MOTHER TO CHILD TRANSMISSION
OF HIV AND ITS PREVENTION
WITH AZT AND NEVIRAPINE

A CRITICAL ANALYSIS OF THE EVIDENCE

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A CRITICAL ANALYSIS OF THE EVIDENCE

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DEDICATION

This work is dedicated *in memoriam* to Huw Christie

Atque in perpetuum, frater, ave atque vale

CONTENTS

PROLOGUE

"We absolutely must leave room for doubt or there is no progress and there is no learning. There is no learning without having to pose a question".

Richard P Feynman, Physicist and Nobel Laureate. Galileo Symposium Address 1964.

The impetus for this review arose as a result of the Presidential AIDS Panel meetings held in South Africa during May and July 2000 under the auspices of the South African Government and President Thabo Mbeki. Our purpose in this publication was not to discuss the HIV theory of AIDS or even the existence of HIV (Those interested in the fundamental question of proving the existence of HIV will find it discussed in Appendix XI). The question this monograph addresses, assuming that HIV does exist, is whether a unique retrovirus is transmitted from pregnant women to their children and whether compounds such as zidovudine (AZT) and nevirapine are able to prevent such transmission.

In Parts I-II we examine the indirect methods said to prove HIV infection and transmission, as well as epidemiological data on mother-to-child transmission. Part III analyses data associated with breastfeeding practices and the possibility of infection. Part IV includes pharmacological data relevant to zidovudine and nevirapine as well as their effects on the several parameters claimed to be indicative of HIV infection and transmission. Included in Part IV is a detailed review of the Pediatric AIDS Clinical Trials Group (ACTG) 076 study which forms the basis of recommending the administration of AZT to all pregnant, HIV positive women and their newborn babies. In Part V we present data on non-retroviral factors which affect the putative mother-to-child transmission of HIV and its prevention, especially the role of nutrition including micronutrients. Part VI consists of a general discussion of the topic.

In reviewing evidence of such a voluminous nature authors face the perennial problem of space and balance. To present too much data is to overwhelm the reader. To present too little is to risk scientific scholarship. Notwithstanding, given the critical nature of this subject to continents of people, and that mother to child transmission is accepted as fact by virtually the whole scientific establishment, we decided to present and discuss at length all the data we could muster. However, with the reader in mind, many of the epidemiological studies are prefaced with a *precis*. We make apology for studies we may have inadvertently omitted.

Scientists who question prevailing theories are under an obligation to present alternatives or, as a minimum, explain particular observations by other means. Consequently, we have included data on the role of cellular oxidation in the genesis of "HIV" phenomenology as well as diseases constituting the clinical syndrome.

It is hoped that this critical analysis of the evidence will prompt a reappraisal of the data interpreted as proof of mother to child transmission of HIV and thereby direct resources towards appropriate efforts to ameliorate factors linked to such biological phenomena.

PART I
TESTS USED TO DETERMINE HIV INFECTION

1.1   Introduction

In 1994 a paper was published by researchers from the UK, Tanzania and the United Nations Children Fund stating "It has been estimated that by the end of 1992, one million HIV-1 infected children had been born in Africa, 600,000 of whom have progressed to AIDS".[1] In 1999 researchers from France and Rwanda wrote that "According to the World Health Organisation (WHO), 1.1 million children were living with human immunodeficiency virus (HIV) infection worldwide at the end of 1997. Of these, the great majority live in sub-Saharan Africa and were infected by their mothers during pregnancy, delivery or breastfeeding".[2] In the same year, two researchers from the University of Zimbabwe wrote: "It is a well established fact that infants born to HIV positive mothers are at risk of acquiring HIV infection from their mothers. HIV infected newborns and infants have a poorer prognosis than those not infected, and therefore, the already existing high infant mortality may significantly rise in sub-Saharan Africa when considering the maternal-foetal transmission rate of 25-35%. With the use of anti-retroviral therapy, HIV transmission to an unborn foetus during pregnancy may be significantly reduced".[3] In 2000 Helene Gayle from the CDC wrote "According to estimates of the global HIV/AIDS situation as of the end of 1999 from the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO), more than 47 million adults and children have been infected with HIV since the beginning of the pandemic, approximately 34.3 million are living with HIV infection or AIDS, and nearly 15,000 people, both adults and children, become infected each day. In 1999, an estimated 5 million people became infected including 620,000 children aged less than 15 years".[4] For such claims to be made by so many leading researchers evidence must exist which proves mother-to-child transmission (MCT) of HIV as well as its inhibition by compounds such as 3'-azido-3'-deoxythymidine (zidovudine, AZT) and nevirapine.

To prove that mother-to-child transmission of HIV takes place first one must have proof that HIV exists. Assuming that HIV does exist then, to prove MCT one must have specific tests to determine the infectious status of both mother and child. At present, infection of the mother is determined by antibody tests and that of the child by antibody tests, "HIV isolation" and measurements of "HIV" RNA or DNA utilising the polymerase chain reaction (PCR). In addition, in many studies children are declared infected with HIV by transmission from their mothers without any laboratory tests but solely if they satisfy clinical criteria encompassed by the Bangui AIDS definition for children in Africa,[5] the Ghent definitions,[6] the CDC 1987[7] or the 1994 CDC revised definition for children aged less than thirteen years[8] (Appendices I-IV).

1.2   Antibody Tests

Antibodies are proteins synthesised in the body as a result of exposure to foreign substances, including infectious agents and proteins, collectively known as antigens (ANTIbody GENerating). Antibodies react with antigens and, assuming the reaction is specific, this property may be used to detect either reactant if the identity of the other is known. Thus, to perform an HIV antibody test to prove a viral infection, two things are required. A sample of blood from the individual thought infected and a test kit containing the virus or its proteins. To date nobody has performed HIV antibody tests using the HIV particles. Rather all the antibody test kits are prepared using approximately ten proteins which the HIV experts claim are those of a unique retrovirus, "HIV". There are two "HIV" antibody tests in common use, the ELISA and Western Blot (WB). The ELISA causes a colour change when a mixture of the "HIV" proteins reacts with antibodies in serum from a patient. In the Western Blot the "HIV" proteins are first separated along the length of a nitrocellulose strip. This enables individual reactions between antibodies and the "HIV" proteins to be visualised as a series of darkened "bands". These bands are referred to with a small 'p' (for protein) followed by a number designating the protein's molecular weight in thousands, for example, p24. In the majority of countries/institutions/laboratories the diagnosis of HIV infection consists of performing an initial ELISA which, if reactive, is repeated. If the ELISA is repeatedly reactive a Western blot is then performed because virtually all experts agree that the ELISA is insufficiently specific. In other words and according to the same experts, the Western blot distinguishes "true" from "false" reactivity in repeatedly reactive ELISAs because, unlike the ELISA, the Western blot assay is highly specific.

It is manifestly obvious that the only way to claim a protein is a viral component is to extract it from a viral particle. However, a single virus particle is a microscope entity of the order of 1/10,000 of millimetre in size and thus it is impossible to obtain proteins in such a manner. The next best thing is to obtain the proteins from material consisting of nothing else but isolated (purified) viral particles. This need is no more than commonsense and was stressed by Luc Montagnier during an interview he gave at the Pasteur Institute in July 1997.[9]

In 1983 Luc Montagnier, and in 1984 Robert Gallo, claimed to have proven the existence of a unique retrovirus, HIV, by purification of particles, which is the standard procedure for proving the existence of viruses belonging to the family of retroviruses. Montagnier and Gallo's claims have been accepted by nearly everybody. However, in his 1997 interview Montagnier admitted he did not purify HIV and, in his view, neither did Gallo. In fact, when asked about the omission of electron micrographic (EM) proof of "purified" virus particles from his 1983 *Science* paper,[10] Montagnier conceded that such EMs were obtained but not published because they did not reveal particles bearing "the morphology typical of retroviruses".[9] The first and only published electron micrographs of "purified" HIV did not appear in the scientific literature until fourteen years later in March 1997. These studies, one from the USA and the other a Franco/German collaboration, clearly show that "purified HIV" is material consisting of abundant cellular fragments and a small number of particles with some, but not all, the morphological characteristics of retroviruses.[11,12] For example, the six "HIV" particles arrowed in Figure 3 of the USA paper have an average diameter of 236 nM, twice that of retrovirus particles including those claimed by many other leading HIV experts to be HIV (see Appendix XI for further discussion on the problematic nature of "HIV" particles and isolation). In other words, the "HIV" proteins have been and still are obtained from particulate material which overwhelmingly consists of cellular fragments in which are interspersed a small number of particles whose morphology more resembles that of retrovirus particles but none of which have all the structural characteristics of retrovirus particles.

Even if there were proof that the proteins in the "HIV" antibody tests are HIV, a reaction with antibodies present in a patient's blood is not proof that the patient is infected. Antibody/antigen reactions can be quite misleading because antibody production may be induced both specifically and non-specifically[13-15] and regardless, antibodies may react, that is, cross-react with non-inducing antigens.[16-23] Cross-reactivity is a property of all antibody molecules including monoclonal antibodies[18,21,24] and there are instances where "cross-reactive antibodies may have higher affinity with antigens other than the inducing antigen".[23] A germane example of cross-reactivity is a study of acute measles infection in non-HIV infected children and young adults reported from the USA and Peru. Here 43/75 (62%) of patients developed antibodies which reacted with one or more of the HIV proteins.[25] In 1995 in Africa there were 551,000 childhood deaths from measles[26] and awareness of this problem may explain why, in one African study of HIV infection, "Children hospitalized with measles were excluded".[27] From the perspective of identifying a designated antibody, for example, an antibody directed against an HIV protein or proteins, cross-reactions are highly undesirable. When they occur in individuals not infected with HIV they are referred to as false-positive reactions. In order to measure the propensity of a test to produce false-positives results, scientists must undertake experiments to determine the test specificity. To diagnose infection with what is almost universally regarded as a lethal human retrovirus HIV, the test should have a false positive rate of zero, that is, the specificity should be 100%. This means the test would never be positive in the absence of HIV.

Before introducing an antibody test into clinical practice laboratory scientists are obliged to determine the test parameters including its specificity by comparing the antibody reactions with the presence or absence of HIV. Since the HIV antibody test cannot act as its own adjudicator scientists must use an independent gold standard. The only scientifically valid procedure for determining the presence or absence of HIV is HIV itself, that is, HIV isolation (purification). However, to date nobody has determined the specificity of the HIV antibody tests in this manner. In fact, as already mentioned, to date nobody has isolated (purified) HIV.[11,12,28-32] Thus it is not possible to prove the specificity of either the ELISA or the Western Blot (WB), the two HIV antibody tests routinely used for diagnosing HIV infection. Manufacturers of HIV antibody tests have long and repeatedly recognised this fact. For example, Abbott Laboratories in their packet inserts state: "At present there is no recognised standard for establishing the presence or absence of HIV-1 antibody in human blood".[33,34] In addition, as can be seen from Table 1.1, the WB is not standardised.

Table 1.1 Criteria used to define a positive HIV Western blot

HIV WESTERN BLOT STRIP*	AFR	AUS	FDA	RCX	CDC 1	CDC 2	CON	GER	UK	FRA	MAC
ENV: p160, p120, p41	ANY 2	ANY 1	ANY 1	ANY 1	p160/p120 AND p41	p160/p120 OR p41	p160/p120 OR p41	ANY 1	ANY 1	ALL 3	3 WEAK BANDS OR ANY STRONG BAND
POL: p68, p53, p32 / **GAG**: p55, p39, p24, p18	ANY 3 GAG OR POL	ANY 3 GAG OR POL	p32 AND p24	ANY 1 AND ANY 1		AND p24	p32 OR p24	ANY 1 GAG OR POL	p32 AND p24	ANY 1 OR ANY 1	3 WEAK BANDS OR ANY STRONG BAND

AFR=AFRICA;[1] AUS=AUSTRALIA;[2] FDA=US FOOD AND DRUG ADMINISTRATION;[3] RCX=US RED CROSS;[3] CDC=US CENTER FOR DISEASE CONTROL;[3] CON=US CONSORTIUM FOR RETROVIRUS SEROLOGY STANDARDIZATION;[3] GER=GERMANY; UK=UNITED KINGDOM; FRA=FRANCE; MACS= US MULTICENTER AIDS COHORT STUDY 1983-1992. * Bands not in electrophoretic order

NOTE:

I. "The Association of Public Health Laboratories now recommends that patients who have minimal positive results on the WB, eg p24 and gp160 only, or gp41 and gp160 only, be told that these patterns have been seen in persons who are not infected with HIV and that follow-up testing is required to determine actual infective status".[4]

II. In February 1993 the US Food and Drug Administration relaxed their criteria in order to "reduce the number of HIV-1 seroindeterminate Western blot interpretations", that is, to increase the number of HIV positive individuals.[5]

1. WHO. (1990). Acquired Immunodeficiency Syndrome (AIDS). Proposed criteria for interpreting results from Western blot assays for HIV-1, HIV-2 and HTLV-I/HTLV-II. *Weekly Epidemiological Record* 65:281-298.
2. Healy DS, Maskill WJ, Howard TS, et al. (1992). HIV-1 Western blot: development and assessment of testing to resolve indeterminate reactivity. *AIDS* 6:629-633.
3. Lundberg GD. (1988). Serological Diagnosis of Human Immunodeficiency Virus Infection by Western Blot Testing. *Journal of the American Medical Association* 260:674-679. (Data presented in this paper reveal that when the FDA criteria are used to interpret the HIV Western blot less than 50% of US AIDS patients are HIV positive whereas 10% of persons not at risk of AIDS are also positive).
4. Mylonakis E, Paliou M, Greenbough TC, Flaninagan TP, Letvin NL, Rich JD. Report of a false-positive HIV test result and the potential use of additional tests in establishing HIV serostatus. *Archives of Internal Medicine* 2000;160:2386-8.
5. Kleinman S, Busch MP, Hall L, et al. (1998). False-positive HIV-1 test results in a low -risk screening setting of voluntary blood donation. *Journal of the American Medical Association* 280:1080-1083.

For example, the criteria that define a positive test in Africa are different from those used in the rest of the world. An African who has antibodies which react with two of three proteins, p41, p120 and p160, is said to be HIV infected. However as far back as 1983,[10] Montagnier accepted that p41 is a normal cellular protein, a view he has not subsequently altered.[35] By 1989 evidence accrued which showed that p120 and p160 in the WB test are composed of subunits of p41.[36,37] In other words, an African is deemed infected with HIV when he or she has antibodies which react with his or her own proteins. In one study "Of 201 laboratories that performed WB and responded, 44 (21.9%) indicated that they used more than one set of WB interpretive criteria; the remaining 157 (78.1%) laboratories indicated that they used only a single set of criteria to interpret WB results. However, discrepancies in WB interpretive practices occurred even among this latter group; when survey analysts compared the interpretive criteria that the laboratory reported using (e.g. ARC [American Red Cross], ASTPHLD/CDC [Association of State and Territorial Public Health Laboratory Directors/Centers for Disease

Control], CRSS [Consortium for Retrovirus Serology Standardization] and Du Pont [FDA licensed]) with the band pattern that same laboratory used to classify a specimen as reactive, only 138 (87.9%) of 157 laboratories indicated a WB pattern that was representative of the interpretive criteria used in that laboratory".[38] In the Genelabs Diagnostic HIV BLOT 2.2 Western Blot Assay Instruction Manual the laboratorian is advised, "Specific guidelines for interpretation may differ depending on the local policies, GENELABS recommends following the accepted policy to be in accordance with local regulations".[37] This is followed by seven different criteria for defining a positive Western blot issued by "different international regulatory bodies". However, GENELABS follow this advice with "We recommend the following guidelines for the interpretation of the Genelabs Diagnostic HIV BLOT 2.2" and list an eighth set of criteria for a positive Western blot. The manufacturer Bio-Rad advises "Each laboratory performing Western Blot testing should develop its own criteria for band interpretation. Alternatively, band interpretation may be left to the clinician" (Bio-Rad Laboratory Manual 1993). The fact that HIV experts do not agree as to which test, the ELISA or WB, should be used to prove HIV infection[39-42] indicates that neither test is conclusive. With two exceptions, nowhere in the world would a positive ELISA be considered proof for infection. The exceptions are Africa and England. According to Philip Mortimer, the "Western blot detection of HIV antibodies" which is used in most countries to prove HIV infection, "began as, and should have remained, a research tool"[39] and should not be used to prove infection with HIV.

1.2.1 Non-specificity

There is ample evidence that the antibody tests are non-specific. Many if not all the studies which claim transmission and inhibition by AZT and nevirapine are reported from Africa or Thailand. Even in studies conducted in the USA and Europe the majority of women originate from Africa or Asia or belong to such communities, that is, geographical regions where the prevalence of mycobacterial diseases (including leprosy and tuberculosis (TB)) is quite high. For example, in the Women and Infants Transmission Study (WITS) from the USA published in 1996, 56% of women were either Black or Hispanic.[43] In another study from the USA published in 1997, 41% of the women were Black and 42% Hispanic.[44] In the Pediatric AIDS Clinical Trials Group Protocol 185, USA, 51% of the women were Black and 35% Hispanic.[45] In the French Perinatal Cohort "40% of the women were born in sub-Saharan Africa or the Carribean".[46] It is accepted that the prevalence of HIV infection in the UK is highest in inner London. By April 1996, 276 of the 389 (71%) children with HIV infection or AIDS reported to the combined obstetric and paediatric national confidential registers were from London, 80% of whom were born to women who had acquired their infection in sub-Saharan Africa.[47]

In 1994 Myron Essex and his colleagues reported that leprosy patients and their contacts "had a very high rate of HIV-1 false-positive ELISA and WB results. Sera from 63.6% of leprosy patients and 23% of their contacts were repeatedly positive for HIV-1 by ELISA…As with ELISA, sera from leprosy patients (83.6%) and their contacts (64.1%) gave extensive cross-reactivities on HIV-1 WB". In fact most of their Western blot patterns satisfy the Australian criteria for a positive WB and these are the most stringent criteria in the world.[48] Essex and his colleagues recognise that *M. leprae* "shares several antigenic determinants with other mycobacterial species, including *M. tuberculosis*". These antigens include the carbohydrate-containing antigens lipoarabinomannan and phenolic glycolipid which are constituents of the cell wall of *M. leprae.* Furthermore, antibodies directed against such antigens cause "significant cross-reactivities with HIV-1 *pol* and *gag* [p32, p55, p68, p24, p18] proteins" used in the HIV antibody tests. This led the authors to warn that amongst leprosy patients and their contacts there is a "very high rate of HIV-1 false-positive ELISA and WB results", that "ELISA and WB results should be interpreted with caution when screening individuals infected with *M. tuberculosis* or other mycobacterial species", and that "ELISA and WB may not be sufficient for HIV diagnosis in AIDS-endemic areas of Central Africa where the prevalence of mycobacterial diseases is quite high".[49] In 2000 the WHO reported that "Someone in the world is newly infected with TB every second…TB kills about 2 million people each year…Over 1.5 million TB cases per year occur in sub-Saharan Africa".[50] In South Africa the TB rate is up to 60 times the rate in the United States or Western Europe with approximately 160,000 new cases reported in 1997. "South Africa's Medical Research Council reports there are about 16,000 new tuberculosis cases among children annually in the country"[51] and "At least one-half of South Africa's 42 million people are believed to be infected with TB".[52]

As far back as 1985 there was evidence that the "reactivity in both ELISA and Western blot analysis may be non-specific in Africa".[53] Increases in antibody levels follow immunisation and immunoglobulin infusions, both of which are recognised to lead to a false-positive antibody test.[54] As all antibodies are potentially cross-reacting the probability of such reactions rises with increasing levels of antibodies. A recent publication reported that 2/33 (6%) of patients with severe *P. falciparum* malaria had a false positive HIV ELISA and warned that "In

tropical countries…where malaria is endemic, results of serological tests should be interpreted with caution".[55] Africans, even healthy African-American blood donors, have higher IgG levels than Caucasians.[56]

Writing in November 1985 on "Serological Studies of HTLV-III [HIV] Antibody Prevalence Among Selected Groups of Heterosexual Africans", Gallo and his colleagues stated, "In other studies, 0% to 20% seropositivity rates among adults from Southern Africa, Zambia, Eastern Zaire and Uganda have been reported, suggesting that the virus is not endemic in South Africa, is new to Zambia, and may have been present in Uganda for a longer time. Surprisingly, a much higher seroprevalence of 35% to 67% has been described among children. Whether this positivity is due to cross-reactivity of antibodies against yet unrecognized antigens, or to the fact that the virus detected may be a predecessor of HTLV-III, clearly requires confirmation".[57]

That cross-reactivity may underlie much if not all "HIV seropositivity" in Africans is strongly supported by Gallo's own data in a study entitled "Evidence for exposure to HTLV-III in Uganda before 1973". Blood samples were collected from "a subsistence-farming environment" in the West Nile district of Uganda. The samples were obtained from healthy children selected as controls for a study of Burkitt's lymphoma, predominantly a disease of young children. In 1985 Gallo tested these samples with the HIV ELISA and Western blot and found that from 75 children of average age 6.4 years, 50 (67%) were positive on both tests. Since the HIV theory of AIDS requires mother-to-child transmission as the cause of HIV antibodies in young children, and since African AIDS is spread by heterosexual intercourse, Gallo expected seropositivity in children to be mirrored by their parents. Gallo was "surprised" by the high level of infectivity and the apparent lack of AIDS in Africa: "If, as we suspect, the antibody reactivities found represent widespread exposure or infection by HTLV-III [HIV], then it must be asked why the incidence of AIDS in the Ugandan population (and neighbouring Zaire) has gone unnoticed for so long…It is possible that AIDS existed in African populations without being recognized as a separate disease entity" or "the virus detected may have been a predecessor of HTLV-III or is HTLV-III itself but existing in a population acclimated to its presence. It further suggests an African origin of HTLV-III".[58] If the antibody tests are proof of HIV infection and HIV causes AIDS then since:

(a) the development of AIDS following infection "may be about 1-2 years shorter than in the developed world";[59]

(b) barrier contraception is problematic even today in Africa;

(c) there is no cure for AIDS;

(d) untreated AIDS is fatal within one to two years even with the best medical intervention;

why are there any Africans alive in Uganda today? Is it possible that AIDS was not "recognized as a separate disease entity" in Africa because AIDS in Africa is nothing else but diseases which have existed there for centuries and which became AIDS as a consequence of the spread of "HIV" testing? In the opinion of researchers from several institutions in the USA, "Considering the lifelong implications of a positive HIV test result, physicians should be aware of the limitations of tests for HIV". In particular, they draw attention to false-positive HIV results which are associated with "autoimmune diseases, renal failure, cystic fibrosis, multiple pregnancies, blood transfusions, liver diseases, parenteral substance abuse, haemodialysis, or vaccinations for hepatitis B, rabies or influenza".[60] Given that a p120 and p160 band is proof of HIV infection in Africa it is particularly significant that "The Association of Public Health Laboratories now recommends that patients who have minimal positive results on the WB, eg p24 and gp160 only, or gp41 and gp160 only, be told that these patterns have been seen in persons who are not infected with HIV and that follow-up testing is required to determine actual infective status. The clinician must judge the test results within the context of other epidemiological and clinical information". However, this reasoning is problematic. On the one hand one cannot use "epidemiological and clinical information" to prove that patients are infected with HIV while on the other, claim that a positive HIV antibody test in AIDS patients proves that HIV is the cause of AIDS, which is the foundation of the HIV theory of AIDS.[60]

It is accepted that parity is associated with repeated false-positive ELISAs and indeterminate WB (IWB).[61] According to the researchers from the University of Washington, "since HIV testing of pregnant women is increasing, and if pregnancy is a risk factor for IWB, rapid evaluation protocols and counselling will be necessary to avoid unnecessary anxiety related to an indeterminate test result and to determine the HIV status of the mother and fetus".[62] However, it is impossible for any scientist to resolve this problem by any amount of "rapid evaluation protocols and counselling" when no gold standard exists for determining the specificity of the

antibody tests in the first place and when one and the same test result may be considered positive in one country or laboratory but indeterminate in another country or laboratory, or even in the same laboratory, and at different times.

1.3 "HIV Isolation"

By "HIV isolation" HIV experts mean detection of the enzyme reverse transcriptase or a reaction between proteins present in cell cultures and antibodies to one such protein, p24, which experts claim is unique to HIV. However,

(a) the reaction between an antibody and an antigen, even given proof that the antigen is an HIV protein and the antibody is directed against it and reacts only with p24, is not isolation of anything, much less a virus or a unique retrovirus;

(b) to date nobody has proven that p24 is an "HIV" protein. To the contrary, there is ample data that p24 is a cellular protein.[11,63-66] Two of the best known experts of HIV and HIV testing, Jörg Schupbach, the principle author of one of Gallo's 1984 *Science* papers where isolation of HIV was claimed, and Philip Mortimer, the Director of the UK Public Health Laboratory Service, do not consider the reaction between the p24 antibody and antigens in cell cultures as HIV specific. Using cultures of unfractionated blood and defining detection of p24 as virus isolation, HIV has been "isolated" from 49/60 (82%) of "presumably uninfected, but serologically indeterminate" individuals and in 5/5 "seronegative blood donors".[67] According to Mortimer, "Experience has shown that neither HIV culture nor tests for p24 antigen are of much value in diagnostic testing. They may be insensitive and/or non-specific".[40] Significantly, using monoclonal antibody to p24, the p24 protein (as well as the p120 and p18 HIV proteins), has been identified in normal, non-HIV-infected placentas, especially "in [chorionic] villi with immunopathological evidence of villitis".[66] ("Several studies conducted among pregnant women who did not receive zidovudine, primarily in Africa, and a recent analysis by the Ariel project…have described an association between clinical or histologically confirmed chorioamnionitis and the risk of transmission of HIV-1 infection".[68,69] In a recent study the presence of chorioamnionitis was associated with an odds ratio of 4.4 for the risk of perinatal transmission of HIV.[68])

1.4 Polymerase Chain Reaction (PCR) and "Viral Load" Tests

With this laboratory technique, fragments of RNA or its complementary DNA (cDNA), primers which are claimed to represent segments of HIV RNA, are used to amplify similar sequences present in tissues. The PCR test is substantially similar to that employed in paternity cases where segments of the DNA in a man are compared with the DNA in a child he may have fathered. HIV experts claim that the PCR proves and quantitates HIV infection by detecting and counting molecules of HIV RNA, or cDNA, in cultures containing tissues from AIDS patients, or in plasma or other tissues from patients. However, before one can claim that a length of RNA is the genome of a unique retrovirus, HIV, one first must have proof such RNA is a constituent of a virus particle. In this regard proof of origin is no different from that which courts of law impose in the resolution of disputed paternity. The child's and the alleged father's DNA are defined solely on the basis that specimens are taken from their respective bodies. Proof of retroviral RNA (its genome) is no different. However, in the case of HIV, the HIV RNA and its cDNA are obtained from material dominated by cellular RNAs and DNAs and scant particles sharing at best only superficial resemblance to retroviral particles.[9,11,12,28,29,31,70] In the case of Montagnier's 1983 "discovery" of HIV, the "purified" virus did not contain any particles "with the morphology typical of retroviruses".[9]

Furthermore, HIV experts, all of whom ignore this most basic requirement of defining the HIV genome on the basis of extracting RNA from purified, infectious retroviral particles, themselves present cogent reasons why the PCR cannot be used to prove HIV infection. In 1989, discussing their studies on human retroviruses, researchers from the University of New York wrote, "Irrespective of the origin of human retroviruses, their presence leads to both practical and theoretical concerns. Presently, the major practical concern is that effective use of PCR as a screening procedure for HTLV-I, HTLV-II and HIV infections must always include appropriate controls to ensure that no endogenous sequences contribute to positive signals. As previously noted, HIV unique primers corresponding to the highly conserved reverse transcriptase region…function well in the PCR amplification of HeLa DNA [a non-HIV-infected neoplastic laboratory cell line] even at annealing temperatures around 60ºC. Another practical concern is that the use of PCR for determining the possible retroviral etiology of a variety of human diseases may be complicated by endogenous retroviruses. Even if cDNAs are used for PCR templates, the transcriptional activities of endogenous sequences must be considered".[71] (Humans are born with DNA nucleotide sequences known as endogenous retroviral sequences. Unlike the genomes of bacteria, viruses other than retroviruses and other infectious agents which, if present in humans are clearly exogenously acquired,

retroviral RNAs, (proviral DNAs) are present in "all of us" and are known to constitute at least 1% of the human genome.[72] When expressed these DNAs give rise to retroviruses known as endogenous retroviruses).

In an article where he discusses the laboratory diagnosis of "HIV infection", Philip Mortimer wrote: "Other diagnostic methods, e.g. p24 antigen testing, and proviral DNA and RNA amplification exist, but these innovations in HIV diagnosis need to be matched against the anti-HIV [ANTIBODY] test and should be rejected unless they fulfil a need that antibody testing fails to meet".[73] According to researchers from the University of London, "The use of polymerase chain reaction (PCR) for the diagnosis of HIV infection is becoming more widespread and although not yet entirely reliable compared with serology, has been of special value in HIV-seronegative intravenous drug users".[74] If PCR needs to be matched against the "HIV" antibody test because it is less reliable than serology then, given the fact that at present there is no evidence which shows that a positive "HIV" antibody test is proof of HIV infection,[29,75,76] one inevitably, although from a different perspective, must arrive at the same conclusion as Shoebridge *et al*. "…until further molecular and biological studies are carried out, it will be unsure as to what detection of HIV-1 DNA, even when shown to be HIV-1 really means".[77]

According to one British researcher, "Those laboratories which undertake HIV screening and confirmation assays understand fully the technical problems associated with PCR and other amplification assays and it is precisely for those reasons that PCR is NOT used as a confirmatory assay (as discussions with any competent virologist would have informed them)".[78] Others agree but for more reasons than mere "technical problems" related to the performance of laboratory procedures. As researchers from several institutions from the USA pointed out in 1996: "To evaluate the sensitivity and specificity of PCR, investigators must ascertain whether study participants are infected with HIV. Typically, a new test is compared with a superior reference (or gold standard) test…The lack of an appropriate reference test substantially complicates evaluation". Even if a gold standard did exist, the specificity of the PCR still could not be determined for the simple reason, that this test, as the same researchers point out, is not standardised: "The criteria for determining when PCR gave positive results varied among the studies".[79] Even when the lack of standardisation is ignored and totally unsuitable gold standards are used, such as the WB, "specificity" of the PCR is still extremely low. In one study, the concordance between HIV serology and "HIV DNA" varied between 40-100%. In "Seven French laboratories with extensive experience in PCR detection of HIV DNA", of 138 samples shown to contain "HIV DNA", 34 (25%) patients had no "HIV" antibodies while of 262 specimens that did not contain "HIV" DNA, 17 (6%) patients had "HIV" antibodies.[80]

Researchers from several institutions in the USA performed a meta-analysis of studies published between 1988 and 1994 that evaluated the sensitivity and specificity of PCR. They "accepted positive results on conventional antibody tests (if they included a confirmatory Western Blot analysis or similar test) or viral cultures as high-quality evidence of infection", that is, as a gold standard. In a search of 17 computer databases, they "identified 5698 titles of potentially relevant articles. After independent review by two readers, 1735 titles were judged to be potentially relevant". Then they "reviewed the associated abstracts and then selected 379 studies published as full articles for further review. Of these 379 articles, 96 met the inclusion criteria and were analyzed". They found that: "Measured performance was extremely variable. When indeterminate PCR results were excluded, sensitivity ranged from 10% to 100% and specificity from 40% to 100%". They concluded "Our investigation produced two main findings. First, the false-positive and false-negative rates of PCR that we determined are too high to warrant a broader role for PCR in either routine screening or in the confirmation of diagnosis of HIV infection. This conclusion is true even for the results reported from more recent, high-quality studies that used commercially available, standardized PCR assays...We did not find evidence that the performance of PCR improved over time".[79]

In regard to the HIV viral load tests, which are used to quantitate HIV in plasma, researchers from the Massachusetts School of Medicine express the problem concisely: "Plasma viral [RNA] load tests were neither developed nor evaluated for the diagnosis of HIV infection…Their performance in patients who are not infected with HIV is unknown" and their use leads to "Misdiagnosis of HIV infection".[81] Researchers from the Service of Infectious Diseases, Institute de Salud Carlos III, are even more decisive: "since their specificity is not well known, these tests [the viral load tests] must not be used for diagnostic purposes".[82] According to manufacturer Roche, "The Amplicor HIV-1 [RNA] Monitor test is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection" (Roche Diagnostic Systems, 06/96, 13-08088-001. Packet Insert).

The inescapable questions then are:

(a) Why do the HIV experts use these tests to prove HIV infection, and in fact to confirm the results of other HIV tests?[60]

(b) Since these tests cannot be used for the diagnosis of HIV how is it possible to use them to quantify HIV as all HIV/AIDS experts advise and currently practise?

How can one even consider using such a test to monitor or diagnose HIV infection when the result varies between zero and one million copies depending which PCR technique or strain of HIV is used but where samples contain the same amount of HIV?

The three assays frequently used to quantify the "viral load" are reverse transcription-polymerase chain reaction (RT-PCR), nucleic acid sequence-based amplification (NASBA) and branched chain DNA (bDNA). To assess the impact of the assays used and of "genetic variability in HIV-1 RNA quantification", researchers from France "evaluated three commercial kits by using a panel of HIV-1 isolates representing strains A to H…These isolates were expanded in culture. Virus was collected by ultracentrifugation and re-suspended in HIV-seronegative plasma. To standardise the quantities of virus to similar levels in each preparation, the p24 antigen was determined and the volume adjusted so that each specimen contained approximately 10pg of p24 antigen per ml". The "HIV-1 RNA copies" per ml of plasma obtained are documented in Table 1.2. These results prove that "quantification of HIV-1 RNA is highly influenced" by the "HIV 1 clade" and the test kit used. Indeed, given their data it is virtually impossible to make any sense at all of "viral load" findings.[83]

Table 1.2   Viral load vs PCR Technique

HIV-1 STRAIN	RT-PCR	bDNA	NASBA
DJ258	<400	111,500	100,000
DJ263	<400	79,800	60,000
SF2	225,500	38,000	240,000
III-B	54,000	17,000	360,000
ZAM18	78,300	70,000	66,000
ZAM20	178,800	125,800	420,000
UG270	179,800	29,200	170,000
UG274	320,000	41,400	32,300
CM241	18,800	72,800	35,000
CM235	4,700	52,000	15,000
163.3069	36,200	94,000	57,000
162.307	2,800	78,100	26,000
G98	254,700	269,000	<400
LBV21	184,500	295,000	<400
VI557	950,000	587,000	125,000

RT-PCR=reverse transcription-polymerase chain reaction
NASBA=nucleic acid sequence-based amplification
bDNA=branched chain DNA
<400 is considered a viral load of zero.

In analysing HIV molecular biology one cannot help reflecting on the words of John Maddox, "Is there a danger, in molecular biology, that the accumulation of data will get so far ahead of its assimilation into a conceptual framework that the data will eventually prove an encumbrance? Part of the trouble is that excitement of the chase leaves little time for reflection. And there are grants for producing data, but hardly any for standing back in contemplation".[84]

1.5   Testing and the AIDS risk groups

The HIV/AIDS risk groups are gay men, IV users, haemophiliacs and individuals born in "Pattern II" countries (the latter include sub-Saharan Africa). In a study entitled "False-Positive and Indeterminate Human Immunodeficiency Virus Test Results in Pregnant Women" published in 2000, the authors from the Departments of Paediatrics and Family Practice, University of Texas wrote: "Under no circumstances should a patient be informed that she is infected unless both the ELISA and WB test results are positive. When the WB is indeterminate, the ambiguity of the test result should be discussed along with methods to clarify the result...Testing for HIV is an emotional experience. An HIV diagnosis may lead to depression, fear, anger and suicidal ideation. Family, friends and community may ostracise infected people, and relationships with spouses or partners may be jeopardised. An indeterminate result can cause the same problems if the physician misinterprets the result as being indicative of infection". Yet in African pregnant women infection is determined by using only one ELISA test.[85]   The researchers from Texas acknowledged that there are several "sets of

published guidelines" for interpreting the WB results. "It is possible for serum to be positive by one set of criteria and indeterminate by another". They also accepted that:

(a) The WB may be positive even if the person is not infected, especially when only two bands are present and, "In rare cases, an uninfected person may even have more than 2 bands present";

(b) "The WB criteria used to define a positive test result were originally designed for use in high-risk individuals…For lower-risk populations, those tests can be problematic".

However,

(i) the only reason gay men, IV users, haemophiliacs and Africans are said to have a high risk of HIV infection is because, when the antibody tests were first introduced, individuals belonging to these groups were found to have a higher probability of testing positive than healthy blood donors;

(ii) the only way to prove these groups are truly at high risk of infection, rather than at high risk of false-positive antibody tests, is to use a gold standard to validate the test. This gold standard can be none other than HIV, that is, HIV isolation. Since no gold standard exists,[29,30] a fact accepted by one of the foremost HIV/AIDS experts, William Blattner,[86] at present there are no data to distinguish between groups of individuals at high risk of infection or at high risk of having a positive test but not infected.

If, as these researchers state, false-positive tests "can be caused by alloantibodies resulting from transfusion, transplantation or pregnancy, autoimmune disorders, malignancies, alcoholic liver disease, or for reasons that are unclear", then it is not possible to say which, if any, pregnant women who test positive are infected.

In situations where the antibody test results are not clear, that is, the WB is indeterminate, the Texan researchers advise that "a polymerase chain reaction (PCR) test for viral nucleic acid sequences should be performed using the PCR-DNA method. Some laboratories may offer only viral load tests such as the PCR-RNA method, which is typically used to follow the course of HIV disease by measuring the amount of virus produced in an infected person. It is reasonable to use this method as a diagnostic tool as well but at present the PCR-DNA is considered the method of choice".[87]

1.6 Discussion

There are three tests which are used to prove HIV infection: the antibody tests, culture (HIV isolation) and PCR. By culture or isolation is meant the detection in cultures of antigens which react with antibodies to a protein, p24, which is claimed to be an HIV protein. Since "isolation", like the antibody tests, is an antigen-antibody reaction, comments on the antibody tests apply equally to "isolation". For this reason we will discuss only the PCR and the antibody test which, at present, is the only test routinely used to prove HIV infection in adults and adolescents.

1.6.1 PCR

(a) At present there is no proof that the primers and probes used in the PCR have been developed from a retroviral particle;

(b) No proof exists that the RNA/DNA amplified in the PCR test is either the genome or part of the genome of a unique retrovirus, HIV;

(c) No direct correlation exists between the PCR and the antibody test. Using the antibody test as a "gold" standard the PCR specificity varies between zero and 100%.

Most importantly, to date nobody has proven the existence of the whole HIV genome in fresh, uncultured lymphocytes from AIDS patients. Furthermore and as we have argued elsewhere, the finding of novel RNAs in human cells, especially those of AIDS patients and those at risk, is not proof that the RNA has been exogenously introduced by HIV or any other infectious agents.[29,31] That this may be the case has of late been accepted even by Luc Montagnier. In a written testimony dated February 2nd 2000 to the US House of Representatives Committee on Government Reform, Subcommittee on National Security, Veterans Affairs and International Relations, in support of the work of his colleague, Howard B Urnovitz, (Montagnier is on the scientific advisory board of a publicly traded biomedical company whose director is Urnovitz), Montagnier wrote: "I have reviewed Dr Urnovitz's published research and the testimony prepared for presentation to this Committee and strongly advise that future research on Gulf War Syndrome should include the study of the detected genetic material".[88]

Urnovitz and his colleagues presented evidence of the existence, in Persian Gulf War veterans, of "novel", "nonviral" RNAs, "possibly induced by exposure to environmental genotoxins". They concluded: "The patterns of the occurrence of RPAs [polyribonucleotides] in the sera of GWVs [Gulf War Veterans] and healthy controls are sufficiently distinct to suggest possible future diagnostic applications…Our studies of patients with active multiple myeloma suggest that patients with individual chronic multifactorial diseases may have unique RPAs in their sera. Validated tests for such putative surrogate markers may aid in the diagnosis of such diseases or in the evaluation of responses to therapeutic modalities".[89]

It is also highly significant that in his "STATEMENT FOR THE DURBAN AIDS CONFERENCE,[90] which begins "What is 'HIV'?", Urnovitz offers no explanation for his parenthetic use of the terms 'HIV', 'HIV genome' and 'HIV biomarker'. In the same document it is also implied that the HIV genome may result from the "reshuffle" of cellular retroelements. That is, Urnovitz agrees with one of several possible explanations put forward by our group to account for the presence of novel RNAs in the cells of AIDS patients but which may not be present in the cells of healthy individuals.[29,31] Urnovitz also agrees with us that "Missing from the landmark 1983 analysis of "HIV" was an understanding of the role "poikilogenic" agents play in the laboratory protocol that is used to study human retroviruses. The term "poikilogenic" is derived from the Greek "poikilo" which means *diversity* and "gen" which stands for *generate*. Poikilogenic agents are those entities–chemical, physical, or biological–that create genetic diversity via genetic recombinatorial events. These events may include the inductive expression of retroelements and the resulting byproducts of newly reshuffled genetic material. One such poikilogenic agent was reported in the 1983 discovery of "HIV". The agent is phytohemagglutinin (PHA). "Using an HERV-H LTR probe, 6 and 4.5 kb transcripts were detected by Northern blot analysis which were induced in normal peripheral T cells after treatment with phytohaemagglutin"[91] (HERV≡ human endogenous retrovirus. PHA has been used as a laboratory reagent not only by Montagnier but by nearly every retrovirologist who claims proof of HIV isolation).

