have done nothing of the sort. As far as is known, none of the vaccine samples in question were prepared for use in Africa, and it is now becoming increasingly evident that most, if not all, of the CHAT vaccine that was sent abroad was passaged again in locally-made tissue culture before being fed to humans.

Some have complained that I was among the most vociferous of those calling for the testing of the samples, and yet now it is done I am still not satisfied. Certainly I called for the samples to be tested – the same samples which the Wistar initially offered (but failed) to have tested back in 1992. And I welcomed the fact that some testing was finally carried out, albeit eight years later. However, I never suggested that such tests would be definitive.²⁶¹

Neither, I thought, did anyone else who knew anything about the background. For instance in February 2000, Simon Wain-Hobson told me that no serious scientist was going to believe that the testing of the Wistar samples would provide proof of the theory either way. Yet apparently he was wrong. Just seven months later, after the Royal Society meeting, Robin Weiss was saying this with regard to the Wistar testing: “I think it was worth doing...I’m slightly surprised Hooper pooh-poohs it now.”²⁶²

At the London meeting, both before and after the announcement of the Wistar results, I continued to stress what I had already been stressing in *The River*: that the CHAT *pool* numbers were not of any intrinsic significance, in that it was now clear that different *batches* of these pools had been prepared in different labs and in different substrates.²⁶³ (A “pool” of vaccine virus indicates all the material produced at a specific passage level, while a “batch” indicates a specific production run of material prepared from a vaccine pool.) CHAT pools 10A-11 and 13 had indeed both been used in Africa, but what really mattered was where and how the specific batches of those pools that were fed in Africa were made.²⁶⁴

It seemed to me that this point had been made clearly enough. And yet, in Robin Weiss's dismissal of the OPV/AIDS theory in his *Nature* commentary in April 2001,²⁶⁵ he referred to 10A-11 and 13 as "batches", and by so doing, sowed confusion around the central issue pertaining to the legitimacy of the CHAT testing. Professor Weiss is not a careless man, and so it was surprising that he had made such a careless mistake.

In the light of the new evidence which indicates that in the late fifties batches of CHAT were made locally in Stanleyville (and perhaps elsewhere in the Belgian Congo too), it is clear that the samples which need to be tested are those which were prepared in the Congo (if any still exist), rather than those from the Wistar.²⁶⁶ I thank the Wistar Institute for arranging for the testing of some of their vaccine samples, but in terms of proving whether or not CHAT vaccine was produced in chimp cells, this testing has (it is now revealed) been of rather limited relevance.

As I write this, in mid-2002, Simon Wain-Hobson has just had yet another paper on the testing of the Wistar vaccines published, this time in the prestigious *Proceedings of the National Academy of Sciences*.²⁶⁷ Papers which appear in the *Proceedings* have to be "communicated" by a member of the NAS, and Wain-Hobson's paper was communicated by Hilary Koprowski. Wain-Hobson claimed that his finding that the CHAT samples released by the Wistar had contained only macaque DNA had "effectively scotch[ed] the [OPV] hypothesis". In a statement quoted by *USA Today*, he amplified his conclusion. "This issue is resolved", he said. "The vaccine lots were made using macaque kidney samples, not chimp, and we know that macaques are not infected by any virus of this ilk."²⁶⁸

We also know (as here the "we" includes Simon, for he sent me a rather brisk e-mail about it)²⁶⁹ that the vaccines that are likely to have been SIV-infected are those that were made in the Congo, not at the Wistar. His testing of the Wistar vaccines has therefore not resolved any issue, and his public claim that it has done raises issues of its own – such as whether he and Robin Weiss are involved in a genuine scientific debate, or in an attempt to persuade the world of a certain version of events.

b) "A totally absurd substrate"?

In a 1994 letter to me, Stanley Plotkin wrote that chimp cells "would have been a totally absurd substrate for a vaccine, considering the difficulty, the expense and the rarity of the species".²⁷⁰

This is simply untrue. In fact, most of the evidence suggests the opposite.

To demonstrate this, I shall examine the rather wide range of arguments which has been advanced by Dr Plotkin and others, as to why chimp cells might have been a poor, an inappropriate, or an "absurd" substrate for an oral polio vaccine, in order to see which, if any, are persuasive.

