New Claims from Paul Sharp -
But Has the Source of HIV-1 Really Been Located?

Introduction

February 2006. In recent days there has been much press coverage about new claims by American, British and Belgian scientists that they have discovered the geographical source of AIDS. These researchers have apparently detected simian immunodeficiency viruses (SIVs) that are closely related to the AIDS pandemic virus, HIV-1, in the stools of wild chimpanzees living in the very south-eastern corner of Cameroon, in western Africa.

Much of this new information is important and valuable. However, much of the accompanying analysis is exaggerated, and not for the first time seems to be driven by an underlying desire on the part of the authors to bolster the bushmeat theory of origin of AIDS, which proposes that humans first got infected through the butchery and consumption of chimpanzee bushmeat.

In fact, as I shall demonstrate, their claims of having established the source of HIV-1 and AIDS are far from convincing. Furthermore, their new findings are as consistent with the oral polio vaccine (OPV) theory of origin as they are with the bushmeat theory. [See note at end of this section for explanation of OPV theory.]

The new information about the Cameroonian chimps was released in February 2006 at the 13th Conference on Retroviruses and Opportunistic Infections in Denver, Colorado, where important speeches were made by Brandon Keele, from the lab of microbiologist Beatrice Hahn at the University of Alabama in Birmingham (US); by Fran van Heuverswyn, from Martine Peeters' lab in Montpellier, France; and by Hahn's long-time collaborator, the geneticist Paul Sharp, who is based at the University of Nottingham, UK.

In a 30-minute speech entitled "Where AIDS Came From" (which is available on a Webcast), Professor Sharp stated that the regions in Cameroon where they had found these chimpanzees were in "the south-eastern extremity" of the country, and claimed that this area represented the "likely origin of HIV-1". He noted that this region was a long way from the city of Kinshasa, in the Democratic Republic of Congo (formerly Leopoldville in the Belgian Congo), where he believes that the AIDS pandemic started. However, he then added that "there is a river that runs most of that distance", by which he meant the 500-odd miles between south-eastern Cameroon and Kinshasa.

Below, I shall summarise the latest findings, and provide my analysis of their significance, before explaining why this new information still poses some real problems for advocates of the bushmeat theory. After that, I shall provide some further background about the history of research into the origins of HIV-1 and AIDS. Finally, I have copied two articles about the new chimp SIV findings that have recently appeared in the press: an instant commentary by Ed Susman of UPI, followed by a more reflective piece by Jon Cohen, for ScienceNOW.]

(Background note. The oral polio vaccine theory, or OPV theory, proposes that the AIDS pandemic was sparked by approximately 30 separate vaccination campaigns that were staged in the Belgian Congo and Ruanda-Urundi between 1957 and 1960, and which employed an experimental OPV called CHAT. Lindi camp, which housed several hundred captured

chimpanzees, was established in the rain forest a few miles outside Stanleyville (now Kisangani), and was set up specifically in order to test, or "perfect", this CHAT vaccine. Despite denials by the vaccine-makers, there is evidence that a significant proportion of the CHAT vaccine that was tested during these years on approximately one million Africans was prepared locally in Africa, in the cells of the Lindi chimps. This means there is a high probability that different batches of the vaccine were infected with different variants of chimpanzee SIV.)

The new information from Cameroon

The most significant discoveries announced by the teams of Hahn, Sharp and Peeters may be summed up as follows:

* They have obtained viral isolates of SIV, simian immunodeficiency virus, from over 30 wild-living chimpanzees (*Pan troglodytes*) from south-eastern Cameroon, thus tripling the number of known isolates of SIVcpz (chimpanzee SIV).

* They have sequenced these isolates, and found sequences that are genetically "much closer" to HIV-1 Group M (the virus that caused the human AIDS pandemic) than any previous sequences.

* They have discovered one group of wild chimpanzees in which 35% of the members are infected with SIV, a higher infection level than in any wild-living chimp group previously analysed.

My analysis of Paul Sharp's findings

The claim by journalist Ed Susman that these new chimp viruses from Cameroon "closely match" the oldest known HIV-1 sequence (ZR59, allegedly obtained in 1959, but see final section below), is not supported by Paul Sharp's Webcast, or indeed by any other documents that I have seen. Unless further information is released, it is probably safer to assume that Susman was mistaken.

Paul Sharp claimed that phylogenetic analysis revealed that the new chimp SIV sequences were "much closer" to the archetypal AIDS virus [HIV-1 Group M, or HIV-1(M)] than any previous sequences, but he has not revealed how close they are. The scientist who actually obtained the sequences, Fran Van Heuverswyn, was more cautious, stating that "two of the isolates more closely matched the HIV-1 causing the human epidemic than any found in the past". Most chimp SIV sequences from Cameroon or Gabon have roughly 80% homology with HIV-1, although this homology varies, depending on which part of the genome is being analysed. I shall be greatly surprised if these latest sequences have more than 90% across-the-board homology with HIV-1, or else the aforementioned scientists would, I suspect, have been more vociferous in their claims. It would require something like 95% to 97% homology before anybody could reasonably claim that these are effectively the same viruses in different hosts … and nobody is claiming that.