Since Montagnier agrees with Urnovitz that novel, nonviral RNAs appear in the Gulf War Veterans, then why should the existence of novel RNAs:

(a) in AIDS patients and those at risk be the genome of a retrovirus HIV and not the result of the many toxins including genotoxins to which they are also exposed?[28,31,92-94]

(b) in cultures containing tissues from AIDS patients be interpreted as HIV RNAs rather than the result of the many toxins including genotoxins[28,94,95] to which both the patients and the cultures are exposed? Especially when both Montagnier and Gallo accept that HIV cannot be detected in cultures which are not treated with such toxins (oxidant agents) including PHA?[89,90,96-98] When hard pressed all the HIV experts will ultimately accept the non-specificity of retroviral-like particles, reverse transcription and antibody/antigen reactions. However, to date no retrovirologists, not even Peter Duesberg, will agree with us that novel RNA may appear in cells without prior exposure to infectious agents. For all such scientists, the presence of novel RNAs in AIDS patients is unambiguous proof for HIV infection although no two identical HIV RNAs have been reported so far. In fact, the difference may be up to 40%. Since now even the discoverer of HIV does not exclude the former possibility, is not the scientific community now under a compelling obligation to recast the "HIV" theory of AIDS in terms of non-infectious factors?[28]

1.6.2   Antibody tests
The HIV antibody tests, the only tests which are routinely used to prove HIV infection of pregnant women, raise several questions. If the tests are non-specific then:

1.6.2.1   If not HIV what leads to a positive test?
As mentioned, at present there is no proof that the proteins employed in the HIV antibody test kit are HIV proteins. However, there is ample evidence that the proteins are not retroviral but cellular in origin.[11,12,29-31,99-102]
This means that anybody who has high levels of autoantibodies, that is, antibodies to his or her own constituents including proteins, would have a high probability of testing positive even if not infected. Also, individuals with high levels of antibodies directed against other antigens may cross-react with the proteins in the "HIV" test kits, again resulting in a positive test, without the patient being infected. Evidence exists which shows that individuals with AIDS and those at risk have circulating immune complexes, rheumatoid factor, anti-cardiolipin, anti-nuclear factor, anti-cellular, anti-platelet, anti-red cell, anti-actin, anti-DNA, anti-tubulin, anti-thyroglobulin, anti-albumin, anti-myosin, anti-trinitrophenyl and anti-thymosin antibodies.[103,104] In a 2001 study emanating from several institutions in the USA, researchers "examined alloreactivity to test the hypothesis that HLA-reactive antibodies might confer some degree of protection from HIV-1 infection…To our surprise…our

12

findings suggest that HLA-reactive antibodies generated in persons with hemophilia–likely as a result of infusion of plasma products–are not protective against acquisition of HIV-1 infection and, in fact, are associated with a heightened risk of subsequent seroconversion". In other words, in haemophiliacs a positive HIV antibody test is directly related to the presence of auto-antibodies induced by infusion of "plasma products" (Factor VIII).[105] Anti-lymphocyte auto-antibodies have been found in 87% of HIV positive patients and their levels correlate with clinical status.[106,107] Pregnant women are typically healthy as are blood donors yet a report of "blood donors, in whom no risk of HIV infection could be ascertained, who were non-reactive for HIV antibodies by ELISA, and for whom all other tests for HIV were negative, revealed that 20% to 40% might have an indeterminate [that is, one or more bands] Western blot".[61] In the scientific literature there are numerous examples where non-HIV-infected humans as well as animals possess or develop antibodies which react with one or several of the "HIV" proteins.[29,75,76] These include individuals at "low risk" for AIDS including "specimens from blood donor centers",[67,108,109] individuals transfused with HIV negative blood or serum,[110,111] patients with measles, mumps, herpes simplex, dengue and other viral illnesses,[112] drug addicts who later reform,[113] patients with primary biliary cirrhosis, systemic lupus erythematosus, chronic viral hepatitis, primary sclerosing cholangitis, biliary atresia and alcoholic liver disease,[114] patients transfused with their own irradiated and thus altered blood,[115] mice injected with allogenic lymphocytes[116] or bacterial extract (see below) and 72/144 dogs tested in the USA.[117] Given that healthy individuals at no risk of AIDS may have one or several or even "diagnostic"[108] bands on the Western blot, and that individuals developing or presenting with illness may develop a range of antibodies thereby increasing the likelihood of additional, non-specific antibody reactivities, it would not be surprising that many patients test "HIV" antibody positive while not being infected with a particular retrovirus. In Africa this is rendered more probable given that the criteria for a positive WB requires only two bands. (These proteins are accepted even by Montagnier to be cellular[10,35-37]). The probability of this outcome multiplies again in pregnant African women who are at risk of mycobacterial and fungal diseases. These diseases lead to cellular activation and antibodies directed against these agents are reactive in the "HIV" antibody tests.[49,75,76,118] Pregnant women also are at risk of developing anti HLA-DR auto-antibodies, long recognised as causes of anti-"HIV" cross-reactivity.[61] They are also exposed to foetal, placental, altered self as well as foreign antigens by virtue of current and prior pregnancies, vaginal and endocervical bacteria,[119,120] faecal soiling, abortions, bacteraemia[121] sexual contact including anal intercourse embracing "Repeated antigenic stimulation by semen or spermatozoa from the same or different sex partners may produce an increase in cell-mediated immune activation in women";[122] and urinary tract infections[123] and chorioamnionitis[124-126] associated with bacterial sepsis including that associated with coitus during pregnancy and lower socioeconomic status.[127,128] Anal intercourse and sepsis are particularly germane given that mice administered extracts of *E.coli*, the predominant aerobic bowel commensal colonic bacterium and foetal membrane and urinary pathogen, develop antibodies reactive with the "HIV" p41 and p120 proteins (V. Colizzi *et al*, personal communication). In Africa such reactivity alone is defined as proof of HIV infection.

1.6.2.2 Why are these tests not more often reported positive in individuals who do not belong to the AIDS risk groups?

Prior to 1987 one "HIV specific" WB band was considered proof of HIV infection. However, since 15%-40% of healthy, at no risk individuals have "HIV specific" WB bands[61,108,129] it became necessary to redefine a positive WB by adding extra and selecting particular bands otherwise at least one in every seven people would be diagnosed infected with HIV. (Notwithstanding, in the MACS one strong band remained proof of HIV infection in gay men until 1990[130]). On the other hand, although AIDS began to decline in 1987,[131-133] this trend was countered by the addition of more and more diseases to each revision (1985, 1987 and 1993) of the first, 1982 CDC definition and, most recently, mere laboratory abnormalities.[134] The net effect of these changes was to maintain a correlation between "HIV" antibodies and "AIDS" amongst the "risk" groups while the risk of an HIV/AIDS diagnosis outside these groups remained slight. This was further accentuated by avoiding testing sick individuals outside the risk groups. However, when such studies are performed a significant number of individuals who are not expected to be infected test positive. For example: (a) amongst 89,547 anonymously tested blood specimens from 26 US hospital patients meticulously chosen at no risk of AIDS, between 0.7% to 21.7% of men and 0-7.8% of women aged 25-44 years were found to be HIV WB positive.[135] At the five hospitals with the highest rates of HIV antibodies, one third of positive tests were in women. Yet, in the USA, men vastly outnumber women as AIDS patients; (b) the US Consortium for Retrovirus Serology Standardization reported that 127/1306 (10%) of individuals at "low risk" for AIDS including "specimens from blood donor centers" had a positive HIV antibody test by the "most stringent" US WB criteria.[108] Thus, the correlation between "HIV" antibodies and AIDS has, over the period of the AIDS era, been selectively adjusted by discriminatory testing and changes in both the definition of a positive test as well as the clinical AID syndrome.

1.6.2.3   Why is there a relationship between a positive test and the appearance of AIDS?

Among the risk groups in North America, Europe and Australia, a positive test confers upon an individual a propensity to develop and die of diseases defined as AIDS. (This relationship is critical because it is the only evidence which HIV experts give as proof that HIV causes AIDS). Furthermore, some including the best known HIV/AIDS researchers, use AIDS as a gold standard for the antibody test.[136,137]   No such relationship exists between a positive antibody test and African AIDS. The HIV/AIDS experts themselves "questioned the utility of WHO clinical definition and showed that it lacked prognostic value".[138]   In one study, 83% of patients with suspected AIDS were HIV positive but so were 44% with malaria, 97% with herpes zoster, 43% with pneumonia, 67% with amoebic dysentery and 41% with carcinoma. In the other study, 42% of women with recurrent abortions, 67% with vaginal ulcerations and 33% with haemorrhoids had a positive HIV antibody test. While the Bangui AIDS definition had a positive predictive value for HIV seropositivity of 62% in one of the studies and 83% in the other, in the same studies the positive predictive value of amenorrhoea was 42% and 89% respectively.[139,140]

In 2000, in a paper entitled "Mortality associated with HIV infection in rural Rakai District, Uganda" by researchers from Makerere University, Uganda Virus Research Institute, Johns Hopkins University and Colombia University, a cohort of 19,983 adults was followed at 10 month intervals for four surveys. "Among 339 infants born to HIV-positive mothers, 71 died within the first year of life (infant mortality rate 209.4 per 1000 live births) and among 2303 infants born to HIV-negative mothers, there were 225 deaths during the first year of life (infant mortality rate 97.7 per 1000 live births)". The causes of death were not given. They reported a 12.9% seropositivity rate in men of which 21.2% have died. The respective values in women were 18.9% and 17.9%. Out of a total of 3210 seropositive individuals 615 have died. "…40.5% (249/615 of all HIV-positive subjects who died reported no symptomatology prior to their demise…Moreover, only 56 out of 615 deaths (9.1%) met the WHO criteria" for AIDS. Discussing their findings they wrote: "However, it is noteworthy that 40.5% of deaths in HIV-positive persons occurred among individuals who were asymptomatic and free of identifiable opportunistic infection at time of interview within 0–10 months preceding death. Other investigations in Uganda also report that a high proportion of HIV-infected subjects are asymptomatic within 1 year prior to death…no particular symptoms or signs, were highly predictive of mortality in the HIV-positive as compared with the HIV-negative populations, which probably reflects the high background levels of these conditions in the HIV-negative population of rural areas".[138]   In other words, the antibody tests are neither specific for AIDS nor for death from AIDS. The authors of this study also cite a paper published in *Lancet* in 1994 by Mulder *et al*. entitled "Two-year HIV-1-associated mortality in a Ugandan rural population". In this study, 9,389 rural subsistence farmers were tested for HIV antibodies and over the next two years 109 out of 9,012 (1.2%) found negative and 73 out of 377 (19.4%) found positive died. However, only five of the positives died of "AIDS".[141]   The authors did not list details of the five "AIDS" deaths but an accompanying commentary from the US Centers for Disease Control implied that they were due to "tuberculosis, other pneumonias, and diarrhoeal diseases". In the same commentary Dondero and Curran wrote: "The study shows that the simple finding of antibodies against HIV in an individual's serum predicts a likelihood of death within the next several years far above that for a seronegative individual. Although most reasonable observers do accept that HIV causes AIDS, even sceptics cannot fail to acknowledge the high prevalence of antibody to HIV in Africa. If there are any left who will not even accept that antibody to HIV indicates infection with the virus their explanation of how HIV sero-positivity leads to early death must be curious indeed".[142]   The explanation may not be that curious if one realises, as many do, that there are many non-specific but nonetheless useful laboratory tests employed in clinical medicine.

A positive antibody test using an extract of ox heart (cardiolipin) as antigen predicts the development of syphilis, including death from syphilis, although the antibodies are not induced by cardiolipin and cardiolipin is not the cause of syphilis. The explanation of how a positive antibody test may predict early deaths is far less curious than the predictions engendered by an increased erythrocyte sedimentation rate (ESR). The ESR, first discovered in 1918 by Fahraeus while seeking an early test for pregnancy, is a common but non-specific test which, when elevated, "is a measure of the presence and intensity of morbid processes within the body". Like a positive "HIV" antibody test, an elevated ESR also has the capacity to predict "a likelihood of death within the next several years far above" a normal ESR. A common cause of elevated ESR is infection and "Elevated ESRs are also seen with pregnancy, malignancy, collagen vascular diseases, rheumatic heart disease, and other chronic disease states, including human immunodeficiency virus infection".[143]   Even asymptomatic, non-anaemic HIV positive individuals may have an increased ESR[144] and the test may be predictive for disease progression.[145]   In HIV positive children a correlation exists between seropositivity, hypergammaglobulinaemia and an elevated ESR.[146]   As far back as 1988 researchers from the Institut National de Transfusion Sanguine, Paris, France, found that: "An increased ESR in HIV-seropositive subjects seems to constitute a predictive marker of

progression towards AIDS before the decrease of the CD4 count".[147] In other words the ESR is a superior predictive marker for the development of the clinical AID syndrome than is a decrease in the CD4 cell count, although the latter is said to be the cause of the syndrome. One important factor which affects the ESR is the size of the red cells, especially rouleaux formation where the red blood cells clump together. Rouleaux formation may result from changes in the negative charge of red cells, caused by "the dielectric effect of proteins in the surrounding plasma", especially by "fibrinogen, immunoglobulins, and other acute-phase reaction proteins", and their increased levels in some disease states.[143] Diseases such as tuberculosis and AIDS are not caused by red blood cell clumping induced by "the dielectric effect of proteins" but the fact this can be demonstrated and measured *in vitro* is of great diagnostic and prognostic utility in clinical practice.

Given that the "HIV" proteins are likely to be normal cellular proteins, cellular proteins with new antigenic epitopes or newly induced cellular proteins, and that individuals who test positive have high levels of auto-antibodies and/or antibodies to many "non-HIV" antigens all or some of which may cross-react with cellular proteins, "HIV" seropositivity, curiously or not, like the ESR, may represent nothing more than a non-specific indicator, serendipitiously discovered in 1983/84, of altered homeostasis connoting a propensity to develop particular diseases. Whether or not a positive antibody test is predictive of such diseases outside the risk groups, including healthy pregnant women remains to be proven. As long as the present interpretation of a positive test is accepted this may never be ascertained because knowledge of seropositivity by both patient and physician attracts multiple confounding factors virtually impossible to eliminate.

1.6.2.4   **If the tests are specific, that is, if the tests prove infection, how did the women acquire HIV?**
World-wide the vast majority of women, including pregnant women, who are reported HIV infected, are said to have acquired the virus through heterosexual contact. A microbe or disease is heterosexually transmitted if, and only if, it is passed from man to woman to man via genital (semen and cervico-vaginal) secretions. Sexually transmitted agents do not have sexual, geographical or racial preferences. Yet HIV is said to be an exception. Its "main mode(s) of transmission" in sub-Saharan Africa is heterosexual, Western Europe MSM (men who have sex with men), IDU (intravenous drug use); Eastern Europe and central Asia IDU; South and South-East Asia, heterosexual, IDU; North America MSM, IDU, heterosexual; Australia and New Zealand MSM, IDU.[4] Indeed, in the United Kingdom and Germany, not only is the number of individuals who are said to have acquired HIV heterosexually negligibly small, they are also said to have been infected in "Pattern II countries", not Europe. By March 2001 in the UK there were only 109 white men and 110 white women who acquired HIV by "Exposure in the UK". At the same time there were 846 and 1008 Black-African, 48 and 30 Black-Caribbean, 99 and 22 Indian/Pakistani/Bangladesh/Asian or oriental men and women.[148] The Robert Koch Institute in Berlin, which is responsible for the evaluation of the German figures, has come to the following conclusion: "The results–HIV prevalence significantly under one per thousand among women giving birth–confirm the assumption of a low distribution of HIV in the general heterosexual population so far…The observed low prevalence might be an overestimation of the true prevalence due to the fact that a high percentage of the HIV-positive women in this study (60%) come from a Pattern II country".[148-151]

1.7   Heterosexual transmission of HIV
To claim heterosexual transmission of HIV one must:

   (a) Prove the existence of HIV;

   (b) Have accurate means of proving that a male (female) is infected with HIV;

   (c) Prove that HIV is present in the genital secretions of infected males (females) at the time sexual contact takes place;

   (d) Prove sexual partners of HIV infected males (females) are free of HIV immediately prior to sexual contact;

   (e) The non-infected partner becomes infected solely following sexual contact and by no other means.

Let us assume conditions (a) and (b) are satisfied. As far as (c) is concerned, there is not one single study from any country proving sexual transmission of HIV based upon evidence of HIV in genital secretions. In fact this may not even be possible because in studies where attempts are made to prove a correlation between HIV in the blood and genital secretions, the correlation is far from 100%.[152-165] Indeed, data from these former studies indicate that approximately 85% and 80% of male and female genital secretion samples respectively obtained from HIV positive subjects do not harbour HIV. The studies also prove that the presence of HIV in genital secretion on one occasion does not predict its presence on another.

15

For example, in one study using a PCR sensitive enough to detect one "HIV infected" cell in 100,000 non-infected semen cells and "occasionally" one "infected" cell in 1,000,000 non-infected semen cells, HIV genetic material could be detected in only 1/25 semen samples from gay men. Culture of the same samples yielded 4/25 samples positive (despite the fact that the PCR could not detect HIV genes in three of the same samples, suggesting "HIV" can appear from nowhere). The authors concluded that "HIV-1 infected cells are not as prevalent in semen as in the peripheral blood".[166]

In another study, Gardner and her colleagues including researchers from the CDC, were able to "isolate" HIV from the semen of only 9/95 (9%) patients. They also reported a longitudinal study of 14 men who between them provided a total of 90 semen samples. "Of the 90 samples collected during the longitudinal study, six (7%) were HIV-1 positive by culture".[157]

In yet another study investigators measured the "viral load" in both blood and non-blood secretions. For men with viral loads greater than 50,000 copies per ml of plasma, 33% had an undetectable virus load in their semen. For men with viral loads less than 10,000 copies, 60% did not register a viral load in semen. When the blood viral load was less than 5000 copies/ml, 15/22 (68%) of semen samples failed to show HIV. For 14 women not taking anti-HIV medications, 12/20 (60%) of samples of cervico-vaginal secretions did not have a detectable virus load.[167]

To have proof for (d) and (e) it is necessary to undertake sexual contact tracing. However, according to Harry Haverkos from the US National Institute on Drug Abuse, "Sexual contact tracing, the standard practice in public health to combat such sexually transmitted diseases as gonorrhoea and syphilis, has been avoided for tracing of HIV infected persons. Health department personnel are concerned about possible discrimination associated with AIDS, plus the fact that there is no cure for the disease".[168] The only evidence said to prove heterosexual transmission is epidemiological, that is, the study of the relationship between seropositivity and sexual behaviours.

To investigate diseases in groups or populations epidemiologists conduct observational and/or experimental studies. The former "allow nature to take its course". The investigator measures but does not interfere with what his subjects are doing or not doing. There are a number of differing techniques for performing observational studies. The most primitive consists of describing one or more characteristics of a number of patients with a certain disease but without making any attempt to link these together. Deaths over time within a community is a common example. When observations go beyond the mere collection and presentation of raw data they are known as "analytical" studies. There are a number of different types which differ in the way cases are selected and whether data are collected now, in the past, or in the future. In regard to proving heterosexual transmission of HIV the most often reported type of study is the "prevalence" study. This means determining the percentage of individuals within a population who have a positive HIV antibody test or AIDS *at a specified time*. Basically the investigator arrives, performs his measurements and then leaves. It is as if time has been "sliced into two parts"–one before the investigator arrived and one after the investigator departed. Hence the use of the optional term "cross-sectional" as a synonym for this type of study. Cross-sectional studies have marked limitations because they make measurements of exposure and effect at the same time. According to one epidemiological text, "It is not easy to assess the reasons for associations [relationships] demonstrated in cross-sectional studies. The key question to be asked is whether the exposure precedes or follows the effect. Cross-sectional studies are less able to prove causation as they provide no direct evidence on the time sequence of events". To prove causation "The temporal relationship is crucial–the cause must precede the effect".[169] In a cross-sectional study this is an impossibility because only one time slice is involved. The problem is best illustrated by an analogy.

An investigator arrives at a cocktail party and observes some guests holding glasses of wine. Furthermore, there are groups consisting of a man and a woman where each partner has a glass. The investigator produces his camera and takes a snapshot of the proceedings and then leaves. Back in his laboratory he studies his one picture in an effort to ascertain the means by which individuals in the picture obtained his or her glass of wine. Or that amongst couples, and regardless of waiters, whether the man gave the woman the wine or *vice versa*. Obviously this is a futile task. Yet by such means HIV epidemiologists claim they are able to prove heterosexual transmission of HIV including its direction. That is, by knowledge merely of the HIV status of heterosexual partners who deny "other" risk behaviours. Indeed, in the majority of such studies the individuals concerned are not even linked. It is as if the investigator obtained a separate photograph of each person, appended particular data, for example, gender or number of drinking partners and, from this collage, attempted to prove the *modus operandi* underlying the transit of wine glasses to and between party goers. As far as Africa is concerned, one must note that "AIDS patients reported to the CDC are classified as HT [heterosexual] if they

(1) report heterosexual contact with a person with HIV infection or at increased risk for HIV infection (US born) or (2) were born in countries where HT is a major route of transmission (non US born)".[170] This means that a man or woman born in Africa can be said to have acquired HIV/AIDS by heterosexual contact even if his/her partner were not proven to have HIV infection, or even if he/she had never had sexual intercourse. Sometimes epidemiologists obtain more reliable data by adding the dimension of time to their studies. Such studies depart from being cross-sectional and are known as "longitudinal" or "prospective". However, even if a non-infected monogamous sexual partner of an infected individual becomes positive over time, it is still not proof that the infection was the result of sexual activity. In other words, although the data from prospective studies may be more reliable than that from the cross-sectional studies, the interpretation of these data can by no means be considered definitive.

In 1983, in an attempt "to evaluate acquired immunodeficiency syndrome (AIDS) in central Africa" a prospective study was performed in Kigali, Rwanda by researchers from Belgium, the Netherlands and Rwanda. A questionnaire was sent "to all clinicians at the Centre Hospitalier de Kigali asking them to make a special note over a 4 week period of new patients who had clinical evidence of opportunistic infections (OI) and/or generalised or multifocal Kaposi's sarcoma (KS) or who had the AIDS prodrome. The prodrome [patients with the prodrome were ultimately classified as AIDS patients] was defined by at least two of the following: loss of more than 10% body weight, diarrhoea for at least 2 months with no pathogen isolated, chronic fever of unknown origin lasting for at least 2 months, and generalised lymphadenopathy". Patients presenting with the above clinical features and a low T4/T8 ratio were said to have AIDS. Twenty six such patients (17 males and nine females) were identified, two of whom were children. The 24 adult patients denied bisexuality or homosexuality or intravenous drug use. The authors wrote: "The study confirms that AIDS exists in Rwanda, a central African country east of Zaire. The detection of 26 AIDS patients in a short period suggests that AIDS may be a public health problem in central Africa…Characteristically, African AIDS affects women as well as men...The low sex ratio suggests that heterosexual contact is the most frequent mode of transmission in central Africa".[171]

In the same year, researchers from Belgium, Zaire and the USA (including the CDC) searched for AIDS in Zaire. During a three week period they identified 38 patients with low T4/T8 ratio. Ten patients had "Chronic mucocutaneous HSV [herpes simplex virus] infection", 14 "bilateral interstitial pneumonia with severe dyspnoea, unresponsive to antibiotics or tuberculostatics", 31 "Oral and/or oesophageal candidiasis" and 6 "Disseminated KS". Regarding the latter, they wrote: "Since KS has long been endemic in Zaire, only patients with fulminant KS were included". Discussing their findings, the authors wrote: "Two important differences between AIDS in Zaire and the disease in patients of European or American origin merit discussion–namely, the sex distribution and apparent lack of risk factors among patients in Zaire…The essentially equal proportions of males and females would require that transmission occurs both male to female and female to male, since one-direction transmission would soon result in an imbalance in the ratio".[172]

In 1984 sera from 37 out of the 38 patients who were diagnosed in Kinshasha were tested for HIV antibodies by Montagnier and 20 associates including researchers from the CDC. The sera were tested by ELISA and then by a RIPA (radioimmunoprecipitation assay), a test similar to the Western blot. The latter was considered positive if a p24 band was present. The p41 band and also an 84-kDa band were not considered diagnostic because "The 43-kD [p41] band and the 84 kDa band are cellular contaminants that are immunoprecipitated in all the tested sera", from both patients and controls. Thirty-two patients (88%) were positive by both tests. So were six out of 26 (23%) controls.[173]

Like Montagnier and the CDC, Gallo and his associates also tested Africans for HIV antibodies. Of 53 patients said to have AIDS, including the first 26 patients reported from Rwanda, 46/53 (87%) tested positive. 67/84 (80%) prostitutes [without any clinical symptoms] and 5/40 (12.5%) and 8/51 (15.5%) healthy controls and blood donors respectively, also tested positive. All blood donors were of good socioeconomic status. Sera which had one positive ELISA were considered proof of HIV infection. Sera which had a borderline ELISA were further tested with the WB. In these tests, "Serum samples possessing reactivity to HTLV-III [HIV] p41 and/or p24 were scored positive". Gallo and his associates concluded, "In Central Africa, as previously noted, the occurrence of the syndrome in young to middle-aged men and women suggests that heterosexual contact is probably the predominant mode of transmission of the AIDS agent. Furthermore, among the 24 adults with AIDS that we saw in Rwanda, 12 of the 17 men had contact with prostitutes and three of seven women were prostitutes".[57]

In 1985 Robert Redfield, from the Walter Reed Army Medical Center, Gallo and his associates published a paper "Frequent Transmission of HTLV-III Among Spouses of Patients With AIDS-Related Complex and AIDS". They reported seven married soldiers who had AIDS-related complex or AIDS, as well as their spouses

and children. Five of the spouses were positive for HIV and so was 1/11 children (a child aged 14 months). In five couples both partners were Black, one couple was Black/Hispanic and one White. Five male partners had AIDS and two had ARC. All men were seropositive and 6/7 had virus "isolated" from their "peripheral blood leucocytes". Three female partners had ARC and four were clinically normal. Four women were seropositive and three seronegative. HIV could be "isolated" from a seronegative woman and could not be "isolated" from two seropositive women. The conclusion of this study was "These data support the opinion that close household contact to patients with AIDS is not an efficient mechanism for virus transmission, while demonstrating that HIV can be transmitted by repeated heterosexual contact". However, the study failed to define or document "repeated sexual contact", and assumed the men were infected first and infected the women because the authors had earlier "failed to identify a defined risk factor other than heterosexual promiscuity in more than 30% of ARC or AIDS cases in US military personnel".[174] Indeed in a statement by Gallo and Montagnier published in 1987 where they "set out a brief chronological history of some critical published facts, in the period up to May 1985, on the discovery and demonstration of AIDS as a retroviral disease",[175] this paper is considered to have been the first to describe "heterosexual transmission of HTLV-III", HIV.[174]

Later in 1985 Redfield, Gallo and their associates published a similar paper presenting data on "Forty-one sequential cases of HTLV-III disease (ARC/AIDS) [that] were evaluated at the Walter Reed Army Medical Center". "All patients were interviewed on multiple occasions by trained investigators to ascertain AIDS risk factors…Any evidence for behaviour characteristics suggestive of either homosexual activity, including asymptomatic rectal carriage of gonorrhoea, or IV drug abuse (evidence of needle abuse) resulted in classification of individual as member of an appropriate risk group". ARC (AIDS related complex) was "defined as chronic lymphadenopathy with a duration of more than three months with nodules of at least 1cm in diameter, involving two or more extrainguinal sites, and an absolute T-helper cell depletion (T-helper cell count of <400/cu mm) persistent for a minimum of six weeks". According to the authors "All patients were exposed to HTLV-III documented by either virus isolation and/or by detection of serum antibody against viral structural proteins...In 15 (37%) of 41 patients with HTLV-III disease (ARC or AIDS), HTLV-III infection appeared to have been heterosexually acquired". Five of the patients were women (3 Black, 2 Hispanics), and 10 were men (1 Hispanic, 2 Whites and 7 Blacks). One man was considered to have been infected by multiple sexual partners in New York City, one by prostitutes in Dallas, one by prostitutes in NYC/Korea, and the other six by prostitutes in Germany.[176]

Gallo claimed this study furnished the first evidence proving "both male-to-female and female-to-male transmission of infection and disease [AIDS]". In all ten men and two of the females there were no data to show that the presumed "infecting" partners were HIV positive or that they were even HIV tested. This included prostitutes who were said to be responsible for "infecting" eight of the soldiers. In addition, the authors determined the sexual preference of the ten men (heterosexual) after "corroboration of patient information was obtained by interviews with family members and other acquaintances and by physical examination, including a rectal culture for gonorrhoea". One wonders how an investigator can expect "family members" including mothers and fathers to necessarily be aware of or report a son's bisexual behaviour. Especially when the son has left home and is posted overseas and may seldom visit his parents. Or how a "physical examination" can prove one's sexuality. Certainly rectal gonorrhoea may suggest a man is gay but its absence does not prove he is not gay. Not all gay men have gonorrhoea. Notwithstanding, without any data whatsoever the authors declared that the alleged prostitutes were also HIV infected and they "were probably exposed to HIV by sexual exposure to [other] HIV infected males". Thus by observing HIV positive soldiers in the US the authors were able to "prove" these men were infected by overseas prostitutes and that the prostitutes themselves were infected by other male clients. Both without any knowledge that either the prostitutes or their other clients were either HIV positive or even tested for HIV.

In the same year, 1985, Gallo and his colleagues published further data from Rwanda. On this occasion the authors tested 33 prostitutes, 25 consecutive male customers of prostitutes who were attending the STD clinic for treatment as well as 27 males who denied contact with prostitutes and 33 females who were not prostitutes. The definition of a customer was "a man who had had sexual intercourse with a prostitute at least once in the previous 3 months". However, no data were presented as to how many if any of the 25 male customers had sexual contact with the 33 prostitutes in the study. Any person who had a positive ELISA was considered infected. Sera with indeterminate ELISA were further tested with WB, which was considered to be positive when there was a reaction with p41 and/or p24. Twenty nine (88%) of the prostitutes, 7 (28%) of the males customers as well as 4/33 (12%) and 2/27 (7%) of the female and male "controls" respectively were found positive. The authors claimed their data "suggests, as previously noted, that HIV may be transmitted directly through heterosexual contact".[177]

The conclusions reached in the studies performed in Africa and the Walter Reed Army Medical Center were criticised by several authors. According to Padian and Pickering, the low male to female ratio of HIV seropositivity in Africa cannot be used as additional evidence for heterosexual transmission of HIV as Redfield,

Gallo and their colleagues have done. "The low African sex ratio does not necessarily confirm that AIDS in Africa is predominantly transmitted by heterosexual contact. The 1:1 sex ratio of AIDS cases can be explained without invoking female-to-male transmission. If female-to-male transmission is rare in Africa, as it appears to be in the United States, then the low African ratios could be explained by higher proportion of bisexual compared with homosexual men in Africa than in the United States".[178]

Schultz and his colleagues from the New York City Department of Health commented "We feel that the study by Redfield et al is based on questionable data and unsound epidemiological reasoning and that the evidence presented for female-to-male transmission unconvincing. Our first criticism is that the means used to exclude persons with known AIDS risks were not sensitive and were subjected to considerable bias...Certainly, discharge from military service for homosexuality and/or intravenous (IV) drug use would dissuade self-reporting of these behaviours; informants are no more likely to reveal "illegal" activity of friends or relatives. A physical examination and single culture for rectal gonorrhoea are not sensitive techniques to identify male homosexual behaviour. The absence of Kaposi's sarcoma in the ten male AIDS patients under analysis (seven of whom are black) is presented as additional evidence against homosexual transmission. However, this cancer is rarely seen among blacks [in the USA] and IV drug abusers…Third, there is no evidence to support the possibility of acquiring HTLV-III infection from German prostitutes. In 1985, nearly 2,000 registered prostitutes were tested for HTLV-III antibodies in Munich, Stuttgart, Berlin, Heidelberg, Frankfurt, and Kiel. Seventeen were positive, and half of these were identified as IV drug users. The current low prevalence of HTLV-III in this population and the greater ease of transmission from males to females suggests that registered German prostitutes are neither a source nor a recipient of the virus. Indeed, a review of the 300 reported cases of AIDS in West Germany indicates no female-to-male transmission within this country. Finally, this study lacks a control group with which to compare the ten male AIDS patients. Without controls, it is impossible to estimate whether prostitute exposure among the cases is any different from that in a comparable group of controls. Such a control group would be men who are HITLV-III negative, matched for age, marital status and race".[179]

According to Wykoff from the South Carolina Department of Health and Environmental Control, "Dr Redfield and his colleagues are to be congratulated for their continued efforts to provide "evidence for both female-to-male and female-to-male transmission of infection and disease. I am not convinced, however, that they have yet completely succeeded in their goal...Part of the difficulty with Dr Redfield's data is that they are presented, in part, to substantiate previous reports from Africa that "support female-to-male transmission" of AIDS. These reports from Africa (and Haiti), however, have themselves fallen well short of documenting female-to-male transmission of HTLV-III…The equal incidence of AIDS in Africa may merely be a reflection of shared risk factors for males and females and may not be due to bidirectional spread of HTLV-III as proposed".[180]

In response to these criticisms Redfield and Gallo defended their position arguing that "We are unaware of any evidence to support the often-repeated statement that soldiers are more likely than civilians to lie to their personal physicians. This statement is presumably based on the hypothesis that military patients with HTLV-III disease who admit to "illegal activity" will be subject to punitive action".[181] Richard Pearce from San Francisco responded to this assertion with "Because there are no data to indicate that soldiers are more likely to lie (about homosexuality or IV drug use) than civilians, Redfield et al concluded that they are telling the truth. Unfortunately, the more absence of data to the contrary does not by itself make the opposite operation true; if it did, science would be a much simpler thing".[182]

Potterat and his colleagues from the El Paso County Health Department, Colo, wrote: "About 30 percent of men with human immunodeficiency virus (HIV) evaluated at Walter Reed Army Medical Center in Washington, DC reportedly acquire the disease heterosexually, according to a study published in THE JOURNAL...Years of experience in the epidemiology of sexually transmitted diseases among military men in Colorado Springs, Colo, (a region hosting about 30,000 active duty personnel, of whom 92% are men) remind us that infected men are likelier to reveal histories of homosexual or drug abuse activity to civilian rather than to military case-investigators. An opportunity to test our anecdotal impressions presented itself a few days after publication of the aforementioned article: Colorado became the first state to add HIV infection to its list of notifiable diseases on Nov 1, 1985. This regulation permitted us to evaluate and counsel HIV-infected military personnel, a service we routinely offer for the other reportable sexually transmitted disease…Between Nov 1, 1985 and Oct 31, 1986, a total of 22 active duty personnel, all men, were reported to us as having serum antibody to HIV structural proteins by Western blot analysis. Our cohort included ten whites, ten blacks, and two Hispanics; the mean age was 26.5 years (range 19 to 39 years); five were known to be married. All had their infection identified as a consequence of screening, including nine blood donors. Ascertainment for six occurred at military installations on foreign soil, whence the patients were repatriated. All had received their risk factor assessment and counselling by military providers. We interviewed 20 of the 22 patients (one was discharged before we reached him, the other refused to meet us). The result of risk factor identification by our health

department case-investigators were virtually the inverse of those obtained by military interviewers. [Table 1.3] Although our cohort is numerically and sociodemographically similar to that reported by the Walter Reed group, risk factor classification in our patients mirrors the national experience: in the United States, 78.7% of men with acquired immunodeficiency syndrome report having sex with men and 14.5% admit to intravenous drug abuse. Of the three patients whose risk factors we classified as "undetermined", one satisfies the Centers for Disease Control definition for a heterosexual case and the other two are regarded as probable interview failures. The 12 patients who admitted classic risk factors to us but not to the military originally claimed heterosexual contact as their source of infection, usually with prostitutes. Of the nine blood donors, at least seven had risk factors that should have provoked self-exclusion for the donor pool. In a subsequent letter to the Editor, the Walter Reed researchers defended their position stating they were "unaware of any evidence to support the often-repeated statement that soldiers are more likely than civilians to lie to their personal physicians". With our soldiers, this often-repeated statement was true".[183]

Table 1.3 Comparison of risk factor classification in 20 HIV-infected Military men interviewed first by military and subsequently by civilian case-investigators

Risk Factor Category	Case-Investigators' Findings, No. (%)	
	Military	Civilian
Homosexual/bisexual	4 (20)	14 (70)
Intravenous drug abuser	1 (5)	3 (15)
Undetermined	15 (75)	3 (15)
Total	**20 (100)**	**20 (100)**

It is of interest to note that in the developed countries, with the exception of Redfield *et al*, "no case has been confirmed of direct transmission from a female prostitute to a male partner", at least till 1989.[184] Nonetheless, the claims that HIV is heterosexually transmitted became virtually universally accepted. However, because the predicted heterosexual spread of HIV/AIDS has not been fulfilled in the developed countries, presently it is claimed that heterosexual transmission is mainly an African phenomenon. In fact the African continent has been subsumed into one immense cross-sectional study for the purpose of proving that heterosexually acquired HIV/AIDS will eventually envelop the West.

HIV experts concede that "most of the persons who have acquired AIDS through heterosexual contact are the female sexual partners of intravenous (IV) drug users", some of whom "may belong to high-risk groups through their own lifestyles, for example, prostitution and IV drug use", but do not admit such practices. These factors seriously confound attempts to prove heterosexual transmission of HIV in either partners of drug users or drug using partners. However, Nature has provided researchers with a "natural experiment" where these factors can largely be ignored. At the beginning of the HIV era it became apparent that approximately 75% of haemophiliacs tested were HIV positive. Virtually all haemophiliacs are men and investigators have taken advantage of HIV positive haemophiliacs to study male to female transmission of HIV because "the number of infected index cases [the males] is easier to define and their female sexual partners in general are healthy and have no other known AIDS risk factors".[185,186]

The largest study of female sexual partners of haemophiliacs was conducted between August 1985 and February 1989 by the US Transfusion Safety Study Group. It enrolled persons with all forms of congenital clotting disorders into long-term follow-up at clinics in New York, Miami, Detroit, Seattle, San Francisco and Los Angeles, as well as 201 female sexual partners. The researchers followed up 151/180 females HIV negative at the beginning of the study for periods ranging from 5 to 47 months. The total period of observation for these 151 females was 351 person-years (2.3 years per person). None became HIV positive despite the fact that 13 (8%) of the 151 women became pregnant. Detailed questionnaires concerning sexual practices were completed by 50 (28%) of the 180 HIV negative women. Of 47 who reported vaginal intercourse, condom use was "always" in 6 (13%), "sometimes or usually in 20 (42%), and "never" in 21 (45%). Commenting on the discrepancy between the findings in the cross-sectional studies conducted by them as well as others and their prospective studies they wrote: "These two circumstances may be explained by awareness of risk earlier in the course of infection and more extensive modification of their sexual practices than among other heterosexual risk groups. The large majority of haemophiliacs treated with factor VIII concentrates in this and other studies become infected essentially as a cohort between 1981 and 1983, a period during which sexual practices would not have been modified. By late 1983 to early 1984, however, haemophilia clinics were aware of risk of infection associated with concentrates and the potential for transmission to sexual partners. Counselling was provided to this group much earlier than other heterosexual risk groups. On the other hand, the information from

our questionnaire indicates that sexual practices capable of transmitting HIV-1 have persisted among haemophiliacs and their partners…In addition, unprotected sexual contact during the period of follow-up was evidenced by the occurrence of 13 pregnancies among women who remained seronegative. Without questioning that sexual practices have been made safer by counselling there has been enough opportunity for sexual transmission to make counselling seem an inadequate explanation for the virtual absence of prospectively observed seroconversions".[187]   (It must be also pointed out that the studies which reported transmission to haemophiliac partners in addition to being cross-sectional, were performed prior to 1987 when a single ELISA or a single band in the Western Blot was sufficient to diagnose infection.)

In 1988 researchers from the Netherlands reported a three year follow-up of 13 HIV positive haemophiliacs and their sexual partners. All couples practised vaginal intercourse, two during menstruation, 4 partners had oral intercourse. One man had used condoms before becoming positive and 4 started to use condoms after being told that the man was positive. No women became positive. They calculated that in 11 couples unprotected vaginal intercourse occurred a maximum of 2,250 times (minimum 1,563) without transmission of HIV. For protected vaginal intercourse the figures were 1,252 and 942 respectively.[188]

In 1988 Brettler attempted to isolate HIV and performed antibody tests on 36 sexual partners and 87 household contacts of 66 haemophiliacs all of whom had been HIV positive "for a minimum of six months before their contacts were studied" (between September 1984 and September 1987). During this time "Thirty-one of the sexual partners have had sexual contact for at least three years and 20 (63%) have had contact with the index hemophiliac since 1980…In our center, 93% (63 out of 68) of the index hemophiliacs in this study were already HIV antibody-positive when first tested in 1983." All partners were HIV negative (ELISA and Western blot). "In addition, virus isolation studies were attempted on 20 randomly chosen sexual partners. Virus could not be isolated from any of the partners, including five who had a spouse from whom virus had been isolated and seven who had a partner with AIDS or AIDS-related complex…Follow-up testing was performed on 18 of the sexual partners to determine whether they had seroconverted with longer heterosexual exposure. Eight partners have been tested twice, five have been tested three times, and two have been tested four times. All follow-up samples have remained seronegative after ELISA and Western blot test. Follow-up virus isolation studies on two members of this subgroup were also negative…Five of the index patients who have AIDS have had a steady sexual relationship for at least five years and one for three years. All partners have been tested since their spouse's diagnosis and remain HIV antibody-negative…Before 1984, six couple used condoms and only two of these used them regularly. Although during 1987 the number of couples using condoms has increased through risk-reduction education, it does not seem that the lack of seropositivity in the spouses is due to a disproportionately higher use of barrier contraceptive devices."   The authors concluded "*The most likely value of the probability of infection within 25.8 months for this group of 36 sexual partners is zero*"[189] (italics ours).