- If Plotkin is suggesting that chimpanzees were too expensive to use, then he is wrong. According to the chief government vet in Stanleyville at the time that Lindi camp opened, Joseph Mortelmans, chimps could be obtained from African

sources for between one and ten U.S. dollars a time during the 1956-1960 period.²⁷¹

- If Plotkin is suggesting that chimps were physically too difficult to handle, then again he is wrong, for the history of Lindi camp has tragically proved how very easy it was to experiment upon, and sacrifice, many hundreds of young chimpanzees and bonobos.
- If he is suggesting they were too rare, then I am surprised. In 1959, Plotkin's Belgian collaborator, Ghislain Courtois, stated publicly that 2,000 litres of OPV would be enough to immunise the whole world against polio,²⁷² which (given the fifties rule-of-thumb that two litres of vaccine could be produced from one primate) would have required the sacrifice of some 1,000 primates. The fact that some 600 chimpanzees and bonobos were sacrificed at Lindi camp in three-and-a-half years gives a fairly clear idea of how Plotkin and his colleagues would have viewed the rarity issue back in the fifties.
- If Plotkin is suggesting that substrates other than rhesus macaque kidneys would not have been considered acceptable, then he is wrong. According to the recommendations of the second and third WHO expert committees on poliomyelitis, which sat in 1957 and 1960, any suitable species of primate could provide cells for a polio vaccine substrate, whether that vaccine be oral or inactivated.²⁷³
- If Plotkin means that Koprowski would never have used an unfamiliar, or poorly characterised, substrate, then this would appear to be a somewhat stronger argument. However, the Koprowski team *was* familiar with chimpanzees. Koprowski and Tom Norton, together with George Jervis (who ran his own lab at Letchworth Village in upstate New York, where he had almost complete freedom), had been doing polio research with chimpanzees since 1949, utilising at least 16 chimps during the period up to 1952,²⁷⁴ and others again in the period up to 1956, when Lindi camp opened in the Congo. It seems probable that most (if not all) of these chimps were eventually sacrificed, and it is not unreasonable to imagine that after they had been killed, the potential uses of all available organs (such as kidneys) would have been investigated. I recently spoke with the respected virologist Robert Hull (who apart from producing inactivated polio vaccine for Eli Lilly during the fifties, was also perhaps the foremost world authority on the identification of adventitious viruses in simian tissue culture during that decade). In the course of the conversation he quite casually mentioned that his lab had also had chimps, and that at one point (perhaps, he said later, around the time that India restricted the export of rhesus monkeys – which was in 1955), they had prepared some experimental tissue cultures from their kidneys, simply because they were available. The cultures were found to be good for growing poliovirus, but the work was not considered important enough to be reported in the scientific literature. These cultures were apparently not used for polio vaccine preparation, but the point was that they *could have been*. It is not unreasonable to propose that a similar process may have occurred with Koprowski's team. Dr Hull, by the way, was shocked to learn that the latter team had been amplifying vaccine direct from vaccine (as has been acknowledged by Dr Plotkin), rather than culturing it from virus seed. "It doesn't make sense to me", he commented. "It would increase your chance of getting an adventitious virus."
- With regard to the previous point, we know from scattered comments in the medical literature (mainly from discussion sessions at different conferences) that

sera from the Lindi chimps had, at least by 1958, been tested for major pathogens like simian B, tuberculosis, Coxsackie B, measles, mumps and influenza by Koprowski, Courtois, Deinhardt and Werner Henle.²⁷⁵ Later, tests for different arboviruses were made by Dr Osterrieth,²⁷⁶ and it is certainly possible that tests were made for other pathogens as well. All this suggests that chimpanzees may in fact have represented a well-characterised substrate to Dr Koprowski and his collaborators. Of course, it was not possible in the fifties to test for then-unknown pathogens, like SIV.