If I am correct, and there is less than 90% homology between these viruses and the AIDS pandemic virus, HIV-1(M), then it is unlikely that the viruses found in these chimpanzee groups actually represent the "origin of HIV-1", as Sharp is claiming. Both Beatrice Hahn and Paul Sharp like to maximise publicity for their scientific work, and are used to playing to the

gallery, and to scratching the backs of favoured journalists. (This is perhaps unsurprising, in an era in which headlines help bolster future grant applications, and in which science journalists are always on the look-out for sexy stories.) The reality, however, is this. Including the latest 30 or so sequences from Cameroon, the various teams researching chimpanzee SIV have to date obtained a total of approximately 40 chimp SIV sequences. Thus far they have sampled certain specific areas of Cameroon, Gabon, Uganda and Tanzania. Only a small amount of chimp SIV research has taken place in the huge area of the Democratic Republic of Congo (DRC), or in Congo Brazzaville, and as far as I know none at all has been staged in Equatorial Guinea or the Central African Republic. (Wild chimps in Rwanda and Burundi are believed to be nowadays extinct, or almost extinct.) Viewed in this context, it is apparent that only a very small proportion of chimpanzee groups have been sampled, which means that Paul Sharp's announcement is at the very least premature, and is quite likely to be inaccurate. There is a high probability that there are further chimp SIVs still waiting to be discovered, including SIVs that are even closer genetically to HIV-1.

The fact that at least one group of chimps had 35% of its members infected with SIV is significant, and it will be interesting to learn the rates of juvenile infection, which I suspect will be over 10%. Lindi camp near Stanleyville, where over 500 chimpanzees were collected between 1956 and 1960, used juvenile chimps almost exclusively, because they were easier and safer for the human experimenters to handle and manipulate. Approximately 400 of those chimps were specifically used in the OPV research staged between 1956 and 1958. The majority of these were sacrificed, and many had their blood and/or organs removed while they were anaesthetised, but not yet killed. If 10% of those 400 juvenile chimps were infected in the wild, this means that 40 of the "polio chimps" would have been infected with SIV upon entry to the camp. Most animals were co-caged, and there was also a play cage where up to 10 chimps at a time were placed. This is likely to have caused further SIV infections through bites, scratches and (perhaps) the licking of open wounds.

What is most intriguing about the latest findings is that Sharp and Hahn have for the first time pointed to a specific region of Africa (south-eastern Cameroon), and said "that's where HIV-1 probably began". They have already pointed to the city of Kinshasa (formerly Leopoldville) as the likely cradle of the AIDS epidemic. And Paul Sharp is quite correct in stating that there is a river link that covers most of the distance between the south-eastern corner of Cameroon and the city of Kinshasa (or that of Brazzaville, across the water from Kinshasa). On the map, the River Congo runs 250 miles north-north-east of Kinshasa and Brazzaville to the small town of Mossaka, after which a tributary of the Congo, the Sangha, runs another 200 miles northwards through eastern Congo Brazzaville to the border town of Ouesso. The Sangha continues north of Ouesso, along the Cameroon/Central African Republic border, and there are also various tributaries, such as the Ngoko and the Boumba, though their relevance depends on the exact locations of the infected chimpanzee troupes, which have not yet been revealed. The river link is navigable between Ouesso and Brazzaville, meaning that there was indeed a viable mode of transport that covered most of the distance between the rural place that Sharp and Hahn posit as the source of HIV-1, and the urban centre that they believe is the hearth of the AIDS pandemic.

At this point, however, it is time to look at some of the counter-evidence.

Inherent Problems with Paul Sharp's Analysis of the Latest Data

1) The great hunter-gatherers of this region of south-eastern Cameroon (like many other parts of central Africa) are the pygmies. However, early studies of HIV infection in pygmies from across the central African rain forest detected zero HIV-1 infection, followed in later years by low levels of infection, which could be traced to sexual encounters between pygmies and local Bantus, who had clearly introduced the virus. One study examined 340 sera that had been obtained between 1975 and 1978 from the Aka pygmies, living on the borders between Congo Brazzaville and Central African Republic; not one sample was HIV-positive. These Aka pygmies would appear to be from the same group as the pygmies of south-eastern Cameroon, who are commonly referred to as the Baka; they live an estimated 100 to 150 miles east of the area highlighted by Sharp. A later HIV seroprevalence study of pygmies specifically from southern Cameroon found that HIV infection was "very rare", even by the 1990s.

