THE ROYAL SOCIETY

doi 10.1098/rstb.2001.0867

Serial human passage of simian immunodeficiency virus by unsterile injections and the emergence of epidemic human immunodeficiency virus in Africa

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There is compelling evidence that both human immunodeficiency virus (HIV) types emerged from two dissimilar simian immunodeficiency viruses (SIVs) in separate geographical regions of Africa. Each of the two HIVs has its own simian progenitor and specific genetic precursor, and all of the primates that carry these SIVs have been in close contact with humans for thousands of years without the emergence of epidemic HIV. To date no plausible mechanism has been identified to account for the sudden emergence in the mid-20th century of these epidemic HIVs.

In this study we examine the conditions needed for SIV to complete the genetic transition from individual human SIV infections to epidemic HIV in humans. The genetic distance from SIV to HIV and the mutational activity needed to achieve this degree of adaptation to human hosts is placed within a mathematical model to estimate the probabilities of SIV completing this transition within a single SIV-infected human host. We found that the emergence of even one epidemic HIV strain, following a single human exposure to SIV, was very unlikely. And the probability of four or more such transitions (i.e. HIV-1 groups M, O and HIV-2 subtypes A and B) occurring in a brief period is vanishingly small. We conclude that SIV cannot become a zoonosis, but requires adaptive mutations to become HIV. Some modern event must have aided in the transition of SIV to HIV.

Our research indicates that serial passage of partially adapted SIV between humans could produce the series of cumulative mutations sufficient for the emergence of epidemic HIV strains. We examined the rapid growth of unsterile injections in Africa beginning in the 1950s as a biologically plausible event capable of greatly increasing serial human passage of SIV and generating HIV by a series of multiple genetic transitions. We conclude that increased unsterile injecting in Africa during the period 1950–1970 provided the agent for SIV human infections to emerge as epidemic HIV in the modern era.

Keywords: acquired immune deficiency syndrome; simian immunodeficiency virus; human immunodeficiency virus; Africa; unsterile injections; zoonosis

1. INTRODUCTION

Multiple lines of evidence indicate that all known human immunodeficiency viruses (HIVs) derive from a group of simian immunodeficiency viruses (SIVs) that pre-date the emergence of the acquired immune deficiency syndrome (AIDS) epidemic by hundreds of thousands, perhaps even millions of years (Sharp *et al.* 1994). Yet, despite centuries of opportunity to emerge (e.g. the African slave trade that went on for over 300 years and dislocated millions (Hochschild 1998)), there is no evidence that HIV ever existed in Africa or reached Europe and the New World prior to the 20th-century epidemic. How, therefore, may we understand the nearly simultaneous emergence of two genetically distinct types of epidemic HIV from ancient and separate simian sources occurring in two different regions of Africa?

Two paths are possible for the emergence of epidemic HIV strains from ancestral SIV. The first is that HIVs evolved in Africa from relatively ancient, multiple and separate cross-species transmissions of SIV to humans. In this model, HIV remained sequestered in remote areas of Africa, without spread to the general population within Africa or to other continents, until the population growth and dislocations of the 20th century enabled HIV to break out as a human epidemic. The second possibility is

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that new ecological factors arose in the 20th century that facilitated the transformation of SIV to epidemic HIV types. However, no models have yet been developed to exclude either scenario or identify a plausible biological mechanism and specific ecological factors, new to the mid-20th century, that could be responsible for multiple transitions of SIV to distinct HIVs.

Here we examine the plausibility of a hypothesis that serial transmission of SIV between humans was the biological mechanism that permitted the accumulation of adaptive mutations of SIV, which led to the emergence of epidemic HIV strains. In this model we make a specific distinction between epidemic and non-epidemic strains of HIV. The hypothesis was assessed in a model that calculates the probabilities for individual non-epidemic SIV human infections completing the genetic transition to epidemic HIV. Using *in vivo* mutation rates and virus burst size data for lentiviruses, we measured the accumulation of adaptive mutations per SIV genome per person. The model shows that even a single epidemic strain of HIV is unlikely to have arisen spontaneously from direct SIV infections of individuals in contact with SIV-infected mangabeys or chimpanzees. Moreover, the probability of several spontaneous transitions, which appear to have occurred within a decade in Africa, is vanishingly small. While many animal viruses can and have made the genetic transition to human pathogens (e.g. smallpox), the rapid transformation of four or five ancient SIVs into the modern HIVs in different parts of Africa suggests a single modern event that occurred in multiple locations. To establish a specific and biologically plausible agent that could adequately amplify the serial transmission of SIV, we focused on the exponential growth of unsterile injections that was associated with the introduction of injectable medications in these regions of Africa in the period after World War II (WWII).