- During my second interview with Gaston Ninane in 1993, he briefly suggested that Dr Koprowski might have been using chimps to make some of his vaccines in the U.S. Thereafter, he declined to elaborate, so there is no way of knowing if he was speaking of a matter which he knew something about, or was merely hypothesising. However, the comment is interesting, given what is now known about the making of chimpanzee kidney tissue culture (CKTC) in Stanleyville. It may of course be that, before that initiative was taken, some experimental batches of CHAT in CKTC were prepared at the Wistar, to check its various qualities. To quote one of Koprowski's former colleagues, who wished to remain anonymous, on the subject of the testing of CHAT samples from the Wistar Institute freezers: "I think that Hilary would have been most eager to have the testing done.... I can see any number of reasons why you would not detect SIV...Let's say that they used 20 kidneys from [primates] that would not produce [an HIV-1-like] SIV, and two kidneys that would produce SIV...." He left the rest of the sentence to my imagination.
- Alternatively, if Plotkin is suggesting that chimpanzees were too close to humans for their cells to be considered safe as a substrate for OPV, then this concept is hardly borne out by the historical evidence. Throughout the fifties, the vaccine developers were rejecting substrates like chick embryo, which were distant from human cells, and which grew poliovirus very poorly (if at all), and were turning instead to primate kidney cells. The best substrate of all, it seems, would have been human cells, but human cell lines such as HeLa were of course deemed too dangerous. Hayflick appears to have developed the first human diploid cell strains in about 1959,²⁷⁷ but in the three or four years preceding, cells from Man's closest relatives (chimps and bonobos) might well have looked like the ideal alternative. In several contemporary articles, bonobos are referred to by members of Koprowski's team as the "closest blood relatives to man".²⁷⁸
- In support of the foregoing analysis is a volume entitled "Experimental medicine and surgery in primates", which was published in 1969 by the society of which Dr Koprowski used to be president, the *New York Academy of Sciences*. The opening paper, entitled "The use of primates in biomedical studies; a review of suitable species" was written by the assistant director of the Yerkes Regional Primate Center, Dr W.C. Osman Hill. He commented: "Naturally, experimental results most likely to be applicable in human medicine...can be expected only from the use of those species having the closest blood-relationship (i.e. phylogenetic proximity) to man. These are the great anthropoid apes of the species *Pan* (chimpanzee), *Gorilla* and *Pongo* (orangutan)...[T]he chimpanzee, [which is] less rare and hardier than the others, is certainly the best choice, providing that financial and housing considerations do not preclude their [sic] use. They are, however, likely to remain the prerogative of relatively few and specialized institutions (primate centers and others), and cannot be recommended for general use."²⁷⁹

- One final point needs to be made. It was common practice in the fifties to use the same species for vaccine substrate that had already been used to test the safety of the polio vaccine strains. (It is said by some scientists that the *same individual animals* were sometimes used, provided they had suffered no ill reactions from the safety testing.²⁸⁰ This, of course, would have reduced the number of sacrifices required.) The only place that CHAT was safety tested exclusively in chimpanzees was the Belgian Congo. This is just one more argument to add to those already cited, and which indicates that *in the Congo, uniquely, chimpanzee cells may have been considered the ideal substrate for preparing oral polio vaccine.*

c) Presence of HIV and SIV in kidney cells.

It has frequently been claimed that HIV-1 cannot survive, or replicate, in kidney cultures. These claims are incorrect, as was demonstrated a couple of months after the Royal Society meeting, by an article entitled: “Renal epithelium is a previously unrecognised site of HIV-1 infection”.²⁸¹ An amusingly-titled editorial (“Much at stake with kidneys?”) co-written by doctors John P. Moore and R.W Doms,²⁸² devoted a lengthy passage to explaining why some persons, “mainly journalists and some laymen on the fringes of AIDS activism...will no doubt cite this article as being supportive of their argument [about how AIDS began]. And so it is, but only to a certain extent.” Why only to a certain extent? Because “OPV was prepared using monkey kidneys, not chimpanzee kidneys, and it is chimpanzees, not monkeys, that harbored the HIV-1 precursor virus”.

I thank Moore and Doms for highlighting the fact that if they are not correct, and chimp kidneys *were* on certain occasions used to make OPV, then this would represent a real opportunity for the crucial zoonosis.

d) Trypsinisation.