2) In the last few years, two American military scientists (doctors Burke and Wolfe) have made an exhaustive search for SIV-like infections (rather than HIV infections) among hunter/gatherers from different parts of Cameroon, with completely negative results for the 76 plasma tested. (They found a host of other viruses, including several infections with a foamy virus which, like SIV, is a simian retrovirus. But they found no SIV.) Burke and Wolfe also tested 1,224 plasma from a general population coming from urban and rural areas of Cameroon, where people "may handle [primate bushmeat] but are unlikely to have repeated contact with the blood or body fluids of freshly killed animals". Just one of these plasma showed some indications of exposure to SIV (in this case SIV from a colobus monkey) by one of the assays used (ELISPOT), but the result was hardly convincing, for the plasma in question showed only weak cellular reactivity, and the authors were unable to amplify any SIV nucleic acids. This might have been a false positive, or it might be that they had genuinely detected one single human exposure to SIV in over 1,300 plasma, but that this exposure had failed to cause any productive human infection. Burke and Wolfe's research, which appeared to be specifically designed to obtain data that lent support to the bushmeat theory of origin, actually did the exact opposite. There is still no data that directly supports the hypothesis that hunters get infected with chimpanzee SIV through the handling and cutting up of chimpanzee bushmeat. [See separate essay about the Burke and Wolfe research, soon to be posted on this web-site.]

3) Paul Sharp apparently claims [see Susman article, below] that in 1959 "there were probably thousands of people in the region" between Cameroon and Kinshasa who were already suffering from AIDS. This is not the first time that Paul Sharp has suggested this, but it gives some idea of the superficial nature of some of his analysis. The reality is that there is zero evidence of either early HIV-1 infection or of early AIDS in either Cameroon or Congo Brazzaville. Now let us look at Africa as a whole. Even after more than twenty years of searching for early African samples in Africa, North America and Europe, only one single sample of HIV-1 has been found that dates from before 1970. (This is ZR59, an HIV-1 sequence that was obtained from plasma sample L70, which was allegedly collected in 1959 from Leopoldville. The portion of the HIV-1 genome of ZR59 that has been sequenced is tiny, representing less than 5% of the entire virus.) Furthermore, there is no convincing report of AIDS-like disease in Africa before 1962 (a woman originating from Lisala, DRC), and no further convincing example prior to 1973 (a child born somewhere in the DRC, perhaps in Kinshasa). These findings conflict sharply with Professor Sharp's claim that "thousands" of Africans were affected with AIDS by 1959. Perhaps he believes that only Western doctors would report such unusual conditions, or record them for posterity. If so, perhaps he should

bear in mind that the Belgian Congo, Cameroon and Congo Brazzaville were still staffed with Belgian and French doctors up to independence in 1960 - and in many cases after that as well.

4) The part of south-eastern Cameroon highlighted by Sharp is, in reality, not very remote or isolated. There are three quite significant roads running from this area to major centres. One road runs north to Yokadouma, and then either west to the Cameroonian capital of Yaounde, or else east to Bangui, capital of the Central African Republic (CAR). A second road crosses over into Congo Brazzaville at Moloundou, and ends up either at Libreville, the capital of Gabon, or else at Yaounde in Cameroon. The third (and largest) road runs due south through Congo Brazzaville from Ouesso to the capital, Brazzaville.

However, Sharp proposes that an SIV acquired by a local citizen from one of the Cameroonian chimpanzees escaped from this area by river, after which the virus got established in only one place, this being Leopoldville/Kinshasa, 450 to 500 miles away by river-boat. To make this happen, an infectee (or chain of infectees) would have needed to carry the virus southwards across two national boundaries (from what was then French Cameroon to French Equatorial Africa, AEF, and from AEF to the Belgian Congo), in an age when few Africans crossed borders, especially those separating different colonial masters. Furthermore, this infectee (or chain of infectees) must not have sparked any outbreaks of AIDS en route. This index virus would (according to Sharp's scenario) have had to have passed through Ouesso, the river-boat terminus just south of the border between Cameroon and Congo Brazzaville. In 1949 Ouesso was a major town of 26,000 people, and the second largest town in Congo Brazzaville (nowadays officially titled the Republic of Congo); it was a trading centre for mahogany, sisal, palm products, copal and rubber. If this was the route whereby early HIV-1 reached Leopoldville, then the bushmeat hunter/butcher/trader would presumably have had to have spent some time in Ouesso waiting for the steamer southwards. Yet there is no evidence of early HIV infection in Ouesso, and the first evidence of HIV-1 in Brazzaville emerges indirectly, when a Soviet man who received a blood transfusion in that city in 1981 returned to the USSR, and sparked a mini-epidemic there. But 1981 is two decades after the first evidence of HIV-1 in Leopoldville/Kinshasa.