In the United States, and in fact anywhere in the world, the most thorough investigation of heterosexual transmission was carried out by Nancy Padian from the Department of Epidemiology and Biostatistics, University of California, and her associates. These studies amply confirm the data reported from patients with haemophilia. Their ongoing studies commenced in 1985 and in 1987 they reported their data on men to women transmission. At entry into the study of 97 women, 22 (23%) were found positive. The rate of positivity was higher (42%) in women who were partners of IV users. Approximately 40% of the 75 seronegative women were re-tested six months after entry into the study; none had seroconverted. Regardless of risk groups of index case, (bisexual, haemophiliac, "men infected from contaminated transfusions"), seropositive women were 4.6 times more likely than seronegative women to have had more than 100 sexual exposures with their infected partner. Anal intercourse significantly discriminated between seronegative and seropositive women. Because three of the women who tested positive admitted to neither IV drug use, oral or anal intercourse, the authors concluded that HIV can be transmitted via vaginal intercourse.[190]   At the Fourth International Conference on AIDS, 1988, Padian and her colleagues reported that at entry to a heterosexual partner study, "Infection rate was 24% (95% confidence interval=18%-32%). In multivariate analysis, only the practice of anal intercourse (p=.003) and non-white race (p=.015) were significantly associated with infection...We have also enrolled male partners of infected women. In spite of repeated unprotected sexual intercourse (median number of sexual contacts=399) none of the twenty male partners was infected".[191]

Because "AIDS epidemiologists may not have the opportunity to collect data on a regular basis and are thus forced to rely on retrospective reports of behaviour even though the accuracy of these reports (especially for a variety of sexual practices) has not been established", Padian attempted to establish a correlation between the answers given by the men and women regarding sexual practices. "The total number of vaginal intercourse contacts reported by the men and the women were highly correlated (r=0.84…). Number of anal intercourse contacts was compared as a continuous variable, with somewhat less strong agreement (r=0.44…) [In fact this is poor agreement] ". In addition, according to Padian, "measurements were not independent because couples may

have discussed responses prior to the interview", the "couples knew they were to be interviewed about their sexual behaviour".[192]

In 1991, six years after the study commenced, Padian and her colleagues reported their data on female to male transmission. Of 72 male "non-drug-using" partners of infected women recruited in the above period, they "observed one probable instance" of female-to-male transmission. They gave a number of reasons for considering this case as only "probable". To this one must add the fact that the criteria they used ("antibodies to p24 (core polypeptide) and or gp41") are at present not considered sufficient to prove HIV infection in most countries, institutions or laboratories. Discussing the discrepancy between their findings and those of other investigators, including those of Redfield and Gallo, they stated that the other "studies may not have adequately controlled for other confounding nonsexual routes of transmission such as risks associated with intravenous drug use. At first blush, cases that appear attributed to heterosexual transmission may, after in-depth interviewing, actually be linked to other sources of risk...because partner studies are by definition not random samples, and most reported results are based on retrospective or cross-sectional analyses, some studies may overselect couples in which both partners in a couple are infected because such couples may be more easily identified, thus biasing transmission rates. Furthermore, it is often difficult to establish the source of infection in such couples".[193]

In 1997 Padian published a paper entitled "Heterosexual Transmission of Human Immunodeficiency Virus (HIV) in Northern California: Results from a Ten-Year Study". The data were divided in two parts, cross-sectional and prospective, and every effort was made to exclude confounding variables such as drug use. In the cross-sectional study they reported an overall 19% male to female transmission from which they estimated that: "infectivity for male-to-female transmission is low, approximately 0.0009 per contact" and "approximately eight-times more efficient than female-to-male transmission". They also reported a 2.4% (two men out of 82) rate of female-to-male transmission. Of these two men one was, the "probable" transmission reported in 1991 and doubts were also expressed about the second man. Using their estimate of male-to-female transmission (0.0009 per contact), it would take 770 or 3333 sexual contacts respectively to reach a 50% or 95% probability of becoming infected. If sexual contact were to take place repeatedly every three days this would require a period of 6.3 and 27.4 years respectively. Based on Padian's estimate of female-to-male transmission it would require 6200 and 27000 contacts and a period of 51 and 222 years respectively (see Figure 1.1, Table 1.4 and Appendix IX).

Discussing their findings they wrote: "To our knowledge, our study is the largest and longest study of the heterosexual transmission of HIV in the United States. The consistency of results over the 10-year duration argues for the validity of our results. For example, the practice of anal sex and lack of condom use have remained strong predictors of transmission since the beginning of the study...Higher rates of heterosexual transmission, particularly from females to males, reported in other studies may be due to a number of factors...Misclassification of mode of transmission may be an especially important factor to consider, particularly when interpreting estimates of the rate of female-to-male sexual transmission, because women who are injection drug users themselves are more likely to have a male injection drug user partner than vice versa. Studies in Europe and the United States which have reported higher rates of female-to-male transmission include relatively higher rates of female injection drug users and their sex partners".

In the prospective study of 175 HIV-discordant couples, tested every six months, not one of the 175 non-infected men or women became HIV positive. Discussing their findings they stated that the absence of infection over the course of the study cannot be entirely attributed to significant behaviour change. Indeed, although the couples received extensive and continuous safe sex education, and although "At last follow-up were much more likely to be abstinent or to use condoms constantly, and were much less likely to practice anal intercourse", even then 25% of the couples were not using condoms "consistently".[194]

In conclusion at present there is ample epidemiological evidence which shows that:

(a) The only sexual act, in both gay and heterosexual sex, which is related to the appearance of AIDS and a positive antibody test is receptive anal intercourse;

(b) The frequency of this practice, by either sex, and not the number of partners (promiscuity) is the risk factor for the development of AIDS and of a positive antibody test.

It is not homosexuality *per se* but the sexual act ("anal intercourse may be practiced by a much larger absolute population of heterosexuals than of homosexuals"[195]) which is of critical importance. Thus AIDS and a positive antibody test, like pregnancy, can be sexually acquired but not sexually transmitted. The difference is that while pregnancy can be acquired by a single act of sexual intercourse, for AIDS to appear a very high frequency of receptive anal intercourse over a long period is necessary. AIDS is more like anal[196,197] and cervical cancer.[198]

The effect is not the result of the act itself but its high frequency. But, as with pregnancy and cervical and anal cancer, other factors may promote or militate against the development of AIDS.

PROBABILITY OF INFECTION AFTER N SEXUAL CONTACTS
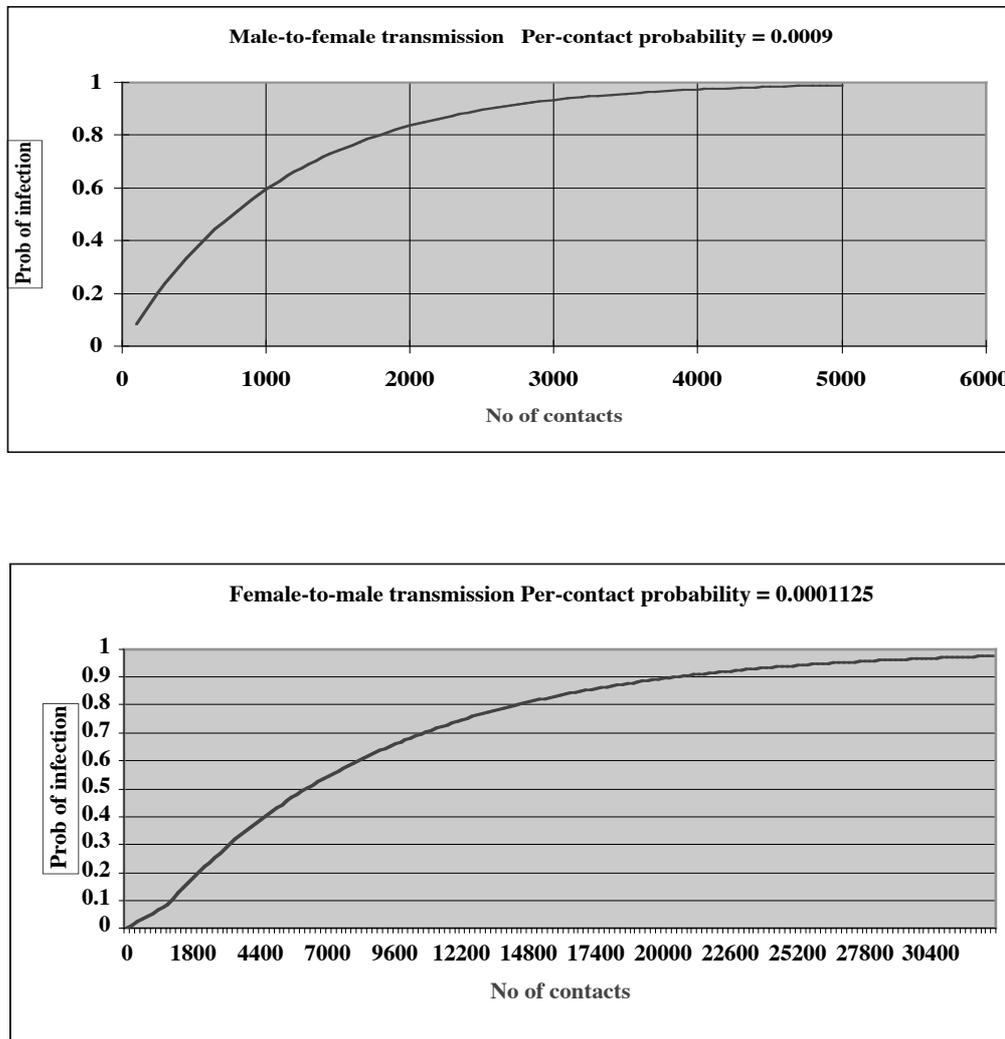
Figure 1.1

As mentioned the claims of heterosexual transmission in Africa are based on evidence from cross-sectional studies. In a paper published in *Lancet* 2001[199] a community based study was reported from Uganda, involving 15,127 individuals aged 15-59 years and followed over 4 years. Gray and his colleagues from Makerere and Johns Hopkins Universities retrospectively analysed data which later identified HIV seroconversions amongst sexual partners where one member was previously known to be seronegative. The results were presented in a paper entitled "Probability of HIV-1 transmission per coital act in monogamous HIV-discordant couples in Rakai, Uganda". In this only study of its kind reported from Africa, "174 monogamous couples, in which one partner was HIV-1 positive, were retrospectively identified from a population cohort". The probability of transmission per sexual contact was 0.0009 for male-to-female and 0.0013 for female-to-male respectively. The former figure is identical to Padian *et al* and for the latter, again assuming sexual contact occurs on average once every three days, it would take 540 and 2380 contacts (4.4 and 19.5 years) to achieve 50% and 95% probabilities of female-to-male transmission (see Figure 1.2, Table 1.4 and Appendix IX). The authors concluded that "The probability of HIV transmission per sex act in Uganda is comparable to that in other populations, suggesting that infectivity of HIV subtypes cannot explain the explosive epidemic in Africa".[200]   In other words, there is no more heterosexual transmission of HIV in Africa than anywhere else, including the USA, Australia and Europe. All that remains is for these scientific facts to become common knowledge.

PROBABILITY OF INFECTION AFTER N SEXUAL CONTACTS

Female-to-male transmission   Per-contact probability = 0.0013
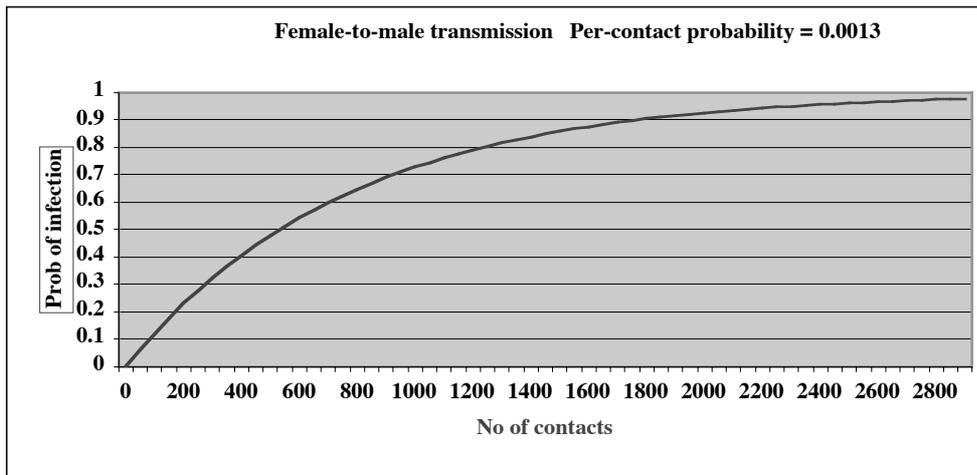
Figure 1.2

Table 1.4   Number of years to attain 50% and 95% probabilities transmission of HIV assuming sexual contact once every three days

STUDY	DIRECTION OF TRANSMISSION	Per contact PROBABILTY	Years for 50% PROBABILTY	Years for 95% PROBABILTY
USA	M to F	0.0009	6.3	27.4
	F to M	0.0001125	51	222
Uganda	M to F	0.0009	6.3	27.4
	F to M	0.0013	4.4	19.5

1.8   Conclusion

Although virtually everybody accepts that the diagnostic tests are based on the proven existence of HIV proteins and nucleic acids, the validity of every laboratory test presently used to prove HIV infection has been disputed by the HIV experts themselves. Yet these tests form the basis for the claims of mother to child transmission of a lethal retrovirus.

PART II
EPIDEMIOLOGICAL EVIDENCE FOR MOTHER TO CHILD TRANSMISSION OF HIV

2.1   Introduction
The way to obtain the most reliable epidemiological evidence of mother to child transmission is to conduct prospective, randomised, blinded, controlled studies. The women who act as controls should, with the one exception of HIV seropositivity, be identical with the test women. In other words, a scientific study should consist of two groups of mother-child pairs incorporating:

(a) children whose mothers are HIV positive;

(b) children whose mothers are HIV negative but are otherwise identical with the HIV positive women.

All the tests and clinical observations must be performed blindly in children born to both groups of mothers.

2.2   Studies from the USA
Children of drug using or economically disadvantaged mothers are of low birthweight and develop immune deficiency and a range of diseases.
In 1982 the CDC reported 4 cases of "Unexplained immunodeficiency and opportunistic infections in infants—New York, New Jersey, California".[201]   At three months of age a Black/Hispanic infant of an intravenous (IV) drug user mother developed oral candidiasis. This was followed by hepatosplenomegaly and staphylococcal impetigo. "Growth, which had been slow, stopped at 9 months". At 17 months of age the infant had progressive pulmonary infiltrates and oral candidiasis and "Mycobacterium avium-intracellulare was cultured from sputum and bone samples". T-cell studies that were initially normal, at 20 months "showed lymphopenia, decreased number of T-lymphocytes, and severely impaired T-cell function in vitro".

A Haitian infant with retarded physical development, developed diarrhoea at 2 weeks and at 5 months was hospitalised because of fever and diarrhoea. He had hepatosplenomegaly, lymphadenopathy and otitis media. While on treatment, he developed pulmonary infiltrates. "An open lung biopsy revealed Pneumocystis carinii, Cryptococcus neoformans, and cytomegalovirus. Serum IgG, IgA and IgM concentration were elevated. The percentage of T-lymphocytes was decreased, but T-cell response to mitogen was normal...The infant died of respiratory insufficiency at 7 months of age...His parents were residents of Brooklyn, New York; their health status is unknown".

A Haitian infant was hospitalised at 5 months with fever and respiratory distress. "Despite antibiotic therapy, the infant's condition deteriorated and an open lung biopsy revealed PCP. Immunological studies showed elevated serum concentration of IgG, IgA and IgM, decreased percentage of T-lymphocytes and impaired T-cell function in vitro". The infant died. The parents' "health status is unknown".

The infant of a white prostitute and IV drug user with a history of oral candidiasis developed vaginal and oral candidiasis at 2 months of age, which responded to therapy. At "5 months, candidiasis recurred and she had hepatosplenomegaly. Immunological evaluation showed that serum IgG, IgA, and IgM levels, normal at 2 months, were now elevated. The percentage of T-lymphocytes was decreased and lymphocyte response to alloantigens was impaired. At 6 months of age, the infant was hospitalised because of fever and cough. Open lung biopsy revealed PCP. Despite appropriate antibiotic therapy, she died".

The above 4 cases were summarised as follows: "It is possible that these infants had the acquired immune deficiency syndrome (AIDS)…Although the aetiology of AIDS remains unknown, a series of epidemiologic observations suggests it is caused by an infectious agent. If the infants described in the four case reports had AIDS, exposure to the putative "AIDS agent" must have occurred very early. Cases 2-4 were less than 6 months old when they had serious opportunistic infections. Case 1 had oral candidiasis beginning at 3 months of age, although M. avium-intracellulare infection was not documented until 17 months. Transmission of an "AIDS agent" from mother to child, either in utero or shortly after birth, could account for the early onset of immunodeficiency in these infants".[201]

Immune deficiency and illness in Black and Hispanic children. Parenteral drug use, neglect and malnutrition or an infectious agent?
In 1983 researchers from New Jersey published a paper entitled "Immune Deficiency Syndrome in Children" They described clinical and immunological findings in 8 infants/children (5 black, 2 black/hispanic, 1 hispanic). The mothers of 4 of them and the fathers of another 2 admitted to IV drug use. All the children had interstitial

pneumonia, 4 had salmonella, 2 PCP and most of them had candida. In 5 children who were tested the T4/T8 ratio was found to be low, and most had increased levels of immunoglobulins. The authors acknowledged that it might "be argued that the illnesses and immune defects in our patients were primarily the result of neglect and malnutrition" and that "*Pneumocystis carinii* pneumonitis has been described in young immunocompetent infants". Nonetheless "It seems more plausible to us that the illnesses in these youngsters were related in some way to household exposure and their residence in communities involved in the current epidemic of AIDS...The early onset of the disease in several patients and the fact that in six of eight instances it was the patient's mother who represented a risk factor do raise the possibility of vertical spread of disease".[202]

Intrauterine-acquired immunodeficiency caused by "reactivated EBV" results in low birth weight, low T4 and low T4/T8 ratios, and AIDS-like diseases in children of Black, promiscuous, drug addicted mothers.
In the same year, 1983, the clinical and immunological findings in 7 black children from New York were described in a paper entitled: "Acquired Immunodeficiency With Reversed T4/T8 Ratios in Infants Born to Promiscuous and Drug-Addicted Mothers". In one case "The clinical history of the mother was unknown", one of the mothers was "alcoholic, and cachectic…and refused medical care". Another "mother was unavailable for medical examination" but, "Heroin withdrawal symptoms were noted in this infant at birth", and 3 of the mothers admitted to IV drug use. All children had interstitial pneumonia, diffuse lymphadenopathy and hepatosplenomegaly. Five had parotitis, 2 chronic gastroenteritis, 6 failed to thrive and 6 were small for gestational age. "All seven children had low percentages and absolute numbers of T_4 cells. T_8 cells were noticeably elevated and the T_4/T_8 ratios were decreased". Six "had a polyclonal IgG elevation with levels up to ten times that of the normal age-matched controls…In five of the children and in three of their mothers, there is evidence of a persistent Epstein-Barr virus (EBV) infection". Commenting on their findings the authors wrote: "We have described seven infants who exhibit a syndrome with clinical, histopathologic, and immunologic features similar to those observed in adults with AIDS…Two observations lend credence to the hypothesis of in utero vertical transmission: (1) the clinical symptoms often started at birth, and (2) six of seven infants were small for gestational age, suggesting an in utero insult…We postulate that this newly described syndrome is related to an intrauterine-acquired infectious immunodeficiency transmitted by reactivated EBV infection in sexually promiscuous and drug-addicted mothers".[203]

Recurrent bacterial, viral and fungal infections in three infants of a drug addicted prostitute. Two children died from Pneumocystis carinii pneumonia.
In a 1984 paper entitled "Maternal Transmission of Acquired Immune Deficiency Syndrome" researchers from the University of California, Davis, described 3 half-siblings born to a 29-year white woman. At 16 she began using marijuana and LSD, at 19 intravenous amphetamines and at 20, heroin. "She has been a prostitute in San Francisco since age 23 years (1976), and estimates that between ages 23 to 27 years she had 1,500 sexual contacts per year. For the past 1 to 2 years she has averaged 200 contacts per year. Her contacts have been exclusively men, but a significant number have been bisexual/homosexual". Her first child, a black female was born at 26 weeks gestation and weighed 860g. "The postnatal period was complicated by bronchopulmonary dysplasia, bilateral ventricular hemorrhage, acquired cytomegalovirus infection, and *Staphylococcus epidermidis* sepsis. During her hospitalization she received more than 40 RBC transfusions. At 11 months of age, she developed intermittent fevers with lymphadenopathy and an erythematous skin rash of unknown etiology, which resolved over a 1-month period. Subsequently, she had two episodes of pneumonia requiring hospitalization". At 21 months of age she was admitted to the University of California, San Francisco (UCSF) "with severe bilateral pneumonia and respiratory distress. In addition, she had otitis media, oral thrush, diffuse adenopathy, and hepatosplenomegaly. Open lung biopsy revealed interstitial pneumonitis and cultures grew cytomegalovirus. In addition, throat cultures were positive for cytomegalovirus, adenovirus, and parainfluenza type III...Over the next 20 months of life, she required several hospitalizations for failure-to-thrive and repeated infections, especially pneumonia. In spite of aggressive pulmonary support and intravenous γ-globulin and prophylactic trimethoprim-sulfamethoxazole, her chronic lung disease worsened until she required continuous oxygen therapy. At age 48 months, she was admitted in respiratory distress to her local hospital where she died in respiratory failure. Autopsy revealed massive pulmonary infiltration with *Pneumocystis carinii*".

The second child was born at 30 weeks gestation. "Her postnatal course was complicated by hyperbilirubinemia which gradually resolved…At 8 months of age, she began experiencing upper respiratory tract infections with fever. She was admitted to UCSF at age 14 months for persistent otitis media and recurrent upper respiratory tract infections. Positive findings included thrush, severe diaper dermatitis, bilateral otitis media, moderate hepatosplenomegaly, and large tonsils and severe diffuse lymphadenopathy. Open lung biopsy revealed interstitial pneumonitis; cultures of lung for bacteria, fungus, and virus, and staining for *Pneumocystis* and acid-fast bacilli were negative. A pharyngeal culture grew EBV. The patient also had significantly increased frequency of EBV-transformed peripheral blood non-T-cells which has persisted for 2 years. Between 15 and 36 months of age, she had several hospitalizations for otitis media and pneumonia".

The third child was born at 35-weeks gestation. "Her postnatal course was uncomplicated. At 2 months of age she had an upper respiratory tract infection associated with *Candida* sp infection in the mouth and vaginal area which responded to antifungal therapy. At that time she had no evidence for lymphadenopathy or hepatosplenomegaly and her immunologic studies were normal". By 5 months of age the child had "developed significant hepatosplenomegaly associated with significant abnormalities in her cellular immunity and polyclonal gammopathy. She was admitted to UCSF at 6 months of age with fever and a dry hacking cough. Open lung biopsy revealed *Pneumocystis carinii* pneumonia and she was treated appropriately…after 2 weeks of therapy she developed bilateral pulmonary infiltrates and respiratory failure, which did not respond to broad-spectrum antibiotics or anti-protozoal and antifungal therapy, and resulted in death". All children had hypergammaglobulinaemia, 2 children (1 and 2) had low T4 and T4/T8 ratio. The third had high levels of T4 and T4/T8 ratio. Summarising their findings the authors of this study wrote: "These findings and the clinical histories indicate AIDS and strongly suggest vertical transmission of an agent(s) during the perinatal period".[204]

MCT of HTLV-I postulated to cause AIDS and AIDS-like diseases in infants born to Black and Haitian mothers.
Fourteen children, 9 with "AIDS with opportunistic infection and/or Kaposi's sarcoma" and 5 with "AIDS-like illness (without opportunistic infection or Kaposi's sarcoma) were "admitted to the pediatric service at Jackson Memorial Hospital in Miami, Florida, during the period November 1980 through June 1983…Jackson Memorial Hospital serves the greater metropolitan Miami area, and Haitian infants accounted for 16.3 per cent of approximately 8000 births at this center in 1981, and 20 per cent of approximately 9000 births in 1982". Eleven of the children were born to Haitian parents, one to black parents and the other two mixed, white/black, black/Haitian. "The most consistent clinical finding in the 14 patients was severe failure to thrive. Persistent infection of the oral mucosa with *C. albicans* was found in 12 patients, and there was extension of the infection into the esophagus in 3 patients…Persistent pulmonary infiltrates were found in 11 patients, and 3 had digital clubbing. Lung biopsies revealed a lymphoid interstitial pneumonitis in six. Patient 7 presented at 13 months of age with a progressive pneumonitis and candida esophagitis. A lung biopsy could not be done because of the patient's deteriorating respiratory status; however, she responded to an antibiotic regimen that included trimethoprim-sulfamethoxazole. This infection was clinically compatible with *Pneumocystis carinii* pneumonitis but remains undocumented. A lung biopsy performed one month later because of persistent pulmonary infiltrates showed changes compatible with respiratory-distress syndrome. Hepatosplenomegaly was present in 13 patients, and elevated liver enzymes were detected in 8. Five patients had generalised lymphadenopathy…Diarrhea occurred in nine patients, and six of these had a protracted course. Seven patients had clinical evidence of atopic dermatitis. *P. carinii* pneumonitis [4 cases] and cytomegalovirus pneumonia were the most common types of opportunistic infection found in these infants. Two patients had histologic evidence of Kaposi's sarcoma in the lymph nodes and spleen at autopsy…Immunological studies showed most of the infants to have inverted ratios of T-cell subsets [two of the children reported with PCP had normal T4/T8 ratios, "at the time of diagnosis"], greatly increased immunoglobulin levels, and circulating immune complexes". Discussing their findings the authors wrote: "It would therefore appear that this series represents a pediatric form of AIDS. The existence of a pediatric form of AIDS transmitted other than by transfusion suggests the possibility of transplacental, perinatal, or postnatal transmission. The mothers of the patients in the present series are being investigated, and one has clinical AIDS…The novel aspects of AIDS and the expanding insights into the epidemiology of the condition strongly support an infectious cause and suggest the involvement of an unusual agent, such as the human T-cell leukemia retrovirus".[205]

45 children and 25 adults. 34 children seropositive (=antibodies reacting with p41). Virus isolation=detection of reverse transcription. Virus "isolated" from 2 seronegative children. Marked hypergammaglobulinemia present in the majority of children.
In 1985 Gallo, Popovic and their associates published a paper entitled "Spectrum of Human T-cell Lymphotropic Virus Type III Infection in Children". "Pediatric subjects in this study consisted of (1) referrals to Medical Centers in Nassau County (Long Island, NY) and Brooklyn, NY, for illnesses subsequently established to be associated with evidence of HTLV-III/LAV infection, (2) healthy siblings of symptomatic children with HTLV-III/LAV infection, and (3) healthy offspring of adults who had been diagnosed as having AIDS or ARC and being HTLV-III/LAV antibody positive. Parents of affected pediatric subjects were studied whenever possible. A total of 45 children and 25 adults were included in this study…Serologic testing for HTLV-III [HIV] antibody was performed by Western blot analysis. Specifically, presence of p41 antibody…was considered as evidence of HTLV-III/LAV viral infection…We identified 36 children with serologic and/or virologic evidence of HTLV-III infection. Sera of 34 children were positive for antibodies to various antigenic components of HTLV-III/LAV virus, including p41 fraction, in all cases…Eighteen children were investigated for presence of HTLV-III/LAV virus in their peripheral blood lymphocytes, virus was recovered in eight of ten children who were antibody positive and in two of eight children who were antibody negative". By the "presence of HTLV-III/LAV virus in their peripheral blood lymphocytes" was meant detection of reverse transcription in co-cultures of the childrens' lymphocytes with cells from the malignant cell line H9. At the time of testing, of the 36 infected children, 14 had AIDS and 7 were asymptomatic.   "Marked

hypergammaglobulinemia involving IgG, with or without increases in other immunoglobulin classes was present in the majority" of children. Twelve of the children had T4 cells counts less than 400/mm^3. "All mothers of affected children who were tested had anti-p41 HTLV-III antibodies". Discussing their findings they wrote: "All of 20 mothers who were studied were HTLV-III antibody positive and had immunologic abnormalities but only nine were symptomatic indicating that apparently healthy women may transmit HTLV-III/LAV infection to their offspring".[206]

Positive PCR in children with and without AIDS born of socio-economically disadvantaged, drug addicted mothers.
In 1985, the Centres for Disease Control (CDC) initiated two collaborative studies to examine the perinatal transmission of HIV. One study, the New York City Collaborative Study of Maternal HIV Transmission, coordinated by the New York City Department of Health, was conducted at five hospitals in New York City (Harlem, Lincoln, Bellevue, Metropolitan, and Mount Sinai Hospitals). The other study was conducted at the Montefiore Medical Centre Bronx, N.Y. "In both studies, women at an increased risk for HIV infection were enrolled in the prenatal period" . Because, "The diagnosis of HIV infection in infants has been hampered by the presence of passively acquired maternal antibody, the difficulty in performing viral culture, the lack of reliable tests for HIV-specific IgM, and the presence of nonspecific signs and symptoms of HIV infection in infants", these researchers used the PCR. "The polymerase chain reaction was performed directly on cells obtained from the infants during the neonatal period and on cells placed in culture from specimens obtained later in life". "As of May 1, 1988, 316 women had been enrolled in the two studies; 110 (35 percent) were seropositive for HIV. These women had given birth to 212 infants; 87 (41 percent) of the infants were born to seropositive women. Most (70 percent) of their mothers were intravenous drug abusers. Most of the infants belonged to minority groups: 31 percent were black, 50 percent were Hispanic, 19 percent were white, and less than 1 percent were of other racial or ethnic groups. Most of the mothers lived in impoverished inner-city environments…All 87 infants of HIV-seropositive mothers were positive for HIV antibody during the neonatal period, according to both enzyme immunoassay and Western blot testing…Thirty of the infants born to seropositive mothers reverted from seropositive to seronegative". In the cells obtained from the infants proviral sequences were detected in 5/7 with AIDS, 1/8 ill with non-specific findings suggestive of HIV infection and 0/9 clinically well. In the cultured cells proviral sequences were detected in 6/6 from children with AIDS, 4/14 "ill with non-specific findings suggestive of HIV infection" and 0/15 clinically well.[207]

HIV Infection more common in first-born than in second-born twins. HIV infection in the child (HIVIC) defined as "HIV-related disorder" at any age or a positive antibody test beyond 15 months of age. MCT=32%.
To obtain information on HIV transmission in twins, "In late 1990, invitations to join the registry and sample data forms were sent to 235 clinicians and researchers including paediatricians, obstetricians, and infectious disease specialists", by researchers from three US Institutions. "40 investigators in nine countries contributed demographic, clinical, and epidemiological data on 100 sets of twins and 1 set of triplets (78 from the USA, 6 from France, 4 each from the Congo and the UK, 3 each from Italy and Spain, and 1 each from Australia, Puerto Rico and Switzerland)". Thirty seven of the mothers were Black, 14 White and 15 "Other/unknown" Of a total of 66 sets with complete data, 22 (32%) were infected. "HIV-1 infection was more common in first-born than in second-born twins". In 22 sets, only one twin was infected (18 first-born, 4 second-born). "The definition of HIV-1 infection was framed to include all children with an HIV-1-related disorder at any age or persistence of HIV-1 antibodies beyond 15 months".[208]

MCT from Haitian mothers. HIV not culturable from all seropositive children until 1-2.5 years of age. MCT=30%.
In Miami, USA, "From August 1, 1986, to December 31, 1988, a total of 4168 live births to Haitian-born women at Jackson Memorial Hospital were identified by hospital birth certificates. Residual serum for 3650 (88%) of the mothers was recovered and screened. Of those screened, 168 (4.6%) were HIV-1 seropositive…The infection status of 82 of the 112 enrolled index infants born between August 1986 and January 1989 was determined. Twenty-five (30%) of the 82 infants were infected. In all 25 infected infants, HIV-1 was isolated from the lymphocytes. Cultures were positive in 21 infants (84%) by 6 months of age and in all but one of the other three infected infants by 12 months of age. In a single infant, virus cultures were not positive until 30 months of age, after four previous cultures were negative during the first 2 years of the study".[209]

HIVIC ≡ "Signs of HIV-1 disease" or a positive antibody test beyond 15 months of age. MCT=29%
In 1989 researchers from seven New York institutions offered counselling and screening for HIV antibodies to "All pregnant women at the State University of New York Health Sciences Center in Brooklyn, New York, who were known to be drug abusers, of Haitian origin, or sexual partners of men at risk of AIDS". They reported that of 55 infants born to these women, "most of whom were drug-users or of Haitian origin, 16 infants were infected with HIV-1, a crude rate of 29%…Infants were considered uninfected only if they had reverted to being

seronegative for HIV-1 and reached the age of 15 months with no signs of HIV-1 disease". Fifteen of the children had "AIDS disorders" (3 PCP, 1 encephalopathy, 3 lymphocytic interstitial pneumonitis, 1 salmonella sepsis, 1 recurrent bacterial pneumonia, "6 had other HIV-1-related clinical disorders"), and 1 had no clinical manifestations, but had a positive antibody test beyond 15 months.[210]

Pre-term delivery and low birth weight related to drug addiction. Low birth weight associated with MCT. MCT=23.1%.
In a study published in 1993, researchers from the University of Maryland, evaluated the infectious status of 134 infants, born to HIV positive women. "Drug use prior to and during pregnancy was high in this population, and approximately a quarter of the mothers were on methadone maintenance". Of the 134 infants "31 have definite serological and/or clinical evidence of infection and 103 are considered noninfected (transmission rate, 23.1%)…Multiple logistic regression analysis indicated low birth weight had the strongest association with vertical transmission of HIV…Pre-term delivery and low birth weight were thought to be related to drug abuse during pregnancy or a direct effect of maternal HIV infection".[211]

HIV culture (p24 detection) "reference standard" for PCR. Positive cultures revert to negative. HIVIC ≡ two or more positive cultures. MCT=18.2%.
According to researchers from several Institutions in the USA, although by 1994 the PCR has been used to diagnose early HIV infection, in many studies "culture of blood or other body fluids remains the reference standard for diagnosis in young infants". Because of this, they "analyzed the interim data from the Women and Infants Transmission study (WITS), a multicenter, prospective "natural history" study of HIV-infected women and their infants to determine the diagnostic utility of HIV culture in the first 6 months of life...For the purposes of this study, a quantitative culture was considered positive if any two wells in the first four of the dilution series contained ≥30 pg/mL p24 antigen at either 14 or 21 days after culture…The analysis included 311 infants for whom evaluable culture data from two or more visits, at least one of which occurred at ≥6 months of age, were available. For the purposes of this study, a baby was considered infected when ≥2 peripheral blood cultures were positive; babies were considered uninfected when ≥2 cultures, all of which were obtained at or after 1 month of age and 1 of which was obtained at or after 6 months of age, were negative and there were no positive cultures. The infection status for 302 of the 311 eligible infants was determined according to this definition", and 55 were infected. Six children had one "positive culture followed by ≥2 negative cultures and negative serology at 18 months".[212]

HIVIC ≡ detection of p24 in culture at least twice. No age limit. Culture not reproducible. Clinical data, serology and PCR needed to "interpret" culture in nine children. Drug using mothers more likely to transmit. AZT not associated with a significant decrease in MCT (18% vs 20%). MCT=20.8%
An update of the Women and Infants Transmission Study (WITS) conducted in Chicago (Illinois), New York City and Brooklyn (New York), San Juan (Puerto Rico), and the Boston-Worcester area in Massachusetts, was published in 1996. Between September 1989 and September 1994, 876 pregnant infected women were enrolled, the majority of which were Black or Hispanic. "Overall, 223 out of 530 (42%) women used hard drugs during pregnancy. Six per cent of women who used hard drugs reported no drug use but had positive urine toxicology". As in the previous WITS study "an infant was defined as HIV-infected if two or more peripheral blood mononuclear cell (PBMC) cultures were positive for HIV (cord blood not included). An infant was defined as uninfected if all PBMC HIV cultures were negative". Of 530 infants included in the analysis, 110 (20.8%) were classified as HIV-infected and 420 as uninfected. Nine children who had 1 positive culture "were subsequently classified by an ongoing indeterminate review committee as uninfected, on the basis of review of laboratory (e.g. serology, DNA polymerase chain reaction) and clinical data". Summarising their findings the authors of this study wrote: "…overall maternal hard drug use and cocaine use in the WITS cohort were associated with maternal HIV culture positivity at delivery, and maternal hard drug use was associated with perinatal transmission…ZDV was not associated with a significant decrease in transmission in this overall cohort (18 versus 20% transmission in ZDV users versus non-users)".[43]

HIV infection in children of 175 Black and Hispanic mothers. HIVIC ≡ two positive PCRs, AIDS or HIV-related disease, positive antibody test at 15 months. Drug using mothers less likely to transmit HIV. Risk of transmission increased by unprotected intercourse during pregnancy. MCT rates vs nil, moderate frequency and higher frequency of intercourse = 9.1%, 22.2% and 39% respectively.
In a retrospective analysis of 175 HIV seropositive women in the prospective Perinatal HIV Transmission Collaborative Study Group conducted at six New York City Medical Centres, 161 of the women were either Black (89) or Hispanic (72). In this study "Infants were considered HIV-1 infected if they (1) were PCR or culture-positive on two different samples, (2) met the 1987 Centers for Disease Control and Prevention criteria for class P2 with either an AIDS defining illness or death attributed to an HIV-related illness, or (3) were HIV-1-seropositive by Western blot at 15 months of age. HIV-1-uninfected infants were children without HIV-1-

related illness who were born to HIV-1-seropositive women and who were either Western blot-seronegative on one or more occasions, or had at least two negative PCR or culture results, with one beyond 2 months of age, and no positive results". Unlike the WITS, in this study "injecting drug users (IDU) were less likely to transmit HIV-1 than non-IDU". The transmission was significantly associated with unprotected sexual intercourse (despite the fact that the "Frequency of unprotected intercourse was associated with ZDV use during pregnancy"). "The rate of perinatal HIV-1 transmission was 9.1% (4 out of 44) among women with no unprotected intercourse during pregnancy, 22.2% (20 out of 90) among those with moderate frequency and 39.0% (16 out of 41) among those with high frequency (P <0.01)". Commenting on their findings the authors wrote: "Repeated antigenic stimulation by semen or spermatozoa from the same or different sex partners may produce an increase in cell-mediated immune activation in women".[122]

Predominantly Black and Hispanic mothers. Prevalent drug use. Birth weight and prematurity common and similar to low income mothers of same ethnicity. HIVIC ≡ two positive cultures, one at 5 months of age. Antibody tests at 15 months to confirm negative status. Unable to determine HIV status in 10%. AZT used in one third of women but no data on effects including transmission. MCT=17%.

The first report from The Paediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted Human Immunodeficiency Virus ($P^2 C^2$ HIV) Infection Study was published in 1996. The infant/children cohort consisted "of 205 infants and children enrolled after 28 days of age (Group I) and 612 foetuses and infants of HIV-infected mothers, enrolled prenatally (73%) or postnatally at age <28 days (Group II). Tests for HIV transmission were conducted only in Group II. In Group II, 49.6% of the mothers were Black, 32.2% Hispanic, 15.8% White non-Hispanic and 2.5% others. 33.2% had a medical history of anaemia; 31.5% of the women self-reported using IV drugs while pregnant and 23.7% the use of cocaine/crack during the same period. 8.1% reported "sex with homosexual men". Of 212 infants and children whose urine was tested "for illicit drugs" and for cocaine/crack" 55.8% and 39.6%, respectively were found positive. "The children born to HIV-infected mothers had a high rate of prematurity (gestational age <37 weeks) and low birth weight (weight <2500g). However, these rates are similar to previously reported rates for low-income African-American and Hispanics in the United States". To estimate transmission rates, "HIV cultures are performed on Group II children at birth (not cord blood), and 3 and 6 months postnatally. Two positive cultures are necessary to assign a patient to Group IIa (infected). Two negative cultures, one of which must be when the child is 5 months of age or older, are necessary to assign a patient to Group IIb (noninfected). An ELISA and, if necessary, Western blot are performed on all Group IIb patients at a minimum age of 15 months to confirm their HIV-negative status…As of March 1, 1996, 62 (10%) of the 612 Group II foetuses and infants were HIV indeterminate, and of the remainder, 95 (17%) were HIV infected". No mention is made if AZT had any effect on transmission, although 34.1% of the women used the drug during their pregnancy.[213]

NIH/CDC study linking transmission to maternal sexual behaviour and drug use. HIVIC ≡ CDC AIDS defining illness or antibodies beyond 15 months or two IgA immunoblots beyond six months of age. Sexual intercourse and IDU during pregnancy but not chronic drug use risk factors. Vaginal vs Caesarean delivery and vaginal infection not risk factors. MCT=24.4%.