Paul Osterrieth states that trypsin was not available in Stanleyville until “several months” after his return from leave at the start of February 1958, and that it took time to set up a tissue culture lab. Thus, even as he denies that polio vaccine ever was made in Stanleyville, he seems keen to establish that it *could not possibly have been made* during the early months of 1958. This, of course, is exactly the time when it seems CHAT vaccine *was* being prepared in his lab, e.g. for the Ruzizi trial.

The claim that trypsin was not available until later in 1958 is very possibly true. On this point, Dr Osterrieth’s claim is supported by the recollection of Dr Ninane, who also stated that, at least in 1957, trypsin was not available at the Stanleyville lab.²⁸³ But it now seems that trypsin is probably irrelevant, because in the fifties it was not mandatory to make polio vaccine with trypsinised cultures. Maitland-type cultures were equally acceptable throughout the fifties, and such cultures could have been produced in Stanleyville from the early fifties onwards – and certainly from the time that Lindi camp opened in mid-1956.

e) Survival of SIVs in polio vaccine preparations.

It has been claimed that the chances of a hypothetical SIV surviving through the various stages of vaccine production to the final vaccine are many trillions to one against.

These well-intentioned but ultimately misleading back-of-an-envelope calculations by professors John Beale and the late Florian Horaud²⁸⁴ are no longer applicable – if, indeed, they ever were. This is because they have been rendered irrelevant by the new evidence indicating that batches of CHAT were prepared locally in Stanleyville – and in all likelihood in primitive cultures made without trypsin, and using cells and sera from locally available primates.

If a proper protocol for the Stanleyville vaccine is ever released, then perhaps some appropriate calculations – or experiments – can be done. However, this seems unlikely, since to date the only CHAT protocol which has come to light (albeit in incomplete form) relates to pool 23, the last pool made in primate kidney culture, this being in 1960 or 1961.²⁸⁵

After speaking with several virologists and a microbiologist, the impression I get is that a live polio vaccine prepared from Maitland-type culture which incorporated SIV-infected cells and sera would be quite likely to retain a significant amount of viable SIV at the end of the vaccine-making process. This is not least because, even when using a good centrifuge and taking great care, it is practically impossible to remove all lymphocytes and – especially – macrophages (those preferred target cells for HIV and SIV) from serum. It may be that some of those chimps which were sacrificed at Lindi were newly-infected (having acquired SIV through the co-caging and group-caging procedures) but had not yet developed antibodies, in which case they would be expected to have had particularly high levels of SIV in their macrophages and lymphocytes at the time of death.²⁸⁶ But in any case, a recent article makes the interesting point that “SIVcpz-infected chimpanzees...produce high infectious virus titres in their peripheral blood”.²⁸⁷

It is worth reemphasising that the “golden age of virology” really had very little in the way of rules and regulations. The tissue culture “bible” of the second half of the fifties was a lengthy chapter by Joseph Melnick entitled “Tissue culture methods for the cultivation of poliomyelitis and other viruses”, which appeared in a book published by the American Public Health Association in 1956.²⁸⁸ It underlines that both (Maitland-type) suspended cell cultures, and trypsinised cultures were considered suitable polio vaccine substrates, and goes on to state that suspended cell cultures can be maintained “for periods varying from 2 weeks to 1 month” by frequent changes of medium, even if “cell growth does not take place in this system”.

This ties in well with the recollection of Osterrieth’s first assistant that polio vaccine orders would come down from the provincial medical director at intervals (presumably in response to requests from doctors in different towns), and that each time Dr Osterrieth would make new (ie a fresh batch of) vaccine.

f) The simian ancestor of HIV-1 Group M.

It has been claimed by some supporters of a west central African origin of AIDS that the wrong species or subspecies of chimpanzee was present at Lindi camp for there to be any chance of the Group M epidemic having emanated from there.