5) Early inklings of these results must have been available for some time, and it would seem that recent attempts may have been made to check the HIV-1 variants currently found in areas bordering the range of the Cameroonian chimpanzees. Research published in late 2005 about HIV-1 diversity in three rural health centres in eastern Cameroon, along the border with CAR, revealed that 27 of 40 HIV-1 viruses detected were classic subtype A, and that one was subtype F2, while the other twelve were recombinant viruses ("circulating recombinant forms", or CRFs) containing traces of subtypes B, E, G and J. The authors described this area along the Cameroon/CAR border as "a potential hotspot of HIV-1 recombination", but this was apparently because the region appeared to lie at the meeting-place of one mini-epidemic spreading westwards from CAR, and another spreading eastwards from Cameroon. However, there were no traces (even as elements of recombinant viruses) of several major subtypes, notably C and D. Moreover, it is not clear just how close any of these health centres were to the range of the chimpanzees.

Further research published in early 2006 (and conducted in 2003-4) concerning five health centres in Congo Brazzaville, including Brazzaville and Ouesso, detected all the major HIV-1 subtypes, plus five of the more common CRFs. However, this survey covered the whole country - and as the pandemic progresses and affects over 40 million persons world-wide, subtypes and CRFs are becoming far more common in every country. By contrast, an article

on genetic diversity in Cameroon published in 1994 reported the presence of only five subtypes in the country. The relevance of these results must be compared to the finding of all eleven of the recognised HIV-1 subtypes A to K (and some CRFs) in sera collected in 1983-6 in a single city (Kinshasa): data that is highly significant with respect to the genesis of AIDS. To sum up, there is no evidence of a wide variety of HIV-1 subtypes emanating from either south-eastern Cameroon or from Congo Brazzaville *at an early stage of the AIDS pandemic.*

6) It becomes apparent that Hahn and Sharp still have no adequate explanation for how the AIDS epidemic that we see today could have been engendered. In fact, for their hypothesis of origin to work, they have to propose a quite extraordinarily far-fetched scenario. They have first to postulate a single chimp virus that crossed (via chimp bushmeat) to humans in south-eastern Cameroon, and which was then transported to Leopoldville/Kinshasa, where it for some reason diversified into many different subtypes at the very start of the epidemic (which, according to them, was in the 1930s and 1940s). They surmise that later on (perhaps in the 1960s or 1970s) these various subtypes began to escape from Kinshasa to establish epidemics of specific subtypes in different locations around Africa, and around the world. Later again, they postulate, various of the subtypes started to meet up again in the cells of individual human infectees, and to create recombinants, which were then passed on to others to become CRFs, circulating recombinant forms.

By contrast, the OPV theory has a much simpler way of explaining the subtypes and recombinant forms. This is that different chimpanzee SIVs, and recombinants thereof, were present in the various batches of OPV prepared in Stanleyville in the late 1950s, and then tested in humans in approximately 30 different vaccination sites around the Belgian Congo and Ruanda-Urundi. The different subtypes did not require extraordinary circumstances to evolve from the index virus, as they do in Sharp and Hahn's theory. They merely needed to become established in different vaccination venues, and thence to begin their worldwide spread.

According to the OPV theory, there are several possible explanations for the fact that Leopoldville/Kinshasa alone has all the subtypes. It could be, for instance, that in Leopoldville the doctors decided to conduct a comparative study of different locally-made batches (for instance by testing different batches in different suburbs). Such a study could have been effected clandestinely; only a very small number of people (perhaps even one individual) would have needed to be in the know. Ghislain Courtois, for instance, could have organised such a trial, and it is perhaps significant that he moved from Stanleyville to Leopoldville in September 1959. (The vaccination of 76,000 children aged five and less in Leopoldville was a gradual process, staged between August 1958 and April 1960.) Alternatively, since it seems that the Koprowski group used a sequential pool system for vaccine manufacture, whereby each new vaccine pool or batch was prepared from the previous one (rather than from a well-characterised seed pool), it may be that Kinshasa received all the HIV-1 subtypes because it was effectively the last place to be vaccinated, by which stage more chimp SIVs and recombinants were present in the vaccine material.

Using phylogenetic data alone, there is no way in which anyone can determine which of the bushmeat or OPV hypotheses is more likely to be correct, for phylogenetic analysis cannot distinguish exactly what occurred at the very beginning of the pandemic. It is only when one analyses the epidemiology of the very earliest cases of HIV-1 infection and AIDS - and discovers that there is a highly significant correlation with venues where CHAT vaccine was administered in the 1950s - that the truth begins to be revealed.