The data from the Mother and Infants Cohort Study in Brooklyn and Bronx, New York was evaluated by researchers from the National Cancer Institute, State University of New York, National Institute of Child Health and Human Development and the Albert Einstein College of Medicine for a possible relationship between transmission, maternal sexual behaviour and injecting drug use. "Infants were classified as HIV-1 infected if they developed an AIDS-defining illness according to Centres for Disease Control and Prevention surveillance criteria or if they remained ELISA and Western blot seropositive at 15 months of age or older or two consecutive anti-HIV-1 IgA immunoblot assays, at least one of which was performed at 6 months of age or later, were positive…HIV-1 transmission occurred in 49 of 201 mother-infant sets, yielding an overall transmission rate of 24.4% (95% confidence interval (CI) = 18.7% to 31.0%). Increased frequency of vaginal intercourse after the first trimester of pregnancy was positively associated with vertical transmission of HIV-1 (trend p = 0.03). A lifetime history of injection drug use was not associated with vertical transmission. However, a history of combined cocaine and heroin injection after the first trimester of pregnancy was associated with vertical HIV-1 transmission, particularly among women with CD4$^+$ lymphocyte levels of 20% or higher (risk ratio = 4.0; 95% CI = 2.0 to 8.1). Cocaine and heroin injection drug use after the first trimester accounted for most of the relation between preterm birth and vertical HIV-1 transmission in this cohort…Route of delivery (cesarean versus vaginal) and any evidence of active genital infection at the time of enrolment were not associated with vertical transmission of HIV-1".[214]

1,702 liveborn deliveries from 1,517 HIV positive Medicaid-enrolled mostly Black or Hispanic women. HIV infection defined clinically by an expert panel, or according to CDC 1987 definition, or seropositivity beyond 18 months, or multiple HIV-associated complications; or were treated with AZT. Maternal smoking increases risk of transmission by 45%. MCT=41% for women with advanced disease; 46% with pneumonia or TB.

In a study published in 1997, "HIV-infected women with at least one liveborn delivery from 1988 through 1990 were identified from New York State (NYS) Medicaid data files". Subsequently, "1,517 HIV-infected, Medicaid-enrolled women with 1,702 liveborn deliveries were identified". The infectious status of the children were identified as follows: "Three HIV/AIDS specialists (two paediatricians and one general internist) identified conditions characteristic of pediatric HIV infection and developed criteria to classify a child as HIV-infected because we did not have access to laboratory testing data on the child's serological or virological status…Because passively transmitted maternal antibody to HIV can persist in infants up to 18 months after delivery and after assessing our classification with primary data as noted above, identification of HIV infection based on a HIV-seropositive test or HIV diagnosis alone was accepted only for children aged ≥ 18 months. A child at any age was considered HIV infected if diagnosed with lymphoid interstitial pneumonitis or an opportunistic infection such as *Pneumocystis carinii* pneumonia (PCP) in the Centers for Disease Control and Prevention's (CDC) 1987 AIDS surveillance case definition. These children delivered by HIV-infected women were also judged infected if they had multiple HIV-associated complications, such as hepatosplenomegaly and pulmonary tuberculosis, or were treated with zidovudine, as our study preceded the 076 trial". The authors reported the following findings: "The maternal-child HIV transmission rate for our cohort was estimated to be 24.5% at 3 years' follow-up. Most study women were either black (41%) or Hispanic (42%)…The transmission rate for women with advanced HIV was 41%; for women diagnosed with pneumonia or pulmonary TB during pregnancy, it was 46%. More than a third of the off-spring of women diagnosed with anaemia were identified as infected…Because cigarette smokers, cocaine users, and women using mixed or unspecified illicit drugs had higher unadjusted transmission rates, we further examined bivariate associations of women engaging in these practices with a variety of demographic, clinical, and health care variables. Black and white women were more likely to smoke than were Hispanic women, and the proportion of black women using cocaine was twice that of any other racial-ethnic group…Our analysis indicates that smoking conferred a 45% increase in the risk of vertical HIV transmission after adjusting for a wide variety of maternal and health care factors. Our results are consistent with the observations of Burns et al. regarding the adverse association of smoking with maternal-child HIV transmission…Smokers in our cohort were much more likely to have a low birth-weight infant and, in general populations, cigarette smoking has been shown to have a dose-related effect on birth weight. Low birth-weight has been associated with transmission by several studies".[44]

Ariel study of 204 mothers and 209 children by David Ho et al. HIVIC ≡ twice detection of p24 in cell culture or once plus a "reproducibly positive" PCR. 85% of mothers took AZT. Transmission rate not significantly influenced by AZT, viral load or T4 cell count. MCT 8.3% vs 13% in mothers who did/did not take AZT.

In the same year, 1997, David Ho and his associates from the Aaron Diamond AIDS Research Centre, the UCLA School of Medicine, the Santa Fe Institute and Los Alamos National Laboratory, the University of Manchester Institute of Science and Technology, England and other Institutions, published data from the Ariel Project for prevention of HIV transmission from mother to infant. In this project in which "women and their infants at seven clinical sites" were enrolled, "Infected pregnant women were eligible for participation in the study if they were >13 years of age, did not breast feed and did not use experimental vaccines, immunoglobulin infusions, or other immunomodulatory therapy". In this study "An infant was considered to be infected if a viral culture was positive and if a PBMC sample from another visit yielded a positive culture or reproducibly positive PCR results". The authors analysed a total of 204 women, 5 of whom gave birth to twins. "Of the 209 children, 19 acquired HIV-1 infection, including one infant from the five pairs of twins, resulting in an overall transmission rate of 9.1%. Transmitting and nontransmitting mothers did not differ statistically in their percent CD4^{+} lymphocytes, Centres for Disease Control (CDC) disease classification, or mode of delivery. This cohort is marked by extensive use of zidovudine: 85% of the women used this antiretroviral drug at some point during pregnancy, including 15 of 19 transmitting mothers. Most initiated zidovudine therapy during pregnancy, but others were receiving it before pregnancy and simply continued the treatment. Five nontransmitters were given didanosine or zalcitabine. No other antiretroviral therapy was given. The transmission rate among mothers who initiated zidovudine use during pregnancy was 8.3% (95% confidence interval (CI) = 5-13%). The transmission rate for mothers who never received zidovudine was 13% (95% CI = 4-30%)", transmission rates which "are not significantly different from one another" Ho and his associates also reported that "Neither CD4^{+} lymphocyte counts nor viral load measurements varied significantly during pregnancy for either transmitters or nontransmitters. The distribution of values for the rate of change in plasma RNA levels scattered equally above and below zero during pregnancy. In contrast, plasma HIV-1 RNA rose significantly after delivery, as is also evident by the larger number of values distributed above zero. Copy numbers of viral DNA in PBMCs increased similarly post partum, concurrent with an appreciable decline in CD4^{+} lymphocytes…The differences between the pre-and post-delivery periods did not appear to result from differing periods of follow up, although the post-partum period of observation (mostly 175-200 days) was somewhat longer than that during pregnancy (typically 80-150 days). These changes cannot be attributed to the cessation of zidovudine since most women continued this therapy post partum".[215]

31

Risk of transmission increased by low T4 cells, rupture of the membranes, maternal bleeding and EBV infection. Minimal effect of AZT in reducing transmission. One third of subjects also reported in WITS.

In a 1997 publication from the P^2C^2 HIV study the authors evaluated the association of maternal and perinatal factors in transmission. "In a multivariate analysis of 74 transmitters and 369 nontransmitters, decreased maternal CD4% (p = .002), rupture of membranes >24 hours (p = .02) and maternal bleeding (p = .03) were found to be significant predictors of increased transmission risk…When ZDV use during pregnancy was included in the model, it was marginally associated with decreased risk of transmission…whereas maternal CD4%, ruptured membranes and maternal bleeding remained statistically significant". In univariate analyses, maternal Epstein-Barr virus shedding was higher among transmitting than nontransmitting mothers and was independently associated with transmission in multivariate logistic analyses. (30% of the infant-mothers pair in the P^2C^2 were simultaneously enrolled in the WITS).[216]

Update of Ariel study. HIVIC ≡ twice detection of p24 in cell culture or once plus a "reproducibly positive" PCR. No significant effect of AZT on MCT. Transmission study site dependent. No relationship between PCR and antibody test even at 18 months. Viral load and T4 cell count not risk factors. MCT=8.6% vs 9.1% with vs without AZT. Extreme variation of MCT between localities.

In 1999, David Ho and his associates published an update on the Ariel project. They reported a transmission rate of 9.1% and "a trend toward a lower transmission rate among women who received zidovudine", 8.6%. "The maternal-child transmission rates at the seven clinical sites varied widely, and the differences were statistically significant (P = 0.026, χ^2 test). For instance, the Houston site, with 33 evaluable mothers, had no infected infants, while New Orleans, with 36 evaluable mothers, had 9 infected infants of 38 (including 2 sets of twins)…However, sequence analysis of PCR-amplified viral RNA from 7 infected infants from New Orleans, one infected infant from Worcester, and three nontransmitting mothers from Houston did not suggest a clustering of related viral genotypes at any site". At one year of age 56% of uninfected children "had a positive or indeterminate" antibody test, and even at 18 months 14% still had a positive or indeterminate antibody test. "By univariate analysis, three factors were significantly associated with transmission; histologic chorioamnionitis, a longer duration of rupture of fetal membranes, and a history of genital warts in the mother...A number of factors (including CD4 and viral load) predictive of maternal-child transmission in other studies were not important in our study".[217]

2.3   Studies from Europe, Australia and Canada

In 1983 researchers from the Sainte-Justine hospital in Montreal, Canada reported an infant born to a Haitian woman who had an undifferentiated lymphoma and miliary tuberculosis, and who died 6 weeks after giving birth. In the 3rd week of life the infant had fever, otitis media, hepatosplenomegaly and anaemia and by 3 months had oral and pharyngal candidiasis, persistent hepatosplenomegaly and cough. The infant was discharged but at the age of 4 months was readmitted with "persistent candidiasis, progressive hepatosplenomegaly, and lymphadenopathy. She had fever and diarrhoea". CMV was isolated from the urine and throat swab. "Epstein-Barr virus infection was documented by an Epstein-Barr virus: viral capsid antigen titer of 80:160...The lymphocyte count dropped to 4% of a total white-cell count of 5000 per cubic millimeter, and the T cells fell to 28 and 16%". The infant did not respond to treatment. "An autopsy revealed generalized cytomegalovirus infection and *Pneumocystis carinii* pneumonia. Cytomegalovirus was isolated from liver and lung specimens". The authors concluded: "These observations may shed new light on a subset of severe combined immunodeficiency diseases with possible infectious causes".[218]

A year later, one of the authors of the above report and a number of other researchers from the same hospital "studied a premature child born by cesarean section after 28 weeks of gestation to a mother with terminal AIDS". The child had no opportunistic infections, had normal T lymphocytes and lymphocytes subset and a negative HIV antibody test. "He died at 20 days of age with severe hydrocephaly and sequelae of intracranial haemorrhages". Anti-HIV-IgG chromatically "purified from plasma obtained from patients with AIDS or normal Haitians…reacted strongly with the thymus of the infant". The authors concluded "these findings strongly suggest transplacental transmission of HTLV-III" (HIV).[219]

A report entitled "LAV/HTLV-III In 20-Week Fetus" was published in the same year, 1985. The pregnancy of a heroin addict who was found to have a positive antibody test, was terminated at 20 weeks. Cells obtained from the foetal thymus, lungs, liver, spleen and kidney were co-cultured with cord blood lymphocytes "derived from placentas of anti-HTLV-III negative mothers". After 11 days supernatants from the co-cultures were added to "Polybrene pretreated H9 and MT-2 cells". "In MT-2 cultures which had been treated with supernatants from co-cultivated thymus, lung, spleen and brain, ballooning, multinucleated cells appeared 48 hr later". No such effects were seen in simultaneously cultured H9 cells even after 3 weeks. The MT-2 and H9 cells cultured with supernatants from the co-cultures containing thymus, lung, spleen and brain cells, "gave a brilliant fluorescence" when reacted with "a pool of human sera containing HTLV-III antibodies". The authors concluded: "Thus from

organs of a 20-week old fetus of an HTLV-III infected mother an agent was isolated by co-cultivation with cord blood lymphocytes which induced in MT-2 cells a characteristic cytopathic effect and led, in H9 and MT-2 cells, to the expression and release of antigens reacting with HTLV-III specific antibodies. This shows that intrauterine infection with HTLV-III can occur as early as the 20th week of gestation".[220]

Comment
It is impossible to claim detection of a virus, much less isolation, based on non-specific phenomena including cytopathic effects or an antigen/antibody reaction no matter how "brilliant" the fluorescence. Even if the authors had proof for the isolation of a retrovirus, since (i) they used lymphocytes derived from the placenta; (ii) placentas harbour endogenous retroviruses;[98,221-227] the retrovirus may be endogenous.

Seventy one infants from Europe. Five with AIDS or ARC. Mothers drug users. Antibody tests not standardised. HIVIC ≡ positive culture or AIDS/ARC. No correlation between "culture" (p24 or RT) and antibodies. Cultures not reproducible. MCT=22%.
The preliminary findings of the European Collaborative Study (ECS) were published in 1987. The authors of this study analysed data obtained on all children born between February 1985 and October 1986 to seropositive women at three European centres; Padua, Berlin and Edinburgh. The median follow-up of the children was 28 weeks (range 3-69 weeks). The infectious status of the children was determined by testing for antibodies, p24 antigen and by culture. The antibodies were detected by ELISA and confirmed by WB, "except in Edinburgh where positive results were confirmed by repeat analyses" with ELISA. Virus "isolation" was determined by "measuring reverse-transcriptase activity in culture supernatants" (RT) although "Virus culture and/or antigen tests were not routinely done. They were carried out for 75% of the Berlin cohort and 16% of the Padua cohort but for none of the Edinburgh cohort". At the time of analysis 71 infants "had been followed-up to a median age of 6 months (range 1-15) months". "13 of the 60 children born to intravenous drug abusers (the risk for the other 11 mothers not given) had symptoms of drug withdrawal…AIDS or ARC had developed in 5 of the 71 children...As well as the 5 children (7%) with AIDS/ARC, a further 11 (15%) are known to be infected since HIV culture and/or antigen tests were positive…The total of 16 (22%) known to be infected is the minimum". 3 of the 5 children with AIDS or ARC have died, one from sudden infant death and 2 from AIDS. 4 of the 5 children, including one who died from AIDS had normal T4/T8 ratio. "9 of the children known to be infected from the results of virus or antigen tests had repeat tests; of their 19 virus cultures, only 7 gave positive results". In other words the culture results were either not reproducible or HIV had disappeared by the time the cultures were repeated. 18 of the 71 children became seronegative, including two who had a positive culture. Discussing their results the authors wrote: "In our study nearly all the children were born to intravenous-drug abusing mothers; thus, as well as being exposed to intrauterine HIV infection, these infants were at increased risk of low birthweight, neurological disorders resulting from drug withdrawal, and other perinatal difficulties. In many, social deprivation was encountered after birth; this factor is also likely to affect the child's development and health adversely...Some of our children had neurological demise which was probably related to other factors, such as prematurity".[228]

Followup report of ECS. Illness in children of drug addicted mothers caused by other factors despite their HIV status. No relationship between MCT and mother's clinical status, mode of delivery or breast-feeding. MCT=24%.
A follow-up report from the European Collaborative Study of mother-to-child transmission was published in 1988. In addition to the children from Edinburgh, Padua and Berlin, this report included more children from the UK, Italy, Germany as well as from Spain, the Netherlands and Sweden. Infant infection "was defined by persistence of antibody (as measured by ELISA, confirmed by western blot) beyond the age of 15 months, clinical AIDS or AIDS-related complex (ARC) or the presence of virus [RT detected in cultures] or p24 antigen...AIDS was defined according to Centres for Disease Control (CDC) surveillance criteria [the 1987 CDC children definition of AIDS]. A child was considered to have AIDS-related complex (ARC) if interstitial pneumonitis, persistent oral candida, or progressive encephalopathy had been present for at least 2 months, with two or more of: persistent general lymphadenopathy, hepatomegaly or splenomegaly, chronic or recurrent episodes of diarrhoea, failure to thrive, or parotid swelling. All such children would be included in class P2 of the CDC classification system for HIV infection in childhood" (see Appendix II). By the end of June, 1988, 271 children born to 264 mothers were enrolled. "All but 12 were caucasians. 85% of mothers, and a further 7% of partners, had a history of intravenous drug abuse...Drug-withdrawal symptoms were present in 68 (25%)" infants. "178 of 268 children who survived the neonatal period have been followed up for at least 6 months and 123 for over 1 year". 10 children "had developed AIDS or ARC, of whom 5 have died: 4 with opportunistic infections, and 1 recorded as a sudden infant death. All the children with AIDS or ARC were antibody-positive, 8 out of 9 tested were virus-positive, but not all had immunological abnormalities...Only the 96 children who were older than 15 months when last tested were included to establish the transmission rate for perinatal infection. 15 (16%) of this group remained antibody-positive and are therefore presumed infected, and an additional 4 children died of AIDS or ARC but would have been over 15 months had they survived. Of the 81

33

who were antibody-negative, 5 (6.2%) are considered infected because of at least 1 virus isolation or positive antigen test…Higher rates based on antibody status at less than 15 months have been reported, but these will tend to be overestimates; for example, had antibody presence after 1 year been used to define infection in our study, the transmission rate would have been 53% not 24%…At first it was believed that maternal antibody would disappear by 6 to 12 months, but with antibody loss reported at ages of 15 months or even later, the definition of infection was revised to include only those children in whom antibody was present after 15 months". (6 of the children in this study "have lost antibody after 15 months"). The researchers of this study "found no evidence that mother's clinical status at delivery, mode of delivery, or breastfeeding influenced transmission rates, but the small sample size and other confounding variables made accurate risk estimates impossible". Commenting on their findings, they again stressed: "Most children in the European study were born to mothers who abused intravenous drugs; as well as exposure to intrauterine HIV infection, these infants were at increased risk of other congenital infections, low birthweight, neurological disorders resulting from drug withdrawal, and other perinatal problems. The observed excess perinatal mortality is probably due to the background of drug abuse–as demonstrated by the association of low birthweight and drug abuse during pregnancy–rather than an effect of HIV infection alone. In many, social deprivation was encountered after birth, which could have adversely affected the child's development and health".[229]

Italian Multicentre Study. Follow-up of 281/486 infants. HIVIC ≡ 1987 CDC definition. Children die of non-AIDS diseases. Maternal drug abuse not HIV "may be mainly responsible for preterm delivery and the low birthweight". MCT=33.9%.

"An Italian register for HIV infection in children was started in 1985 by the Immunology Study Group of the Italian Association of Paediatrics. All children born with HIV infection or born to seropositive mothers in 52 centres were enrolled in the study". The first four cases of mother to child transmission were recorded in 1982 and by the end of January 1988 a total of 486 children were born to HIV positive mothers, of which "281 were followed up from birth". "A standard registration form was used which listed risk factors for HIV infection, patient's anagraphic data, sex, gestational age, mode of delivery, birthweight, type of feeding, age at each follow-up, age at onset of symptoms, and age at death. The questionnaire contains specific requests for details of growth failure, fever, hepatomegaly, splenomegaly, lymphadenopathy, parotitis, diarrhoea, neurological disorders, lymphoid interstitial pneumonia, cancers, secondary infectious diseases (recently split into 3 categories according to Centers for Disease Control [CDC] criteria), other associated diseases, and congenital malformations. Laboratory investigations required include HIV antibodies, viral markers, anaemia (<8g/dl), thrombocytopenia ($<10^{11}$/l), plasma aspartate aminotransferase and alanine aminotransferase levels, total and differentiated white blood-cell count, lymphocyte subset analysis by monoclonal antibodies, serum immunoglobulin levels, and proliferative response to mitogens. The CDC recommendations[6] were followed to define infection status and to classify children". (Reference 6 cited by the authors is the 1987 CDC classification system for HIV infection in children under 13 years of age[230] see Appendix II). The term "viral markers" is undefined. Of the 486 enrolled children "165 (33.9%) were considered infected…Of the 165 infected children, 33 (20%) were symptom-free (11 P1A, 18 P1B, and 4 P1C): 27 were over 15 months, of whom 7 were virus-positive, antibody-negative, the 6 younger children had viral markers. 132 children (80%) had symptoms or signs of HIV infection". 130 "Nonspecific findings", 26 "Progressive neurological disease", 16 "Lymphoid interstitial pneumonia", 43 "Secondary infectious diseases", 1 "Secondary cancers", 46 "had other conditions possibly related to HIV infection (P2F): 17 had thrombocytopenia, 15 had anaemia, 1 had neutropenia, 17 had hepatitis and 13 had dermatological diseases". 28 of the 132 children with symptoms "have died, with a higher mortality rate in patients with severe secondary infectious, neurological disorders, and hepatitis...The higher frequency of hepatitis in children who died is unexplained; the mechanism of liver damage in HIV infection is still poorly understood (hepatitis is presently not an AIDS defining illness). In contrast, frequency of lymphoid interstitial pneumonia did not appear to affect mortality in these children, although it is included in the CDC surveillance definition for AIDS". The authors of this study reported "a higher rate of mother-to-child transmission of infection in children born by vaginal delivery and breast-fed, but when each factor was assessed independently no significant difference was found. Although in this study "laboratory investigations required included…lymphocyte subset analysis" and "proliferative response to antigen", the authors did not publish any immunological data. Discussing their findings they wrote: "Maternal drug abuse is associated with a shorter gestational age and a low birthweight, and most of our HIV-positive mothers for whom a full history is available abused intravenous drugs during pregnancy: this, rather than HIV infection, may be mainly responsible for preterm delivery and the low birthweight".[231]

308 infants from France. Two thirds of mother drug addicts. HIVIC ≡ positive WB beyond 18 months of age or AIDS. Mode of delivery unrelated to risk of infection. Nine infants (8%) seronegative with non-specific clinical and laboratory abnormalities "but possibly related to HIV infection". MCT=27%.

The findings of the French Collaborative Study Group of HIV infections in newborns, which commenced in January 1986 and included 51 obstetrical and paediatric centres, were published in 1989. "To be included in the study, the mother had to be known to be seropositive before delivery...At the time of the birth and at regular

intervals thereafter, each physician completed standardized questionnaires about the mother and child...In the absence of a history of drug addiction or of previous blood transfusion in the mother, the infection was considered likely to have been transmitted heterosexually". "…the diagnosis of HIV infection was made on the basis of a positive Western blot test at the age of 18 months. In infants who died before 18 months, the diagnosis required evidence of an opportunistic infection or the isolation of HIV...Western blotting was considered negative when no HIV antibody of any type was present". By June 1988, 308 mother-child pairs had been studied. "The probable mode of infection with HIV was determined in the case of 303 of the women (in the five others, a history of drug use was suspected but not certain). In 176 of the 303 women (58 percent), the main risk factor was intravenous drug addiction. A heterosexual route of infection appeared most likely in the cases of 93 women (31 percent), most of whom came from countries or regions such as Haiti (6 percent) and Central Africa (12 percent). Nine women (3 percent) had received blood transfusions before 1985. At least two risk factors were noted in each of the other 25 women (8 percent), 12 of whom were drug addicts. Hence, a total of 188 women (62 percent) were drug addicts". Three infants, two born to drug-addicted mothers, died suddenly and without apparent cause between the age of two and four. When the authors of this study performed their analysis "128 children had been born at least 18 months earlier. Of them, nine were lost to follow-up, and two had died suddenly and without known cause. The remaining 117 infants were classified. Eighteen months after birth, 32 infants (27 percent) had had serologic or other evidence of HIV infection; of these, 6 had died of AIDS. Seventy-six infants (65 percent) were symptom-free and seronegative. Nine infants (8 percent) were seronegative but had persistent or transient clinical signs (hepatomegaly, splenomegaly, or adenopathy) or abnormalities (hypergammaglobulinemia or a decrease in the CD4 lymphocyte count) that were nonspecific but possibly related to HIV infection (*sic*). The mode of delivery was unrelated to the risk of infection in the infant…The high incidence of unexplained sudden death reported in this study can also be attributed to maternal drug addiction".[232]

Update of ECS. Authors criticise methods used by other researchers. 52% of mothers drug users and 600 children (28%) suffered drug withdrawal symptoms. HIVIC ≡ AIDS, "death considered to have resulted from HIV infection", antibodies beyond 18 months, culture or antigen detection in 2 samples. Children with "AIDS', although seronegative, considered infected. Positive cultures revert to negative. Signs and symptoms of HIV infection as well as low T4 counts present in both seronegative and seropositive children. Low T4 cell count follows and does not proceed the clinical syndrome. MCT=13%.

An up-date of the European Collaborative Study was published in 1991. By June 15, 1990, 600 children born to HIV seropositive mothers had been recruited and followed in the ten European centres. "Many of the children had been born into deprived circumstances: more than 20% had been adopted, fostered, or institutionalised; 28% had drug withdrawal symptoms at birth; and a further 52% had mothers with a history of intravenous drug use (IVDU). 4 children had congenital syphilis, and 2 had symptomless congenital CMV, though ascertainment of these infections was not complete…The criteria for HIV infection were: acquired immunodeficiency syndrome (AIDS), death considered to have resulted from HIV infection, persistence of antibody beyond 18 months, positive virus culture, or antigen detection in two samples. Lymphoid interstitial pneumonitis (LIP), as judged by severe respiratory symptoms confirmed by radiograph, was regarded as an AIDS indicator disease, but radiographic findings alone were not…Children who died with AIDS/HIV are assumed to be antibody positive at the time of analysis, and the 2 children with AIDS who subsequently lost antibody are assumed to have remained seropositive". Of the 353 children who lost antibody, and in whom AIDS did not develop "virus isolation had been attempted in 163; in 10 (6.1%) positive results were obtained at least once...In 4 children the initial positive culture was confirmed on subsequent samples by repeat virus culture and/or polymerase chain reaction (PCR). But in the remaining 6, a total of eight attempted cultures were negative…Of those who will lose antibody, an estimated 10.2% lose it after 15 months, and 2.5% after 18 months". Although some of the "signs and symptoms associated with HIV infection" occurred more often in the positive children, they were present in the negative children as well. "None of the clinical findings in the perinatal period discriminated between infected and uninfected children" yet the study permitted a clinical diagnosis of HIV infection. "The incidence rates show that oral candidosis and parotitis [neither are AIDS defining] were highly discriminating indices, occurring many times more often in HIV-infected than in presumed uninfected children. Septicaemia and pneumonia with gram-negative bacteria were also strongly associated with HIV infection, although only 4 of the 11 episodes contributed to a subsequent diagnosis of AIDS. Encephalopathy, other neurological abnormalities, and failure-to-thrive were uncommon in the pre-AIDS period; all were associated, but not significantly, with infection. Although there was a highly significant statistical association between many of the signs and HIV-infection status, only a few were specific. For example, of the 67 children with episodes of non-cryptosporidium diarrhoea as many as 47 (70%) were uninfected. Lymphadenopathy, hepatomegaly, and splenomegaly were common findings in infected children, but, as evidenced by the low rate ratios, were relatively non-specific on their own, as were eczema, fever, rhinitis, otitis, and pneumonia (except when associated with gram-negative bacteria)". Low T4/T8 ratio was found in 15 of 343 uninfected children and in 32 of 64 children "judged to be HIV infected". In contradiction to the HIV theory of AIDS, the low T4/T8 ratio "came after the onset of AIDS in 44% of those in whom AIDS developed". This means that either the cause of

immune deficiency follows the effect (HIV) or the diseases themselves cause the immune deficiency. Discussing their findings the authors wrote: "…during the second and third years of life, the progression of HIV disease seems to be slow, with more children improving than deteriorating. Long-term treatment of symptom-free children or those with mild symptoms may be necessary to show any benefit; this not only creates difficulties in the design of trials for treatments with side-effects, but also raises doubts about earlier, open therapeutic studies that attributed to treatment improvements that might have occurred spontaneously. Our 13% estimated transmission rate is lower than rates published in all but one study, which was a subset of the ECS…estimates in many earlier studies may have been biased upwards. For example, in one study, virus cultured from cord blood was taken as proof of infection. In a study citing 39% transmission, 47% of the cohort had been lost to follow-up; and those remaining may have been more likely to have been ill. A 29% rate was cited in a New York study. However, that analysis included antibody-negative children only if they had been followed for 15 months, whereas infected children were included with shorter follow-up. In studies in which some children are recruited retrospectively, the requirement that the mother should be seropositive may be insufficient to prevent a bias towards selective recruitment of sick children. A further requirement is that collaborating centres report all children born to seropositive mothers. This type of bias may have occurred in the Italian Multicentre Study (33% transmission), which is register based; in the French Study, which has 51 collaborating centres (27% transmission); and also in earlier reports from the ECS. The requirement for inclusion in the transmission rate that children should have an antibody test after 18 months rather than have a birth date 18 months earlier also biases estimates upwards because parents of children who lose antibody earlier are less likely to bring the children back for continuing follow-up. Even if all the children who were lost to follow-up were infected, the transmission rate would still only be 23%. Deaths in children of indeterminate infection status, of which there were 7 in the ECS, also make the calculations of transmission rates difficult. The mortality rates we found [17% of children "died of HIV-related diseases"] are higher than the 1/600 SIDS and the 5/1000 neonatal mortality rates in the UK, though the social deprivation of our cohort must be taken into account".[233]

1005 infants and children from 19 European centres MCT related to prematurity, maternal p24 antigenaemia and T4 cell count but not mode of delivery. MCT=14.4%.
In 1992 there was another update of the European Collaborative Study. By August 1991 there were 1005 children enrolled. The collaborative centres increased from 10 in 1991 to 19 in 1992. HIV-infection status was determined in 721 (87%) children of 701 mothers. In this update it was reported: that the rate of transmission was 14.4% and was higher "in children born before 34 weeks gestation...Transmission was associated with maternal p24-antigenaemia and a CD4 count of less than 700/µl...The balance of evidence suggests that mothers with established infection can transmit HIV infection through breastmilk, although the relative importance of this route remains to be defined…Although the relative importance of transmission at delivery is becoming apparent, prospective studies have shown similar rates of transmission in children delivered vaginally and by caesarean section. In our study, there was some evidence of a protective effect of operative delivery but the result was not statistically significant".[234]

Swiss study of 286 children. HIVIC ≡ AIDS, positive WB beyond 2 years or other laboratory data. Advanced maternal disease, prematurity, mode of delivery not risk factors. Infants persistently PCR positive but culture and antibody negative. Positive PCR reverts to negative. False positive PCR "not rare". Caveat "not to rely on PCR as the only virus detection test for the diagnosis of HIV infection". MCT=14%.
In yet another study published in 1992, "Epidemiology of vertically transmitted HIV-1 infection in Switzerland: results of a nationwide prospective study", the participants were "Physicians from all regions of Switzerland and from all paediatric hospitals of the country...Registration of children occurred where possible at birth, otherwise on recognition of maternal HIV seropositivity. Children with known maternal HIV infection before or within 14 days of birth were included in cohort A, those registered later in life constituting cohort B...Follow-up data were collected on questionnaires mailed every 3-6 months to the treating physicians. Signs and symptoms characteristic of paediatric HIV infection according to Centers for Disease Control (CDC) criteria were specifically requested. The age when symptoms according to stage P-2 were first recognised was defined as the age at onset of symptomatic HIV infection. Blood samples for virological examinations (western blot for HIV antibodies [WB], p24 antigen, if possible virus culture [VC] and starting from 1988 polymerase reaction [PCR] were taken at the same intervals, if consent was obtained…Children were considered to have vertically transmitted HIV infection if they met at least one of the following criteria: (1) AIDS as defined by CDC; (2) HIV seropositivity by WB beyond the age of 24 months; (3) positive virus detection tests (Antigen, VC [reverse transcriptase assay/p24], PCR) in at least two different blood samples in a seropositive child of any age…Intravenous drug use (IVDU) was reported as risk factor for acquisition of HIV infection for 68% of mothers; 46% of them were reported to have used drugs during pregnancy. Twelve percent had a sex partner with a history of IVDU. Eleven per cent came from and 2% had a sex partner from a country with high prevalence of HIV infection (mainly Central Africa). One woman had been infected by transfusion (0.4%) and the others (6.6%) had none of these risk factors". Under the subheading "Vertical transmission rate": "Of 201

infants in cohort A, 27 have documented HIV infection. The ratio of 14% (95% C1 9%-19%) should be regarded as a minimum estimate of transmission rate, as 37% of children still have their infection status undetermined". One hundred and eighteen children were classified non-infected. "They all lost maternal antibody, had no symptoms of stage P-2", and "all tests for antigen, VC (118 attempts in 59 children) and PCR (81 tests in 54 children showed negative results…Twenty children who lost maternal antibody and showed no symptoms of stage P-2, had at least one atypical test result and were classified as being of questionable infection status…Two children had a single positive VC (positive result by reverse transcriptase and p24 assay). One was from cord blood of a now seronegative child (patient 2) without symptoms and a negative antigen test…The second positive VC was at age 6 months from a now seronegative asymptomatic 41 month old child with three subsequent negative cultures and two negative PCR results. A further four children had a total of nine positive PCR results. Two of them with repeatedly positive tests had transient questionable symptoms but consistently negative VC. Both lost antibodies against HIV and eventually their PCR tests were negative". Survival at 12 months was 80%, with 5 "HIV-related deaths". "Overall infant mortality rate in these children of HIV-infected mothers was 4.6% compared to 0.7% in the general Swiss population…and post-neonatal mortality rate was 4.0% compared to 0.3%". Discussing their findings the authors wrote: "Diagnosis of vertically transmitted HIV infection still relies mainly on observation of infants for the development of clinical disease and persistence of seropositivity during the first 2 years of life. However techniques for virus detection by culture or PCR have improved recently, so that now early diagnosis during the first months of life seems feasible...Single positive virus detection tests in seronegative asymptomatic infants can possibly be explained as false positive results. The fact, that their occurrence (especially for PCR) is not rare in children who lost maternal antibody, must be borne in mind in the interpretation of attempts at early diagnosis. The repeatedly positive PCR results in two children with negative VC and losing their antibodies is especially puzzling in this context...In view of these difficulties it seems prudent to always use two successive blood samples and not to rely on PCR as the only virus detection test for the diagnosis of HIV infection during the first months of life. Even in very carefully conducted prospective studies calculation of an exact vertical transmission rate is impeded by the inevitable incompleteness of follow up and by diagnostic difficulties in some children. For these reasons we attempted to estimate a minimum and maximum value rather than a single point estimate. The range of 14%-20% is somewhat higher then the transmission frequency of 12.9% found in the European Collaborative Study but still at the lower end of the spectrum of published figures, which range between 10% and 50%. Previously suggested associations of transmission rate with advanced maternal disease, prematurity or vaginal birth and breast feeding could not be confirmed in the present study". Neither could they find a significant reduction by caesarean delivery. They concluded: "The fact, that 20% of children of HIV infected mothers in Switzerland have to be placed in foster care by 1 year of life and 50% by 3 years clearly shows that, even though vertical transmission frequency is lower than previously thought, the prospects for a harmonious development are very limited for these babies".[235]

320 HIV infected women from 18 centres. HIVIC ≡ positive antibody test or culture one beyond 3 months. Maternal viral load a transmission risk but African women with lower viral loads just as likely to transmit. MCT=19%.

In a study published in 1997 entitled "Maternal Virus Load during Pregnancy and Mother-to-Child Transmission of Human Immunodeficiency Virus Type 1: The French Perinatal Cohort Studies", the relationship between maternal viral load and transmission was studied in 320 HIV infected women from 18 centres. "The mode of infection was thought to be intravenous drug use for 17%, blood transfusion for 7%, and sexual contact for 76%. Among the latter, 49% were from sub-Saharan Africa…Among the 236 evaluable children, 191 were uninfected and 45 were infected (19%±5%)...The diagnosis of HIV infection was based either on HIV antibody status or on the results of two virological tests (culture or PCR), at least one of which was done after age 3 months". "Zidovudine was prescribed during pregnancy for 66 of the 320 women" but for some unknown reason these women were excluded from the analysis. The authors of this study reported that "Maternal virus load appears strongly related to HIV transmission to the child...MCT was correlated with HIV RNA levels in African and in non-African mothers. However, Africans had lower RNA levels. [46% African mothers had RNA<1000; 30% between 1,000-10,000 and 24% ≥10,000. The corresponding distribution for the 156 non-African mothers were 16%, 43% and 41%]...The clinical characteristics and CD4 cell counts of African women did not differ from those of the rest of the population under study, and African mothers were as likely to be transmitters".[236]

UK study but 81% of mothers African

In a 1998 publication by researchers from London, UK, the authors reviewed 57 consecutive mother-infant pairs who presented for infant diagnosis of HIV infection between January 1994 and July 1997. "The mother-infant pairs fell into two groups: those in which mothers were known to have HIV infection in pregnancy or before and could consider options to reduce transmission and those in which a family member, most often the infant (17 of 24), presented with HIV related symptoms". Of the "57 consecutive mother-infant pairs, 81% of mothers (46) were of African origin…Antiretroviral therapy was taken by 69% (39/57) of mother-infant pairs. Twenty six (46%) took a full 076-type course of zidovudine and seven took an incomplete course with only one or two parts of the regimen (antepartum, intrapartum, or postpartum)". In the text one reads: "Transmission rate was 8%

(3/39) for mothers who took any kind of antiretroviral therapy and 22% (4/18) for those who did not". In the abstract one reads: "With antiretroviral therapy or caesarean section or both, transmission occurred in 6%" of pairs.[237]

2.4   Studies from Africa and Asia

475 infants from Zaire. HIVIC ≡ antibodies between 12-18 months of age, positive culture (cord blood) or an "in house" clinical classification. Socioeconomic differences between the two hospitals and socioeconomic factors as well as non-HIV-related events major determinants of outcomes. No correlation between culture and antibody tests. MCT=39%.

The first of such studies was published in 1989 by researchers from the USA (the CDC, National Institute for Infectious Diseases, the Boston University, Tulane University and the Armed Forces Institute of Pathology); Zaire (Mama Yemo Hospital and Cliniques Ngaliema); The Global Programme on AIDS, WHO; and Belgium (The Institute of Tropical Medicine, Antwerp). The study was conducted at two hospitals in Kinshasa. Hospital A, served patients "of low socioeconomic status", and B, "of higher socioeconomic status". The mothers who were found seropositive were matched for age and parity with seronegative mothers. Of a total of 8108 women screened, 466 (5.8%) were found positive. To establish infection of the infants, the authors "used both clinical and laboratory criteria…The laboratory criteria consisted of a positive cord-blood culture or the detection of HIV-specific IgG antibodies at 12 months that persisted to the age of 18 months". The clinical criteria were: "major criteria: fever (documented temperature higher than 37.5ºC) for 15 days during a month; persistent diarrhea (more than two stools a day that took the shape of a container) for 15 days during a month; and failure to thrive (weight-for-age ratio below the 10th percentile on the scale of the National Center for Health Statistics) for two consecutive months. Minor criteria in the definition were generalised lymphadenopathy, oral-pharyngeal candidiasis after the age of six months, pneumonia, and a generalised papular dermatitis. We assigned a point score of 3 for each major criterion and a score of 2 for each minor criterion except dermatitis, which was given a value of 1 because of the relative frequency of various nonspecific skin conditions. Any child with a seropositive mother and a cumulative score of 10 or more points was considered to have clinically defined AIDS. Any child who died during the first year of life whose AIDS score was at least 7 and whose mother was seropositive was considered to have had AIDS". (Thus not only did the authors invent an "in house" definition of HIV infection they modified their definition according to whether a child lived or died). In the case of cord blood samples, "92 samples from infants with seropositive mothers [not seronegative mothers] were randomly selected for isolation of HIV-1 at the Centers for Disease Control" after "shipment to the United States". Of the 92 cord blood samples randomly selected for viral culture, 48 from Hospital A and 44 from Hospital B, overall 19 (21 percent) were positive for HIV-1 (13/48 from Hospital A and 6/44 from Hospital B). "The presence of HIV-1 antibodies in children who survived the first year of life did not correlate with the results of their cord-blood cultures. Only 1 of the 11 infants (9 percent) who had a positive cord-blood culture and who was alive at the age of 12 months had detectable IgG antibodies in the 12 month blood sample". The mother to child transmission "Incidence rates were calculated for the 92 children with complete clinical follow-up and cultures of cord blood for HIV-1 (48 at Hospital A and 44 at Hospital B). Thirty-six of these 92 children (39 percent) had evidence of perinatally acquired infection". The transmission rate of 39 percent was based on the finding of one of the following: clinical signs and symptoms; a positive culture; or a positive antibody test.

Comments

The authors themselves admit that:

1. No correlation exists between the culture and antibody test results.

2. "diagnosis of perinatally acquired HIV infection based solely on clinical criteria is very difficult to make in developing countries". Indeed, all the signs and symptoms present in the children considered infected and said to signify AIDS were also present in non-infected children, although at a lower frequency.

3. "As compared with the mothers at Hospital B, more mothers at Hospital A reported the death of a previously born child. The rate was higher among seropositive than among seronegative mothers at each hospital. At Hospital A, the rates were 43 percent for seropositive mothers and 32 percent for seronegative mothers (P<0.01). At Hospital B, the rates were 30 percent and 13 percent for seropositive and seronegative mothers, respectively…At Hospital A, 9.9 percent of the children born to seropositive mothers had a five minute Apgar score of less than 8, as compared with 2.2 percent of infants to control women (P=0.002), but these differences were not noted in study infants at Hospital B...Among the infants of seropositive women there were 29 neonatal deaths (6.2 percent), as compared with 8 among the infants of seronegative women (1.3 percent)…During the first year of life, a total of 123 infants (11.5 percent of the original cohort of 1072 live births) died. There were 100 (21 percent) deaths among the 468 children of HIV-1 seropositive mothers and 23 (3.8 percent) deaths among the 604 children of seronegative mothers". The causes of post-

neonatal death of the children born to the non-infected mothers were 27% diarrhoea, 7% pneumonia, 20% meningitis and 40%, "others".