This is unproven. Beatrice Hahn and Paul Sharp quite strenuously argue that the true ancestor of HIV-1 Group M is the SIV of the *Pan troglodytes troglodytes* chimpanzee from west central Africa (Cameroon, Equatorial Guinea, Gabon and Congo Brazzaville), and not that of the *Pan troglodytes schweinfurthii* chimpanzee from central Africa (the DRC, Uganda, Rwanda, Burundi and Tanzania) . However, so few SIV-infected chimps have thus far been sampled that this seems little more than a hunch. [Figure 6]

As I understand it, this is how the debate stands at present. The only known close relatives to the human virus, HIV-1, are the SIVs from common chimpanzees. Of the two SIV-positive *schweinfurthii* chimps which have been reported to date, one comes from Jane Goodall's Gombe Stream camp in north-western Tanzania, while the other (Noah) hails from an unknown location in the DRC (which may or may not be close to the Tanzanian border, and Gombe). Phylogenetic analysis reveals that both of the *schweinfurthii* SIVs are rather less closely related to the HIV-1(M) branch than are the *troglodytes* SIVs from west central Africa.²⁸⁹

However, we still know very little about levels and types of SIV infection in chimps coming from the rest of the *schweinfurthii* range, such as the vast body of the rainforest in the DRC, to the north of the Congo river. Chimps from around Kisangani (Stanleyville), or Ango in the far north, or Gemena further west, may carry very different strains of SIV. Further sampling and testing – of the type which has recently been done so successfully in Cameroon and Gabon – is clearly needed.

So far, the sampling of chimps and bonobos from the central African rain forest has been very limited, and the reporting of tests on those samples has been even more so. In other words, there is insufficient data for theories about which chimp populations hosted the precursor virus to Group M to be advanced on anything more than a tentative basis.²⁹⁰

Of course, the discovery of even one SIV sequence from the DRC which sits closer to the M group than do the present SIVcpz sequences from Cameroon and Gabon would transform the picture. This would be even more dramatic if the SIV in question branched off from *within* Group M.

Probably the best riposte to the assumption that the *P. t. troglodytes* SIV is the “only true ancestor” of Group M comes from the primatologists, and in particular the paper presented by Pascal Gagneux and colleagues at the Royal Society meeting.²⁹¹ They emphasise that recent genetic studies of chimp populations have revealed just how much remains to be discovered. Such studies have resulted in the recognition of a new subspecies (*Pan troglodytes vellerosus*, from Cameroon and Nigeria), but have “called into question the long-accepted genetic distinction between eastern chimpanzees (*Pan troglodytes schweinfurthii*) and western equatorial chimpanzees (*Pan troglodytes troglodytes*)”. The paper features a phylogenetic tree that demonstrates a striking interweaving of mitochondrial DNA sequences from the two latter subspecies, and bears the legend “There is no support for monophyly of [either]

subspecies.” In other words, it seems possible that *troglydytes* and *schweinfurthii* may in future be redefined as a single subspecies.

The paper explains that chimpanzee gene flow in equatorial Africa is still little understood, and that “it seems reasonable to speculate that a chimpanzee population or populations may exist which both harbor the putative HIV-1 ancestor, and which have remained reproductively isolated from other chimpanzee populations over the time-scale relevant to the evolution of the SIVcpz/HIV-1 complex of viruses”. In other words, the ancestral host to today’s pandemic AIDS viruses may exist, or may have existed, in an isolated pocket somewhere in central Africa, and may either not have been sampled yet, or may have died out in recent times.

To highlight the fact that atypical chimp populations exist, Gagneux points to a group of chimps from Mambasa, in the eastern Congo, which appeared to be unlike any other known group in terms of the pattern of blood group antigens. The 1961 paper on these blood group studies²⁹² is written by, among others, Osterrieth and Ninane, and records that 21 of these Mambasa chimps were among those housed at Lindi. (Its lead author, Dr André, told me in 1994 that he believed that none of these chimp bloods are still in existence.) Other chimps from Mambasa *territoire* were supplied to Alexandre Jezierski for his OPV and IPV research.

Even leaving aside the question of whether *troglydytes* and *schweinfurthii* should be defined as one, or two, subspecies, there is a far more basic question to ask with respect to the OPV theory. Can we be certain which apes were present at Lindi?

Despite Ghislain Courtois’ summarising article from 1967, which states that only *Pan troglydytes schweinfurthii* and *Pan paniscus* were present at the camp, the truth is that we simply don’t know (and for that matter, neither would Courtois have known for sure).²⁹³ We do have some clues, however, which suggest that *Pan troglydytes troglydytes* may also have been present.