An Alternative Explanation of the New Information that is Consistent with the OPV Hypothesis

In the 1950s, there was a small but significant steamship landing on the Belgian Congo shore called Lukolela, which was situated some ten miles from the mouth of the Sangha river, where it debouches into the River Congo. Just a few miles across the river was another landing called Mossaka, situated on the Congo Brazzaville shore just downstream from the Sangha's mouth. These were the landings where people transferred between the River Congo steamers and the River Sangha steamers. Both these steamship landings were situated some 2 days from Brazzaville and Leopoldville to the west, or 5 days from Stanleyville to the east. A further 115 miles north of Lukolela on the Congo river was Coquilhatville, capital of Equateur province; both Lukolela and Coquilhatville were situated in the same district, variously called Coquilhatville or Equateur district. There are anecdotal stories from persons who travelled on the Congo steamers in both the 1950s and the 1980s indicating that chimps were frequently purchased at stops along the route, usually to be sold on at the end of the journey. In the late fifties, it was apparently quite widely known that "white doctors" were purchasing chimpanzees in Stanleyville, and this may well have stimulated local trade in the species. In support of this scenario is the fact that one of the 54 Lindi chimps for which we have a documented place of origin, (Ikela Marie), came from "Coquilhatville". This could well mean that she (or other Lindi chimps) originated from Cameroon or Congo Brazzaville, and was/were brought down by steamship to Lukolela or to Coquilhatville town, before being sold on to persons travelling eastwards to Stanleyville. Ikela Marie lived in Lindi camp for an unusually long time; she arrived in April 1957, and was still alive in mid-1959. She (or other chimps obtained in similar areas) could therefore have been infected with the Cameroonian SIV strain highlighted by Paul Sharp, or a similar virus, and she/they could have passed such a virus on to other chimps at Lindi, for both co-caging and group-caging were routine at the camp.

It is important to note that contrary to what many have claimed, Ghislain Courtois, the Belgian doctor who ran Lindi camp in conjunction with the American vaccine-maker, Hilary Koprowski, always carefully avoided stating whether or not any *Pan troglodytes troglodytes* (*Ptt*) chimps (the subspecies coming from Cameroon, Gabon and Congo Brazzaville) were present at Lindi camp. Furthermore, there is documentary evidence indicating that in 1957, the polio group in Stanleyville was keen to acquire *Ptt* chimps. This and further evidence which I shall reveal in due course strongly suggests that at least some *Ptt* chimps were sacrificed in the course of the OPV research at Lindi.

I continue to believe that the crucial element that allowed the chimp virus, SIVcpz, to transfer to humans and to prosper in its new host was not the preparation or consumption of chimpanzee bushmeat. After all, such activities did not, to anyone's knowledge, result in any outbreaks of AIDS by 1959, despite Professor Sharp's unconvincing claim that thousands of Africans would have had the disease by then. As proposed above, it is entirely possible that one or more chimpanzees infected with a virus close to HIV-1 (such as chimps from south-eastern Cameroon) could have ended up at Lindi. I believe that two or more chimp SIVs were present in the tissue cultures that were used to create CHAT vaccine, and that the vaccine therefore contained both chimp SIVs and various recombinant strains created from them. [Geneticist Mikkel Schierup has pointed out that initial recombination between just two SIVs would have been sufficient to create all the viral subtypes and variants of HIV-1(M) seen today.] I further believe that an OPV administered orally via a high-pressure squirt from a

syringe, as CHAT was, would have provided an effective (and completely novel) route of transfer for these viruses from chimpanzee to human.

In support of this is the fact that the earliest examples of HIV-1(M) and of pandemic AIDS occur nowhere near south-eastern Cameroon, or indeed anywhere in Cameroon or Congo Brazzaville. Instead, they occur almost exclusively in those cities, towns and villages where CHAT vaccine was administered in what is now the Democratic Republic of Congo, Rwanda and Burundi.

To sum up: Paul Sharp believes that there was a single crucial transfer of SIV from chimp to human in around the 1930s that was the source of HIV-1(M), and that this virus quickly moved to Leopoldville/Kinshasa, which thus became the cradle of the AIDS epidemic. I believe that there were several separate (but nearly simultaneous) transfers of chimp viruses to humans in the late 1950s, and that Leopoldville/Kinshasa is only one of several vaccination sites that served as cradles for AIDS.