4. "The mortality rates among the children born to seropositive and seronegative mothers at the two hospitals differed markedly, partly because of differences in *socioeconomic* status and in the proportion of mothers with AIDS in the study at the two hospitals...Our finding that seropositive women at Hospital A were more frequently symptomatic with their HIV-1 infection than seropositive women at Hospital B may also have played a part in the high mortality rate among the children born to the seropositive women at Hospital A…Finally, women with symptomatic HIV-1 infection may be less able to nurture their children during the first several months of life, when the risk of acquiring other, often fatal, childhood illnesses is great" (italics ours).

Since,

(a) No correlation exists between the result of the cultures and the antibody tests, one or both tests cannot be considered specific;

(b) The clinical signs and symptoms considered to prove the existence of AIDS are non-specific and may be the result of the socioeconomic status of the mothers and their less than adequate ability and means to "nurture their children", it is not possible to say how many, if any, of the children had HIV infection on the basis of diseases.

The inescapable conclusion is that the evidence presented in this study cannot be considered proof of a 39% transmission of HIV from the mother to the child, or indeed, any transmission.[238]

12% of 1954 mothers from Lusaka HIV positive. HIVIC ≡ positive antibody test beyond 24 months or CDC definition. MCT=39%.
In the same year, 1989, researchers from Zambia and the USA published a study conducted at a teaching hospital in Lusaka. During February to May 1987, 1954 women were tested for HIV at delivery and 227 (12%) were found positive. There were 205 live births. Of 109 children available for follow-up, 19 died of "clinical AIDS" as defined by the CDC (1987 children AIDS definition), and thus were considered to be HIV infected, and 23 had a positive antibody test at 24 months. Thus, "The overall rate of perinatal transmission was 42 out of 109 (39%)...Before the age of 1 year infected children had pneumonia and recurrent coughs, thereafter symptoms included failure to thrive, recurrent diarrhoea and fever, pneumonia, candidiasis, and lymphodenopathy. All babies had received live attenuated vaccines before 8 months with no adverse affects".[239]

4588 pregnant Haitian women with 9.7% HIV positive. HIVIC ≡ positive WB beyond 12 months of age or by excess mortality over children of non-HIV positive mothers. Transmission rate estimated to be 25%. Socioeconomic factors acknowledged as significant confounders in this estimate.
In 1990 a study was published by researchers from The Johns Hopkins University, Baltimore; The National Institute of Allergy and Infectious Diseases; Bethesda and the Complexe Medico Sociale de la Cité Soleil, "a periurban slum outside Port-au-Prince, Haiti". Of 4588 women from Cité Soleil tested at 6 to 7 months of gestation, between August 1986 and August 1988, 443 (9.7%) were found to have a positive antibody test. By January 1990 when the offspring of these women were 12 months of age or older, outcomes were available for 308 (71.7%) of 377 infants born to women who were HIV-1 seropositive and for 3360 (75.4%) of 3589 infants born to women who were seronegative. "The nutritional status of infants born to women who were seropositive was significantly lower (P<.01) than that for infants born to women who were seronegative at birth, 3 months, and 6 months of age…Infants born to women who were HIV-1 seropositive had significantly (P<.001) lower survival rates than infants born to women who were seronegative. This difference in mortality was significant by 3 months of age when 13% of the infants born to women who were HIV-1 seropositive had died as compared with 6.1% of the infants born to women who were seronegative (P<.001). The infant mortality rate was 23.4% for infants born to women who were seropositive as compared with 10.8% for infants born to women who were seronegative…Serum specimens were available for 230 infants (61%) born to women who were HIV-1 seropositive…Fifty percent of the infants had lost maternal antibodies by 7.6 months of age…Of 150 infants for whom blood specimens were available beyond 12 months of age, 17 were HIV-1 seropositive, three were indeterminate, and 130 had lost maternal antibodies. Of the 80 children for whom no blood specimens were available after 12 months of age, 42 had lost antibodies before dying or becoming unavailable for follow-up and 38 were HIV-1 seropositive at their last clinic visit. Two methods were used to estimate the rate of mother-to-infant HIV-1 transmission. First, the excess infant mortality rate for infants born to women who were HIV-1 seropositive was assumed to be due to HIV-1 infection in the infants. The difference in mortality rates at 12 months of age (12.6%) was added to the 11.3% of infants with persistent HIV-1 antibodies after 12 months of age, resulting in an estimated 23.9% rate of mother-to-infant HIV-1 infection transmission. In the second

method, 172 children were considered to be free of HIV-1 infection because they had lost HIV-1 antibodies prior to death, reaching 1 year of age, or loss to follow-up. These infants were subtracted from the total 230 infants born to women who were HIV-1 seropositive, thus leaving about (25.2%) of 230 infants probably infected". The authors concluded: "The estimated mother-to-infant HIV-1 transmission rate in these breast-fed infants was 25%, similar to the rates reported for non-breast-fed population in the United States and Europe". Discussing their findings they wrote: "The calculation of mother-to-infant HIV-1 transmission rates in developing country settings has been complicated by high background rates of infant mortality and malnutrition due to other causes…The increased mortality in the first 3 months of life in the current study could have been secondary to complications of prematurity and not necessarily due to HIV-1 infection in the infants...Both methods of calculating the mother-to-infant transmission rate could have overestimated the rate because children who were dying (before the age of 12 months) while HIV-1 seropositive were considered to be infected".[240]

Two year follow up of children of 215 seropositive and 216 seronegative mothers respectively. HIVIC ≡ antibody at 15 months or "HIV related death", or AIDS, or excess mortality or if fulfilling an "in house" definition. Two methods used to calculate MCT. MCT=27.7% or 21.1%.
In a study conducted in Kigali between November 1988 and June 1989 by researchers from Rwanda, Belgium and France, 218 children of 215 HIV antibody positive mothers and 218 of 216 seronegative women of the same age and parity were followed-up for 2 years. Three criteria were used "to define children born to seropositive mothers as infected with HIV-1". "HIV-1 antibody positive test at age 15 months *or* HIV-related death *or* acquired immunodeficiency syndrome (AIDS)…Death was defined as HIV-related either in a child with AIDS or in a child with at least one HIV-related sign/symptom when last seen and dying from severe infection or persistent diarrhea beyond the first 4 weeks of life. The following signs and symptoms were regarded as HIV-related: persistent diarrhea (≥14 days), failure to thrive (<80% weight for age), persistent generalised lymphadenopathy, oral candidiasis (beyond the neonatal period), severe or recurrent pneumonia, chronic parotitis, and herpes zoster infection. Children with at least two major and two minor signs of the World Health Organization (WHO) clinical case definition of pediatric acquired immunodeficiency syndrome (AIDS) were considered to have AIDS. However, one modification was made to the WHO definition: if present, severe pulmonary infection (respiratory rate ≥50 in children 1-11 months old or ≥40 in children 12-23 months old) or recurrent pulmonary infection (≥2 episodes during follow-up) was considered as a major sign in the place of chronic cough as a minor sign". Of the 218 children born to the positive mothers 40 (18.3%) died and 21 (9.6%) were lost to follow-up. Of these 40 children only 23 were considered to be infected, out of the 218 children born to HIV seronegative mothers 10 (4.6%) died and 15 (6.9%) were lost to follow-up. The causes in both the children born to HIV positive (including those who died from AIDS) and negative mothers were the same, only the frequencies were higher in the former. Two methods were used to calculate MCT transmission rate.

"Method 1. A combination of the information provided by the persistence of HIV-1 antibodies at 15 months of age in children born to HIV-1 seropositive mothers and by the excess mortality in this group compared with the reference cohort constituted of children born to HIV-1 seronegative mothers.

Method 2. A case by case evaluation of all the children born to HIV-1 seropositive mothers according to the classification presented in table 1 ["HIV-1 antibody positive test at age 15 months *or* HIV-related death *or* acquired immunodeficiency syndrome"] yields a direct estimate".

The "estimated rate of mother-to-child transmission calculated with Method 1, was 27.7%…With Method 2, 46 (21.1%) of the 218 children born to HIV-1 seropositive mothers, were classified as HIV-1 infected". Discussing their finding the authors wrote: "This cohort study shows that in Rwanda, mortality among children born to seropositive mothers is high in the first 2 years of life, occurs early, and is principally due to diarrheal diseases and pulmonary infections. This mortality rate (19% for the first 2 years of life), although lower than in other African studies is two-to threefold higher than among European children born to HIV-1 seropositive mothers. Diarrheal diseases and pulmonary infections, the principal causes of death in children under age 5 years in the developing world, are also the major causes of death in children born to seropositive mothers in this study...Among the children born to seronegative mothers, the mortality during the first 2 years is lower than expected in Rwandan children".[241]

HIVIC ≡ AIDS before 12 months or death before 12 months with "probable" cause "severe infection or persistent diarrhea" or positive antibody test at 12 months. Two methods used to calculate MCT. MCT "between 20 and 29%" with a maximum of 34%.
In yet another study from Rwanda published in the same year, 1993, this time by researchers from the Johns Hopkins University and the National University of Rwanda, the authors evaluated MCT in "all singleton infants born at least 12 months prior to the date of analysis who survived the neonatal period". A child was considered HIV infected if one of the following was fulfilled:

(a) the child developed "clinical AIDS" (The WHO 1986 clinical definition) during the first 12 months. (Appendix I);

(b) The child died before 12 months of age and "severe infection or persistent diarrhea was the probable cause of death";

(c) The child had a positive antibody test at 12 months of age.

"Two methods were used to estimate the rate of MCT of HIV-1. First, the minimum MCT rate was estimated by dividing the number of infected children by the total number of infected and uninfected children. With this method, children with indeterminate HIV-1 status were excluded. The second method estimated the maximum MCT rate by dividing the number of infected children plus the number of children with indeterminate HIV-1 status by the total number of infected, indeterminate, and uninfected children". The MCT rate was reported to be 20% by the first method and 29% by the second. The authors of these studies analysed the risk factors for MCT transmission and reported that: "The vertical transmission rate was estimated to be between 20 and 29%. Unprotected sexual intercourse with increased number of partners during the past 5 years was strongly associated with mother-to-child transmission (*P*<0.001), even after adjustment for maternal CD4/CD8 ratio, parity, history of sexually transmitted diseases, and evidence of genital infection during pregnancy…Women with more than one sexual partner during the first trimester of pregnancy were at particularly high risk of transmitting the virus". Discussing their finding the authors wrote: "This report includes only infants who survived the neonatal period. If we assume all foetal and neonatal deaths to be HIV-1 infected offspring, the maximum transmission rate would become 34%".[214]

HIVIC ≡ positive PCR or culture in two specimens or antibody at 15 months. Risk factors for MCT low T4 cell count, high T8 cell count, p24 antigenaemia, chorioamnionitis. MCT=26%.
Similar to the report of the researchers from Rwanda and the Johns Hopkins University, in a study published in 1993 researchers from the CDC, National Institute of Allergy and Infectious Diseases and Kinshasa also attempted to determine the risk factors for MCT. In this study "Human immunodeficiency virus infection in children was defined as a positive virologic assay (PCR or viral culture) on two or more specimens drawn on different days or a persistently positive HIV antibody assay at 15 months or later". They reported a 26% transmission rate. "In women with neither p24 antigenaemia, high CD8$^+$ or low CD4$^+$ lymphocyte counts, nor placental membrane inflammation, the transmission risk was only 7%. Additional correlates of transmission included maternal anaemia and fever, but not maternal sexually transmitted diseases".[242]

Harvard study of 114 HIV positive mothers and their infants from the Congo. HIVIC ≡ positive antibody test at 15 months or excess mortality compared to children of HIV negative mothers. Mother's health and duration of relationship with father risk factors. MCT=40.4%.
In a study published in 1994 by researchers from the Harvard School of Public Health, USA including Myron Essex and several from Brazzaville Congo, including members of the Congolese Research Group on MCT of HIV, it was pointed out that "Published rates of perinatal transmission vary widely, from 13 to 45%. Direct comparison of one study with another is difficult, however, because of differences in study design, infant HIV infection criteria, and methodology for calculating the risk of transmission". In their study "Sera were considered positive if they showed antibodies against at least two envelope glycoproteins…A positive serological test for HIV antibodies at 15 months was considered decisive evidence of HIV infection. Infants who died prior to 15 months were classified as infected if they either had AIDS according to the WHO case definition or met the EEC/WHO Ghent workshop criteria for HIV-related death. Infants who died during the neonatal period, were lost to follow-up, or could not be classified clinically were considered indeterminate. The rate of transmission was calculated by the direct method based on the case-by-case analysis of the outcome for all children born to HIV-1 positive mothers. An indirect transmission rate was also calculated based on the assumption that, from birth to 15 months, the mortality difference between children born to HIV-1 positive mothers and controls was due to HIV infection. This indirect rate was obtained by combining the mortality difference with the proportion of seropositive children among those who survived to 15 months…A total of 114 HIV-1 positive women and their 118 infants were enrolled in the cohort study. Five mothers had twins, including one stillborn child. Seventy-three children could be tested for antibodies to HIV at 15 months…Seventeen children were seropositive at 15 months. Thirty-three children died before 15 months, of whom eight had AIDS. Of the remaining 25, 15 were considered infected because they met the criteria of HIV-related death and 10 could not be classified". When the infants born to the seropositive mothers were compared to 208 infants born to seronegative mothers, the former were found to have a lower birth weight and were more likely to be premature. "Probability of death in the two groups differed significantly as early as 3 months of age...By the direct method, the estimated transmission rate was 40.4% (95% CI, 30.7-50.1). The upper and lower estimates of risk of transmission, assuming that all indeterminate infants were either infected or uninfected, were 33.9% (95% CI, 25.4-42.4) and 50.0% (95% CI, 41.0-59.0), respectively. The indirect

transmission rate was 42.7% (95% CI, 32.7-52.7). Since twinning only occurred among HIV-1 positive mothers and three of the five deaths associated with prematurity occurred in twins, we also calculated the rates of transmission after excluding twins. Point estimates were slightly lower 38.7% (95% CI, 28.1-48.0) by the direct method with upper and lower limits of 47.2% (95% CI, 37.8-56.6) and 32.4% (95% CI, 23.6-41.2), respectively. The indirect estimate was 39.9% (95% CI, 29.5-50.2)...The authors concluded that in their study "The transmission rate was 40.4%".   ADDIN ENRfu   243

*561 Rwandan women. HIVIC ≡ positive antibody test at 15 months or PCR or clinical diagnosis. MCT=26.7% with chorioamnionitis; 10.5% without chorioamnionitis. Overall MCT 12%. **No antiretroviral treatment**.*
In a study entitled: "Chorioamnionitis and Pregnancy Outcome in HIV-Infected African Women", researchers from Rwanda and France conducted HIV antibody testing in women between 24 and 28 weeks of gestation. "Within 2 weeks after the HIV screening test, HIV-positive women and an equivalent number of HIV-negative women were enrolled". In this study which was designed to evaluate the rate of chorioamnionitis on pregnancy outcome in HIV-1-infected women; "The children and their mothers had follow-up observation until 15 months after delivery. In the group of children born to HIV-positive mothers, HIV status was determined either by HIV antibody testing at 15 months or by polymerase chain reaction...at 3 and 6 months of age. Each child's HIV status was classified using the 1993 classification of the Ghent International Working Group [see Appendix I]…Among the children born to HIV-positive mothers, presence of HIV infection was assessed in 158 children, 15 in the CAM-positive group, and 143 in the CAM-negative group. Overall, 19 children were diagnosed as HIV-infected (12.0%; 95%CI = 6.2%-17.8%); four children (26.7%) were HIV-infected in the CAM-positive group and 15 (10.5%) in the CAM-negative group.[69] (Note: In African women even with no treatment and with the Ghent Classification only 12% transmit HIV).

295 HIV positive women from Bangkok. HIVIC ≡ two positive PCRs, or one PCR and AIDS. High viral load and low birth weight risks for transmission. Mode of delivery and other obstetric variables including maternal immunological parameters not a risk. MCT=24.2%.
During 1992-1994 The Bangkok Collaborative Perinatal HIV Transmission Study conducted by researchers from Thailand and the CDC, USA, recruited HIV-infected women from two large hospitals in Bangkok. "Infants were considered to be HIV-1 infected if they had two HIV-1 positive DNA PCR tests, or one positive PCR test and a CDC AIDS-defining condition. Infants were defined as uninfected if they tested PCR HIV-1 negative on two samples, including one obtained at 6 months of age or older, or if they seroreverted to HIV-negative status on EIA testing. Infants not meeting these criteria were considered to have unknown infection status…Of 295 HIV-infected pregnant women who delivered during the study, 281 (95.3%) had infants with known HIV-infection status. There was a total of 68 infected infants and the overall transmission rate was 24.2%...High maternal viral load at delivery and low birth-weight (<2500 g) were independently associated with *in utero* transmission…Pre-term infants (<37 weeks gestational age, by modified Ballard) had a 25% risk for *in utero* transmission compared to 5% for full-term infants...Maternal immunological markers, such as CD4+ T-lymphocyte and NK cell percentage and absolute count, and obstetric factors such as mode of delivery and duration of membrane rupture and labour, were not associated with *in utero* transmission…Maternal delivery viral load was also strongly associated with intrapartum transmission". The authors concluded: "In summary, this study demonstrates that maternal viral load, as measured at delivery, is strongly associated with both *in utero* and intrapartum HIV-1 perinatal transmission, and suggests that interventions aimed at reducing maternal viral load may be effective in reducing transmission at both of these times".[244]

Study from Thailand with above principal author as co-author. Viral load, mode of delivery and some immunological variables in the mother are risks for transmission. MCT=24%.
The same authors analysed the relationship between the viral load in the 281 mothers, as well as other factors, and the transmission rate. Their findings were published in the same year, 1999 in a paper entitled: "Maternal Virus Load and Perinatal Human Immunodeficiency Virus Type 1 Subtype E Transmission, Thailand". "Before virus load measurements were available, CD4+ was used as proxy for viral activity as well as an important immunological index...In our study, CD4+ T cell count and percentage were only weakly correlated with virus load and were not related to transmission...Higher transmission rates were associated with prematurity, low birth weight, vaginal delivery and low NK cell percentage...In this non-breast feeding Asian population with predominantly HIV-1 subtype E infection, we found the perinatal HIV transmission rate to be ~24% and maternal plasma virus load at delivery to be an extremely strong predictor of transmission. Transmission risk was low (8.5%) at virus loads below 10,000 copies/mL, and no transmission occurred below 2000 copies/mL…These findings may be useful in designing simplified interventions to reduce perinatal transmission in developing countries and may help explain the 50% reduction in perinatal HIV transmission recently announced at these same study hospitals, at which a short, oral zidovudine regimen late in pregnancy was used".[245]

2.5   Discussion

There are several reasons why the presently available evidence cannot be considered proof of mother to child transmission of HIV.

2.5.1   Epidemiology

It is well known that AIDS was first diagnosed in white gay and bisexual men and up to 20% of men who consider themselves gay have sex with women.[246,247]   In 1986 Nancy Padian and her colleagues at the University of California presented data that "A possible chain of transmission for ARV [HIV] infection would be through bisexual men. The potential for such transmission can be estimated from data available from the San Francisco Men's Health Study, a prospective study of the epidemiology and natural history of the acquired immunodeficiency syndrome. Because the cohort is a probability sample, inferences can be extended to the entire population of single men in the study area. Of the 1,035 men aged 25 to 54 years in the cohort, 169 (16.3%) classified themselves as bisexual and 108 of them reported one or more female sexual partners during the previous two years". By extrapolating these data to their "entire population, 23,000 single men", the authors calculated "the total number of women exposed by these men is approximately 3,200" and concluded "A substantial potential clearly exists for epidemic spread of ARV infection to women in San Francisco. To date, there is no evidence that such extension has occurred".[247]   In the 1996 P^{2}C^2 study group of MCT in the USA, 8.1% of the women had "sex with homosexual men".[213]   By 1982 the vast majority of haemophiliacs tested in the USA were HIV antibody positive while education in regard to safe sexual practices was introduced only in 1985-86. Even by 1997 with no effort in focused, personalised, safe sex education spared, 25% of partners of infected individuals enrolled in one on-going study conducted by HIV experts did not practice safe sex.[194]   The birth rate in haemophiliacs did not decrease in the AIDS era. In fact "the HIV epidemic will produce small reductions in the expected numbers of hemophilic (1.79%) and carrier (2.63%) births in the next 2 centuries. More substantial reductions in the numbers of hemophilic and carrier births required extreme assumptions regarding the fertility of hemophilic men, the extent of HIV infection among hemophilic men, and the proportion of hemophilic births that arise from spontaneous mutation".[248]

Anyone attempting to analyse the presently available data is confronted with an unexpected finding. If HIV was sexually transmitted and from mothers to their children then:

(a) by now there should have been an epidemic of HIV infection of infants, in fact this should have been well under way by the mid-1980s;

(b) children of white parents should have been among the first reported infected and even today should form the majority of children infected by this route.

However,

(i)   to date, in the USA and Europe the number of children reported HIV infected either by MCT or by any means remains very small. So much so that the same children are reported in different studies while in some countries such as Australia and Canada, the numbers of children are so small epidemiological studies cannot be generated. For example, in Australia the cumulative total of perinatally infected children between 1982-2000 number 46, less than three annually.[249]   This means that even if MCT of HIV does take place, it must be negligible;

(ii) the first cases reported suggest the claims of MCT of HIV were from non-white, mothers-infants pairs;

(iii) with the exception of a small number of children born to white drug using mothers, even today and even in the USA and Europe, the vast majority of children reported infected by this route are born to non-white mothers.

This means that either HIV, unlike any other infectious agent is able to discriminate between races, or there is a significant problem with the methods used to prove infection.

2.5.2   Methods used to prove MCT

Four methods have been used to prove infection of children: antibody tests, culture, PCR and the clinical AID syndrome. Of these the antibody test and the clinical syndrome are the two most often used tests to prove MCT. Even if one considers these modalities specific for HIV infection in adults this cannot be the case for children.

2.5.2.1   Antibody tests

As previously discussed, the validity of all the laboratory tests, including the antibody tests, used to prove HIV infection has been disputed by HIV experts themselves. There are ample scientific reasons why a positive antibody test, even if specific in adults, cannot be considered proof for HIV infection in these children:

(a)   Since the vast majority of mothers said to transmit HIV to their children are poor non-white women or drug users or both, they are at high risk for many diseases including those caused by infectious agents even in the absence of HIV. This means that they will possess antibodies that react with many antigens other than HIV including auto-antigens. These antibodies, like the putative HIV antibodies, may also cross the placenta and, like all antibodies, cross-react in such a manner to produce a positive antibody test in the absence of HIV infection;

(b)   It is accepted that the children born to women similar to those reported in the MCT studies, that is, poor and drug using women, are at high risk of ill health and death, than children born to socio-economically advantaged and non-drug using women. This means that the children themselves will develop antibodies to many non-HIV antigens which in turn may cross-react with the antigens in the HIV test, resulting in a positive test even if the children are not infected. For example, septicaemia is "strongly associated with HIV infection".[233]   Children are particularly prone to infection with *E. coli* which could invade the blood stream, especially from a compromised intestine. However, antibodies to *E. coli* lipopolysaccharide react with the HIV proteins (V. Colizzi *et al*, personal communication);

(c)   The child's weight is one of the factors which has been most often reported a risk factor for transmission. However, at least in adults, weight loss leads to the development of a positive test and not vice versa[250] (see Part V for full discussion);

(d)   In children, using WB as a gold standard, hypergammaglobulinaemia identified HIV infected children with a specificity of 97%.[233]   Sixty-three sera obtained from 23 patients before and immediately after immunoglobulin infusion were tested for HIV antibodies using WB. Of the 63 sera, 52 (83%) were found positive. "Several samples tested in an HTLV-III p24 radioimmunoassay were also positive. The amount of antibody detected was greatest immediately after infusion and decreased between infusions". An individual was given six 5ml injections of donated Rh+ serum, administered at 4 day intervals. "The donor serum was shown to be negative on HIV antibody and antigen ELISA, so was blood taken from his wife and child". "Blood taken after the first immunisation was shown to be negative on HIV antibody ELISA and immunoblot assay. After the second immunisation a weak signal on ELISA, slightly above the cut-off level, was monitored. After the third immunisation the signal was strong and immunoblot revealed distinct interaction with p17 and p55 proteins. An even stronger signal was monitored after the fifth immunisation. Interaction with p17, p31, gp41, p55 and some other proteins was evident".[111,233]   This means that immunised children may have positive HIV antibody tests even if not infected.

For some unknown reason not one of the researchers who performed MCT studies questions the specificity of the antibody test. In all studies the antibody test results are considered 100% specific although there are scarcely any two studies that employ the same criteria to define a positive WB. In fact in some studies from Africa and Asia the test used is ELISA and not the WB.[244,251]   Even in the latest publication by the DITRAME Study Group, "Serum samples collected between 9 and 15 months of age were screened for HIV-1 and HIV-2 antibodies by commercial enzyme-linked immunosorbent assay (ELISA...). Confirmation on the same samples was obtained with a synthetic peptide ELISA".[252]   However, according to the CDC, 2000, "Revised Surveillance Case Definition for HIV Infection", "repeatedly reactive enzyme immunoassay" may be used only as "a screening test for HIV". Infection must be confirmed "by a positive result on a confirmatory (sensitive and more specific) test for HIV antibody (e.g. Western blot or immunofluorescence antibody test)".[253]   Yet in a 1997 study from Zimbabwe, one ELISA (using blood from children who "were certified dead on arrival in hospital or who died shortly after in the casualty department") in children as young as 1 month was considered proof for HIV infection.[254]

(e)   As far back as 1988, there was evidence that the antibody tests in children are non-specific. It is accepted by all HIV experts that a child can have positive antibody test without being infected. This is because maternal antibodies cross the placenta as early as the 12th week of gestation.[255]   As

44

a result of normal catabolism, the level of these antibodies decreases post partum and by 9 months of age they are no longer present in the child[256] (Figure 2.1). In other words, if the HIV antibody test is specific, any child who has a positive HIV antibody test beyond 9 months should remain positive for the remainder of his or her life. In the only study providing a detailed analysis of *post partum* loss of infant HIV seropositivity, the European Collaborative Study, approximately 23% of the children became seronegative between birth and 9 months. However, 59% became seronegative between 9 and 22 months (Figure 2.2). Since the latter cannot be due to loss of maternal antibodies, the only explanation is that either: (i) the antibody test is non-specific or; (ii) the children managed to clear HIV infection without treatment. If 23% of children test positive because of maternal antibodies and in 59% the test is non-specific, how certain can one be that in the remaining 18% of children the test will not also serorevert after 22 months? Or if the test remains positive it is a true positive?[229] In the Ariel project a child was defined as positive "if a peripheral blood sample was positive for HIV-1 by culture and a second sample from a separate blood drawn was positive by either culture or HIV-1 DNA". "Of the 117 uninfected children with serological testing done at 1 year of age, 66 (56%) had a positive or indeterminate result". Even by 18 months of age 14% of uninfected children tested had a positive or indeterminate antibody test.[217] The only conclusion one can draw from these data is that either, one, two or all of the tests (antibody, culture, PCR) are non-specific.
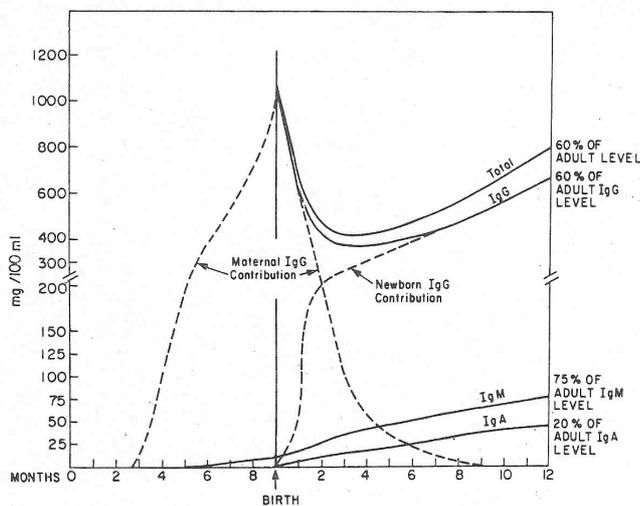
Figure 2.1
Time vs loss of maternal antibodies (ref 256)
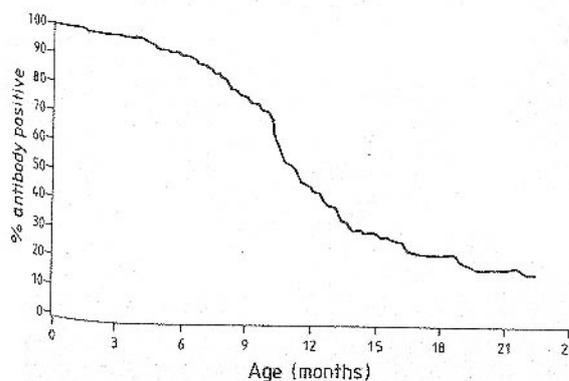
Figure 2.2
Loss of "HIV" antibodies post partum (ref 229)

2.5.2.2 Culture

In some studies including the ACTG076 study, proof for transmission is obtained by performing "viral" cultures where cells from children are co-cultured with other cells. Cultures which exhibit one of the following: (i) reverse transcription of the synthetic primer template An.dT$_{12-15}$; (ii) a reaction between culture constituents and antibodies directed against the p24 "HIV" protein; are considered proof that the child is infected with HIV. Even if one were to accept that such findings do prove infection of adults they cannot be used in children. In the McIntosh *et al* study from the USA,[212] 6/302 children had one "positive culture followed by ≥2 negative cultures and negative serology". In the 1996 WITS study, nine children who had 1 positive culture "were subsequently classified by an ongoing indeterminate review committee as uninfected, on the basis of review of laboratory (e.g. serology, DNA polymerase chain reaction) and clinical data".[43]

According to both the Pediatric AIDS Clinical Trials Group (ACTG) and the 1995 "Methodology of intervention trials to reduce mother to child transmission of HIV with reference to developing countries", to claim proof for MCT of HIV, it is necessary to have two positive cultures from two independent blood samples.[257,258] Yet in the vast majority of the studies one positive culture is considered proof for MCT.

In the 1991 publication of the European Collaborative Study, of 353 children whose antibody test became negative and in whom AIDS did not develop, "virus isolation had been attempted in 163; in 10 (6.1%) positive results were obtained at least once...In 4 children the initial positive culture was confirmed on subsequent samples by repeat virus culture and/or polymerase chain reaction (PCR). But in the remaining 6, a total of eight attempted cultures were negative".[233] The evidence from the Ariel study, the European Collaborative Study and the Italian Study shows that no correlation exists between antibody and culture tests.[217,228,229,231] Similar findings were reported in the 1992 study from Switzerland.[235]

In the first study published from Africa the result of the culture depended on the socio-economic status of the mother.[238] This means that HIV discriminates between poor and privileged children or the reaction between the antibody to p24 and antigens present in the culture is non-specific.

In the Multicenter Quality Assurance Program for the Pediatric AIDS Clinical Trials Group it was shown that the result of isolation depends on:

(a) the length of time of the culture. If a culture is tested repeatedly over a long period it is more likely to get a positive result;

(b) the culture conditions;

(c) the efficacy of HIV isolation is laboratory dependent.[257]

Even the CDC has reservations regarding the use of culture (HIV isolation) to prove MCT. In the Revised Surveillance Case Definition for HIV Infection, 2000, one reads: "HIV nucleic acid (DNA or RNA) detection tests are the virologic methods of choice to exclude infection in children aged less than 18 months. Although HIV culture can be used for this purpose, it is more complex and expensive to perform and is less well standardized than nucleic acid detection tests".[253]

2.5.2.3 PCR

Because it is accepted that in most children an early positive antibody test reverts to negative, PCR has been used to prove MCT. However, already by 1995 numerous studies[259-262] revealed that in children a positive PCR, like a positive antibody test and culture, may revert to negative. While an excuse can be found for the antibody test, no explanation, other than that the test does not detect the HIV genome, can be found for PCR. In a report published in 1995 by French researchers, in a six year cohort of 188 "infected" children analysed retrospectively, 12 (6.7%) "cleared HIV infection". Each child had at least two positive PCR results at two separate time points in the first year, followed by numerous (up to 7) negative PCR results. For PCR the investigators used primer pairs for the *gag, pol*, and *env* gene regions; and the test was considered positive "if at least two genes were amplified". Commenting on their results the authors wrote, "Three different rooms with separate air-conditioned circuits were used for DNA extraction, PCR-buffer preparation, amplification and blotting. Amplicons were never transferred in the area reserved for unamplified sequences. Thus, positive PCR results are unlikely to be due to contamination…Nevertheless, as our PCR assays are performed on unmanipulated cells, culture contamination leading to false positive PCR results is impossible…We therefore consider that the probability of repeated contamination on successive samples from the same child is scarce". The authors "could not find any correlation between either neutralising or antibody-dependent cellular cytotoxicity-mediating antibodies and

HIV clearance". Of 139 children born to HIV positive mothers but who were "clearly negative", eight were PCR-positive once for a single viral gene (*pol),* three were positive twice for the *pol* gene, and once of the three was also positive for the *gag* gene in a single assay".[263] Because of this, Ho and his colleagues said that to use PCR for the diagnosis of HIV infection in children it is necessary to have "reproducibly positive PCR results".[215] The CDC also asserts the need for two positive PCRs in two separate specimens.[253] The authors of the 1992 study from Switzerland pointed out that: "Single positive virus detection tests in seronegative asymptomatic infants can possibly be explained as false positive results. The fact, that their occurrence (especially for PCR) is not rare in children who lost maternal antibody, must be borne in mind in the interpretation of attempts at early diagnosis. The repeatedly positive PCR results in two children with negative VC and losing their antibodies is especially puzzling in this context…In view of these difficulties it seems prudent to always use two successive blood samples and not to rely on PCR as the only virus detection test for the diagnosis of HIV infection during the first months of life. Even in very carefully conducted prospective studies calculation of an exact vertical transmission rate is impeded by the inevitable incompleteness of follow up and by diagnostic difficulties in some children".[235] Yet not only is PCR used to prove MCT, in most of the studies in which PCR was used to prove infection the PCR was the only test used, and a single positive PCR test is considered proof of infection. That difficulties exist with the use of the PCR for defining HIV infection in children is accepted by the authors of the "Report on the Meeting of the Technical Working Group on HIV/AIDS in Childhood, Geneva, 27 February - 1 March 1989" organised by WHO where it is stated: "Among the problems are false positive reactions and the technical complexity of the test".[264]

In the 2000 Revised Surveillance Case Definition for HIV Infection (Appendix IV) it is stated: "In adults, adolescents, and children infected by other than perinatal exposure, plasma viral RNA nucleic acid tests should **NOT** be used in lieu of licensed HIV screening tests (e.g., repeatedly reactive enzyme immunoassay)".[253] This raises the following questions:

(a) why a test (PCR RNA), the basis of which is claimed to be recognition of nucleotide sequences specific to an exogenous retrovirus HIV, "should NOT be used" even as a screening test in adults and adolescents and infants infected via blood transfusion yet the same test is approved to prove perinatal transmission?

(b) why the test cannot be used even as a screening test to prove infection of children by means other than perinatal transmission but can be used to prove MCT? How it is possible for the test to discriminate between children infected perinatally and by other means?

(c) how is it possible to claim that a PCR RNA test cannot be used to prove HIV infection but can be used to quantify HIV?

Answers to these questions have been actively sought from the CDC (see Appendix X). Their response included "The issue of testing for diagnosis of HIV infection is a clinical question. The Food and Drug Administration is responsible for setting standards for diagnostic tests for HIV infection. CDC recommendations are only for surveillance purposes". This raises at least one additional question: Why, on the basis of tests recommended by the CDC for surveillance purposes only, mothers and children are diagnosed as being infected with HIV? Comments on the CDC response are given in Appendix X.

2.5.2.4   Clinical symptoms and signs

The only evidence in the initial reports claiming proof of MCT of HIV is the presence in children of AIDS or AIDS-like diseases. Even after the development of the HIV tests (antibodies, cultures, PCR), in the vast majority of studies the diagnosis of HIV infection in a child and transmission from its mother is determined not by virological but clinical data, and consists of one of the following:

(a) AIDS;

(b) death from AIDS;

(c) death from "HIV related disease" without defining what is meant by "HIV-related disease".

Such assumptions may be considered valid if and only if:

(a) there is definite proof that HIV causes AIDS (immune deficiency, that is, T4 decrease; and the clinical syndrome) and "HIV related disease";

(b) there is definite proof that the children had AIDS or have died from AIDS or "HIV related disease";

(c) there are no other causes for AIDS or "HIV related disease".

It is accepted by all HIV experts that the proof that HIV causes AIDS is based solely on a correlation between a positive antibody test and AIDS. This being the case AIDS or death from AIDS cannot be considered proof for HIV infection. It is not possible to claim on the one hand, that a positive antibody test is proof that HIV causes AIDS, and on the other that the presence of AIDS is proof that the patient is infected with HIV. In very few of the studies in which proof for MCT transmission of HIV is claimed are there data on the immunological (T4 cell) status of the children. It is possible this may be due to the fact that by 1986 evidence existed that "the effect of HTLV-III infection on the immune system of children is somewhat different than on adults. Early in the course of pediatric AIDS, lymphopenia may not be noted, and lymphocyte mitogenic responses to phytohemagglutinin (PHA) and Concanavalin A (Con A) as well as in the mixed lymphocyte culture are normal…Total T4 cell numbers and percentages may not be altered, and interleukin-2 as well as interferon production are normal. These normal phenotypic and functional properties of T cells may be maintained for months and years, even in the presence of opportunistic infections".[265] This was confirmed in the European Collaborative Study where it was shown that low T4/T8 ratio was present in less than half of the infected children. Furthermore, in complete contradiction to the HIV theory of AIDS, the low T4/T8 ratio "came after the onset of AIDS in 44% of those in whom AIDS developed" and was also present in non-infected children.

Since,
> according to the HIV theory of AIDS infection with HIV leads to destruction of the T4 cells, (AID);
> the decrease in T4 cells leads to the appearance of the clinical syndrome (S);
> in the vast majority of children with the clinical syndrome the T4 cell number is either normal or if decreased it follows the appearance of S;

then, it follows that HIV cannot be the cause of AIDS in children.

In an extensive review of Pediatric AIDS, Rubinstein, Professor of Pediatrics, Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York, it is stated: "The differential diagnosis of pediatric AIDS encompasses disease entities unique for this young age group. The two most confounding disease processes in infants are various congenital infections and primary congenital immunodeficiencies". The latter included cytomegalovirus, Epstein-Barr virus,, Herpes virus, Rubella virus, Toxoplasmosis and "Immunodeficiency in infants born to drug-abusing mothers".[265] In other words, at the beginning of the AIDS era it was accepted that what became known as AIDS in children existed long before 1980.

At present there are two principal definitions of AIDS for use in children. The WHO clinical definition, the Bangui definition and its modified version, the Ghent definition, and the CDC definition, (Appendices I-III, VII-VIII). As can be seen in these appendices, what the WHO considers AIDS are largely symptoms and signs, and a few diseases, all non-specific. The CDC definition, which purports to be more rigorous, omits symptoms and signs and consists of diseases but these are also non-specific. The diseases which the CDC define as indicative of AIDS are not only diagnosed in individuals said to be infected with HIV. This is accepted by HIV experts. In the "Report on the Meeting of the Technical Working Group on HIV/AIDS in Childhood: Geneva, 27 February - 1 March 1989", one reads: "Indicator diseases seen in immunocompromised HIV-infected children overlap with diseases commonly seen in children not infected with HIV".[264] This is the case even with the two AIDS indicator diseases considered to be the most specific for HIV infection, PCP and KS (the latter now accepted even by HIV experts as not being caused by HIV yet KS is nonetheless an AIDS indicator disease and AIDS is said to be caused by HIV). Furthermore, in the vast majority of studies, no mention is made as to what methods were used to diagnose AIDS, death from AIDS or "HIV related disease".