Fritz Deinhardt’s hepatitis databook from 1959 mentions 54 apes, of which 41 are identified by species, but not subspecies – all 41 were common chimps, *Pan troglydytes*. (That thirteen apes were not identified is probably due to the fact that species was apparently not documented for the earliest arrivals at Lindi, from June 1956 to early 1957.) Two of these 54 apes (one common chimp; one unidentified) came from zoos (with the previous history unrecorded), and one unidentified ape, Ikela Marie, came from the district of Coquilhatville (now Mbandaka), about 1,000 kilometres downstream from Lindi and Stanleyville. However Ikela Marie came to be at Lindi, it seems likely that the River Congo would have played some part in her journey there. She may have been brought upriver by a trader travelling on one of the huge Congo steamers, in which case her precise origin, other than “Coquilhatville”, would probably have been unknown.

Although Ikela Marie was not identified by species, it seems probable that she was a common chimp.²⁹⁴ Several doctors recall that the bonobos were only present at the camp during the first year or so of its existence – ie up to 1957, or, at latest, early 1958 – and a newspaper article written in March 1959 describes the bonobos as having been already “used” for the Lindi “experiments”.²⁹⁵ Ikela Marie arrived at

Lindi in April 1957, and was still alive in April 1959, so it seems unlikely that she was a bonobo.

But which type of common chimp was she? The former district of Coquilhatville (now Mbandaka) was situated on the south bank of the River Congo, directly opposite the natural territories of both *troglydytes* and *schweinfurthii* chimpanzees. The very real possibility that Ikela Marie was not just a common chimpanzee, but a *Pan troglodytes troglodytes* chimpanzee, has significant implications because of the co-caging that was routine at Lindi.

Dr Deinhardt's databook documented rather less than 10% of all the chimps that were "guests" at Lindi camp, so it may well be that not just one, but several *Pan troglodytes troglodytes* were incarcerated there.

So even if Beatrice Hahn is right with her hunch about the precursor virus coming from *P. t. troglodytes*, the real possibility that one or more representatives of this subspecies were present at Lindi is enough to dispose of the argument that the Group M precursor could not have existed at the camp.²⁹⁶

One of the most exciting developments in AIDS research in the last two or three years has been the acceleration of investigations into African SIVs. Recently there have been some particularly interesting discoveries.

In April 2001, Sentob Saragosti's team reported the sequencing of an SIV from Wolf's monkey, *Cercopithecis mona wolffi*, and the fact that this SIV contained a *vpu* gene which is characteristic of the SIVcpz/HIV-1 lineage, and not found in other lineages (such as SIVsm, SIVagm, SIVmnd and SIVsyk). This important finding, which to date is only available as a conference abstract,²⁹⁷ is made all the more significant because Wolf's monkey is found on the southern side of the Congo river, where it shares the greater part of its range with the bonobo, *Pan paniscus*.²⁹⁸

The recent helpful contribution to the debate by Anne-Mieke Vandamme's group, reports on the SIV testing of 26 *Pan paniscus* (14 wild-caught, and 12 born in captivity), and the finding of no positive samples.²⁹⁹ However, the paper goes on to state that bonobos "are known as a very social and peaceful species, and hunting or aggressive interactions with sympatric primates have not been observed. Therefore, the chance of SIV interspecies transmissions to bonobos seems very low." This conclusion is then used to cast doubt upon the OPV theory.

I am surprised by this line of reasoning. *Pan paniscus* is omnivorous, and there can be little doubt that (despite their passivity) bonobos do on occasions eat, or come into contact with, other monkeys, as when they come across individuals that have been wounded or recently killed by other predators. And whether or not it has been observed, it is certainly possible that there may be occasional fights between bonobos and other primates (just as we know there were fights, admittedly under artificial conditions, when chimps and bonobos were caged together at Lindi). The very fact that a *vpu* gene has been found in a monkey that shares its range with *Pan paniscus* should only encourage further interest in sampling the bonobo for SIV.

One looks forward to further reports, since it is known that at least three other teams have conducted similar surveys of *Pan paniscus* in recent years.