Further Background

The discovery that viruses related to HIV were found naturally in healthy African primates was first made in the mid-1980s, and in 1989 came the first publication (by Martine Peeters' group) demonstrating that SIVs that were related to HIV-1 were found in common chimpanzees, *Pan troglodytes.* (The actual genetic sequences were published by Simon Wain-Hobson's group in 1990.) Given this sound start, it is rather surprising that so little progress was made in SIV research in central Africa during the next nine years. Early in 1999, the Hahn/Sharp group claimed that they had found the most closely related SIV to HIV-1 in *Pan troglodytes troglodytes* (*Ptt*), a chimpanzee subspecies found in Congo Brazzaville, Gabon, Cameroon and Equatorial Guinea, and drew a distinction between these SIVs, and others SIVs found in *Pan troglodytes schweinfurthii* (*Pts*), the chimp subspecies that is located further east, in the DRC, Uganda and Tanzania. Superficially, this seemed reasonable, in that those *Ptt* SIVs that had been sequenced by then seemed to be roughly 80% homologous with HIV-1, whereas *Pts* SIVs were roughly 70% homologous. (All the other SIVs found naturally in African primates, and there are now about 30 in all, are far more distantly related to HIV-1. And the evidence to date suggests that the two other chimpanzee subspecies, *Pan troglodytes verus* and *Pan troglodytes vellerosus,* are not naturally infected with SIV.) However, Hahn and Sharp's 1999 hypothesis about a *Ptt* source was based on only four chimp SIV sequences, and there were unexpectedly large differences between those sequences, suggesting that other SIVs that were far more similar to the human virus might still be out there in the forest, waiting to be discovered. Furthermore, some primatologists argued that *Ptt* and *Pts* were effectively the same subspecies, and should never have been split into two. This was supported by early research from some of the Lindi scientists in the Belgian Congo, which found unexpected similarities between the blood components of some of the Lindi chimps and those of *Ptt* and *Pan troglodytes verus* chimps from the west.

An example of those chimp SIVs that were still waiting to be discovered has just been reported from south-eastern Cameroon, but this does not exclude the possibility that other chimp SIVs will subsequently be found that are even closer to HIV-1. So far, only one SIV sequence has been obtained from a wild chimp originating from a known location in the DRC (the Parisi Forest, about 100 miles south-east of Kisangani), and that apparently had only about 70% homology with HIV-1. However, chimps used in the 1950s OPV experiments were obtained from a huge area (some 300,000 square miles) of the northern Belgian Congo,

extending at the minimum from Coquilhatville (now Mbandaka) in the west to Zapai in the north and Mambasa in the east, and chimps from these areas have not yet been sampled.

What is worrying about Hahn and Sharp's research is that from the start it seems to have been driven by a determination to prove the bushmeat theory right and the OPV theory wrong. There may be many reasons for this, some of which may be subliminal, but one reason is simple: the medical research community desperately wants the OPV theory to be wrong, because if proved right they fear it will shake confidence in that central support of all public health programmes: vaccination. Moreover, they fear that a proven OPV origin would spark public outrage, and a backlash that would involve huge law-suits. In the light of this, it is hardly surprising that research that might end up offering support to the OPV hypothesis does not readily engender governmental or private support or funding.

But for Sharp and Hahn there may be another, even simpler reason. If the OPV theory proves to be correct, as I believe it will do, then it will hugely undermine the last 15 years of their research. Some of that research will still be of very considerable value, but its central tenets will have been largely destroyed.

Neither Sharp nor Hahn are known as shrinking violets, and neither of them countenances opposition easily. (By contrast, Martine Peeters has assumed a consistently low-key role in this research, despite which her team has come up with some of the most significant breakthroughs. These include the latest Cameroonian chimp SIV sequences, and her important and well-researched confirmation, in year 2000, that the DRC was indeed the hearth of the AIDS pandemic.) Professor Hahn, in particular, has something of a reputation. On one level she is committed and hard-working, she is an excellent organiser, and she deserves great credit for her determination in tracking SIVs in Africa since the mid-1990s. However, she is also recognised as being domineering, and as having an overweening self-image. This is despite the fact that she sometimes makes quite fundamental mistakes in her research: mistakes or misinterpretations which she is loath to acknowledge. Hahn appears to be a skilled practitioner of spin, and she is therefore adept at massaging analysis in such a way that it glosses over shortcomings or errors in her previous arguments.

In 1995, having already interviewed Paul Sharp in the UK, I travelled to Birmingham, Alabama to interview Beatrice Hahn. She expounded her theories for a couple of hours or so. However, when it came to my turn to expound some of my ideas, she gave me the floor for less than 60 seconds before she again took over, with all the finesse of a bulldozer.

Five years later, at the Royal Society meeting on the origin of AIDS, Dr Hahn tried a different tack, when she approached me during a break and tried to be chummy. However, it took only a few minutes for me to realise that her chumminess was as loaded as her high-handedness. Since then, I tried once more to approach Hahn by e-mail, offering a sort of truce, and asking her a question about the possibility of recombination between different SIVs in a tissue culture based on cells from two or more chimpanzees. Her response was back to her old familiar self, for she simply refused to answer my question: "I am not going to discuss this, because this is going nowhere", she wrote, "but don't kid yourself (or others) into believing that you have the necessary scientific background or expertise to make a judgement call in this matter."