2.5.2.4.1   Pneumocystis carinii pneumonia in children
Virtually no physician actively involved in clinical practice has either memory or experience of patients with PCP prior to the AIDS era. Yet this disease is neither new nor specific to AIDS and in fact was described and researched at length in relation to poverty and malnutrition prior to the AIDS era.[266,267] In a paper published in 1956 in the *American Journal of Pathology*, H. Hamperl, Director of the Institute of Pathology, Bonn University, wrote, "Knowledge of pneumocystis pneumonitis has developed far more rapidly in Europe than in the United States or elsewhere in the world…The disease scarcely ever occurs before the 6th week of age or after the 6th month, and is evident most frequently between the 10th and 14th weeks. It appears primarily in premature or in mature dystrophic infants, in hospitals and nurseries…The etiology of the disease was unknown until van der Meer and Brug, Vanek and Jirovec, and Giese recognized foamy material in the alveolar lumina as parasites. Vanek and Jirovec, together with most of the other authors, believed the parasites to be *Pneumocystis carinii*, a protozoan. They spoke of pneumocystis pneumonitis. The term *Pneumocystis carinii* had been coined by

Delanoes to designate a parasite previously described by Carini. Giese, on the other hand, considered the parasite to be yeasts…The fact that therapy has not been successful with the drugs employed against protozoa but with those against fungi could be interpreted in favor of Giese's opinion…There is a remarkable frequency of combination of cytomegalic disease and interstitial plasmacytic pneumonitis…It seemed worth while, therefore, to consider the possibility that pneumocystis pneumonitis may occur also in adults with cytomegalic disease much more frequently than is suspected".[268]

Discussing PCP in his book Viral Sex, Jaap Goudsmit wrote: "As far as we know, the largest epidemic which struck very young children, emerged just before World War II and lasted about twenty years. It affected only children in their first year of life, only in continental Europe, but thousands of children died...Adults were affected but only sporadically". According to Goudsmit, the cause of the PCP epidemic in children even before the AIDS era, was HIV. However, he fails to explain why:

(a) the epidemics appeared only in "institutionalized, debilitated children…premature, malnourished infants";[269]

(b) such epidemics occurred in widely separated hospitals of Eastern and Western Europe only in children, without parents or any other adult group being affected;

(c) "In the laboratory the most striking observation was a hypergammaglobulinaemia. This means an abnormally high level of antibodies–a sign of chronic infectious disease and a hallmark of AIDS in children". Yet, "the 1950s disease was importantly different from paediatric AIDS as seen since the 1980s";

(d) "No children who recovered from early PCP (with or without CMV) died years later of other manifestations of immunodeficiency";

(e) the epidemic stopped and the children cleared HIV without any treatment while in the AIDS era with tens of billions of dollars spent on anti-HIV treatment, HIV has not been cleared even from one individual.[270]

In a study published in 1964 entitled "Endemic Pneumocystis Carinii Pneumonia in South Iran" the authors pointed out that *Pneumocystis carinii* "has been described in many animals, and infections in dogs, rabbits, cats, mice, sheep, goats, monkeys and pigs have been reported…*Pneumocystis carinii* was first seen in a human being in Brazil by Chagas [1909] during his search for the life-cycle stages of *Trypanosoma*. However, the first clear description of the pathological anatomical picture and the association of pneumocystis with plasmacellular pneumonia was published during the last war in 1942 by Meer and Brug. Since then most of the cases described have been in Europe especially in Germany and Czechoslovakia, where much attention was paid to this entity after the early studies by Vanek and Jirovec. For the years 1951 to 1952 Dvorák and Jirovec reported 160 cases from Czechoslovakia alone, of which 86 were fatal and confirmed by autopsy. Recently it has become apparent that the disease has a world-wide distribution".

Between July 1961 and March 1963, the authors conducted clinical and epidemiological observations on the children in two nurseries in Iran. All the children who died during this time "were brought to autopsy". Of these 40 children, "six died with pneumocystis carinii infections". Discussing their findings they wrote: "There are two predisposing factors in our series of cases, namely prematurity and dystrophy. It has nearly always been shown that only those who are weakened by prolonged ill health are, with certain well-known exceptions, susceptible to the infection. Of our total of 40 deaths, 20 were premature and most of them were foundlings. Only five fatal cases showed good nutritional condition…These factors were greatly aggravated by the problems existing in the nurseries, where an insufficiently trained staff was unable to cope with anything but the most basic rules of antiseptic care…The personnel used to take their own nursing infants with them to work, but among these children there were no symptoms attributable to pneumocystis". They concluded: "From the published material, weakness and ill health are serious contributions if not necessarily the decisive factors in this disorder. We think, therefore, that in areas where hygienic conditions are poor and where debilitated children are treated and kept in institutions, the pneumocystis rate will be greater than previously expected. Similarly, among premature and malnourished children as well as among pregnant women in their homes, this disease must be regarded as a serious problem".[271] At least as far as children are concerned, researchers from Chile, expressed a similar view: "One thing seems clear. The disease attacked only infants with poor nutrition. An adequate consideration of these factors ought to be of paramount importance in the prevention and treatment of this disease.[272]

In a study published in 1974, the authors from St Jude Children's Research Hospital, Memphis, USA and the University of Natal, Durban, South Africa, wrote: "*Pneumocystis carinii* pneumonitis occurs almost exclusively in patients with serious underlying diseases. The primary diseases include leukemia, sarcomas, Hodgkin disease, neuroblastoma, histiocytosis, multiple myeloma, Waldenström macroglobulinaemia, thymic dysplasia, rheumatoid arthritis, rheumatic fever, Schönlein-Henoch disease, thrombotic thrombocytopenic purpura, haemophilia, haemolytic anaemia, aplastic anaemia, Albers-Schönberg disease, nephrosis, cryptococcosis, tuberculosis, hypoglycaemia and adrenogenital syndrome. This disease is also seen in patients after organ transplantation, in debilitated infants, and in children with agammagolbulinaemia, hypoproteinaemia, and Wiskott-Aldrich syndrome. Rarely is it observed in otherwise healthy people…We decided that protein-calorie malnutrition might play a role in *P carinii* pneumonitis because malnutrition is associated with the various predisposing disease entities, nursery outbreaks of *P carinii* pneumonitis occur in "debilitated" infants, severe emaciation is observed in the cortisone-treated rat before fatal pneumonitis ensues, and undernutrition is associated with impaired resistance to a number of infectious diseases". The study was done in children from the USA and South Africa. In the USA "data from 225 cases of *P. carinii* pneumonitis revealed mean body weights and serum protein values that were below normal…Of 39 South African children who died with kwashiorkor, three (7.7%) were found to be infested with *P carinii*, whereas no organisms were found in the lungs of 21 well-nourished and geographically matched children. In Sprague-Dawley rats fed a 23% protein diet for normal growth, none of 15 acquired *P carinii*; whereas, 13 of 15 fed a protein-free diet died infested with *P carinii*". Discussing their findings they wrote: "The finding of *P carinii* infestation and pneumonitis in rats maintained on protein-free diets, with and without vitamin supplements, and the absence of the organism in animals under the same experimental conditions that were fed a 23% protein diet, clearly demonstrates that *P carinii* infestation and infection can be induced solely by protein-calorie malnutrition. Furthermore, deaths from *P carinii* pneumonitis were greatly increased when cortisone-treated rats were fed protein deficient diets rather than the 23% protein diet. Rescue of protein deficient rats with infestation by dietary-protein replenishment, demonstrates further the role of nutritional status in *P carinii* infestation and pneumonitis … Malnutrition may affect the host's resistance to infection by influencing phagocytosis, endocrine function, the natural microbial flora, tissue integrity, humoral and cellular immunity, wound healing, and collagen formation as well as a number of nonspecific resistance factors. Perhaps the greatest impact is on humoral and cellular immunity".[273]

A follow-up study of PCP in the two Iranian nurseries was published in 1976. "Despite the provision of adequate pediatric nutrition, infants had considerable diarrhea in the first months of life, due to improper feeding methods and lack of food sterilisation. The crowding prevented complete control of this problem for some time…The development of infants was severely hampered, and practically none of the infants reached the Boston 3d percentile in weight and height for the first year of life. Babies with marasmus, due to constant diarrhea rather than inadequate nutrition, were frequently observed…Further morphologic studies revealed that all infants with severe pneumocystosis had marked flattening of the spruelike bowel mucosa due to the repeated intestinal infections and persistent diarrhea, which accompanied the marasmus and the immune deficiencies. Severe thymic atrophy was also found in most patients, with thymus weights of less than 3 g wet weight at autopsy and accompanying absence of the T-cell rim in mesenteric and tracheopulmonary lymph nodes; in other patients the thymus appeared normal…The infants, relatively well-nourished and close to the Boston 3d percentile–albeit under it–showed less severe changes than the marasmic ones with weights less at 3-4 months of age than at birth, among whom most of the IPCP occurred. These children looked like skeletons; no trick of the pediatric feeding armamentarium helped to improve the nutritional status, since the atrophy of the mucosa by itself interfered with absorption. When X-rayed, the thymus had disappeared in these infants by the second to third month and the cell-mediated immunity lagged in development. It appears from most recent studies that cell-mediated immunity in the survivors never recovered completely".

The study was divided into 4 periods, and the mortality from PCP and other infections was as in table 2.1.

Table 2.1

Period	Date	Total No. of deaths	Non-P. Carinii Infections*	P. Carinii*
1	9/61- 3/63	40	25.0	4.96
2	3/63- 3/64	36	33.6	7.35
3	3/64- 9/65	82	35.0	25.49
4	9/65-12/68	75	9.15	4.57

*Death rates/100 beds for 1 year

According to the authors of this study, "despite all efforts in care, a hardcore 4-5% incidence of pneumocystosis deaths remained before prophylaxis was introduced…Care and supervision are usually improved during scientific studies. This factor must be carefully evaluated in any therapeutic or prophylactic trial, since

improved care alone may be responsible for the amelioration of an IPCP [interstitial plasma cell pneumonia=PCP] epidemic".[274]

Thus before the AIDS era there was ample evidence of a relationship between malnutrition and PCP. Yet in a study published in 1997 by researchers from Zimbabwe, 106/184 (58%) children were malnourished but PCP was diagnosed in 19 (16%) and only in those HIV positive. There can be only three reasons for this:

1. The study was biased;
2. The positive HIV antibody tests in PCP children are false-positives;[75,76]

3. In the HIV era malnutrition abruptly ceased to cause PCP.[254]

In a paper published in 1980 entitled "Pneumocystic Carinii Pneumonia in Young Immunocompetent Infants" the authors from the University of Alabama, Tennessee and Johns Hopkins wrote: "Previous reports have already shown that *P carinii* infection is highly prevalent among normal, young children and that sporadic or epidemic forms of pneumonitis, occurring primarily between 6 and 16 weeks of age may be seen in patients with no coexisting disease". Summarising their findings they wrote: "The findings of this prospective study clearly indicate, however, that *P carinii* is also etiologically linked to pneumonitis in young immunocompetent hosts without underlying illnesses. In fact, evidence of active infection, as demonstrated by antigenemia (ten cases) and histopathology (one case), occurred in 14% (10/67) of a group of infants less than 3 months of age who were enrolled in a prospective study of infant pneumonia...None of the ten infants with *P carinii* pneumonia had evidence of a primary immunodeficiency and no patient had received immunosuppressive medication prior to the onset of his illness. Of these ten infants, nine had a history of poor weight gain…These results indicate that *P carinii*, singly or in combination with other infections may be an important cause of pneumonitis in young, immunocompetent infants with no underlying illnesses".[275] Yet enigmatically the CDC clinical definition of AIDS requires individuals with PCP to be classified as AIDS even when there is "laboratory evidence against HIV infection".[134] That is, these individuals have a syndrome, AIDS, caused by HIV even when HIV is proven to be absent from such individuals. In the MCT studies, PCP without further laboratory tests is considered proof that the child is infected with HIV.[276]

It must also be pointed out that prior to the AIDS era children with pneumonia were seldom tested for PCP. This was due either to lack of awareness by physicians or because the most specific method for diagnosis required an invasive procedure, that is, open lung biopsy. On the other hand, in the AIDS era no effort is spared to diagnose PCP in children of mothers who are at risk of AIDS but, instead of open lung biopsy other methods are used. However, even with the bronchoalveolar lavage (BAL), which is most often used and which HIV experts consider extremely specific, "one might expect to find *P carinii* in fluids from bronchoalveolar lavage in about 40% of patients with AIDS who present with symptomatic pneumonia caused by other organisms".[277]

2.5.2.4.2   Kaposi's sarcoma in children
In the early 1980s, a high frequency of Kaposi's sarcoma was identified in gay men. For several years and for all intents and purposes, this malignancy and PCP constituted what became known as AIDS. Indeed, KS was the basis for Gallo's hypothesis that the cause of KS and thus of AIDS is a retrovirus. KS is not a new disease. A fitting description is included in the Ancient Egyptian Ebers Papyrus dating from 2500 BC while its modern description dates from Moritz Kaposi in 1872. Whilst relatively rare in the rest of the world, KS was prevalent in Africa long before the AIDS era. In Africa, KS affects young adults (25-45 years of age) and children between the ages of 2 and 15. In this group, as in gay men, there is an appreciable incidence of generalised and aggressive KS with most of those affected dying within two years of diagnosis.[95,278]
There are many AIDS risk groups, gay men, haemophiliacs, transfusion recipients, heterosexuals, Africans and Asians. However, with rare exception, KS is limited only to gay men and Africans. In the US by 1990 there were only 13 cases of KS/AIDS in children and all but one of these US born children were offspring of Haitian women. In that year researchers from the CDC stated that these cases of AIDS were "atypical" and that "diagnostic biases might exist". The uneven distribution of AIDS among the risk groups, its diagnosis in many gay men who are not immunosuppressed and who test negative for HIV, eventually led to the conclusion that KS is caused by an infectious agent but the agent is not HIV.[279,280] Although at present everybody accepts that the cause of KS is not HIV, for unknown reasons KS remains an AIDS-defining disease, a syndrome caused by HIV. In studies of mother-to-child transmission, KS, a disease not caused by HIV, is considered proof that a child is infected with HIV and that HIV infection was acquired from the child's mother.

In 1974 researchers from the University of California described "The syndrome of cellular immunodeficiency with immunoglublins…a distinct primary immunodeficiency". This report consisted of the clinical and immunological features of 6 cases of their own as well as 28 cases of other workers. The children had cellular immunodeficiency with elevated levels of immunoglobulins, including auto-antibodies. The clinical manifestations included recurrent pulmonary infections, including 4 cases of *Pneumocystis carinii* infection, failure to thrive, hepatosplenomegaly, oral or cutaneous candidiasis, chronic diarrhea, recurrent skin infections, severe varicella, otitis, gram-negative sepsis. "Onset of symptoms is generally by six months of age, although some patients were asymptomatic through the first year". The authors concluded that the immunological and clinical manifestations in these children had "sufficient features to characterise them as a distinct syndrome", a syndrome which is indistinguishable from what nowadays is called AIDS.[281]

It is accepted that maternal drug addiction has detrimental effects on the infant both before and after birth. In 1977 a study was published from New Jersey USA documenting "Experiences with 118 infants born to narcotic-using mothers". The author described, "as have others, the deleterious effects of maternal addiction on the infant both before and after birth…The perinatal mortality rate for the study group was 3.4%, compared with 2.32% for the controls. The narcotic users had almost a 5-fold greater number of small-for-date babies and 3 times as many prematures…Infants born to narcotic addicts appear to run an excessive risk of infections, both acute and chronic. However, the tremors, diarrhea, vomiting, tachypnea, hypertonicity, and other withdrawal symptoms often suggest the possibility of infection in these infants".[282]

The effects of heroin addiction on 149 newborns of 101 women were evaluated in a Canadian study entitled: "Narcotic addiction, pregnancy and the newborn". They reported that: "Average birth weight was 2,710 ± 760 gm (SD), compared with an average of 3,420 ± 225 gm (SD) for all newborn infants at this hospital, and a mean 3,325 gm for the Province of British Columbia. Thirty seven per cent of the babies born to heroin-addicted mothers were of low birth weight, compared with an overall hospital rate of 6.7%. Two thirds of them were preterm and one third small for gestational age". 6.7% of newborns had hypoglycaemia and 14.4% hypocalcaemia. "Increased respiratory rate as part of the withdrawal symptomatology was present in 16 of the babies. In an additional six it was associated with pneumonia or cardiac anomaly. There were seven infants with idiopathic respiratory distress syndrome (IRDS), four of the six under 28 weeks gestation, and three of the 30 at 29 to 36 weeks". In the same study "Details were obtained about an additional 140 children born to these same mothers either previous to the 149 births in this study or subsequently at various hospitals. Of the resulting total of 289 children, 199 were born at a time of maternal narcotic intake: 90 while their mother was allegedly not taking drugs or at least not taking narcotics. The average age of these children at the time of last contact with social agencies was 4 years. Only 72 of the 289 or 25% were then still living with their mother. 44 or 15.2% were with relatives: 143 or 49.4% had been relinquished for adoption or foster care and 30 or 10.4% were dead".[283] In 1979 researchers from the USA presented evidence that 17 of 688 (2.5%) infants of drug dependent mothers born at the Hutzel Hospital over the three year period September 1974-September 1977, compared to 2 of 388 (0.5%) of controls, died from sudden infant death syndrome.[284]

According to a paper "Drug Use in Pregnancy: Parameters of Risk", published in the Paediatric Clinics of North America, 1988, "Numerous reviews of drug use during pregnancy show that most drugs taken by the mother during pregnancy freely cross the placenta. Drugs that act on the central nervous system are usually lipophilic and are of relatively low molecular weight, characteristics that facilitate the crossing of the substance from maternal to fetal circulation...Most drugs that have been studied have a longer half-life in the fetus than in the adult. This is also true in the neonate since the enzymes involved in the metabolic process of glucuronidation and oxidation are not fully developed in the fetus. In addition, the immature renal function of the newborn may delay the excretion of drugs that have been metabolized to an excretable form...Multiple other factors in the environment of the pregnant substance-abusing woman, including poor nutrition, lack of prenatal care, maternal psychopathology, and the drug-seeking life-style, affect the ultimate outcome of the passively exposed infant and must be considered in the interpretation of clinical and research information...The most common features of the neonatal abstinence syndrome mimic aspects of an adult withdrawing from narcotics. Most significant for the neonate are the high-pitched cry, sweating, tremulousness, excoriation of the extremities, and gastrointestinal upset. Withdrawal from narcotics persists in a subacute form for 4 to 6 months after birth, with a peak in symptoms at around 6 weeks of age...Infants born to mothers maintained on methadone throughout pregnancy continue to be significantly smaller in weight and length compared to drug-free infants through 6 to 9 months of age but usually catch up in weight and length by 12 months of age...Of concern, however, is the fact that the infants demonstrate a downward trend in developmental scores by 2 years of age, a phenomenon not uncommon in infants from low socioeconomic groups".[285]

In summary, in the early 1980s there was ample evidence that "AIDS" in children existed long before the HIV/AIDS era. This was especially the case in children born to mothers living in poverty or using drugs. That

what is called AIDS in offspring of these mothers may be present in both HIV infected and non-infected children is best illustrated by an analysis of the data from a few studies.

In 1991 researchers from the North Central Bronx Hospital, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, and the CDC published the results of a study, "conducted in the Bronx, New York, of 70 infants of human immunodeficiency virus (HIV)-infected (n=33) and uninfected (n=37) mothers who had a history of intravenous drug use or of intravenous drug-using sex partners.[260] Because "clinical evaluation of infants for early signs of HIV infection may be complicated by the morbidity associated with maternal drug use...long-term follow-up of infants of HIV infected mothers remains necessary to ascertain the presence of HIV disease". In their study, "seventy infants born between July 1985 and December 1989 to women of known HIV serologic status were followed from birth for a median of 24 months (range, 3 to 54 months)". Of a total of 70 mothers (33 infected and 37 uninfected) only 10 were white, the rest were black or Hispanic. "Western blot analysis was performed on all specimens of infants of seropositive mothers, including specimens non-reactive by enzyme-linked immunosorbent assay…Confirmed infection in infants was determined by one or more of the following criteria: (1) conditions meeting the current (1987) CDC surveillance definition for AIDS; (2) the presence of HIV in blood or tissues as detected by culture or by PCR on two or more specimens; (3) the presence of HIV antibody and HIV-related symptoms, as outlined in the CDC Paediatric Classification, in infants more than 15 months of age...HIV infection was confirmed in 7 of 33 (21%) infants of seropositive mothers". All infants "with confirmed infection", and without confirmed infection had mothers who reported drug use, including "intravenous and intranasal cocaine and heroin, cocaine smoked as crack, and alcohol" during pregnancy. "Infants of mothers who used drugs during pregnancy had lower birth weight and shorter gestation than infants not exposed ($P < .05$), independent of maternal HIV status. All infants of seropositive and seronegative mothers who were born ≤37 weeks gestation had in utero drug exposure. Ten of 12 infants (83%) of seropositive mothers and 8 of 9 infants (89%) of seronegative mothers born ≤ 37 weeks had mothers who reported cocaine use during pregnancy. Among infants of seropositive mothers, mean head circumference was smaller in infants with reported in utero drug exposure than in those who were unexposed ($P=.02$). A similar trend was observed in infants of seronegative mothers ($P=.07$)". Of the 26 infants who were uninfected, one had a positive PCR followed by two negative. The clinical features of the children are presented in the three tables below.

Concerning the data in the three tables the CDC researchers and their associates wrote: "Infants with confirmed infection tended to have a higher incidence of events in the first year of life ($P = .07$) and had significantly higher rates of HSM, PGL, chronic otitis media, persistent anaemia, persistent oral candidiasis, and failure to thrive. Loss of developmental milestones was observed only in infants with confirmed infection. There were no statistically significant differences among the three groups in the incidence of recurrent upper respiratory tract infections or in the incidence of bronchiolitis and pneumonia, excluding *P carinii* pneumonia and lymphoid interstitial pneumonitis. Compared with infants of seronegative mothers, infants of seropositive mothers without confirmed infection had significantly higher rates of PGL ($P = .01$), chronic otitis media ($P = .05$), and persistent anaemia ($P = .05$). HSM, PGL, persistent oral candidiasis, and failure to thrive were not observed in infants of seronegative mothers. Infants of seropositive mothers had higher incidence of growth retardation ($P = .04$) and developmental delay ($P = .08$) than infants of seronegative mothers."

TABLE 2.2 HIV infected children

Patient	Sex	Age at Last Follow-up, mo	Age at 1st Symptoms, mo	Clinical Signs and Illnesses	T-Cells ‡	
					CD4/CD8	CD4
1	M	Died at 3	2	Oral candidiasis, PGL, HSM, PCP athymic	1.66	1676
2	M	8	2	Oral candidiasis, FTT, chronic otitis, PGL, bronchiolitis, HSM, HT, WT < 5th	2.76	1493
3	M	Died at 9	2	Hypertonic, FTT, PGL, oral candidiasis, HSM, HIV+, pneumonia, anaemia, LIP, HT, WT <5th, loss of milestones	2.81	1129
4	M	25		Chronic otitis	2.05	2462
5	M	36	7	Sepsis, FTT, HT < 5th, PGL, oral candidiasis, HSM, chronic otitis, loss of milestones	0.30	376
6	M	Died at 36	5	PGL, FTT, HT, WT <5th, chronic otitis, HSM, hypothyroidism, oral candidiasis, MAI, LIP, nephrosis, anaemia, loss of milestones	0.02	58
7	M	38	7	PGL, FTT, HSM, anaemia, HT, WT <5th, pneumonia, chronic otitis, loss of milestones, LIP, parotitis	0.48	729

Abbreviations: PGL, persistent generalised lymphadenopathy; FTT, failure to thrive; HSM, hepatosplenomegaly; LIP, lymphoid interstitial pneumonitis; PCP, *Pneumocystis carinii* pneumonia; H zoster, disseminated herpes zoster; MAI, Mycobacterium avium-intracellular in blood; anaemia, persistent anaemia; HT, height; WT, weight; HC, head circumference.
‡ At last follow-up.

TABLE 2.3 HIV negative children of HIV positive mothers

Patient	Sex	Age at Last Follow-up, mo	Clinical Signs and Illnesses	T-Cells ‡	
				CD4/CD8	CD4
8	F	16	Chronic otitis, persistent oral candidiasis	2.95	1607
9	M	21	PGL, chronic otitis, anaemia, parotitis,bronchiolitis, HSM	0.79	1938
10	M	28	PGL, FTT, WT <5th, developmental delay	1.33	3868
11	F	37	PGL, WT, HC < 5th, developmental delay, chronic URI	1.80	1755
12	M	37	PGL, chronic otitis, HT, WT <5th, anaemia	0.80	1085
13	F	48	PGL, anaemia	2.94	1394
14	M	54	HT <5th, anaemia, PGL, developmental delay	0.84	663

Abbreviations: PGL, persistent generalised lymphadenopathy; FTT, failure to thrive; HSM, hepatosplenomegaly; URI, upper respiratory tract infections; anaemia, persistent anaemia; HC, head circumference; HT, height; WT, weight; <5th, <5th percentile;
‡ At last follow-up

TABLE 2.4 Incidence of Clinical Signs and Symptoms in Infants >15 Months of age by Maternal Human Immunodeficiency Virus (HIV) Status*

Clinical Signs and Symptoms	Infants of HIV (-) Mothers (n=21)	Infants of HIV (+) Mothers	
		Without confirmed infection (n=21)	With confirmed infection (n=4)
Hepatosplenomegaly	0	1.4 (1)	61.2‡ (3)
Persistent generalised lymphadenopathy	0	10.8 (6)	63.8‡ (3)
Chronic otitis media	7.5 (4)	22.8 (10)	100.0‡ (4)
Recurrent upper respiratory tract infections	11.7 (6)	16.4 (8)	25.6 (2)
Bronchiolitis/pneumonia§	17.0 (8)	13.4 (7)	8.7 (1)
Persistent anaemia	1.6 (1)	10.2 (6)	76.9‡ (3)
Persistent candidial dermatitis	1.6 (1)	3.1 (2)	9.9 (1)
Failure to thrive	0	2.9 (2)	40.0‡ (3)
Growth retardation¶	8.1 (5)	20.3 (10)	33.7 (3)
Development delay	5.0 (3)	12.2 (7)	20.6 (2)
Loss of developmental milestones	0	0	28.3‡

* The values stated are the rate per 1000 infant person months in infants >15 months old (number of infants).
‡ *P* <.05 (infants with confirmed infection vs infants without confirmed infection). § Excludes *Pneumocystis carinii* pneumonia and lymphoid interstitial pneumonitis. ¶Head circumference, height and/or weight <5th percentile.

However,

1. Only one of the "infected infants" was reported with PCP. No mention is made if the diagnosis of PCP was definite or, as permitted by the CDC, only presumptively diagnosed and, if the former, what method was employed. Nor do they mention if all the infants, including the non-infected infants born to positive mothers and the infants born to negative mothers with pneumonia, were tested for PCP;

2. Lymphoid interstitial pneumonitis (LIP) was diagnosed in 3 "infected" infants. Its diagnosis "was based on chest roentgenogram", while in the European Collaborative Study, "radiographic findings" are not sufficient to diagnose the disease.[233] Furthermore, in one of the infants with LIP, infant 3 in Table 2.2, who is said to have died from AIDS, the initial positive WB reverted to negative by 5 months, and remained negative till his death at 9 months of age. Again, no mention is made if all the children were examined for LIP. In this regard, it is significant that in Table 2.4 the highest incidence of pneumonia is reported in infants of HIV-negative mothers, followed by non-infected infants of HIV positive mothers;

3. The reported higher incidence of some of the diseases and signs and symptoms in the "infected" infants compared to the non-infected and those of the HIV-negative mothers, as stated in Table 2.4, may be due to factors other than HIV:

    (a) In this study there were a total of 70 women, of which 33 were HIV positive. "HIV infection was confirmed in 7 of 33 (21%) infants of seropositive mothers". (Seven is also the total number of infected children given in Table 2.2). Elsewhere in the text one reads: "Although the size of this study sample is small, 7 of 25 (28%) infants older than 15 months had confirmed infection, within the range of rates reported in larger studies". However, as can be seen from Table 2.2, only 4 infected infants were over 15 months of age when last seen, which is the same number of similar aged children listed in Table 2.4. In the same table there are 21 non-infected children born to HIV positive mothers, that is, 4 out of a total of 25 (16%) of infants more than 15 months of age were infected. No mention is made why the 8 additional children born to HIV seropositive mothers and the 16 additional children born to HIV-negative mothers were not included in Table 2.4. These children may have been excluded because they were younger than 15 months. Nonetheless, since the mortality rate up to 1 year of age in infants of drug abusing mothers has been reported to be between 7.4–33.8%,[283] the possibility cannot be excluded that some of these children may have died. Be this as it may, since as the authors themselves point out "the size of this study sample is small", it is exceedingly difficult to draw statistical conclusions from such a study. In particular there were only 4 infected children older than 15 months, of which one, at the last follow-up, had only otitis, and in the other 3 the symptoms appeared between 2 and 7 months of age. Furthermore, as the authors of this study mention, the "clinical evaluation of infants" for "signs of HIV infection may be complicated by the morbidity [and mortality] associated with maternal drug use". Yet for unknown reasons which nonetheless have the potential to completely alter the validity of the

conclusions, there are no data on the clinical status before the age of 15 months of the infants of HIV-negative mothers, or the non-infected infants of the HIV-positive mothers;

(b) Since,

    (i)   "All infants with confirmed infection had mothers who reported drug use, including cocaine, heroin, and alcohol, during pregnancy";

    (ii)   "18 (62%) without confirmed infection had mothers who reported drug use" during pregnancy;

    (iii)   65% of the HIV negative mothers were using drugs;

the possibility cannot be excluded that any significant statistical difference, if present, either quantitative or qualitative between the three groups of infants, was due to differences in the use of drugs, not the presence or absence of HIV;

(c) Since the study was neither randomised nor blind, biased design, execution and data analysis cannot be excluded.

According to the HIV theory of AIDS, HIV does not cause clinical manifestations directly. These effects are all indirect. The virus destroys the T4 cells, which in turn leads to the signs, symptoms and indicator diseases that constitute AIDS.

As can be seen from Table 2.2, 4 out of the 7 infected infants, including the child with PCP who died at 2 months of age, had normal T4/T8 ratios. Three of the infants, including the child with PCP, also had normal absolute T4 cell numbers. In Table 2.3 the clinical and immunological data of the 7 infants of infected mothers, who themselves were negative, but nonetheless, were said to have "morbidity suggestive of HIV infection", four infants had normal T4/T8 ratios and all had normal T4 counts. Furthermore, the abnormalities in T4 cells, when present "were concurrent with or subsequent to the appearance of HIV-related illness". That is, the abnormalities of T4 cells were the result not the cause of the illness. This means that regardless of the clinical status of the drug using mothers, the diseases and the signs and symptoms in their children cannot be caused by HIV.[260]

As mentioned, high frequencies of KS existed in African children long before the AIDS era. In 1951 in South Africa the prevalence of TB, the most frequently reported AIDS indicator disease in Africans, was 1477 Whites, 1084 Asian, 4586 Coloured and 19,392 Black. The corresponding figures in 1968 were 921, 990, 7481 and 61,292. In 1953-54, the infant mortality per 1,000 live births in South Africa was 33 Europeans, 66 Asian, 134 Coloured and 210 Black. The commonest causes of death among the black infants were recorded as bronchopneumonia, dehydration and diarrhoea. In 1964 there were 410 cases of kwashiorkor amongst Coloured children, 40 Asians and 13,358 Blacks. In 1988, the organisation Operation Hunger was quoted as showing that "rural South Africa's malnutrition problem was worse than many other countries in Africa". 58% of the children in Eastern Cape, 80% in Northern Cape and 40% in Transvaal, showed stunted growth[286,287] (Tables 2.5 to 2.10). Thus what is surprising in South Africa is not the high frequency of AIDS indicator diseases and the signs and symptoms which are said to indicate AIDS but the fact that many consider these to be a new phenomena in Africa caused by HIV.

Table 2.5   Tuberculosis reported cases 1951 whole country

Africans	Whites	Coloured	Asian
19,392	1477	4586	1084

Table 2.6   Tuberculosis: reported cases, 1968

Africans	Whites	Coloured	Asian
61,292	921	7,481	990

Table 2.7   Leprosy, polio and typhoid: reported cases 1964

	Africans	Whites	Coloured	Asians
Leprosy	501	6	20	5
Polio	86	2	15	4
Typhoid	3,027	74	123	19

Table 2.8   Infant mortality per 1000 live births, 1953/4

Africans	Whites	Coloured	Asians
210	33	134	66

Table 2.9   Kwashiorkor: reported cases, 1964/5

Africans	Whites	Coloured	Asians
13,358	Zero	410	40

**Table 2.10   Percentage of children under 12 with stunted growth
(including kwashiorkor) 1988**

South Africa		Neighbouring countries	
Eastern Cape	58%	Mauritius	21%
Northern Cape	80%	Swaziland	10%
Transvaal	49%	Zambia	19%

Data in Tables from Survey of Race Relations in South Africa[286-287]

In 1986 researchers from the Department of Public Health, Kinshasa, Zaire; the Institute of Tropical Medicine Antwerp, Belgium; the CDC and the National Institute of Allergy and Infectious Disease, Bethesda Maryland conducted a study to evaluate the clinical case definition of AIDS in African children[27]. They reported that "The positive predictive value for HIV seropositivity was 25%" and concluded: "This study suggests that the utility of the proposed WHO clinical case definition of paediatric AIDS for surveillance of cases of AIDS in African children is limited as clinical criteria are not adequately predictive of HIV seropositivity. For surveillance, and certainly for diagnostic purposes, laboratory tests need to be performed to confirm a clinical suspicion of AIDS in a child".

In 1999, researchers from France and Rwanda conducted a study "To compare morbidity and mortality of human immunodeficiency virus type 1 (HIV-1) infected and HIV-1 uninfected children and to identify predictors of acquired immunodeficiency syndrome (AIDS) and death among HIV-1 infected children in the context of a developing country…The children and their mothers were followed every 2 weeks during the first 2 years of life and every 4 weeks thereafter. Thus, during 5 years, they were visited regularly at home by a social worker or they attended the outpatient clinic organized within the Mother and Child Health Clinic of the Centre Hospitalier de Kigali. In addition, the children were systematically examined by a pediatrician every 3 months and assessed for HIV-related signs and symptoms. The following conditions were regarded as HIV-related: chronic diarrhea ($\geq$ 14 days); chronic cough ($\geq$14 days); chronic fever ($\geq$14 days); severe or recurrent pneumonia; hepatomegaly; splenomegaly; generalised dermatitis; lymphoid interstitial pneumonitis; oral candidiasis (beyond the neonatal period); chronic parotitis; persistent generalised lymphadenopathy (lymph nodes measuring $\geq$0.5cm and present in two or more extra inguinal sites); and failure to thrive (<80% of weight for age…Children were considered HIV-1 infected (group 1) if they had at least one positive PCR result during follow-up, regardless of clinical and serologic criteria…Children were considered HIV-uninfected if they had no positive PCR test result and either 1) they were negative for HIV antibody at 15 months of age and did not fulfill the modified WHO clinical case definition of pediatric acquired immunodeficiency syndrome (AIDS), in which severe and/or recurrent pulmonary infection is considered a major sign in place of chronic cough as a minor sign during the follow-up, or 2) they died before 15 months of age, they were HIV-seronegative and their death was not considered HIV-related (death was defined as HIV-related either in a child fulfilling the modified WHO clinical definition of pediatric AIDS or in a child with at least one HIV-related condition when last seen and

dying from severe infection or persistent diarrhea beyond the first 4 weeks of life). Uninfected children were either born to seropositive (group 2) or seronegative mothers (group 3)". Of "436 eligible children, 54 were diagnosed as infected (group 1); 45 of these children were born to HIV-infected mothers, and the remaining 9 children were born to women who were uninfected at delivery but who became HIV-seropositive during the follow-up, and thus became themselves infected too. A total of 347 children were considered uninfected; 138 were born to HIV-infected women (group 2) and 209 to HIV-uninfected women (group 3)…In HIV-1 infected children, the most frequently observed clinical signs were chronic cough, failure to thrive, and generalised lymphadenopathy, which also were reported among the most frequent HIV-related conditions in other developing countries. Chronic cough and failure to thrive were present in almost half of the initial patterns of symptomatic disease in our series. However, these three conditions were also common in uninfected children. In our cohort, the most specific findings of HIV-1 infection were oral candidiasis and chronic parotitis, a pattern similar to the one observed in the New York City cohort…Twenty-eight (52%) infected children and 13 (4%) uninfected children died during follow-up…The risk of death was not significantly higher in children who had developed AIDS in comparison to the other children. No specific combination of clinical manifestations was associated with differences in survival (data not shown). Biologically, neither the maternal CD4 cell count at day 15 nor the child's CD4/CD8 ratio at 6 months of age was predictive of death".[2]

In a 2000 study from Malawi, the authors from Malawi and France reported that HIV infected children had a lower number of T4 cells than non-infected children. However, since in the infected children the T4 cell number was 1323 (± 907) and since according to these authors, any child with a T4 cell number higher than 1000 is not immunosuppressed, many, if not all of infected children were not immunosuppressed. Clinically, "age-adjusted recurrent fever, chronic diarrhea, vomiting, ear infections, skin conditions, oral thrush, and cough were significantly ($P < .05$) more commonly reported among HIV-infected children than among HIV uninfected children. On clinical examination, otitis media, dermatitis, oral candidiasis, signs of active chest problems, lymphadenopathy, and development delay were significantly more frequent among HIV-infected children…In proportional hazards models that simultaneously adjusted for the first occurrence of all the AIDS-related conditions, developmental delay, fever, oral thrush and splenomegaly were the major morbidity predictors of child mortality among HIV-infected children. Among HIV-uninfected children, the major predictors of mortality were splenomegaly, developmental delay, and cough". In other words, the same signs and symptoms were present and were predictors of death in both infected and non-infected children. The higher frequency in the children reported infected may be explained by means other than HIV. For example, the "HIV" tests may be a non-specific marker for morbidity and mortality although not proof of HIV infection (see Part I). The authors of this study admitted that:

(a) "biases related to selection of children for enrollment and their follow-up might have occurred";

(b) they "relied primarily on clinical diagnoses and did not perform blood cultures or chest radiography to confirm clinical diagnoses";

(c) "Use of maternal history might have overestimated or underestimated some conditions".

Discussing their findings they wrote: "Of the AIDS-related conditions, developmental delay, fever, oral thrush, and splenomegaly were the strongest predictors of mortality among HIV-infected children…Among HIV-uninfected children, splenomegaly, developmental delay, and cough could be attributed to malaria, malnutrition and respiratory tract infections, respectively. These are the commonest causes of childhood deaths in Africa".[288]

The questions which arise are:

1. Why are the commonest causes of death in African children in the AIDS era the same commonest diseases that caused childhood deaths in African children prior to the AIDS era? Why have the causes which operated in the pre-AIDS era suddenly disappeared to be replaced by HIV?

2. Why do malaria and malnutrition cause splenomegaly and development delay in HIV-negative African children but not in HIV positive children? Especially given, as the authors report, the frequency of malaria is if anything higher in HIV positive children (8.4% v 6.5%)?

3. Since K*lebsiella* is the most common post mortem detected cause of pneumonia in both HIV positive and HIV negative children, how is it possible to claim that the former children die because of HIV and the latter because of malnutrition?[288]

In the 1989 "Report on the Meeting of the Technical Working Group on HIV/AIDS in Childhood", World Health Organisation, global program on AIDS, it is stated: "Many infections that are common in children, particularly in developing countries (such as pneumonia and diarrhoea), mimic the HIV infection in their clinical

signs". In studies conducted in Africa "only half of the children meeting the WHO Bangui case definition were seropositive. Of the HIV seropositive children fewer than half met the Bangui case definition".[264] If this is the case, then, obviously, the clinical status or death of a child cannot be used to prove HIV infection of the child and much less proof for MCT of HIV . In the same report it is also stated: "Because of the incompletely defined spectrum of disease in infancy and childhood and the limitation of correct laboratory diagnostic tests [antibody, PCR, virus culture, HIV antigens, in vitro antibody production] there is no clear standard against which to measure existing or proposed paediatric AIDS definition". If this is the case, that is, if there are limitations in both the definition of AIDS and the tests, then it is not possible to determine MCT.

2.5.2.5   Experimental design

The only way to obtain the most reliable epidemiological evidence to claim proof for MCT is to conduct randomised, double-blind controlled studies. The women acting as controls should be those who, with one exception, are identical with the test women (HIV positive women). The exception is HIV seropositivity. In other words the study should consist of two groups of mother-child pairs:

1.   Children whose mothers are HIV positive;

2.   Children whose mothers are HIV negative, but otherwise identical to seropositive women;

All the studies, including testing for HIV of the children of both, HIV positive and HIV negative mothers and the clinical follow-up, should be performed blindly.

Such studies are no more difficult to perform than the many studies performed so far. Yet to date not even one single, randomised blind-controlled study has been published. In fact no study of any kind has been performed in which children of HIV negative mothers but otherwise identical with the positive mothers, have been tested for HIV (antibodies, culture, PCR). Since no such studies have been performed, from this fact alone, it follows that it is impossible to draw any valid conclusion regarding MCT from the presently available data. Some of the best experts in MCT accept that not only are the epidemiological data not obtained from double-blind controlled studies but they are also biased. For example: "Among the children born to seronegative mothers, the mortality during the first 2 years is lower than expected in Rwandan children".[241] "In most studies, mortality in the comparison group (children born to HIV-seronegative mothers) was lower than anticipated, due to surveillance bias".[6] To draw valid conclusions from epidemiological studies they must, in addition to being randomised and blind-controlled, also have low rates of loss to follow-up. Yet in many of the studies of MCT of HIV "One major problem is that many mothers and their infants are lost to follow-up" necessitating "Strictly defined exclusion criteria should be used to reduce the loss to follow-up". According to many experts "Loss to follow-up is a major problem in prospective studies and may bias the estimates of MTCT rates".[6]

The presently available data on MCT show that:

1.   To date not one single randomised blind-controlled study has been published - the only way to obtain useful information regarding MCT of HIV;

2.   There is no valid method to prove infection of the child with HIV. There is no proof that any of the tests used to prove infection are HIV specific. Neither are the tests reproducible or standardised. This is well illustrated by the fact that there are very few studies in which the same methodology and tests are used (in some studies an "in-house" definition of MCT is used[238]) and the extreme variability in the findings of different groups, the findings are not consistent even within one and the same group or even with the site in the same study. This is best illustrated in the ECS and the Ariel project studies.[217,229] There is nothing specific either in regard to the immune deficiency (T4 decrease) or the clinical signs, symptoms and diseases, that is, AIDS reported in infants and children of HIV positive mothers and used to prove MCT of HIV. Such abnormalities existed in infants and children long before the AIDS era and, in fact, in high frequencies in the two groups in which the claims for MCT of HIV have been obtained, that is, in women living in poverty and using drugs.