Bill Hamilton, in particular, always stated that his best hunch for the immediate ancestor to HIV-1 Group M would be an SIV in *Pan paniscus*. He also pointed out that even if such an SIV had existed among the *paniscus* populations which supplied Lindi camp in the fifties, it might be that such populations were now extinct. Sadly, his attempt during his final expedition to arrange for the collecting of faecal samples from these places proved to be unsuccessful, because the samples that finally arrived in the U.K. were revealed to be from *Pan troglodytes*, and not *Pan paniscus*.³⁰⁰ However, it is hoped that another, more recent expedition may have enjoyed more success.

With regard to the faecal and urine samples from *Pan troglodytes schweinfurthii* which were collected by Bill Hamilton in July 1999 and January 2000, portions of which were then delivered to Simon Wain-Hobson, it is regrettable that we still do not have any formal results. This is despite the fact that one of the published reasons for the postponement of the Royal Society meeting from May to September 2000 was to allow time for a report to be prepared on these samples. All we have is Wain-Hobson's verbal answer to a question posed at that meeting by Stanley Plotkin, in which he stated that he had found no evidence of SIV in the Hamilton chimp samples, but gave few further details.³⁰¹

However, a final point. I have over the past year been informed from several different sources of instances in which, allegedly, SIV-like results have been obtained from samples derived from the two anthropoid apes (*Pan troglodytes schweinfurthii* and *Pan paniscus*) which made up the bulk of the experimental population at Lindi camp. (These reports do not refer to the two SIV-positive *schweinfurthii* chimps which have already been identified, but to other apes.) I do not know what credence to place in these reports. I fully accept that they may be incorrect, and that even if they are real, that the alleged findings may be inaccurate. None the less, this does emphasise the crucial importance of the SIV testing of the anthropoid apes, and the desirability of its being conducted in an open and transparent manner. I believe that all the results of such "sensitive" testing should be fully reported – whether they be positive, indeterminate, or negative.

g) The question of HIV-1(M) diversity.

In the recent past, it has been proposed that not only is the greatest diversity of SIVs seen in Gabon and Cameroon, but that these countries also contain the greatest HIV-1 Group M diversity, meaning that they represent the hearth of the AIDS pandemic.

The first contention (regarding SIV) is unproven; the second (regarding Group M) is now generally accepted as wrong.

The range of SIVs identified in west central Africa during the last few years is wide, culminating in the recent impressive report by Peeters et al, who detected SIV in 13 of 16 primate species tested, and in 16.6% of all primates tested.³⁰² The research was especially valuable in that blood samples were taken both from pets and from

bushmeat found in local markets, revealing that seroprevalence in the wild was rather higher (18.4% versus 11.6%).

However, this comprehensive study was possible largely because Cameroon and Gabon are stable and viable places in which to conduct research, and because there are supportive organisations and institutions functioning in the region, such as PRESICA (*Projet Prevention du SIDA au Cameroun*) in Yaounde, Cameroon, and CIRMF (*Centre International de Recherche Medicale de Franceville*), with its large primate facility, in Franceville, eastern Gabon. There are currently no comparable bases in the DRC, which has been riven by civil war in recent years, with the result that relatively little sampling of primate populations has taken place. Had there been a similar level of sampling, one suspects that a similar level and range of SIV infection might have been detected.

It may be for similar reasons that the two minor groups of HIV-1, Group O and Group N (which are commonly considered to represent separate transfers from chimpanzees to humans), have mainly been encountered in the same two countries, Cameroon and Gabon.

As for the perceived “centre” of HIV-1 diversity, this has changed over the years. In 1996, a team of French and Belgian researchers from the Institute for Research and Development, Montpellier, France (led again by Martine Peeters, and by her husband, Eric Delaporte) proposed that there was a relatively low seroprevalence, but high diversity, of M group subtypes in Gabon, with five subtypes detected there, and that this diversity, together with the presence of HIV-1 Group O, in Gabon, Cameroon and the Central African Republic, could indicate that the epidemic in this region was older, and that “the HIV viruses [might] somehow originate from this part of Africa”.³⁰³ Even as late as 2000, Beatrice Hahn, Paul Sharp and Kevin De Cock were still claiming that it is “within west equatorial Africa that the greatest diversity of HIV-1 Group M viruses has been found”, citing studies in which seven of the M clades had been detected in Gabon and Cameroon.³⁰⁴ Of course, this tied in with their belief that all three groups of HIV-1 (M, N and O) had transferred from different SIVs found in *Pan troglodytes troglodytes*.