2. THE GENETIC TRANSITION OF SIV TO HIV

SIV occurs as five or six different genetic lineages distributed throughout sub-Saharan Africa (Fukasawa *et al*. 1988; Peeters *et al*. 1989; Hirsch *et al*. 1989; Tsujimoto *et al*. 1988; Emau *et al*. 1991; Georges-Courbot *et al*. 1998). Recombination (Robertson *et al*. 1995), phylogenetic data (Fukasawa *et al*. 1988; Peeters *et al*. 1989; Hirsch *et al*. 1989; Tsujimoto *et al*. 1988; Emau *et al*. 1991; Georges-Courbot *et al*. 1998) and the continent-wide distribution of naturally infected simian hosts (Georges-Courbot *et al*. 1998; Muller *et al*. 1993; Gao *et al*. 1999) provide strong evidence that SIVs in mangabeys, African green monkeys and chimpanzees are ancient, up to one million years old. Other SIVs have spread across Africa through more recent cross-species transmissions (Jin *et al*. 1994). SIV is therefore ancient in Africa and all six SIV lineages predate the AIDS epidemic by many thousands of years. Although the ancestral origin of both HIV types is well documented (Hirsch *et al*. 1989; Gao *et al*. 1999; Chen *et al*. 1996), the mechanism for the transition of these SIVs to epidemic HIV strains is still unexplained. Most importantly, the simian hosts of the HIV ancestor lineages, *Pan troglodytes troglodytes*, and *Cercocebus torquatus atys*, are not found in the same areas of Africa, but exist in natural, non-contiguous ranges over 1500 km apart (Kingdon 1997).

Of the SIV lineages, four are divergent from HIV while the remaining two, from chimpanzees (SIVcpz) and sooty mangabeys (SIVsm), are closely related to HIV-1 and -2, respectively (Peeters *et al*. 1989; Hirsch *et al*. 1989; Gao *et al*. 1999; Chen *et al*. 1996). Since HIV-1 and -2 are genetically closer to naturally occurring SIVcpz and SIVsm lineages, respectively (Peeters *et al*. 1989; Hirsch *et al*. 1989; Gao *et al*. 1999; Chen *et al*. 1996), than they are to each other, types 1 and 2 therefore must have separate ancestral origins.

HIV-1's closest known relatives SIVcpzGab1, SIVcpzGab2 and SIVcpzUS, occur in *P. t. troglodytes*, a chimpanzee subspecies whose natural range (Kingdon 1997) coincides with the occurrence of all three HIV-1 groups (M, N, O) found thus far (Gao *et al*. 1999). In contrast, SIVcpzAnt from the *P. t. schweinfurthii* of eastern Africa has no known HIV counterpart (Gao *et al*. 1999) (figure 1*a*). A similar phylogenetic relationship was established for sooty mangabeys, *C. t. atys* (Hirsch *et al*. 1989; Georges-Courbot *et al*. 1998; Chen *et al*. 1996), in that HIV-2 clusters with SIV from only the sooty mangabey subspecies. The sister taxon of sooty mangabeys, *C. t. torquatus* the red-capped mangabey (Georges-Courbot *et al*. 1998), harbours an SIVsm-related virus (Georges-Courbot *et al*. 1998; F. Gao, personal communication) that has not been found in humans (F. Simon, personal communication) (figure 1*b*).

3. SIVsm AND SIVcpz AS DEAD-END HUMAN INFECTIONS WITH LIMITED PATHOGENESIS

The genetic lineages of the HIV-1 and -2 are shown in figure 1. A striking feature of HIV trees is the discordance in prevalence of HIV genetic variants. Of six HIV-2 subtypes, only A and B are epidemic (Chen *et al*. 1997; Gao *et al*. 1994). The rest, subtypes C–G (Yamaguchi *et al*. 2000), are non-epidemic HIV-2 strains that are weakly pathogenic, replicate poorly in infected humans and are found only within the range of the sooty mangabey (Chen *et al*. 1997; Gao *et al*. 1994). In conserved genes, the least divergent SIVsm is *ca*. 7.0% divergent from non-epidemic HIV-2 strains and 12.1% from epidemic strains (Chen *et al*. 1997). SIVsmH4 is *ca*. 25% distant from epidemic HIV-2 (Hirsch *et al*. 1989; Chen *et al*. 1997). Non-epidemic subtypes are very rare, found only in individuals who either live in or emigrated from western Africa (Chen *et al*. 1997; Gao *et al*. 1994). For example, HIV-2 subtype F was found in only one person among 9306 individuals living in the same area of rural Sierra Leone (Chen *et al*. 1997) where sooty mangabeys harbour natural SIVsm infections in this same region.

SIVcpz strains from the *P. t. troglodytes* subspecies show a similar relationship with epidemic and non-epidemic HIV-1. The HIV N group, which is also rare in humans (Simon *et al*. 1998) (figure 1*a*), has infected humans for at least a decade (F. Simon, personal communication). Group M occurs as the major epidemic group in the world, whereas groups O and N are relatively rare and are found within the range of the Central African chimpanzee (Gao *et al*. 1999). We used the prevalence of rare HIV-1 and -2 strains as the basis to test a mechanism that produces epidemic HIV subtypes from poorly adapted, non-epidemic HIV subtypes, and employed a broad range of mutations, from 20 to 2000 nucleotides, to