It would be easer to accept such implied criticism if Dr Hahn were willing to apply the same yardstick to herself. Having studied her work for the last 15-odd years, I believe that she has

considerable scientific background and experience, but I'm far less confident about her expertise and sound judgement. Hahn did her early research in the lab of Robert Gallo (the American scientist who wrongly claimed that the AIDS virus was a C-type retrovirus, and who tried to get credit for its discovery, even though the original virus had been obtained in Luc Montagnier's lab), and she appears to be every bit as ambitious and driven as her former boss, and suffers just as frequently from blinkered vision. To my mind, this renders her ill-equipped to make *impartial* and *well-balanced* judgement calls on this subject.

Let me give an example of what I mean. Apart from the "geographical source" argument, the other major argument repeatedly propounded by doctors Hahn and Sharp (and others, such as Bette Korber) against the OPV theory is the "dating argument", in which they propose that the Most Recent Common Ancestor (or, in effect, the index case) of HIV-1 was infected in 1931, or 1940 … or, in any case, some years before the OPV trials in Africa in the 1950s. However, these dates of 1931 and 1940 are simply theoretical estimates and, moreover, estimates that are based on a false premise.

This false premise is that it is possible to back-calculate the age of an HIV, or SIV, by using a constant molecular clock, calibrated according to the virus's rate of mutation. However, the major form of evolution of immunodeficiency viruses such as the HIVs and SIVs is not mutation, but the completely different process of recombination. With respect to SIV and HIV, mutation is the process of gradual change caused by errors when individual nucleotides of a specific virus are incorrectly copied during the replication cycle. Recombination is the process whereby two different viruses meet within a cell and produce a new virus or viruses through "viral sex", this being the exchange of entire slabs of genetic material. The SIVs and HIVs evolve more rapidly, and are more recombinogenic (prone to recombination) than any other known organisms. Indeed, recent research suggests that recombination is approximately ten times more significant than mutation in the evolution of HIVs and SIVs. In short, the phylogenetic daters are trying to measure the wrong process. Their arguments about dating HIV-1 via its mutation rate are built on sand.

One argument they frequently use in support of phylogenetic dating relates to ZR59, a short sequence from an HIV-1 isolate that apparently dates from 1959. The daters claim that this sequence ties in remarkably well with an MRCA date of around 1931. As it happens, I was the person who made the initial moves, in 1995, to get this earliest of HIV-1 isolates analysed by PCR techniques, and as a result I was a co-author of the paper that was subsequently published about the ZR59 sequence in *Nature* in 1998. Paul Sharp and Bette Korber were brought in later on to help with the phylogenetic analysis, and I know that there was some considerable controversy about how to interpret the partial sequence (representing less than 5% of the HIV-1 genome). Having done further research since the publication of that article, I now also know that there is considerable doubt about the provenance of L70, the plasma sample that provided the ZR59 sequence.

It is not yet time to tell the full story, but what I can state is this. There is a striking lack of precise information about the L70 sample. All that can be stated with certainty is that it was obtained from a male subject from Leopoldville who had the sickling trait and G6PD deficiency. However, I now know that this sample was not obtained in 1959, but rather at some time between 1960 and 1963. Moreover, the male may have been a boy, rather than an adult man (as has always been assumed). If indeed L70 came from a boy who was aged nine or less in 1963, then that boy would almost certainly have been immunised with CHAT vaccine between 1958 and 1960. Furthermore, the L70 sample was originally obtained not by

one of the two scientists so credited in the initial paper, but almost certainly by one of their collaborators, a Belgian scientist who, from mid-1960 until early 1961, was also responsible for looking after the final 50 or 60 chimpanzees from the group that was experimented upon at Lindi and Stanleyville between 1956 and 1960. Whether or not that last detail is relevant is not clear. What is clear is that the HIV-1 sample that significantly predates all others has an unexpectedly opaque history.

To sum up, the true facts surrounding ZR59, the earliest sequence of HIV-1, are extremely unclear, but it is certainly possible that the L70 sample (which produced ZR59) was obtained from the arm of a young CHAT vaccinee. If so, then ZR59 may have been just one of a number of early HIV-1 variants that arrived in humans via CHAT vaccine in the 1957-60 period. There is no way of knowing if this particular variant was ever passed on to others, either via the vaccine or later on, via the L70 donor. If it was not, then ZR59 was effectively a dead-end infection.

Ed Hooper, 20th February, 2006

Analysis: Tracking the source of AIDS

By Ed Susman
United Press International

Published February 8, 2006

The work was gross, smelly and messy, but when the scientists were finished their dirty work was rewarded with a gem of research: the origin of the virus that causes AIDS.