It was actually the same Peeters/Delaporte team that proved the flimsiness of this line of reasoning, when Martine Peeters announced at the Royal Society meeting that 247 HIV-1 sequences obtained in 1997 from three cities in the DRC (Kinshasa, Mbuji-Mayi and Mbandaka),³⁰⁵ had demonstrated an “unprecedented diversity”³⁰⁶ Many of these sequences appeared to be deep-rooted in the phylogenetic tree, and another article by the same team acknowledged in its title that the AIDS pandemic seemed to have originated in “central Africa”.³⁰⁷ This subtle change from “west central Africa” indicated that the DRC, rather than the former French colonies to the north (Cameroon, Gabon and Congo Brazzaville), was now becoming accepted as the likeliest hearth of the Group M epidemic. Further analysis of the Peeters dataset is eagerly awaited.

Other recent studies of HIV-1 sequences from the DRC have been equally remarkable, including one which reported the presence of seven Group M subtypes in a single small town, Kimpese, situated some 200 kilometres west of Kinshasa.³⁰⁸ (It is

worth noting that the nearest known CHAT vaccination site to Kimpese was just 50 kilometres away, at Mbanza-Ngungu, formerly Thysville.)³⁰⁹

The most dramatic evidence, however, has been the recent detection of *all ten recognised Group M subtypes* in a group of 70 HIV-1-positive samples which were originally obtained from Kinshasa women at the start of the recognised African epidemic, in 1983-1985.³¹⁰ Just six of the 70 Kinshasa sequences did not cluster with any of the known subtypes; and three of these six clustered together as what is referred to as a “recombinant form”. In February 2002, one of the lead authors, Tom Folks, chief of the HIV and retrovirology branch at the CDC, informed me that the sequences of the ten subtypes and the recombinant form were “present at nearly the same numbers [ie frequencies] as now”.

The fact that one of these samples was a HIV-1(M) subtype B, the so-called “Euro-American strain”, is especially interesting, for the only evidence of African subtype B infection prior to this report related to white gay South African males in the eighties, and a scattering of isolated African cases in the nineties – all of which appeared likely to be the result of “reimported” infections from the West. Many had begun to suspect that indigenous African clade B might have died out, and that this subtype existed only in Western countries. This Kinshasa sequence may possibly represent the first evidence of indigenous subtype B infection in the African continent, but it still remains to be seen how closely the Kinshasa sequence resembles typical Euro-American sequences from the same time frame.

In his helpful communication, Professor Folks also provided some indication about how his team was interpreting these remarkable findings: “We are stunned by the high diversity and low prevalence situation that we continue to encounter in that region. Obviously I don’t know the answer, but we now hypothesise that multiple events had to occur for HIV to adapt and evolve into a transmissible agent, as well as one that is pathogenic. Whether this is because multiple viral infections were followed by recombinations or whether infections were followed by behavioural events is unclear, but we think that a single spark probably did not happen.”

Tom Folks also wrote that he did not think this supported an iatrogenic theory of origin, but rather one in which only a certain sort of SIV isolate (those resembling HIV-1 Group M) had managed to transfer successfully to humans.

However, having discussed this with others (a geneticist and a molecular biologist), I believe that a far more parsimonious explanation might apply. It seems possible that the microcosm of the Group M epidemic which is represented by the 1983-1985 data from Kinshasa may indicate that a number of individual chimp-to-human transfers occurred in Leopoldville/Kinshasa and its hinterland. These might, for instance, have occurred through the immunisation campaigns in which some 75,000 Leopoldville children (the entire population of under-fives between August 1958 and April 1960) were vaccinated with CHAT, or they might have occurred through the campaigns in which nearly a million others were vaccinated elsewhere in the Belgian colonies.

Urban drift to Leo/Kinshasa was pronounced, for the population seems to have increased almost four-fold in six years – from 350,000 in 1958, to 1.3 million in 1964.³¹¹ The early 1980s population was apparently over three million. So not only