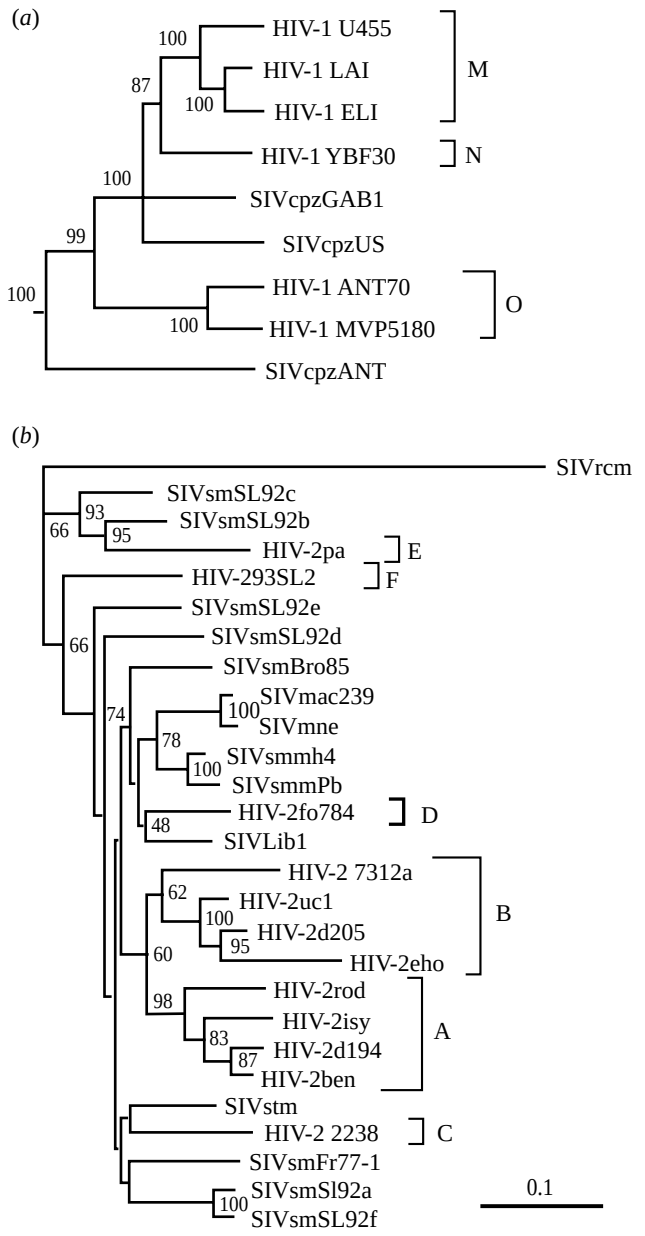

Figure 1. Phylogenetic analysis was performed on a 453 bp fragment of the *gag* gene. Nucleotidic sequences were aligned using the CLUSTAL W v. 1.7 program (Higgins *et al*. 1996), the final alignment being adjusted by eye. Genetic distances between pairs of DNA sequences were calculated using Kimura's two parameter model. Phylogenetic analysis of sequences consisted of minimum evolution estimated by the neighbour-joining method of Saitou & Nei (1987) implemented in the CLUSTAL program, without taking gaps into account. The reproducibility of the branching order was estimated by applying a bootstrap procedure to 100 replicates of the original data set. Phylogenetic relationships between SIV and HIV. (*a*) SIVcpz strains found in Central African chimpanzees (GAB1 and US) are closest to HIV-1 groups M, N and O (Gao *et al*. 1999). SIVcpzAnt, from the East African chimpanzee, is more distant. (*b*) Distinct SIV strains occur in two mangabey subspecies, and only one is closely related to HIV-2. SIVrcm, found in the red-capped mangabey of equatorial Africa, is more distant from HIV-2 when compared with SIVsm, which is found in mangabeys from western Africa (Georges-Courbot *et al*. 1998). Close ancestral relationships between SIVsm and HIV-2, and between SIVcpz and HIV-1, are each confined to one subspecies of mangabey and chimpanzee, respectively. Although both subspecies gave rise to HIV-1 and -2 at about the same time, they are found in different parts of Africa.

test the genetic transition of SIV to epidemic HIV strains. Therefore, it is not necessary to know the precise number of mutations required for SIV to epidemic HIV genetic transitions, because the model allows for very few genetic changes, as well as large numbers of mutations.

4. DETERMINING THE PROBABILITIES OF SPONTANEOUS SIV MUTATION TO HIV

A key issue in understanding the origin of HIV is whether or not SIV has the potential to become a zoonosis by making the transition from SIV to HIV in a single human exposed to simian blood. Because of our data showing multiple examples of dead-end SIV infections (figure 1), we assume that adaptive mutations are necessary for SIV to become epidemic. If this were true, then SIV is not a zoonosis. Normally, human host defences would suppress poorly adapted SIV strains within a few weeks of infection. If the requisite genetic events needed for the transition to HIV would have taken a longer time to accrue than is normally available in a human SIV infection, then the single-infection zoonotic theory would be less tenable.