The higher frequency of the signs, symptoms and indicator diseases reported in "infected" children or children born to "infected" mothers may be due to factors other than HIV including bias in the design and analysis and interpretation of the results. According to the CDC, children with decreases in T4 cells and opportunistic infections, by definition have AIDS, "even when HIV serologic findings are negative".[289,290] Indeed, under many circumstances the CDC require AIDS to be diagnosed in the proven absence of HIV.[134] In other words, children can be said to have a syndrome caused by a virus, HIV, even if not infected with this virus.

These, taken together with the publicity surrounding AIDS in children, the vast number and complexity of the MCT studies and the reluctance of experts to participate in fundamental debate and not least, the understandable trust people place in experts, have led, despite lack of scientific proof, to the near universally accepted belief that millions of children are infected with a deadly retrovirus HIV and suffer and die from AIDS. And that both HIV and AIDS can be prevented and cured with nothing else but antiretroviral drugs.

2.6   Conclusion
At present there is no proof that HIV, even if it is assumed to be present in pregnant women, is perinatally transmitted to their offspring.

PART III
BREAST FEEDING AND TRANSMISSION OF HIV

3.1 Introduction

There is unquestionable evidence that breast-feeding protects babies against morbidity and mortality from infectious diseases.[291] "Breast-feeding provides protection to babies in many ways. It provides ideal nutrition to the infant at no cost and gives an immunologic protection against agents responsible for diarrheal and respiratory diseases, as well as other infections. Breast-feeding also plays an important role in birth spacing, mainly in the developing world. Finally, breast-feeding is important as favoring mother-child interactions and the psychosocial development of the child".[292] Given the importance of breast-feeding for the child's well being one would need to have extremely well founded reasons to advise against it. The minimum absolutely necessary but not sufficient reasons for advising mothers against breast-feeding their infants because of HIV is proof that:

1. HIV exists.

2. HIV is present in the milk of infected women.

3. HIV can be transmitted to the child by breast-feeding.

To prove this one must conduct prospective, randomised, blinded, controlled studies in which the possibility of HIV infection by means other than breastfeeding has been rigorously excluded.

3.2 HIV in Breast Milk

Assuming the existence of HIV is proven the only way to prove the presence of HIV in breast milk is to isolate it from breast milk. In this regard, from 1985 till the present, HIV scientists cite two publications. The first was published in 1985 by researchers from Belgium, including Institut Pasteur du Brabant where the authors claim isolation of HIV from the milk of three healthy women. The first woman was born in Zaire and had a positive ELISA. The second woman was born in Belgium but she and her husband had lived in Zaire during the previous ten years. Her ELISA was negative. The third woman was from Rwanda and she had a positive ELISA. The lymphocytes from these women as well as the child of the first and third were stimulated with PHA and cultured for four weeks with interleukin-2 (IL-2). They were then reacted with "rabbit hyperimmune serum against HTLV-III" (HIV). The authors also mention that the supernatants were tested for reverse transcriptase but only the reaction with the rabbit serum is given. The cultures of both children as well as the three women were reported as positive. Milk extract from the three women was added to H9 cell cultures as well as lymphocyte cultures stimulated with PHA and cultivated with IL-2. The cultures were treated with polybrene and some cultures were also treated with antibody to α-interferon. All the H9 cultures were reported to react with the rabbit antiserum. Only the lymphocytes cultured with the milk extract from the third woman reacted with the rabbit antiserum and then only when the antibody to α-interferon was added. Supernatants from the H9 cells were added to cultures of "HUT 78 cells", "with the appearance of RT activity", which was interpreted as proving transmission of HIV from the H9 to the HUT78 cells.[293]

Comments
(a) reaction between some proteins found in cells and rabbit hyperimmune serum against HIV is not proof of viral isolation;

(b) The reaction may be considered proof for detection but if and only if there is proof the reaction is specific. No such proof exists. To the contrary even Gallo and Montagnier admit that many cellular proteins react with the "HIV" antibodies;[10,294,295]

(c) According to the authors, the immunoassays were conducted using "rabbit hyperimmune serum against HTLV-III, monoclonal antibodies directed at HTLV-III p24 and p15". However:

   (i) The p24 and p15 are said to be proteins coded by the *gag* gene of HIV;

   (ii) According to one of the best known HIV experts, William Blattner, "it may be feasible to use viral antigen probes to look for cross-reactive antibodies, since certain viral proteins may be highly conserved between subtypes of viruses";[86]

   (iii) Under appropriate conditions normal, non-infected cells release retroviruses, endogenous retroviruses.

61

Thus even if the rabbit antiserum was reacting with retroviral proteins, the proteins could have been those of endogenous retroviruses and not HIV.

(d) Even if the Belgian researchers had proof for the isolation of a retrovirus from their cultures the retrovirus may not have been HIV because:

(i) the phenomena may have been the result of an endogenous retrovirus. Naomi Baumslaf from Georgetown University Medical School pointed out in her comments regarding HIV transmission by breast- feeding, "Retroviruses are commonly found in breast milk"[296,297];

(ii) the H9 cell line originates from a patient with adult T4-cell leukaemia which, according to Gallo, is caused by a retrovirus HTLV-I. According to Montagnier, in HIV cultures that utilise these cell lines, there will be "a real soup" of retroviruses.[9] In 1983 Gallo himself reported that HUT78 "contained HTLV proviral sequences"[299] (HTLV=HTLV-I);

(e) The finding of RT activity in the HUT-78 cell cultures cannot be considered proof for transmission for many reasons including the following:
(i) The HUT-78 cell line is the same as H9;[298]
(ii) Given the appropriate conditions RT activity can be found in all cells.[300-303]

(f) The authors had no controls.

In the second study to date said to present proof for HIV isolation from breast milk, no data are given. The paper merely asserts that "HTLV-III/LAV was isolated from the cellular fraction of colostrum from one of these women (unpublished)";[154]

One wonders why, for the past 14 years, nobody has reported further data on HIV isolation from breast milk. Could it be, as researchers from the Paediatric Epidemiology Department, Institute of Child Health, London and from the University of Padua, Italy, pointed out, "unsuccessful attempts at isolation may have gone unreported"?[304]

In 1993 researchers from Australia examined the milk of a IV drug using woman and reported: "Retrovirus was visualized in the cellular and cell-free fraction of her milk by electron microscopy".[305] However, (a) no electron micrographs were published; (b) there was no description of the retrovirus particles; (c) retrovirus particles in milk were reported long before the AIDS era. For example in 1969 Chopra *et al* reported that retrovirus "particles have been observed in the density gradient purified fractions of milk samples obtained from women having breast cancer and from milk of a normal woman with a family history of breast cancer. A few particles have also been detected in tissue-culture of a breast cancer biopsy".[306,307]

In 1996 researchers from the USA and Uganda published a paper entitled "Detection of Human Immunodeficiency Virus Type 1 (HIV-1) DNA and p24 Antigen In Breast Milk of HIV-1 Infected Ugandan Women and Vertical Transmission". "HIV-1 DNA was detected in the breast milk in 72% of nontransmitter mothers (75/104) and 80% of transmitter mothers (16/20)...After elimination of 6 infected children with evidence of HIV-1 infection present at birth from the analysis, there remained no significant difference in the transmission rates for mothers with a detectable HIV-1 DNA by PCR in the breast milk (11/86, 13%) compared with those with no detectable HIV-1 DNA in the breast milk (2/30; 7%)". According to the authors: "The presence of HIV-1 DNA in breast milk cells does not necessarily indicate the presence of actively replicating, infectious virus. Therefore, we examined the breast milk cell-free supernatants for the presence of p24 antigen, which is considered a better indicator of the presence of replicating virus". No milk had detectable p24. Discussing their finding and that of others the authors wrote: "other studies have reported much lower rates of detectable HIV-1 DNA in the breast milk of HIV-1 infected mothers. In a small study in Thailand, Buranasin et al found PCR-positive breast milk in 8 (44%) of 18 HIV-1 infected women between 1 and 9 days postpartum. In Rwanda, Bulterys et al detected HIV-1 DNA in 36% of breast milk specimens obtained 14 days after birth and in 5% of those collected 14 weeks postpartum. Van de Perre and colleagues detected HIV-1 DNA by PCR in breast milk in 47% of 129 women 15 days postpartum and in 21% at 6 months". As far as p24 is concerned they wrote: "A similar result was obtained in the Haiti study in which ICD [immune complex dissociated] p24 antigen was present in the breast milk of 9 (27%) of 37 women tested in the first 4 days after delivery, but was not detectable in any later specimens. This may indicate that early breast milk from HIV-1 infected women may be more infectious than later milk; however no data were given on the transmission status of those mothers with and without detectable p24 antigen". They concluded: "In summary, our data suggest that the extent of

postnatal HIV-1 infection through breast milk may not be significant in our cohort of Ugandan women infected with HIV-1 before delivery. No correlation was found between the detection of HIV-1 in breast milk by PCR or HIV-1 p24 antigen or the duration of breastfeeding and transmission of infection. These findings are further supported by the similar overall vertical transmission rate in our breastfed cohort to that found in several exclusively bottlefed United states and European studies…Given the substantial benefits of breastfeeding, it may be more reasonable to direct public health efforts in developing countries toward preventing seronegative breastfeeding mothers from becoming infected with HIV-1 rather than preventing HIV-1 infected women from breastfeeding".[308]

In 1998 researchers from the University of Washington, USA and the University of Nairobi, Kenya published a paper entitled "Cell-free Human Immunodeficiency Virus Type 1 in Breast Milk".[309] After noting that:

(a) "there are few convincing reports that HIV-1 can be cultured from breast milk [14,15]". (References 14 and 15 are the 1985 study of Thiry *et al* in Belgium and the 1986 statement of Vogt *et al*)*;*

(b) "The overall risk of transmission of HIV-1 through breast milk is likely to be related to the quantity of HIV-1 in cell-free milk in addition to other factors";

(c) "There are no published data on the prevalence or quantity of cell-free HIV-1 in breast milk";

the authors then determined the HIV RNA content in the milk of 90 women from Nairobi using "a highly sensitive PCR assay for viral RNA". They reported that "HIV-1 RNA was detected in 29 (39%) of 75 specimens tested. Of these 29 specimens, 16 (55%) had levels that were near the detection limit of the assay (240 copies/mL), while 6 (21%) had >900 copies/mL. The maximum concentration of HIV-1 RNA detected was 8100 copies/mL".

In summary, to date there have been only two papers which claim isolation of HIV from breast milk. In one paper no data are presented and in the second, with the methods used, it is not possible to prove isolation or even detection of HIV. A few studies have reported the finding of HIV-1 DNA and HIV–1 RNA in breast milk However,

(a) There are large differences between the data reported in these studies;

(b) To date nobody has proven the specificity of the tests used;

(c) There is no correlation between HIV-1 RNA and HIV-1 p24 although both are said to prove the presence of active virus.

3.3   HIV Transmission Via Breast Feeding

According to researchers from the Epidemiology and Biostatistics Unit, Institute of Child Health, London "Human Immunodeficiency virus type 1 (HIV-1) has been detected in breast milk by both culture and polymerase chain reaction (PCR). However, this finding does not necessarily mean that breastfeeding is a route of transmission".[310] Like HIV isolation, for some unknown reason almost all the evidence which is said to prove HIV transmission through breast milk comes from studies of African and Australian women. In 1985 researchers from Australia published a paper entitled "Postnatal Transmission of AIDS-Associated Retrovirus from Mother to Infant".[311] The mother of a child was transfused with two units of blood immediately after delivery. Thirteen months later one of the blood donors, a gay man, developed KS and PCP. No mention is made if the donor was ever tested for HIV. Following the development of AIDS in the donor the child and mother were tested with an ELISA and "by a radioimmunoprecipitation assay for antibodies to the LAV antigen p25 [p24] which is less sensitive but has greater specificity". The authors interpreted their data as proof that the mother was infected via the donor's blood and the child through breastfeeding that took place for 6 weeks. However,

(a) the authors themselves admit the ELISA is non-specific;

(b) it is accepted that antibodies to p24 are non-specific;[40,114]

(c) more than 30% of individuals who are transfused with HIV negative blood develop antibodies which react with the p24 protein;[110]

(d) antibodies that react with the "HIV" p24 protein have been detected in 1 out of 150 healthy individuals, 13% of randomly selected otherwise healthy patients with generalised warts, 24% of patients with cutaneous T-cell lymphoma and prodrome and 41% of patients with multiple sclerosis;[312]

(e) p24 seroreactivity "was found in 27 (35%) of 77 patients with primary biliary cirrhosis, 14 (29%) of 48 patients with systemic lupus erythematosus, 14 (50%) of 28 patients with chronic viral hepatitis, and nine (39%) of 23 patients with either primary sclerosing cholangitis or biliary atresia, compared with only one (4%) of 24 patients with alcohol-related liver disease or alpha 1 - antitrypsin-deficiency liver disease, and only one (4%) of 25 healthy volunteers".[313]

In other words there was no proof that either the child or the mother was infected with HIV.

In 1987 researchers from Belgium (including the Institut Pasteur) and Rwanda reported "two cases of post-partum transmission of HIV from mother to infant observed in Kigali, Rwanda". The mother of the first child received two units of blood on post-partum day one. One of the two donors tested 14 months after transfusion was found "HIV seropositive". It is not stated when the mother and the child were tested. They only say that: "The child was well until 10 months, when she presented with failure to thrive, chronic cough, and recurrent fever. At 12 months she had generalised lymphadenopathy, oral thrush and hepatomegaly, moderate psychomotor delay, and lobar pneumonia on chest x-ray. She had received twelve intramuscular injections in infancy" and that "The child died from chronic diarrhoea at age 19 months". The second mother also received two units of blood. "The child was well and thriving until 5 months of age when he had severe diarrhoea. At 7 months he had bouts of nocturnal fever and generalised lymphadenopathy. At 10 months, he was wasted (6.2kg) with enlarged lymph nodes, liver, and spleen and atopic dermatitis. Chest x-ray and fundi were normal. He had received four intramuscular injections. He was HIV seropositive. He is now 16 months old and has chronic diarrhoea and persistent failure to thrive with the same physical signs. He is still being breast-fed. The 27 year old mother was examined when her son was 10 months of age. She had had recurrent diarrhoea for 3 months and was 14kg less than her pre-pregnancy weight. Anti-HIV was detected in her serum".

The mothers and the children were tested using ELISA and "all results were confirmed by western blot". However, the WB results are not given and since up until 1987 everybody but especially the Institut Pasteur considered a p24 band sufficient to prove infection, it is impossible to claim that either the children or the mothers were infected.[314]

Discussing the Australian finding as well as their negative findings, researchers from the Paediatric Epidemiology Department, Institute of Child Health, London, UK and from the Institute of Clinical Paediatrics, University of Padua, Italy wrote: "At least 6 of our 11 breast-fed babies have apparently escaped HIV infection despite feeding for up to 7 months. Although these numbers are too small to make any definitive statement, they do add to the impression that the relative contribution of breast-feeding to HIV transmission is probably small compared with that of intrauterine transmission. Breastfeeding shortly after acquisition of HIV, as in the case of post-partum transfusions, may be a special case carrying a higher risk of viral transmission. To extrapolate from this special group to HIV-positive mothers who were seropositive before delivery is not justified in the light of current knowledge".[304] Similarly, Naomi Baumslag wrote: "This is not the first time that the finding of a virus in breast milk has initiated scientifically unsubstantiated panic decisions...In developing countries the major cause of death is diarrhoeal disease not AIDS".[296]

In 1988 in a paper published in *Lancet* the authors from the CDC USA, Belgium and Zaire, reported data on three seronegative women who received HIV ELISA positive blood transfusions, two immediately and the other during an operation 11 months post postpartum. All three women were seropositive (by ELISA) 6 months post transfusion. The children of the first 2 women who were breastfed for 9 months remained negative. The child of the 3rd woman was negative at 14 months and positive at 15 and 18 months. The child of a woman who "died during caesarean section delivering her fifth child" was breastfed for 12 months. "At 1 year of age generalised lymphadenopathy was noted. The child and the wet nurse were both HIV-1 seropositive. The child had been given injections because of his illness but had never received a transfusion or scarification". No mention is made as to what antibody test was used.[315]

In 1991 researchers from Rwanda, France and Belgium reported the results of a prospective cohort study conducted in Kigali, Rwanda. Two hundred and twelve infants born to seronegative mothers were followed up for a mean of 16.6 months; 16 of the mothers became seropositive. Of their 16 infants, 9 became positive. One of them had a positive PCR at birth. "Postnatal seroconversion to HIV-1 occurred in four of the five infants born

to the mothers who seroconverted during the first 3 months post-partum" and thus, according to the authors, the mothers may have been infected during their pregnancy and could have transmitted the virus during pregnancy or delivery. The other four infants were born to the 10 mothers who seroconverted between month 4 and month 21. "In all cases, the infant seroconverted during the same three-month period as the mother".[316]

In a paper published in *Lancet* in 1992 data from USA, France, Switzerland, Zaire and Australia were used to determine the "Estimates of additional risk of HIV-1 transmission through breastfeeding a mother infected prenatally". Extreme estimates were observed in the studies from Australia (33% additional risk) and Miami (5% lower infection rate among breastfed children). The data from Switzerland also showed a lower (1%) infection rate among breastfed children.[310]

In 1992, using the limited data from Australia and Africa, Dunn *et al* from the Institute of Child Health in London wrote, "Based on four studies in which mothers acquired HIV-1 postnatally, the estimated risk of transmission is 29%…Analysis of five studies showed that when the mother was infected prenatally, the additional risk of transmission through breast-feeding, over and above transmission in utero or during delivery is 14%".[310] The Dunn *et al* estimates and their conclusions were criticised by Wendy Holmes from Teaching Aids at Low Cost, UK[317] and Tim Cullinan, College of Medicine, Chichiri, Blantyre, Malawi.[318] In the same year, Sally Ann Lederman from the Center for Population and Family Health, School of Public Health and Institute of Human Nutrition, Colombia University, wrote: "In some cases, the transfusion was tracked when the infant was found to be infected. In others, the mother was tracked (usually many months later) because a blood donor was found to be infected. In one Australian case, the child may not have been infected, but the virus was shown to be present in breast milk. In other cases the infants were found to be HIV infected (or antibody positive); breastfeeding *may* have been the route of transmission to the infected children. Although suggestive, these reports provide no insight into the *probability* of infant infection, even from women infected by transfusion after delivery. An unspecified number of the infants of women transfused and followed up had not developed infection".[291]

In a study published in 1993 from Australia, "The data were collected retrospectively and verified by the attending physicians of mothers and infants". Eleven women who acquired HIV postnatally and who breastfed a mean of 3.7 months were identified. Ten received "blood products post-partum". "Thus none had been tested for the presence of HIV antibody in the pre- or intrapartum period. No predelivery sera was available for retrospective testing". The other woman, an IV drug user who shared needles "seroconverted 6-10 months postpartum". All partners, except 1 who refused testing, remained negative. Of the 11, 3 infants, including the child of the IV user, who were tested from 1-2 years after delivery, were found positive. The author concluded: "Whether HIV-positive mothers should breastfeed remains a dilemma".[305]

In 1995 Philippe Van de Perre from Brussels, Belgium, and Kigali Rwanda, one of the researchers who has most thoroughly studied HIV transmission by breastfeeding wrote: "The estimation of the additional risk of mother-to-child transmission of HIV-1 attributable to breast-feeding from mothers who were infected before becoming pregnant is much more problematic. Dunn et al performed a meta-analysis of five observational studies from industrialised countries and one case-control study from Zaire by comparing the mother-to-child transmission rates in breast-fed versus artificially fed infants. According to them the additional risk of transmission attributable to breast-feeding is 14% (95% confidence interval 7% to 22%). However, this meta-analytic approach is probably weakened by the observational and retrospective nature of the pooled studies and by the fact that studies with negative results may have been potentially omitted". And he continued "Assessments of the risk of mother-to-child transmission of HIV-1 by breast-feeding have many drawbacks. They have been drawn from observational studies to date, no intervention trials, randomised or not, have been reported on this subject. Also, many of these estimates have been based on studies with a limited sample size".[292]

In 1997 a study was published entitled "Late postnatal mother-to-child transmission of HIV in Abidjan, Côte d'Ivoire", with authors from the National AIDS Control Program, Côte d'Ivoire, the CDC, USA, and London School of Hygiene and Tropical Medicine, UK. "A child's HIV status was defined by a combination of serological and PCR criteria. HIV infection in children was classified as either early infection (in utero or intrapartum transmission) or late postnatal infection. Children whose mothers were seropositive, and for whom a PCR result on a sample taken at or before age 6 months was available, were classified as having early infection if the PCR result was positive. They were classified as having late postnatal infection if they were followed for more than 6 months and had a negative HIV-1 PCR at 3 or 6 months of age, followed by either or both a positive HIV-1 PCR at 9 months of age or older, and a persistently positive HIV-1 serology at 15 months of age or older. For children who were born to seropositive mothers but had no PCR result, HIV seroconversion after the disappearance of maternal antibodies was also defined as late postnatal infection. In children born to mothers who serostatus changed after delivery (either from HIV seronegative to HIV seropositive, or from single HIV

reactivity to dual HIV reactivity), late postnatal infection was defined as seroconversion observed in the child during the follow-up period. HIV-infection time (early *vs* late) could not be determined for children who had no available PCR results and who were persistently seropositive during follow-up…All children were breastfed…Among the 138 children born to HIV-1 seropositive mothers, 82 had a PCR result from a sample taken at or before age 6 months. 23 (28%; 95% CI 19-39) were HIV positive, and 59 were HIV negative. Of these 59 children, 45 were followed beyond 6 months, and four (9%) were classified as having late postnatal transmission". They concluded: "Our results show that most late postnatal transmission is likely to have occurred through breastfeeding and not through transfusions, injections, or other exposures to HIV-infected blood". They also added: "The validity of our results depends on the reliability of the PCR testing, though we are confident that our PCR results are reliable".[319]

3.3.1   The first Durban study

One of the few studies from Africa in which the authors are exclusively Africans was published in 1997 by researchers from South Africa. In a large urban hospital in Durban, between October 1990 and April 1993, 234 black infants and their 229 HIV positive mothers were entered into the study. No women refused entry into the study. Fifty-three patients did not attend a single follow-up visit and were subsequently excluded. The remaining 181 infants "were tested for HIV-1 antibodies by a commercial enzyme-linked immunosorbent assay (ELISA; Abbot Laboratories, North Chicago, Illinois, USA), by a confirmatory Roche ELISA (Cobus Core, Basel, Switzerland) or an immunofluorescent assay (IFA; Virion, Cham, Switzerland). Samples were considered positive if the second ELISA or the IFA was positive. The same method was applied for maternal samples...Infants were regarded as infected if they were antibody positive at 15 months or had an HIV-related death. They were classified as non-infected if the antibody test was negative from 9 months of age, or if death was non-HIV-related. Those infants who were lost to follow-up before the age of 9 months whilst still antibody-positive and those whose cause of death could not be determined, were classified as indeterminate. The diagnosis of AIDS was based on the revised WHO 1989 criteria". The "181 infants were classified as 48 infected (including 17 deaths); 93 non-infected, and 40 indeterminate (including eight deaths). The median vertical transmission rate was 34% [confidence interval (CI), 26-42%]. The upper estimate, which assumes all indeterminates were infected, was 48% (40-60%) and the lower estimate, which assumes all indeterminates were non-infected, was 26.5% (20-33%)…There were 133 infants for whom adequate feeding information was available. Twenty-one infants (16%) were fed exclusively on formula, 36 infants (27%) were exclusively breastfed and 76 (57%) received both breast and formula (mixed) feeds…Feeding method was defined as: exclusive breastfeeding, where the child was on breast-feeds only from birth (these infants received no supplementary milk feeds); mixed feeding, where the child was receiving both formula feeds and breast milk (the period of exclusive breastfeeding and the age at which formula was commenced were noted, as well as the duration during which the infant received both breast and formula feeds); and exclusive formula feeding, where the infant received formula feeds only. All groups of infants would have received other complementary foods for varying periods". The authors reported the following findings in the 133 infants: "The HIV transmission rate was 39% in those exclusively breastfed, 24% in those fed exclusively on formula and 32% in those receiving mixed feeding…There was a stepwise increase in the transmission rate with duration of exclusive breastfeeding of 1, 2, and 3 months (45%, 64% and 75% respectively). Of the infected infants, seven (50%) exclusively breastfed, 13 (51%) of those on mixed feeds and none on formula only developed AIDS; exclusively breastfed infants had a slower rate of progression to AIDS (mean age, 7.5 months versus 5.0 months, *P*=0.2242) than those on mixed feeds. Mortality (which occurred in the infected infants only) was 19% in the exclusively breastfed infants; 13% in those on mixed feeds and 0% in those exclusively formula-fed. The frequency of failure to thrive and episodes of diarrhoea and pneumonia were not significantly different between the three groups in both the infected and non-infected infants". They concluded: "Exclusive breastfeeding by HIV-infected women does not appear to protect their infants against common childhood illnesses and failure to thrive, nor does it significantly delay progression to AIDS. The implication of the trend towards differential mortality rates according to feeding groups is uncertain and requires further investigation".[251]

3.3.2   The second Durban study

In another study published in 1999 by the same South African group, they pointed out that all other studies including their own from 1997, which analysed "HIV-1 transmission via breastmilk are flawed because they have failed to account for the effects of different types of breastfeeding practices: exclusive or mixed (without or with water, other fluids, and foods that might contaminate and injure the immature gastrointestinal tract). Two studies have attempted to examine the effect of different breastfeeding patterns on mother-to-child transmission, but both have limitations. The most widely quoted meta-analysis on the risks of mother-to-child transmission by breastfeeding depended on studies with small sample sizes, short breastfeeding durations, and studies that do not distinguish exclusive from mixed breastfeeding". The mother-infant pairs enrolled in this study were participating in a Vitamin A intervention trial to study its effect on HIV transmission. In the analysis there were 156 children never breastfed, 103 exclusively breastfed for at least 3 months ("no other liquids, including water, or food had been given to the child before 3 months of age") and 288 received mixed feeding.

To determine the HIV status of the children "Venous blood was drawn from the infant on the first day after birth and again at 1 week, 6 weeks, and 3 months of age, and every 3 months thereafter until age 15 months. If the mother was still breastfeeding, venous blood was drawn from the infant at 3 months after the end of breastfeeding. Plasma was separated within 5 h and stored at -70ºC for possible subsequent quantitative assay of HIV-1 RNA by PCR (Roche Molecular Systems, Branchburg, NJ, USA) at the South African National Institute of Virology...We did RNA tests in children who had two positive ELISA tests at 9 months and 15 months, starting with the earliest sample and testing sequentially until the first positive test. In children who had not yet reached 15 months of age but who had reached 9 months, an ELISA (Abbott Laboratories, Chicago, IL, USA) was done at 9 months and an RNA-PCR test at 6 months; if these results were positive, we did RNA tests on the stored plasma samples starting with the earliest sample until the first positive test. Children who had not yet reached 9 months had an RNA-PCR test on each of their last two samples, and if either of these were positive, samples were tested from the earliest". In this study the authors did not find that Vitamin A supplementation had any effect on transmission. (However, there were no data to prove the women given supplements were Vitamin A deficient). On day 1 after delivery 6.4% of the never breastfed children, 6.8% of the exclusively breastfed and 5.2% of those who received mixed feeding were estimated to be positive. The estimated infection by one month of age was 14.8%, 8.7% and 14.2% respectively. By 3 months the corresponding values were 18.8%, 14.6% and 24.1%. Of the infants who were negative on day 1 and that were never breastfed 8.3% became positive by 1 month and 13.2% by 3 months. The corresponding values for exclusively breastfed were 2.1% and 8.3% and for those who received mixed feeding 9.5% and 19.9%. These differences in MCT were found even when the children were followed up for longer periods.[320] Summarising their results, the South African researchers wrote: "The estimated proportion (Kaplan-Meier) of infants HIV-1 infected by 3 months was significantly lower for those exclusively breastfed to 3 months than in those who received mixed feeding before 3 months". Although in their summary no mention is made regarding the higher seropositivity in infants who were never breastfed compared to those who were exclusively breastfed, in the discussion they wrote: "Among infants not already HIV-1 infected at birth, those who were exclusively breastfed had a lower probability of infection than those never breastfed".

Table 3.1   INFECTION ACCORDING TO THE TYPE OF BREAST FEEDING

TIME POST PARTUM	NEVER (%)	EXCLUSIVE (%)	MIXED (%)
1 DAY	6.4	6.8	5.2
1 MONTH	14.8	8.7	14.2
3 MONTHS	18.8	14.6	24.1

Table 3.2   INFECTION FOLLOWING A NEGATIVE PCR ON DAY ONE

TIME POST PARTUM	NEVER (%)	EXCLUSIVE (%)	MIXED (%)
1 MONTH	8.3	2.1	9.5
3 MONTHS	13.2	8.3	19.9

Comparing their 1997 with their 1999 results it is obvious, although not mentioned by the authors, that the findings are contradictory. In the 1997 study The "HIV transmission rate" was highest (39%) in those exclusively breastfed, 32% in those receiving mixed feeding , and lowest in those receiving only formula, 24%. In the 1999 study the "HIV transmission rate" was lowest in those children exclusively breastfed, 14.6%.

In an effort to explain the higher transmission rate in infants who had mixed feeding the South African researchers hypothesised: "Ingestion of contaminated water, fluids, and food may lead to gut mucosal injury and disruption of immune barriers. Since mixed feeding is unlikely to involve hygienic food preparation practices, bacteria and other contaminants may be introduced into the gut and result in inflammatory responses and subsequent damage to the mucosa. HIV-1 is less likely to penetrate intact and healthy gastrointestinal mucosa than damaged mucosa". However the hypothesis that HIV is likely to penetrate the intestinal "damaged mucosa" also applies to many other agents both living and non-living, each with antigenic potential for producing cross-reacting antibody reactivity. Significantly, there is proof that germ free laboratory animals produce between 10-20% of the amount of antibodies as normal animals[321,322] and antibody levels normalise when the intestinal flora is restored.[323] Regarding the lower probability of infection of children exclusively breastfed compared to those exclusively formula fed, they wrote: "it raises a possibility that virus acquired during delivery could have been neutralised by immune factors presented in breastmilk but not in formula feeds". However, although this may explain the higher rate by 1 month, it cannot explain the higher rates by 3 months (18.8% vs 14.6%) and even beyond 3 months. The only alternative explanation is that either the formula is infected with HIV or that the tests used in this study, antibody and PCR, are non-specific.[324]

3.3.3 Other studies

In yet another study on HIV transmission via breastfeeding published in 1999 and conducted in Malawi, the authors, (from the Johns Hopkins School of Hygiene and Public Health, USA, Malawi College of Medicine and the National Cancer Institute, National Institute of Health, USA) "investigated the risk of HIV transmission through breastfeeding in an urban setting in Malawi, where HIV prevalence in nursing women is approximately 30%, and breastfeeding is the recommended method of infant feeding…Infants found to be HIV-positive by polymerase chain reaction (PCR) at their first postnatal visit, scheduled at age 6 weeks (n=355), were excluded from the study, because most of them would have been infected in utero or perinatally. A small proportion, however, would have been infected through colostrum or early milk. To be included in the study, breastfed infants had to be HIV-negative at their first postnatal visit, have breastfeeding data (n=1012), and have a second follow-up visit (n=672)". The interval between the 2 visits was the observation period during which breastfeeding risk was assessed. In this study "The cumulative infection rate while breastfeeding, from month 1 to the end of months 5, 11, 17 and 23, was 3.5%, 7.0%, 8.9% and 10.3%, respectively. Incidence per month was 0.7% during age 1 to 5 months, 0.6% during age 6 to 11 months, and 0.3% during age 12 to 17 months". Commenting on their findings the authors wrote that:

1. "Supplemental foods were introduced well before weaning occurred (median, 4 months)".

2. "Our study showed that an uninfected infant, breastfed by an HIV-positive mother for 23 months, had at least a 10.3% risk of becoming infected. This rate of postnatal infection does not include postnatal transmission in the first month of life, which could not be reliably distinguished from intrapartum transmission in this study. The transmission rate in the first month could be substantially higher than in later months, because it includes feeding with colostrum and early milk, which are rich in cells. Our trend data showed a higher risk in the 1-to 5 month period than after 5 months".

Yet, the transmission rate at 5 months via breastfeeding in this study (3.5%), is lower than the transmission rate by 3 months in the infants who were negative on the first day post partum in the South African 1999 study. (8.3% in exclusively breastfed, 13.2% in never breastfed and 19.9% in the mixed feed infants[325]).

In 2000, a study entitled "Effect of Breastfeeding and Formula Feeding on Transmission of HIV-1. A Randomised Clinical Trial", was published by researchers from the USA and Kenya. Of 16,529 women attending four antenatal clinics in Nairobi, 2,315 (14%) were HIV-1 seropositive (2 positive ELISAs). Of the positive women 1,708 returned for results of which 425 were enrolled in the study. Of the 212 mothers in the breastfeeding arm there were 197 liveborn infants (there were 2 miscarriages, 1 maternal death, 8 lost to follow-up before delivery, 4 singleton stillbirths). Eight of the 197 infants were lost to follow-up. Of the 213 mothers in the formula feed arm there were 204 liveborn infants (1 miscarriage, 5 lost to follow-up before delivery and 3 singleton stillbirths). Seventeen of the 204 infants were lost to follow-up. The median follow-up time was 24 months. The HIV status at study end was available only for 333 infants, 171 (87%) in the breastfeeding group and 162 (79%) in the formula group. "A child was determined to be HIV-1 infected if PBMC or filter paper blood samples from 2 consecutive dates had positive test results for HIV-1 DNA by PCR, if a single blood sample had a positive test result for HIV-1 DNA if the sample was obtained at last visit seen, or if a serum sample had a positive HIV-1 ELISA test result if the sample was obtained at the last visit of a child 15 months or older with no sample from that date available for PCR testing…For a child determined to be HIV-1 infected because of 2 consecutive positive PCR test results, time of infection was defined by the first test result".

At 24 months, 61 (35.7%) of the breastfed children and 31 (19.1%) of the formula fed infants were infected. "Eighty-four children died during the study, 45 in the breastfeeding arm and 39 in the formula arm…The mortality rates we observed were much higher than those for the Nairobi population as a whole; the infant mortality rate was reported to be 4.1% in 1998". Discussing the findings they wrote: "In our trial, mortality rates in the formula and breastfeeding arms were similar, but participants had access to clean water and extensive instruction in safe use of formula. In developing country communities in which clean water and formula-feeding knowledge are limited, the balance of risks and benefits could be shifted".[326]

Comments
1. 9/61 (14.7%) of children reported infected were already positive at birth, compared with 2/31 (6.4%) of the formula fed children. That is, a two fold difference in HIV infection existed between the two groups before feeding commenced.
2. To prove infection "Nested PCR that detects a single HIV-1 provirus copy was used to detect HIV-1 in all specimens. Primers that recognised highly conserved gag gene sequences were used".
However, since:

(a) endogenous retroviruses are present "in all of us";[72]

(b) it is accepted by some of the best known HIV/AIDS experts including Hans Gelderblom and William Blattner that, "certain viral proteins [genes], particularly the polymerase and gag proteins, may be highly conserved between subtypes of viruses";[86,327]

the possibility cannot be excluded that the positive PCR results were the result of endogenous retroviruses and not HIV infection.

3.4   Discussion

The first step in claiming that HIV can be transmitted via breastfeeding is to have proof that the virus is present in milk. This proof can only be obtained by isolating the virus from milk which to date nobody has achieved. Several studies have reported the finding of positive PCR, DNA or RNA. However, the DNA detection rate is low and decreases with time and, more importantly as researchers from the USA and Uganda point out, "The presence of HIV-1 DNA in breast milk cells does not necessarily indicate the presence of actively replicating, infectious virus".[308] As far as RNA PCR is concerned it suffices to mention that the CDC does not recommend the use of this test to prove HIV infection, or even as a screening test, for "adults, adolescents, and children infected by other than perinatal exposure".[253] Furthermore, if the virological tests indeed proved HIV infection of breast milk a correlation should be observed between DNA PCR, RNA PCR, p24 and transmission. This is not the case.[308]

The second step is to conduct prospective, randomised, blinded and controlled studies in which the possibility of HIV infection by means other than breastfeeding has been excluded. Although many epidemiological studies have been published claiming infection of children through breastfeeding, none to date fulfil these criteria. Suffice to quote some of the most important experts in this field. As mentioned, in 1995 Van de Perre acknowledged that the conclusions were drawn from "observational studies", and that "to date, no interventional trials, randomised or not, have been reported on the subject". In 1999 the researchers from Durban, South Africa expressed the view that the studies conducted up till then on "HIV-1 transmission via breastmilk, are flawed". There is no doubt that the 1999 study by the Durban researchers is the best designed and executed study conducted to date. However, since their own evidence also indicates that the test they used, PCR, is not specific, it means that their study, like all the others, is also "flawed", that is, it does not prove HIV transmission via breastfeeding.

According to these researchers, of the children never breastfed and who were negative at day 1, 8.3% became positive by 1 month and 13.2% by 3 months. According to David Dunn from the Department of Epidemiology and Biostatistics, Institute of Child Health, London, UK, and his associates from the United Kingdom, USA, France, Canada, Italy, Sweden, Switzerland, including researchers from the CDC, "92% of infected infants will have detectable virus if tested [DNA PCR or culture] at age 14 days"[328,329] (and David Dunn, personal communication).

Since:

1. Elsewhere the Durban researchers wrote: "The study protocol required sample collection for HIV-1 testing on the day of birth, at 1 week, 6 weeks, and 3 months. A blood sample was not specifically collected at 1 month; thus, the 1-month transmission rates we reported can be misinterpreted";[324]

2. The never breastfed children could have become infected only *in utero* or at delivery;

3. They used the RNA PCR which is said to be "more sensitive" than the DNA PCR or culture;[330]

then no child PCR negative on day one and never breastfed should have first tested positive after 1 month, and certainly not by 6 weeks. Instead, 37% of the children reported positive were found to be positive between 6 weeks and 3 months. Since the children had no risk factor for HIV infection, it follows that the positive PCR results between 6 weeks and 3 months were false positives. This means that the possibility cannot be excluded that some, or all of the positive PCR results obtained between day 1 and 6 weeks in the never breastfed children, as well as the positive results in the other two groups of children at anytime, were also false positive. In other words, the data of the Durban researchers, cannot be considered proof that HIV can be transmitted by breastfeeding.

3.5 Conclusion

The presently available data do not prove that HIV can be transmitted by breastfeeding.

PART IV
EVIDENCE CLAIMED TO PROVE AZT AND NEVIRAPINE REDUCE MCT OF HIV

4.1 Introduction

At present, it is generally accepted that both AZT and nevirapine prevent mother to child transmission of HIV. The only way to prove that a drug inhibits mother to child transmission of HIV is to conduct prospective, randomised, double-blind controlled studies. Being double-blind ensures that bias is minimised by denying both doctor and patient all knowledge of which drug, active or placebo, the patients are receiving. The study should compare two groups of seropositive women and their infants matched in all respects in which one group of mother-child pairs receive the drug and the other the placebo. Since the tests used to prove MCT and the putative effects of the drug are non-specific, it may be necessary to introduce an additional control group of children, these being children of non-infected women who do not receive the drug.

4.2 The ACTG 076 study
4.2.1 Importance of the ACTG 076 study

In 1994 researchers from 59 centres in the USA and France published a paper entitled "Reduction of Maternal-Infant Transmission of Human Immunodeficiency Virus Type 1 with Zidovudine Treatment". This study, known as the Pediatric AIDS Clinical Trial Group 076 (ACTG 076), is the basis for all subsequent use of AZT in HIV positive, pregnant women.[331] According to David Wilkinson and James McIntyre, in their article published in the *South African Medical Journal* in 1998, "the landmark ACTG 076 trial done in the USA and France showed that a lengthy, complex and expensive regimen of zidovudine (ZDV) given through pregnancy, in labour and to the newborn reduced MTCT of HIV by 67% in women who did not breast-feed. Widespread implementation of this regimen in the USA and other countries, including France had led to a marked reduction in the incidence of paediatric AIDS–a public health triumph".[332] (In fact it is accepted that the "decrease" in mother to child transmission began well before the introduction of AZT[231]). Given the importance attached to this study, and that to date it is the only study which the authors claim as "randomised, double-blind, placebo-controlled", a thorough analysis of its data is warranted.

4.2.2 Patients and Methods used in the ACTG 076 study

In the text the authors state: "From April 1991 through December, 1993, 477 pregnant women were enrolled at 59 centers. Of the eligible women, 409 gave birth during this period to a total of 415 live-born infants, including 403 singletons and 6 sets of twins. Two women had a history of HIV seropositivity but were later found not to be infected. These two women and an infant born to one of them were excluded from the analysis. Twelve women (one of whom had a creatinine concentration outside the specified range) withdrew from the study before delivery; data on these women were included up to the time of withdrawal".

In Table 2 entitled "Characteristics of the Women and Infants in the Study" there are only 461 women, 86 white (48 treated with AZT and 38 placebo); 234 Black (107 AZT and 127 placebo); 132 Hispanic (71 AZT, 61 placebo) and 9 others (6 AZT, 3 placebo). In Table 1, "Status of Mothers and Infants in the Study, as of December 20, 1993", the authors state that of the 477 mothers enrolled they ended up with 409 "Eligible mother-infant pairs", of which only 363 were used in their statistical analysis". The racial distribution of the 363 mother-infant pairs is not mentioned. Neither in this regard are differences between the AZT and placebo groups.