A quarter of a century after the syndrome was first recognized, doctors said Tuesday they believe they have pinpointed the roots of the epidemic that has killed millions and now infects 40 million others worldwide.

The virus appears to have originated in a community of chimpanzees that lived in southern Cameroon, near the border of present day Gabon and the Congo Republic, said Paul Sharpe, professor of genetics at the University of Nottingham in the United Kingdom.

Sharpe and his colleagues spent 15 years digging in mounds of old chimpanzee feces and then in laboratories when high technology genetic testing and sequencing tools were used to unravel the clues found in West Central Africa.

It took the researchers 15 years of searching to closely match the genetic structure of an animal virus to that of the earliest known example of human immunodeficiency virus, the pathogen that causes AIDS.

That human sample dates to 1959 and was found in a serum sample in Kinshasa, the capital of the Democratic Republic of the Congo, just down a river from where he believes the virus

jumped from chimpanzee to a human being. "That jump most likely occurred in the 1930s, our research and that of others indicates," he told United Press International.

"By the time the man in Kinshasa had HIV infection, there were probably thousands of people in the region with the disease," Sharp said Tuesday in discussing his findings at the 13th Conference on Retroviruses and Opportunistic Infections in Denver.

The closest virus in structure to HIV in the animal kingdom is SIVcpz. SIVcpz is found in a subspecies of chimpanzees, *Pan troglodytes troglodytes*. Sharp tracked families of these chimpanzees in the isolated area of Cameroon, taking samples from dried mounds of feces and then performing molecular sequencing of the samples.

He said the techniques used were completely non-invasive. None of the chimpanzees had to be captured, shot or injected for scientists to retrieve the biological specimens needed to connect the dots and lead to the location of the chimpanzee troop.

Using sophisticated molecular sequencing techniques, Sharp was able to pinpoint a clustering of these early viruses, determining the area where the epidemic appears to have begun.

He said the sequencing of the virus of the man in Kinshasa, with its resemblance to the SIVcpz strain, "is clear evidence that the virus had been circulating in that area 30 years earlier."

"This study is really a remarkable achievement," said John Coffin, professor of molecular biology at Tufts University in Boston. "They have shown the way to settle one of the many issues in HIV research."

ScienceNOW Daily News 10 February 2006

Tracing HIV's Steps

By Jon Cohen

DENVER, COLORADO--Clarifying the origin of AIDS won't prevent or cure any HIV infections, but the mystery has long gripped the field. Two groups studying wild chimpanzees in Cameroon reported progress on that front here this week at the 13th Conference on Retroviruses and Opportunistic Infections.

These teams earlier had found persuasive evidence that chimps harbor a simian immunodeficiency virus called SIVcpz that became HIV-1--the predominant cause of AIDS in humans (ScienceNOW, 1 February 1999,)--but they had discovered precious few infected animals to make the case. Their next step was to analyze 1300 fecal samples of wild apes (some turned out to be from gorillas) and identify several places in which SIVcpz was widespread in chimp communities. Then they genetically characterized dozens of new isolates.

The new work found more than 30 strains of SIVcpz, tripling the number previously discovered. The researchers took advantage of the fact that chimps cannot swim, which means

that rivers block the spread of viruses. For the first time, they found chimp communities in which SIVcpz infection was widespread--in one, up to 35% of the animals analyzed had the virus in their feces. "Our eyeballs popped out of our heads," said Brandon Keele, who works with Beatrice Hahn at the University of Alabama, Birmingham.

In a separate presentation, Fran Van Heuverswyn, part of a team headed by Martine Peeters of the Institut de Recherche pour le Developement in Montpellier, France, described how two of the isolates more closely matched the HIV-1 causing the human epidemic than any found in the past. (HIV-2, which infects humans much more infrequently, clearly comes from an SIV in sooty mangabeys.)

Building on the new data, Paul Sharp, who studies molecular evolution of pathogens at Nottingham University, United Kingdom, explored the origin of SIVcpz. Sharp's new analysis suggests that the SIVcpz closest to HIV- 1 is a combination of SIVs isolated from red cap mangabeys and monkeys from the Cercopithecus genus.

Putting in the final piece of the puzzle, Sharp said the virus must have reached a major city to start the AIDS epidemic. He posited that a person became infected in rural Cameroon and then traveled by river to what is now known as Kinshasa, Democratic Republic of Congo. Kinshasa has the greatest genetic diversity of HIV-1, suggesting that the virus has been there longer than anywhere else. It also was home to the first known HIV-infected person, a Bantu man who had his blood sampled in 1959 for a malaria study.

"They're closing in on some very hot stuff," said James Hoxie, who studies HIV and SIV at the University of Pennsylvania, Philadelphia. "It's compelling genetic evidence."