Therefore, we sought to establish a probability distribution for the timing of the SIV to HIV transition following a single human infection. In that way, the plausibility of the scenario by which SIV to HIV transition might occur in a single infection could be examined. Very low probabilities would cast doubt on the underlying theory (i.e. single infection) and lead to a search for new theories that would allow for serial passage of SIV in transition to HIV.

We used a stochastic approach with continuously valued generation times, similar to the treatment of the coalescent theory approach taken by Rodrigo & Felsenstein (1999). Our goal was to generate a probability distribution for the elapsed time to produce HIV, given different values of the proportion of the SIV genome that must change in order to produce HIV.

Specifically, we assumed a human is infected with wild-type, replication-competent SIV. We then used binomial theory to estimate the probability of one or more progeny SIV virions in each generation carrying a genetic change in the direction of HIV. We used the error rate of the reverse transcriptase as the underlying change probability and conditioned on the length of the SIV genome. We allowed the number of virions produced per generation to vary (table 1). Treating this probability as a hazard rate allowed us to estimate the time (in number of generations) to the next HIV-productive genetic change. Finally, we cumulated the estimated times over the total number of required changes to reach the total time for SIV to HIV transition.

In order to capture the full sampling variability in the process, we used resampling with a range of possible values of generation size in calculating per-change hazards. Using the exponential distribution to determine

Table 1. *Estimation of the number of acute infection days (period of relatively high virus load) required for m mutations*

(CI = 90% confidence interval, expectation is median to *m* mutations (Rodrigo & Felsenstein 1999).)

	m					
	20	40	61	100	200	1000
upper bound of CI	77	87	96	104	119	234
expectation	43	52	57	65	80	192
lower bound of CI	23	30	35	42	57	166

time from one change to the next allowed for continuous generation times and avoided heavier parameterizations which might induce bias. Finally, we made assumptions that would be most favourable to a rapid SIV to HIV transition. The most significant of these was our assumption that SIV plasma virus loads approached human HIV-1 loads during the acute infection period, before immune responses inhibited SIV replication. In this way, we would be assured that a finding of a low probability of the single-infection theory was not due to bias.

The number of mutations required for SIV to HIV transition is unknown, so we took a broad approach. In this mutation-only illustration, there is almost no ($< 5\%$) transition-probability density at $\leqslant 35$ days post-infection, when the number of mutations required is greater than 60. At $m \leqslant 60$ mutations, the probability of successful transition in $\leqslant 35$ days remains small ($\ll 50\%$). The probability density for duration of transition at $m = 100$ mutations is centred at 65 days and at $m = 200$ mutations is centred at 80 days. The immune responses would greatly suppress an unfit virus, making the SIV to HIV transition highly unlikely during the chronic infection period.

5. SERIAL PASSAGE OF SIV

Serial transfer of SIV between humans would have a markedly different outcome. In that case, *m* mutations would be achieved by allowing them to accumulate in an incremental and additive fashion in several persons. We conclude that a single, initial human SIV infection would not accumulate sufficient mutations before host immune responses largely suppressed growth of the poorly adapted viruses. These findings explain the rare occurrences of non-epidemic subtypes of HIV-2 and suggest a similar phenomenon for HIV-1 group N (Simon *et al.* 1998), which has existed in humans for over a decade, but has not emerged as a significant epidemic strain thus far (F. Simon, personal communication). Additional serial passage would be required before these viruses could become epidemic.

6. MASSIVE UNSTERILE INJECTIONS: A MECHANISM FOR AMPLIFYING SERIAL PASSAGE OF SIV IN HUMAN POPULATIONS

While a precise determination of the date for the emergence of the HIV-1 M group is not yet possible (Goudsmit & Lukashov 1999; Korber *et al.* 1999), sequence data from the earliest known HIV infection in Central Africa by Zhu *et al.* (1998) place the first known case no later than 1959. For HIV-1 group M to emerge during this time-period, the event(s) associated with an increased risk of serial passage of human SIV infections in the area of Central Africa must have occurred prior to that date. But not by many years, given HIV-1's clinical latency and time of progression to AIDS, i.e. less than a decade, or else we would have had earlier evidence of it. For western Africa and the emergence of HIV-2 the timing is perhaps a decade later.

We therefore searched for a biologically plausible event that occurred in this region of Central Africa within the decade before 1959 and continued to operate through the 1960s elsewhere in Africa. A massive increase in the number of unsterile injections in sub-Saharan Africa in this period qualifies on all points—it is a specific and parsimonious explanation for significantly increased serial transfer of SIV, in both Central and western Africa during the 1950s and 1960s, respectively. We propose that this event greatly increased the probability of serial transmission of partially adapted SIV during acute, but normally time-limited, SIV infections in humans.

To document this event and address the regional and temporal specificity of HIV's emergence, we assembled and reviewed the available literature on the changes in the supply and demand for injection equipment worldwide during the 20th century. We also examined changes in the uses, costs, demand for, and availability of syringes and their effects on injecting practices in sub-Saharan Africa during this period.

(a) *The global market in injection equipment (1898–1998)*

The history of the hypodermic syringe is punctuated by important manufacturing and drug developments that dramatically affected their availability, price, demand and use worldwide over the past century (table 2). Following their invention in 1848 and until the end of World War I (WWI), sterile syringes were considered precision medical instruments and individually hand-made from glass and metal. The cost was high, about $50 per unit in 1900 (adjusted to current dollars), and even by 1920, after the considerable increases associated with WWI, production was still very limited (*The Echo* 1991*a*)—only about 100 000 syringes per year worldwide.

However, beginning in the period between the World Wars, syringe manufacture was increasingly mechanized, using interchangeable components and mass production methods. Global production reached two million per year (by 1930) and eight million per year by 1952. Throughout this period, the unit price declined steadily, by 80% between 1920 and 1950. At the same time the number and significance of clinical applications grew, e.g. for the increased injection of insulin in the USA and Europe in the 1930s and 1940s (*The Echo* 1991*a*). But it was penicillin that drove the greatest increase in demand for injection equipment worldwide, and it did so in the 1950s.

(b) *The role of penicillin*

While penicillin was first manufactured during WWII, it did not become generally available (especially outside the USA and Europe) until the early 1950s. By that time

Table 2. *History of injectable medications and needle reuse in Africa: 1900–1998*

year	injectable drugs and indications	clinical settings	regions affected	injections yr^{-1}	comments
1900–1920	syphilis	missionary clinics, colonial and military units	all	— [a]	injections[b] of arsenic, methylene blue, mercury, silver, potassium permanganate, methenamine; ante-dates awareness of transmission of infectious diseases by unsterile injections;
1920–1938	VD[c], diabetes	colonial, missionary and military clinics	western and Central Africa	0.5 million yr^{-1}	salvarsan use in the first widespread campaigns to eliminate VD, yaws and kala-azar; half doses for prophylaxis. Injections within clinic settings, sterile practice good
1939–1945 (WWII)	VD eradication programmes in military; penicillin first used by military in 1943	military clinics and limited programmes in colonial Africa	western and Central Africa	reduced availability of injection equipment	USA penicillin production and cost: 1942, total production 1 dose; 1943, 5–25 doses; 1944, 100 000 doses[d] at \$20 per 10^5 units; 1945, \$6 per 10^6 units; 1946, less than \$1 per 10^6 units; 1947, 0.30 per 10^6 units
1946–1951	injections for VD; chloroquine	colonial clinical programme re-establishment for VD and malaria	western and Central Africa	—	penicillin at lower prices; introduction of new antibiotics
1952–1958	polio vaccine used in USA and Europe; penicillin available for UN programmes (yaws); injections for malaria	first UNICEF mass injecting campaigns in Africa, through expanded colonial clinics and missions	western and Central Africa	UNICEF yaws campaign; 2.5×10^6 injections yr^{-1} with reuse of injecting equipment and inadequate sterilization facilities	UNICEF programmes account for over 3×10^7 injections in region (1952–1960) for 5×10^7 population. Yaws campaign focused on British, French and Belgian colonies, other programmes using injections in other areas
1959–1964	increase USA penicillin production and use; vaccination programmes for polio and smallpox	yaws campaign; other injections increased in WHO vaccination programmes	Central Africa	plastic disposable syringes widely reused, with increase in unsterile injections	US penicillin production and prescriptions: 50 prescriptions per 100 persons. 1960–1964, 3×10^6 lb yr^{-1}
1965–1975	production and use of penicillin increase	WHO programmes;	post-colonial decline in medicine, e.g. in Uganda 1970–1991 80–90% less for health	increase use of injection; 78–94% of households in Africa received injections in two-week period	of 10^7 US prescriptions: 1970–1971 (89 prescriptions per 100 persons)
1976–1998	production and use of penicillin increase, plus growth of illicit injecting of heroin and other opiates	WHO programmes; antibiotics; quinine; vitamins	—	10% of healthy children receive injections. $> 10^8$ immunizations (WHO 1997)	WHO extended immunization programme guidelines call for reuse of polypropylene (i.e. single use) syringes up to 200 times without adequate facilities

[a] Not available.
[b] Including injections into the urethra.
[c] Venereal disease.
[d] First documentation of transmission of VD and hepatitis from unsterile injections of penicillin.

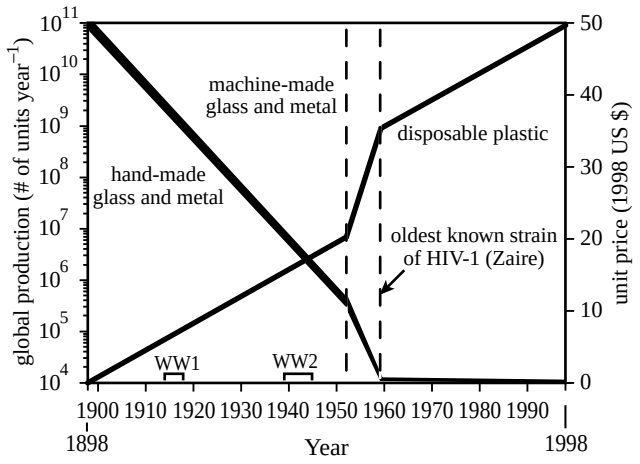

Figure 2. The growth in global production of injecting equipment in relationship to unit price from 1898 to 1998. Cost was adjusted to unit prices of syringes in US dollars for the year 1998. The almost total replacement of reusable glass syringes occurred in the period from 1950 to 1960 and was associated with a 100-fold growth of production from seven million to a thousand million. The decrease in unit price was \$3.75 to \$0.18 in the decade. A comparable increase in production and decline in unit prices for penicillin occurred in the same period, which was the initial decade of mass availability following World War II (WWII). WWI, World War I.

the mass production of antibiotics was substantially lowering prices for these drugs (table 2) (Mahoney *et al.* 1943; Brandt 1987) and increasing their global availability (Parascandola 1980). As the efficacy of these new drugs became apparent, popular demand increased worldwide (Wyatt 1984; Reeler 1990; UNICEF 1987). But, even with declines in the cost of antibiotics, the injection equipment needed to administer them was relatively expensive and the safe reuse of glass syringes was still dependent on a costly infrastructure to ensure sterilization (Van der Geest 1982) (figure 2). And, even at the production rate of 150 000 reusable syringes a week (in 1952), it was impossible to meet the growing demand (*The Echo* 1991*b*,*c*).

By the 1950s manufacturing injection equipment was increasingly a centralized industry. While even at the time prior to WWII there were scores of companies making injecting equipment, by the 1950s Becton-Dickinson were acquiring other smaller manufacturers and (by 1960) were making over 50% of all the injecting equipment manufactured in the world.

This increased demand was anticipated by the industry and led to a series of important changes in the conception of injecting equipment, i.e. the development of inexpensive, disposable 'single-use' syringes. And with it a mass manufacturing technology for plastic injection equipment, dramatically lowering prices, and massively increasing availability worldwide (*The Echo* 1991*a*–*c*) (figure 2). During the decade 1950–1960 sterilizable glass and metal units were largely replaced with single-use plastic syringes. This change represented a 100-fold increase in global production of syringes, up to one billion units per year in 1960 and was coupled with a 56-fold decline in price—down to \$0.18 per unit when adjusted for inflation (figure 2) (WHO 1997).

These 'single-use' syringes were never intended for sterilization or reuse. The material from which they are fabricated, polypropylene plastic, does not maintain integrity of the seals and deforms at autoclave temperatures and they cannot be effectively sterilized unless disassembled, heated at temperatures above 80 °C, and reassembled under sterile conditions (*The Echo* 1991*c*).

Nonetheless, in much of the developing world, especially in sub-Saharan Africa, these new syringes flooded the market in the 1950s adding significantly to the pool of injecting equipment already in circulation. And as more injectable medications became available (especially the antibiotics) multiple reuse quickly became common practice, often without even attempts at sterilization (Wyatt 1984; UNICEF 1987; Van der Geest 1982; Whyte & Van der Geest 1994; CIBA 1977) (table 2).

(c) *Mass injection campaigns*

Although the use of injectable medications in Africa and elsewhere was common by the late 19th century (*The Echo* 1991*a*), and always included some unsterile use (Sheehan 1944; Nickum 1933), the period after WWII saw a dramatic surge in the frequency of medical injections worldwide—and especially of injecting in the developing world—most of which were performed under unsterile conditions (Wyatt 1984; WHO 1997; Mollaret & Reilly 1947) (table 2). The consequences of this huge increase in unsterile injecting (Parascandola 1980; Reeler 1990) were soon evident in the worldwide rise of injection-related transmission of infectious diseases—including hepatitis (Sheehan 1944), malaria (Nickum 1933), syphilis (Mollaret & Reilly 1947) and possibly poliovirus (Wyatt 1984). Sub-Saharan Africa, then entering the last decades of the European colonial period (Wyatt 1984; Reeler 1990; Whyte & Van der Geest 1994), was particularly affected by these developments.

In the 75 years prior to WWII, a network of colonial and missionary clinics was the principal base of Western medical practice in this region of Africa (Wyatt 1984; Reeler 1990; Whyte & Van der Geest 1994). Specific practices varied, depending on the medical traditions of French, British or Belgian colonial powers (UNICEF 1987), but most administered injectable drugs. This was done on site and under medical supervision, while closely controlling access to the relatively costly drugs and injecting equipment (UNICEF 1987), using sterile injecting procedures and with access to sterilization equipment on hand. There is little evidence of injection-related transmission of disease in this pre-WWII colonial period (Alland 1970).

However, in the period following WWII, with independence movements sweeping across Africa, Europe's control of civic affairs in the region began to weaken (Alland 1970), including its controls on medical practice (Whyte 1982). And despite substantial new investments by some colonial powers (Britain and France) in educational and administrative preparation for independence, the shrinking colonial medical care system was not quickly replaced by the newly independent, but impoverished, African states (UNICEF 1987; CIBA 1977; Alland 1970; Whyte 1982).

Soon the old medical care system was supplemented by a growing number of indigenous practitioners with

varying degrees of training and minimal controls, e.g. in 'country clinics' using Western practices, injecting equipment and medicines. Medications and injecting equipment (most previously used) were easily and often diverted or salvaged from the former system. These formed the basis for an 'informal' parallel system of injecting with little or no awareness of the need or the capability for sterilization procedures (UNICEF 1987; Van der Geest 1982; Whyte & Van der Geest 1994; CIBA 1977; Alland 1970; Whyte 1982).

(d) *Reuse of single use syringes in mass public health campaigns*

In the 1950s, the first mass campaigns using injectable antibiotics took place in India and Africa. In Central Africa (table 2) between 1952 and 1959, there were 35 million injections under UNICEF's yaws eradication campaign (UNICEF 1987). In this programme, and in many subsequent antibiotic and anti-malaria treatment campaigns in sub-Saharan Africa, the mass administration of injectable medications by poorly trained local aids using unsterile practices became the norm (Wyatt 1984; Reeler 1990; UNICEF 1987; Van der Geest 1982; Whyte & Van der Geest 1994; CIBA 1977; Alland 1970; Whyte 1982).

Further, throughout this period and in the following decades, there was a sharp growth in the use of injections throughout Africa (Wyatt 1984; Reeler 1990; UNICEF 1987; Birungi *et al*. 1994; Gumodoka *et al*. 1996). They were expected at every medical visit and for the treatment of any condition (Alland 1970). While earlier documentation is sparse, by the mid-1960s, several studies establish that more than 75% of households in sub-Saharan Africa had received an injection within the previous two-week period (Birungi *et al*. 1994). Ethnographic and public health surveys conducted in several parts of Africa (and India) in the 1960s found very high levels of injecting—in one study in Uganda 80% of households owned their own syringe. Under these conditions, new to Africa at the time, the probabilities of serial passage of any infectious agent multiplied rapidly and presented a plausible mechanism in the right place at the right time to facilitate the mutation of SIV to HIV in this region of Africa in the latter half of the 20th century.

7. DISCUSSION

Any theory of the origin of HIV must explain how distinct strains of SIV, which were native to dissimilar Central and West African simian species, and to which humans were routinely exposed for thousands of years (through bites, scratches and butchering of monkeys for food), could evolve into all known variants of epidemic HIV-1 and -2 in such a relatively brief evolutionary timeframe. And, of equal importance, why they did not emerge earlier.

There is a challenge to the timing of HIV-1's emergence only in the 1950s or slightly before. Korber *et al*. (2000) proposes a model using a molecular clock to place the date of the emergence of HIV-1 group M in the 1930s to the early 1940s. The establishment of an epidemic HIV long before the large increase in needle reuse would be evidence against the hypothesis. However, the assumptions used to create and validate the model may have adversely affected its accuracy. For example, the model ignores natural selection, which is certain to play a role. The model may overestimate the age of HIV-1 by missing mutations that were under negative selection. Moreover, the model relies on steady-state infections. Mutations during serial passage to induce epidemic strains would be strongly driven by natural selection. Finally the model is only for HIV-1 group M, and does not deal with the emergence of other HIV-1 and -2 viruses. The theory does not take into account that shorter times to transition could be achieved by serial passage between humans.

The number of mutations needed for an SIV to HIV transition is unknown. However, recent findings in animal models strongly support the serial passage mechanism for HIVs' emergence and give an estimate of the number of mutations for one gene, *env*, to adapt to a new host. Several *in vivo* passages of SHIV clone HxB2 were needed to achieve necessary adaptive mutations. SHIV clone HxB2, although containing non-*env* genes adapted for macaques, replicated to low levels *in vivo* (Cayabyab *et al*. 1999). Replication of the parental virus was low throughout the acute infection and was undetectable at about 15 weeks. Low levels of replication by poorly adapted viruses, and strong suppression after an acute replication phase are key concepts in the serial passage mechanism for HIV emergence from SIV. Pathogenicity and replication increased 1000-fold after serial passage in three macaques and animals developed AIDS. Cayabyab *et al*. (1999) showed that only 12 amino-acid changes in *env* were required for enhanced pathogenicity. Serial passage, therefore, enhanced the *in vivo* replicative capacity and persistence of SHIV clone HxB2 *in vivo*. The relevance of these findings is that three serial passages were required to effect even a small number of adaptive changes in the *env* gene of this poorly adapted SIV–HIV hybrid virus. Poorly adapted SIV infections would face similar limitations.

Alternative events in post-colonial Africa, such as population growth, changing sexual practices, migration, social upheaval, increased hunting and deforestation have also been considered as primary causes for the emergence of epidemic HIV (Pela & Platt 1989). Yet, despite centuries of forced migration and social upheaval among African peoples (Hochschild 1998; Hunt *et al*. 1997), epidemics of HIV did not emerge. The slave trade is ancient in Africa, and even ante-dates the massive European and Arabic slave trade that took hold in the 17th century (Hochschild 1998; Hunt *et al*. 1997). Ultimately, over 30 million rural people were displaced over a period of 400 years. Further, displacement did not stop with the end of the American Civil War in 1865, but continued into the early 20th century in the form of rubber slavery (Hochschild 1998).

This slave trade originated in the same areas of Africa where epidemic HIV-1 and HIV-2 emerged in the 20th century. Had HIV existed, even in small numbers of people, it is likely that HIV would have spread as a sexually transmitted virus. In contrast to HIV, human T-cell leukaemia virus appears to have spread during the period of slave trade (Gallo *et al*. 1983), showing that retroviruses were disseminated during those voyages.

Deforestation and hunting practices may have promoted HIV's emergence by increasing contact between humans and SIV-infected simian species. However, these social factors could have increased opportunities for newly emerged epidemic strains to spread, but were not first causes since no mechanism for SIV adaptation was present.

Traditional practices, such as tattooing and clitoriectomy, existed in Africa long before the advent or rise of unsterile injecting and have been discussed as being related to the emergence of HIV. However, these procedures were limited in number (occurring once a year in many areas), have been declining in post-colonial Africa, and have involved use of fresh razor blades in modern times (Pela & Platt 1989; Hunt *et al.* 1997). The limited and declining prevalence of ritual cutting must be contrasted with our striking finding that 80% of African households had experienced needle use in a two-week period by the 1960s (Birungi *et al.* 1994).

Finally, in recent years, SIV contamination of oral polio vaccine (OPV) and subsequent OPV mucosal exposure has been invoked as a first cause of HIV's emergence (Gallo *et al.* 1983). This mechanism is not plausible because kidneys from Asian macaques and African green monkeys were used, and neither species naturally harbours SIVs closely related to HIV type 1 or 2 (Hirsch *et al.* 1989). Therefore, contamination of OPV with both mangabey and chimpanzee kidneys must be invoked. However, these species were not routinely available for vaccine production. Furthermore, contamination with at least four, and as many as nine SIVs (Chen *et al.* 1996; Simon *et al.* 1998) (consider six HIV-2 subtypes and three HIV-1 groups) from separate chimpanzee and mangabey sources would be required. Lastly, if OPV theories are correct, then it must be explained why HIV did not emerge earlier from mucosal exposures to blood and tissue of hunted chimpanzees and mangabeys.

Unsterile needle reuse was the only mechanism common enough to cause serial passage of SIV. The invasive procedures in needle reuse are primarily percutaneous needle sticks. The risk of percutaneous needle transmission of HIV is 0.25%, or more (Goudsmit & Lukashov 1999; McCray 1986). In Ippolito *et al.* (1993), HIV was transmitted to a student nurse from a seronegative, acutely infected patient. This is the precise mechanism implicated in this study for SIV serial transmission. Therefore, percutaneous needle reuse would be capable of serially passaging SIV, if the injection event occurred during the acute phase of the SIV human infection, when virus load would be relatively high. Thus, serial passage of SIV during acute infections is also rare and plausible only with the advent of large increases in needle reuse.

(a) *Implications*

Beyond proposing an explanation of the modern origins of HIV, this study calls attention to a mechanism for continued acceleration of the adaptation of other animal viruses to a human host. It also establishes a critical need for alternative drug delivery systems and better control of needle use, e.g. through development and use of single-use or auto-destruct injecting equipment. Otherwise, this model suggests, new HIV strains will continue to emerge and may thwart vaccine programmes by adding diversity to an already diverse group of epidemic strains of HIV. Our findings also indicate that future studies should be directed toward tracking SIV-like infections in areas where needle reuse is widespread.

Most significantly, the use of unsterile injection practices is still widespread and is increasing each year. Global production of injection equipment is currently at 40 billion units per year (up from one billion in 1960), more injectable drugs are now available, and the intravenous use of illicit drugs (WHO 1997) is burgeoning worldwide—responsible for igniting explosive AIDS epidemic outbreaks in many regions of the developing world (Birungi *et al.* 1994; Gumodoka *et al.* 1996). And while there are a few needle exchange programmes for heroin injectors in Asia, and there has been some attempt to introduce safer injecting equipment in immunization programmes, WHO immunization guidelines (as recently as 1998) still proposed up to 200 reuses of (resterilized) plastic syringes, despite the common lack of adequate facilities for such sterilization throughout the developing world (WHO 1997). If we are to avoid the emergence of new human pathogens, it is critical that we take measures to curtail unsterile injecting worldwide and limit the serial passage of pathogens that it makes possible.

Evidence for proof or disproof of the model will require tracking the emergence of new HIV strains. This will require studying regions where needle reuse and exposure to SIVsm or SIVcpz are common. Tracking and characterization of SIV infections in these populations could lead to proof of the cut-hunter hypothesis (that direct exposure to SIV through contact with SIV-infected blood is sufficient to launch an epidemic strain) or the needle reuse hypothesis.

The research of P.A.M. was supported by a grant from the US National Institutes of Health, no. AI44596. We thank Phillip Ye for programming assistance, Lisa Chakrabarti for help with the phylogenetic trees, and Dr Sol Levinson for his advice and data on the history of injecting equipment. We thank Theresa Secrist for editorial assistance.

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