Again, given that:
1. AIDS was first diagnosed in 1981 in the USA, in gay and bisexual men;
2. Up to 20% of men who consider themselves gay have sex with women;[246,247]
3. By 1982 the vast majority of haemophiliacs in the USA were infected with HIV;
4. Education in regard to safe sexual practices was introduced only in 1985-86;
5. Even by 1997, with no effort in safe sex education spared, 25% of partners of infected individuals enrolled in on going studies conducted by HIV experts did not practise safe sex;[194]
6. Women who are pregnant have not consistently practised safe sex;

it follows that there should have been no difficulties in the 1990s to recruit HIV infected mothers. Instead,

   (a) The ACTG076 researchers required 59 institutions in two continents to recruit 477 mothers;

   (b) Of the mothers recruited only a minority (18.6%) are white.

As far as antibody testing is concerned the authors state: "Enzyme immunoassays and Western blot assays for HIV antibodies were performed in certified laboratories by commercially available methods". The criteria considered to constitute a positive test are not mentioned. Given that the results of the WB depend on the commercial test kit used[333] and that the criteria for a positive WB vary between countries and laboratories,[29,108] and even within the same laboratory[38] including in the USA, this omission is of crucial significance. "Infants with at least one positive HIV culture of peripheral-blood mononuclear cells were classified as HIV-infected…HIV cultures of peripheral-blood mono-nuclear cells…were performed in certified laboratories according to published standard methods.[22]…The French sites used an equivalent program to ensure quality.[24]". In reference 24 one reads: "A culture was considered positive when the optical density was higher than the cut-off value supplied by the manufacturer.[334] Reference 22 is a paper entitled "Standardization of Sensitive Human Immunodeficiency Virus Coculture Procedures and Establishment of a Multicenter Quality Assurance Program for the AIDS Clinical Trials Group" published in 1992.[335] There one reads: "An independent quality assurance program has been established by the Division of AIDS, National Institute of Allergy and Infectious Diseases, for monitoring virologic assays performed by nearly 40 laboratories participating in multicenter clinical trials in the United States. Since virologic endpoints are important in evaluating the timing and efficacy of therapeutic intervention, it is imperative that virologic measurements be accurate and uniform". In this quality assurance program the criteria for a positive result of "isolation" were based on HIV p24 antigen results obtained from biweekly culture samples according to the following scheme:

"(a) two consecutive HIV p24 antigen values of ≥30 pg/ml, of which the second value is at least four times greater than the first value or is out of range;

(b) two consecutive HIV p24 antigen values that are out of range; or

(c) three consecutive increasing HIV p24 antigen values of ≥30 pg/ml, where neither consecutive value is four times or more that of the previous sample but the third value is at least four times greater than the first".

"When the quality assurance program was initially created, [1988], fewer than 40% of the laboratories could consistently recover human immunodeficiency virus (HIV) from peripheral blood mononuclear cells (PBMCs) of HIV-infected patients. By comparing coculture procedures in the more competent laboratories with those in laboratories who were struggling to isolate virus, optimal conditions were established and nonessential reagents and practices were eliminated. Changes were rapidly introduced into a laboratory when experience dictated that such modifications would result in a favourable outcome".

In this program it was shown that the HIV isolation result:

(a) depended on the length of time the culture was maintained. If a culture is tested repeatedly over a long period a positive result is more likely;

(b) varied according to the culture conditions;

(c) was laboratory dependent.

Although at the end of the quality assurance program, by implementing a "Consensus culture protocol" the agreement between laboratories had improved, there were still significant differences. So much so that 11% of the AIDS Clinical Trials Group (ACTG) laboratories remained uncertified. Since there are significant differences between the ACTG laboratories in the USA and, given that the French use different criteria for isolation, the probability cannot be excluded that even bigger differences may exist between the USA and the French laboratories. More importantly, while the quality assurance program for monitoring virological assays in laboratories participating in the ACTG in the USA require two consecutive positive values to prove "isolation", in the ACTG 076 mother to child transmission study, "A single positive culture was used to define an infant as HIV-infected". In other words, although HIV isolation in the mother to child 076 ACTG transmission study was performed by ACTG laboratories, the criteria which defined HIV infection were inferior to those endorsed in the quality assurance program for monitoring virological assays in the ACTG.

As mentioned in Part II, the need for two isolations, from two independent blood samples is also emphasised in the 1995 "Methodology of intervention trials to reduce mother to child transmission of HIV with reference to developing countries". Here an infant is said to be infected if "a positive viral culture or polymerase chain reaction is obtained for the first time and at least one subsequent sample is also positive".[257] Furthermore, in an update of the ACTG 076, published in 1999, "Infants were identified as infected if assays for HIV-1 were

positive on 2 separate occasions or if a condition considered to define the presence of AIDS was diagnosed".[258] (Because the conditions "considered to define the presence of AIDS" in children are totally non-specific (see Part II and Appendices I-IV) and they are not uncommon in children born to mothers similar to those who participated in the ACTG 076 study, (poor, Black and Hispanic), one must wonder if these criteria are used as proof for infection, how great a percentage of poor, Black and Hispanic HIV negative women "transmit" HIV to their children?)

4.2.3   Experimental design of the ACTG 076 study

According to the authors of the ACTG 076 study,[331] their study was a "randomized, double-blind, placebo-controlled trial". However, although it is customary in even the simplest epidemiological study to define the means by which the study is rendered double-blind, no such information is volunteered by Connor *et al*. Neither do they mention how their study was randomised. Given that randomisation is the *sine qua non* of epidemiological studies and that in this study there were 59 centres in two countries from two continents including hundreds of researchers, such an omission is difficult to comprehend. Equally as puzzling is the fact that Connor *et al* do not mention if the placebo was administered in the same manner as the AZT, what substance was used as placebo, whether the mode of administration and the placebo were identical for all patients or what measures (if any) they undertook to prove women were taking either AZT or placebo. They only state: "The women were stratified according to gestational age (from 14 to 26 weeks or greater than 26 weeks) and were randomly assigned to receive zidovudine or placebo. The zidovudine regimen consisted of antepartum zidovudine (100 mg orally five times daily) plus intrapartum zidovudine (2 mg per kilogram of body weight given intravenously for 1 hour, followed by 1 mg per kilogram per hour until delivery) plus zidovudine for the newborn (2 mg per kilogram orally every 6 hours for six weeks, beginning 8 to 12 hours after birth)...The women received the study drug for a median of 11 weeks (range 0 to 26) before giving birth". Under "Results", Connor *et al* wrote: "Forty-six infants (22 in the zidovudine group and 24 in the placebo group) stopped treatment before completing six weeks of therapy…22 stopped because of toxic effects (11 in each group)". Given that AZT is generally considered a toxic drug (evidenced here by the necessity to terminate treatment in 11 children within 6 weeks) one is astounded by the nature and choice of a placebo with toxicities identical to AZT.

4.2.4   HIV status of the infants in the ACTG 076 study

Summarising their results, Connor *et al* wrote: "HIV infection status was known for 363 births (180 in the zidovudine group and 183 in the placebo group). Thirteen infants in the zidovudine group and 40 in the placebo group were HIV-infected. The proportions infected at 18 months, as estimated by the Kaplan-Meier method, were 8.3% (95% confidence interval, 3.9% to 12.8%) in the zidovudine group and 25.5% (95% confidence interval, 18.4% to 32.5%) in the placebo group. This corresponds to a 67.5% (95% confidence interval, 40.7% to 82.1%) relative reduction in the risk of HIV transmission (Z = 4.03, P = 0.00006)". (In Table 3 where some of the results are given they reported that in children older than 32 weeks, 29 in the AZT group and 22 in the placebo group, had "indeterminate" results. Similarly in children one year or older, 12 in the AZT group and 15 in the placebo group had an "indeterminate" result. However, nowhere in the paper one can find a definition of "indeterminate" "Infection status"). In fact, since the infectious status of the children was determined by the success or failure of virus "isolation" from cultures, it is not possible to have "indeterminate" findings. HIV isolation was attempted at birth, 12, 24 and 78 weeks. Testing was stopped if the child was found positive or if the end point (78 weeks=18 months) was reached.

Since,

1. "Extremely high levels of viraemia are observed during primary human immunodeficiency virus type 1 (HIV-1) infection", that is, the viral "burden" (HIV DNA) and viral "load" (HIV RNA) are maximum immediately after infection;[336] because of this and because it may take three months to develop an antibody response, only HIV "isolation" and PCR are used to prove early HIV infection;

2. "…the lymphoid system matures relatively late in embryonic development"[337] thus newborn children, exposed *in utero*, would regard HIV as "self" and not inhibit its multiplication;

    (a) the immune system in children is immature;

    (b) for retroviral replication, and thus for HIV, cellular division is necessary and in infants there is a very high level of cellular division;

3. As mentioned in Part III, according to David Dunn from the Department of Epidemiology and Biostatistics, Institute of Child Health, London, UK and his associates, from the United Kingdom, USA, France, Canada,

Italy, Sweden, Switzerland including researchers from the CDC, "92% of infected infants will have detectable virus if tested [DNA PCR or culture] at age 14 days"[328,329] (and David Dunn, personal communication);

It follows that from the moment of infection infants can be regarded as capable of producing abundant quantities of HIV.

It was reported that "Thirteen children in the zidovudine group had at least one positive HIV culture and were classified as infected, as compared with 40 children in the placebo group. None of the twins were infected. On the basis of the Kaplan-Meier analysis at 18 months, the estimated proportion of infants infected was 8.3 percent in the zidovudine group (95 percent confidence interval, 3.9 to 12.8 precent) and 25.5 precent in the placebo group (95 percent confidence interval, 18.4 to 32.5 precent)…The estimated absolute difference between the two study groups in the percentage who were infected was 17.2 percent (95 percent confidence interval, 8.9 to 25.5 percent), corresponding to a 67.5 percent relative reduction in the risk of transmission (96 percent confidence interval, 40.7 to 82.1 percent…HIV infection was detected by culture within the first six months of life in nearly all the infants. Only 2 of the 53 infants classified as infected (both in the placebo group) had their first positive culture reported after the first 24 weeks". From an inspection of the Kaplan-Meier Plots of percent probability of transmission and the 95% confidence intervals bars in their Figure 1 (below), it is obvious that at 14 days and 6 weeks of age there was only a small probability (approximately 7.5% and 10% in the placebo and 3% and 4% respectively in the AZT group) of finding a positive HIV "isolation" result and there appears to be no significant difference between the two groups. In other words, by the time the virus can be detected in at least 92% of infants, there was no difference between the two groups of children. Thus, the authors of the ACTG 076 study group did not present evidence which proves reduction of HIV transmission by AZT treatment of the mother and the child. Furthermore and most importantly, there can be only two explanations for the infants who were found to have a positive "HIV isolation" test after 14 days and certainly after 6 weeks:

1. These infants were infected after delivery;

2. "Virus isolation" does not prove infection with a retrovirus HIV.
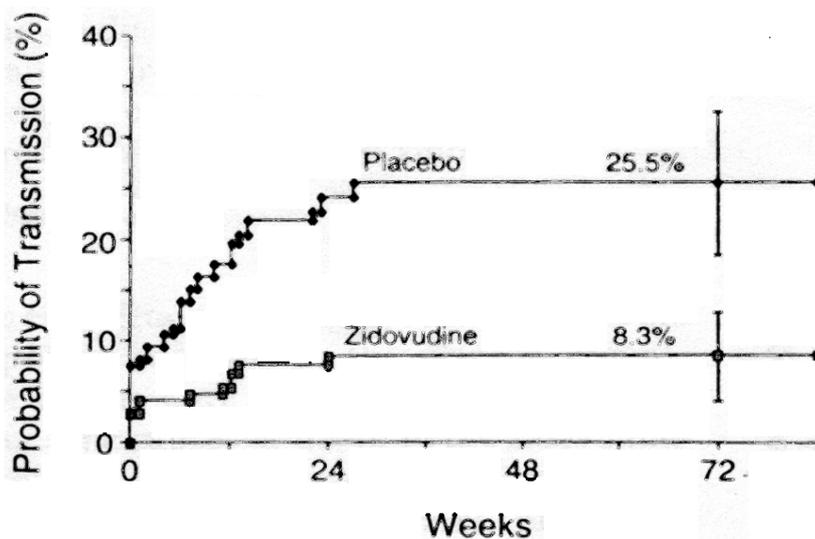
Placebo	183	84	42	37
Zidovudine	180	105	51	43

Figure 1. Kaplan–Meier Plots of the Probability of HIV Transmission, According to Treatment Group.
The estimated percentages of infants infected at 72 weeks are shown with 95 percent confidence intervals. The numbers of infants at risk at 24, 48, and 72 weeks are shown below the figure.

74

However, according to the authors of the 076 study, with the exception of pregnancy and delivery, the children had no other risk for mother to child transmission (only one woman breastfed and her child was negative), nor did they have other risk factors for infection. Thus one cannot but conclude that any positive "HIV isolation" test obtained after the first few weeks of life, certainly after 6 weeks, was a false positive. This being the case, the possibility cannot be excluded that at least some, if not all the positive "isolation" results obtained between delivery and 6 weeks were also false positive.

If both the detection of p24 in the culture (HIV isolation, positive culture result) and the HIV RNA prove the presence of active HIV, then one would expect a one to one correlation between a positive culture and a positive RNA PCR. In a 1999 update publication from the 076 study the plasma HIV-1 level at birth in the AZT group was 0.400×10^3 and in the placebo group also 0.400×10^3 copies/mL. The corresponding values at ≤ 6 weeks of age were 659×10^3 and 673×10^3 respectively and at 12 weeks of age 401×10^3 and 265×10^3 respectively. The authors concluded: "The high rate of viral replication and the lack of apparent effect of zidovudine on the plasma viral RNA level [in fact the RNA level at week 12 was higher in the AZT group] in these infants, provided potential for the selection of resistant virus".[258] If HIV had a high rate of replication in all of the children from both groups in the 076 study then it should have been possible to isolate the virus from all of them, or at least from the same proportion of children in both groups. Given many questions remain in regard to the tests used to prove the infectious status of the mother and the infants, the design and execution of the ACTG 076 study, as well as the data collection and interpretation, the inescapable conclusion is that the ACTG 076 did not provide any proof that AZT decreases mother to child transmission during pregnancy or delivery, or even that such transmission takes place.

4.3   Other Studies on the Effect of AZT on MCT
4.3.1   Studies from Part II

1. In 1996 the authors of the WITS study reported that "ZDV was not associated with a significant decrease in transmission in this overall cohort (18 versus 20% transmission in ZDV users versus non-users)".[43]

2. In the same year, the authors of the Perinatal HIV Transmission Collaborative Study Group, reported that MCT was significantly associated with unprotected sexual intercourse despite the fact that unprotected sexual intercourse was associated with AZT use during pregnancy.[122]

3. Also in 1996 the P^2C^2 failed to mention any effect of AZT on transmission although 34.1% of the women used the drug during pregnancy.[213] However, in a 1998 publication from the same study "When ZDV used during pregnancy was included in the model it was marginally associated with decreased risk of transmission".[216]

4. In 1997 David Ho and his associates from the Ariel project of MCT reported that: "The transmission rate among mothers who initiated zidovudine used during pregnancy was 8.3%...the transmission rate for mothers who never received zidovudine was 13%" and these transmission rates are "not significantly different from one another".[215] In 1999 the same authors reported that the transmission rate was 9.1% in non-users and 8.6% in users.[217]

5. Because New York State has the highest HIV seroprevalence rate among childbearing women in the USA, in 1995 the New York State Department of Health established a paediatric diagnostic service that offered PCR (DNA) testing. "Physicians were made aware of the service through the distribution of written materials and through clinical conferences". Researchers from the AIDS Institute, Wadsworth Laboratory, and Preventive Medicine Residence Program, New York State Department of Health and the Department of Epidemiology, School of Public Health, University of Albany, New York, requested numerous information from "the pediatric care provider". They included, PCR results, the infant's date of birth, age, sex, race or ethnic group "as well as information on whether antiretroviral prophylaxis was administered during the prenatal or intrapartum periods, within 48 hours after birth, or from 3 to 42 days of life...infants were classified as infected with HIV if they had had at least one positive PCR test at any age, and as not infected with HIV if they had had no positive PCR tests and at least one negative PCR test after the age of one month". Of a total of 939 infants, 10.5% were White, 58.3% Black, 27.1% Hispanic and 4.2% other. Using the data obtained from the paediatric care providers, the New York researchers "examined whether the use of abbreviated zidovudine regimens could reduce perinatal HIV transmission." They reported that the transmission rate depended on when AZT treatment began. When the treatment was begun in the prenatal period, it was 6.1%, when begun intra partum, 10.0%, within the first 48 hours of life, 9.3% and when begun on day 3 of life or later, 18.4%. In the absence of AZT treatment it was 26.6%. The authors of this

study concluded: "These results confirm the efficacy of zidovudine prophylaxis and suggest that there are reductions in the rates of perinatal transmission of HIV even with the use of abbreviated regimens that are begun intra partum or in the first 48 hours of life".[338]

6. In a 1998 publication by researchers from London, UK, of the 57 consecutive mother-infant pairs, 26 (46%) took a full 076-type course of zidovudine and seven took an incomplete course with only one or two parts of the regimen (antepartum, intrapartum, or postpartum). In the text one reads: "Transmission rate was 8% (3/39) for mothers who took any kind of antiretroviral therapy and 22% (4/18) for those who did not". In the abstract one reads: "With antiretroviral therapy or caesarean section, or both, transmission occurred in 6%" of pairs.[237]

4.3.2   Further studies

The Pediatric AIDS Clinical Trials Group Study 185 was conducted between October 1993 and March 1997 "at 53 clinical sites in the mainland United States and Puerto Rico". The study was designed to evaluate "whether HIVIG [hyperimmune immunoglobulin] infusions administered to the mother monthly during pregnancy and to the neonate at birth compared with zidovudine prophylaxis would significantly lower perinatal HIV transmission compared with infusions of intravenous immunoglobulin (IVIG) without HIV antibody combined with zidovudine prophylaxis…Eligibility criteria included laboratory documentation of maternal HIV infection, current receipt of zidovudine, baseline CD4 cell count ≤500µL. gestational age between 20 and 30 weeks, hemoglobin level ≥ 8 g/dL. serum creatinine level ≤ 1.5 mg/dL. and urine protein grade <2+ by dipstick or level <4 g in 24 h of urine collection…Exclusion criteria included evidence of pre-existing fetal anomalies with high probability that the fetus or infant would not survive the study period (e.g. anencephaly), chorionic villous or percutaneous umbilical blood sampling during the current pregnancy, illnesses associated with extensive protein loss, preexisting conditions that required IVIG treatment, receipt of HIV vaccine or passive immunotherapy during the current pregnancy, and severe pre-eclampsia…Median maternal age was 26 years and 87% of women were of minority race/ethnicity" (51% Black, 35% Hispanic, 1% other, 13% White, non-Hispanic). 24% of the women required AZT treatment for maternal health before pregnancy. After entry into the study "Women continued receiving their physician-prescribed antepartum antiretroviral regimen and received intrapartum zidovudine (intravenous loading dose of 2 mg/kg followed by a continuous infusion of 1.0 mg/kg/h until the umbilical cord was clamped); infants received the standard 6 week course of zidovudine prophylaxis (2 mg/kg zidovudine syrup orally every 6 h) starting within 8-12 h of birth". The infant status with respect to HIV-1 infection was based on the results of the HIV cultures (number of cultures required not given). "Four hundred fifty-nine women gave birth to 468 infants, 1 of whom was stillborn, resulting in 467 live-born infants and 458 mother-infant pairs (9 women gave birth to twins) eligible for analysis. Four infants did not have an HIV culture available, leaving 454 mother-infant pairs with at least one viral culture result available. Twenty-two infants were determined to be HIV-infected. The overall Kaplan-Meier transmission rate was 5.0% (95% confidence interval [CI], 3.0%-7.1%). Thirteen infants (6 in HIVIG, 7 in IVIG) were unable to have infection status classified definitively because of loss to follow-up after an HIV culture was obtained but before definitive infection status could be determined at age 6 months. Restricting analysis to the 441 mother-infant pairs with definitive infant infection status determined, the overall transmission rate was similar: 5.1% (95% CI, 3.0%-7.2%)". The authors reported that transmission rate was related to the T4 cell counts "but not with time of zidovudine initiation (5.6% vs. 4.8%, if started before vs. during pregnancy". The transmission rate for HIVIG recipients was 4.1% and for IVIG 6.0%. They concluded: "The unexpectedly low transmission confirmed that zidovudine prophylaxis is highly effective, even for women with advanced HIV disease and prior zidovudine therapy, although it limited the study's ability to address whether passive immunization diminishes perinatal transmission".[45]

One year later, 1998, several French researchers evaluated the affect of AZT and mode of delivery on mother to child transmission. In 85 perinatal centres "From September 1, 1985, through December 31, 1996, the participating sites reported 3474 deliveries of HIV-seropositive women. The study group comprised 2834 mother-child pairs after excluding 174 mothers infected with HIV-2, 44 breast-fed children, 114 twins, 43 children who died before HIV status could be determined, 61 children not enrolled because of lack of parental consent, 84 children lost to follow-up, and 120 children with indeterminate status at the time of analysis…Mother's mean age at delivery was 28 years, 31% were past or present intravenous drug users, and 40% were born in sub-Saharan Africa or the Caribbean...The HIV infection status was determined for children born at least 3 months prior to April 1, 1997. A child was considered infected at 18 months if HIV-1 antibodies persisted or in the case of death from HIV-related disease, and considered uninfected if the findings of 2 HIV antibody tests were negative. Antibody testing was carried out with both of 2 commercial enzyme-linked immunosorbent assays, and positive results were confirmed by immunoblot. Immunoblot findings were considered negative when no antibody was directed against the HIV envelope glycoproteins. For children aged 3 to 18 months, infection status was determined using DNA-polymerase chain reaction and/or HIV culture. The

child was considered infected when 2 different sample test findings were concordantly positive, and was considered uninfected when 2 different sample test findings were concordantly negative (at least 1 of which was administered at or beyond age 3 months)". The authors of this study reported that: "Transmission occurred in 58 (6.4%) of 902 treated mothers and 329 (17.2%) of 1917 untreated mothers...In the presence of zidovudine prophylaxis, we observed only 1 child infected with HIV-1 in 133 delivered by elective cesarean...Because untreated women showed decreased transmission risk, from 20% from 1986 through 1991 to 15% from 1992 through 1996, we also tested in the model interaction between period of birth and mode of delivery. This interaction term was not significant ($P = .97$). In the final multivariate analysis for the untreated group, 4 variables remained significantly related to transmission rate: p24 antigenemia ($P<.001$), cervicovaginal infections ($P = .008$), amniotic fluid appearance ($P <.001$), and birth year ($P = .008$)".

Commenting on their data the authors wrote: "Whether cesarean delivery has a protective effect independent of zidovudine prophylaxis can be further investigated by a large, international, individual patient data meta-analysis of observational studies. However, a definitive answer to the question will require a randomised clinical trial, which is the only method to ensure that women who undergo an elective cesarian delivery do not differ from those with other types of delivery for any known or unknown confounding factor. The mother-to-child HIV transmission rate started to decrease in France before the introduction of zidovudine prophylaxis. A decrease in transmission independent of zidovudine prophylaxis has also been reported in the United States. Changes in clinical management over a decade may have played a role, possibly regarding risk factors such as cervicovaginal infections during pregnancy, chorioamnionitis, fever in labour, pre-term delivery, and above all, prolonged membrane rupture, all of which may involve subclinical bacterial infection…Assuming that a planned cesarian delivery with zidovudine monotherapy can decrease the risk of transmission from 6% to 1%, 20 operations would be necessary to prevent 1 case of transmission. Maternal mortality with cesarian delivery is increased 5-fold (from 7 to 40 per 100 000 deliveries in Sweden) compared with vaginal delivery, and postoperative complications have been reported in 31% of HIV-infected women, 3 times more than in HIV-negative subjects. In most of the developing world, where preventive measures are desperately needed, the mortality associated with cesarean delivery is much higher".[46]

Because of its complexity and cost of the ACTG076, "this regimen has not been implemented in most developing countries, and no other intervention had been efficacious in reducing perinatal HIV transmission. In 1996, the Ministry of Public Health of Thailand and Mahidol University, in collaboration with CDC, initiated a randomized, placebo-controlled trial of a simpler and less expensive regimen of ZDV to prevent perinatal HIV transmission". "HIV infected women in antenatal care were recruited" at the same two hospitals, where they conducted their other studies, in which they examined the relationship between viral load and transmission. Of a total of "1140 HIV-1-positive women screened", only 423 were enrolled in the study and of these 397 were randomised 199 received placebo of which 193 were followed-up for more than 2 months and 198 received AZT of which 187 were followed-up for more than 2 months. "The zidovudine regimen consisted of 300 mg tablets taken orally twice daily from 36 weeks' gestation until onset of labour, taken once at onset of labour, and then every 3 hours until delivery. The placebo regimen consisted of tablets identical in appearance taken according to the same schedule…Mothers were given infant formula and asked not to breastfeed [but were not excluded if they breastfed]...Babies were taken to be HIV-1 infected if any PCR test result was positive, and uninfected if their last available PCR test result at 2 months or older was negative…Of 392 babies with at least one PCR test, 55 tested positive: 18 in the zidovudine group and 37 in the placebo group. The estimated transmission risks were 9.4% (95% CI, 5.2-13.5) on zidovudine and 18.9% (13.2-24.2) on placebo (p=0.006; efficacy 50.1% [15.4-70.6]). Between enrolment and delivery, women in the zidovudine group had a mean decrease in viral load of 0.56 log. About 80% of the treatment effect was explained by lowered maternal viral concentrations at delivery".[339,340]

Comments
(a) The placebo is not identified;

(b) The transmission rate in the AZT group varied according to when children were born in relation to the study midpoint, that is, the April 23rd , 1997. For children born before this date the transmission was 10.6%. On or after April 23rd the rate was 8.5%. The respective transmission rates in the placebo group were 14.4% and 23.5%;

(c) The authors reported that "In the placebo group, transmission risk was lower" at one of the hospitals, 14.3% vs 23.7%. No explanation is offered;

(d) In their 1998 study, women given neither placebo nor AZT, had a higher transmission rate compared to the placebo treated group in this study. The authors admitted that the "Reasons are unknown for the lower transmission rate in the placebo group (18.6%) than in untreated women (24.2%) studied in the

same hospitals during 1993-1994. The lower than expected background transmission rate highlights the importance of having included a randomised, concurrently enrolled, untreated control group. Had the test regimen been inactive, a transmission rate of 18.6% may have suggested some efficacy when compared with historical data".[339]

In the 1998 paper in which the CDC researchers presented their preliminary findings from the Thailand study on the effect of AZT on MCT they wrote: "CDC has sponsored another placebo-controlled trial of the same regimen of ZDV in collaboration with the Ministry of Public Health in Côte d'Ivoire in West Africa, where most HIV-infected women breastfeed their infants. Because the trial in Thailand demonstrated that the short-term regimen is efficacious in reducing transmission around the time of birth, and because preliminary data from the trial in Côte d'Ivoire have shown the regimen to be safe in this population, enrolment in the placebo group of the Côte d'Ivoire trial has been stopped".[339] When the findings from the Côte d'Ivoire were published in a paper entitled "Short-course oral zidovudine for prevention of mother-to-child transmission of HIV-1 in Abidjan, Côte d'Ivoire; a randomised trial", a placebo is included although neither its composition nor its means of administration is identified. Of 1601 women found positive, 618 did not return for counselling, of 983 who received counselling they reported "127 excluded" and "576 not enrolled". Two hundred and eighty women were enrolled, 140 received AZT and 140 placebo. Five women in the AZT group and 2 in the placebo were lost to follow-up. One in the AZT group and 3 in the placebo did not give birth before the end of the study. Of 134 infants in the AZT group and 135 in the placebo, from each group "124 children had at least one PCR result". The authors reported that: "Among babies with known infection status at age 3 months, 30 (26.1%) of 115 babies in the placebo group and 19 (16.5%) of 115 in the zidovudine group were identified as HIV-1 infected. The estimated risk of HIV-1 transmission in the placebo and zidovudine groups were 21.7% and 12.2% (p=0.05) at 4 weeks, and 24.9% and 15.7% (p=0.07) at 3 months. Efficacy was 44% (95% CI -1 to 69) at age 4 weeks and 37% (-5 to 63) at 3 months".[341] (Note: Although the same AZT regime was used in both the Thailand and Côte d'Ivoire studies the transmission rate in Thailand was 9.4% and in Côte d'Ivoire 16.5%).

In the same year, 1999, in the same issue of *Lancet* in which the Thailand and Côte d'Ivoire CDC collaborative studies were published, a third study, this time by researchers from France, Côte d'Ivoire and Burkina Faso, was also published,[342] the DITRAME study. Of the 14,385 women tested, 1,579 had positive antibody test. Of these 931 returned for enrolment. Of 873 eligible for enrolment 431 were enrolled, 214 in the AZT group and 217 in the placebo. Women who had a pregnancy of 36-38 weeks duration "were randomly assigned zidovudine (n=214; 300 mg twice daily until labour, 600 mg at beginning of labour, and 300 mg twice daily for 7 days post partum) or matching placebo (n=217)". The placebo and its method of administration were not identified. Eight women in the AZT group and 6 in the placebo were lost to follow-up. Two hundred infants of live-births in the AZT group and 200 in the placebo were used for analysis. The "diagnosis of HIV-1 infection in the child was defined on the basis of one positive PCR. The first positive test in the series allowed estimation of the timing of infection. Absence of infection was defined by a negative PCR on the last available sample or by a negative serological result with the techniques reported for maternal HIV screening and applied to samples collected every 3 months between 9 and 18 months of age. Children who had no sample available for PCR and could not be followed up beyond 6 months of age were classified as having unknown HIV status...The Kaplan-Meier probability of HIV infection in the infant at 6 months was 18.0% in the zidovudine group (n=192) and 27.5% in the placebo group (n=197; relative efficacy 0.38 [95% CI 0.05-0.60]; P=0.027). Adjustment for centre, period of recruitment, mode of delivery, maternal CD4-cell count, duration of labour, prolonged rupture of membranes and duration of breastfeeding did not change the treatment effect".[342] (Note: The transmission rate in the AZT group, 18%, is the same as the placebo group in the Thailand study).

In 1999 researchers from Johns Hopkins University, University of Washington, Fred Hutchinson Cancer Research Center, Family Health International, Durham, Boston University, NIAID/NIH, NICHD/NIH, Boehringer Ingelheim Pharmaceuticals, USA, Makerere University, Kampala, Uganda and Glaxo-Wellcome, UK, published a study in which the effect of AZT was compared with nevirapine. Of 13,839 pregnant women tested in Kampala, 2,144 had a positive test, 313 were given AZT and another 313 nevirapine. "The nevirapine regimen included oral administration of a single 200 mg tablet given to the mother at the onset of labour, and a single dose of nevirapine suspension of 2 mg/kg for the neonate, administered by study staff with a calibrated oral syringe at 72 hours after birth or at discharge from hospital, whichever occurred first. The zidovudine regimen included administration of two 300 mg tablets at onset of labour, followed by one 300 mg tablet every 3 hours during labour and, for neonates, administration with a calibrated oral syringe of zidovudine syrup, 4 mg/kg twice daily for 7 days after birth. The neonates zidovudine dose was given by the study staff and mothers together in the hospital and by mothers at home". In infants, "HIV-1 infection was defined as a positive qualitative HIV-1 RNA assay confirmed by quantitative HIV-1 RNA assay or HIV-1 culture on a second blood sample. If babies died after only one positive RNA assay on the sample, we classified the baby as being infected…The estimated risks of HIV-1 transmission in the zidovudine and nevirapine groups were: 10.4% and

8.2% at birth (p=0.354); 21.3% and 11.9% by age 6-8 weeks (p=0.0027); and 25.1% and 13.1% by age 14-16 weeks (p=0.0006)".[343] (Note: The transmission rate in the AZT group in this study is higher than that of the placebo in the Thailand study[340]).

In 2000 researchers from several institutions in the USA, France and Thailand published a study entitled "A trial of shortened zidovudine regimens to prevent mother-to-child transmission of human immunodeficiency virus type 1". In this study "Infants were considered to be infected with HIV if the results of the PCR test were positive for blood samples obtained on two separate occasions". The trial was designed to compare the effect of 4 regimens of AZT. AZT "starting in the mother at 28 weeks' gestation, with 6 weeks of treatment in the infant (the long-long regimen), which is similar to protocol 076; zidovudine starting at 35 weeks' gestation, with 3 days of treatment in the infant (the short-short regimen); a long-short regimen; and a short-long regimen. The mothers received zidovudine orally during labour. The infants were fed formula and were tested for HIV DNA at 1, 45, 120 and 180 days. After the first interim analysis, the short-short regimen was stopped…At the first interim analysis, the rates of HIV transmission were 4.1% for the long-long regimen and 10.5% for the short-short regimen (P=0.004); at this point the short-short regimen was stopped. For the entire study period, the transmission rates were 6.5% for the long-long regimen, 4.7% for the long-short regimen, and 8.6% for the short-long regimen".[344] (Note: There is no placebo. This is the lowest transmission rate ever reported and is also one of only two studies (the other is the Ariel) which required two positive PCRs to determine infection and two negative PCRs after the age of one month to determine the absence of infection).

In 2001 the researchers from the DITRAME study published a paper entitled: "Maternal plasma viral load, zidovudine and mother-to-child transmission of HIV-1 in Africa : DITRAME ANRs 049a trial".[252] In this study, using the data from their 1999 study, they analysed the effect of AZT on the viral load and the relationship between viral load and MCT. In this study "421 women were enrolled and delivered 401 live infants (200 ZDV, 201 placebo)". The study is said to be "randomised double-blind placebo-controlled trial". However, "Maternal RNA levels were available and measurable for 55 cases (23 ZDV, 32 placebo) of the 94 mothers diagnosed as infected up to 15 months of age…Among the 290 mothers of children uninfected at 15 months of age, 117 controls (47 ZDV, 70 placebo) were selected for comparison with the 55 infected cases". No mention is made as to the selection criteria or why such selection was necessary. At entry into the study mean plasma viral load in the transmitting mothers was 4.61 $\log_{10}$ At the end of the study it was 4.72 $\log_{10}$, an increase of 0.10 $\log_{10}$. The corresponding values in the non-transmitting mothers were 3.69 $\log_{10}$, 3.73 $\log_{10}$. Although the study is said to be "randomised double-blind", in the transmitting mothers at entry into the study, there was a significant difference in viral load between the AZT and the placebo groups, 4.82 $\log_{10}$ *versus* 4.45 (p=0.01). No such difference was found in the non-transmitting mothers, 3.73 $\log_{10}$ v 3.66 $\log_{10}$ (p=0.70).

At the end of the study, in the transmitting mothers the viral load was 4.70 $\log_{10}$ in the AZT recipients and 4.74 $\log_{10}$ in the placebo. The corresponding values in the non-transmitting mothers were 3.29 $\log_{10}$ and 4.03 $\log_{10}$. In their final multivariate model the only variables significantly associated with the risk of MCT were: maternal CD4 cell count at entry, maternal plasma RNA at entry, absolute difference between end and entry of study viral load count and low birth weight. They also reported that "Our maternal short ZDV regimen decreased significantly the maternal viral load at day 8 post-partum from its baseline level when compared with the placebo group". Discussing their findings they wrote: "By selecting mothers who had a more severe immunodeficiency compared with those not included in this study, this potential bias could have overestimated the fraction of MTCT attributable to this risk factor…Our findings support the role of high viral load in overall MTCT of HIV-1 in Africa but also suggest the role of ZDV in decreasing the maternal plasma viral load and consequently reducing the risk of MTCT even after a few weeks of treatment".[252]

In an analysis of this study it was our view that the claims in regard to the relationship between viral load and MCT as well as the effect of AZT on viral load were not supported by the data. According to the opinion of an independent statistician: "With respect to the maternal HIV transmission paper I can't see any particularly outlandish statements drawn from the data, except, I suppose, we need to carefully keep in mind that the viral load reduction between ZDV and placebo groups was due (I suspect wholly) to the non-transmitting group who had a much lower baseline load to start with [and who "were selected for comparison with the 55 infected cases"]. In the transmitting mothers, the ZDV group did not show a reduction and it seems imprudent to make one common statement about both groups. While some comment was made about the different effect on the transmitting/non-transmitting groups, I feel that a greater distinction should have been drawn between the two and I agree that the statement "Our maternal short ZDV regimen decreased significantly the maternal viral…when compared to the placebo group" does not give an accurate picture of the results. The qualifier "when compared to the placebo group" attempts to give the statement an illusion of legitimacy but does not really disguise the fact that they are playing fast and loose with the term "decreased". I think the authors should have been a bit more careful in their phrasing, bearing in mind that ultimately only their concluding statement will be quoted, suggesting that ZDV will decrease viral load, without the benefit Table 2 to alert you to the

distortion. In statistical terms, there is some evidence to suggest that the change in viral load due to ZDV treatment is not independent of the baseline load and hence you can't combine effects across the different baseline load categories, i.e. you can't draw a common inference about change by combining both ZDV groups as a "treated" group–you need to describe each group separately".(L. Mina, personal communication).

4.4   Discussion

4.4.1   AZT

The presently available data show that:

1. Although many studies have been published which claim that AZT inhibits MCT of HIV, not one of the studies is a randomised double-blind controlled study.

2. Many of the studies do not have controls. When controls are used no information is provided as to their nature and controls appear to suffer toxicities similar to those caused by the active drug.

3. The reported effects of AZT on MCT vary widely from study to study and even between participating centres and stage of study within the same study. For example, although some studies in the USA including the ACTG076 claim a substantial reduction, in other studies including the Ariel, AZT had no significant effect on transmission. In the Thailand study in which identical women were recruited from two hospitals, the transmission rate in the placebo group in one of the hospitals was 14.3% and in the other 23.7%. In the same study, in the children given AZT and born before April 23rd 1997, the study midpoint, the transmission was 10.6%. On or after April 23rd the transmission was 8.5%. The respective transmission rates in the placebo group were 14.4% and 23.5%. The reported transmission rate in the Thailand study was 9.4%, in the Côte d'Ivoire 16.5%, although the same regime of AZT was used. In the 1998 Rwandan study of MCT discussed in Part II, the MCT was 12% without the use of any antiretrovirals.[69]

4. Although it is accepted by leading authorities including the CDC[253] that one virological test is not sufficient to confirm HIV infection, in the vast majority of studies one positive virological test is considered proof of infection.

5. Since not one of the tests used to prove HIV infection of the children is of proven specificity let alone 100% specificity, it follows that the greater the number of samples tested the higher the probability of obtaining a positive result even though a child is not infected. However with very few exceptions, no mention is made if the same number of samples were tested from both groups of children, those whose mothers received AZT and those whose mothers received placebo. It is also a fact that the "isolation" results depend on the length of time of the culture.[335] Yet in no study are there data presented that cultures of the children in both groups were tested the same number of times and over the same length of time.

In summary, given that:

  (i) not one of the studies is randomised, double-blind, the placebo is not identified or is not used;
  (ii) the reported effects vary from study to study;
  (iii) in the majority of studies the virological tests performed lack the rigour which HIV experts themselves assert essential to prove HIV infection;
  (iv) there are other problems associated with these studies;

the inescapable conclusion is that at present no proof exists that AZT inhibits MCT of HIV. While no experimental proof exists that AZT inhibits MCT there are basic scientific reasons why AZT cannot have such an effect.

4.4.2   AZT and Viral Load

In the studies on MCT of HIV and its inhibition by AZT conducted in Thailand by researchers from the CDC and Thailand the authors claim that "clear evidence was found that elevated maternal viral load is a strong risk factor for both in utero and intrapartum transmission", and in fact that "about 80% of the treatment effect was explained by lowered maternal viral concentration at delivery". In their view, intervention to reduce maternal viral load should be effective in reducing both in utero and intrapartum transmission and that this intervention should result in reduction of at least 0.5–1 log.[244,339,340] Indeed, according to the most eminent experts on anti-HIV drugs, a drug that does not induce a sustained decrease of HIV RNA of at least 0.5–1 log cannot have an anti-HIV effect.[345,346] At present there is no evidence that AZT has such an effect (Appendix VI). In pregnant women the AZT effect on viral load is "only a minimal (i.e. 0.24 $\log_{10}$) reduction".[215,347] This failure of AZT to lower "viral load" accords with the pharmacological data. HIV experts agree that only the triphosphorylated form of AZT can inhibit the replication of HIV. However, AZT underwent clinical trials and was introduced as a specific anti-HIV drug many years before there were data proving that the cells of patients are able to triphosphorylate the parent compound to a level considered sufficient for its putative pharmacological

action.[348,349] Furthermore, since 1991 it has become apparent that no such phosphorylation takes place which means AZT cannot possess an anti-HIV effect and thus reduce mother to child transmission[348] (Appendix V).

Even if there was proof beyond reasonable doubt that AZT decreases the frequency of a positive PCR and WB test in children, such a finding cannot be considered proof for an inhibitory effect of AZT on MCT. In Part I we have mentioned that:

1. at present there is no proof that the primers and probes used in the "HIV" PCR test are fragments of HIV RNA (cDNA). All the available data indicate that they are cellular RNAs (cDNAs);[31]
2. there is evidence that chemical agents (drugs) are able to modify cellular RNAs (cDNAs) resulting in the appearance of novel RNAs (cDNAs). This is a fact presently accepted even by Montagnier, the discoverer of HIV.

Evidence also exists that AZT is an oxidising, mutagenic and carcinogenic agent.[348,350-355] All HIV experts accept that AZT induces changes, that is, mutations in the HIV genome, which means that AZT is a "genotoxin". Since the "HIV" PCR primers are universally obtained from cell cultures derived from antiretroviral (including AZT) drug naïve invididuals, "HIV" nucleic acids will be less efficiently amplied in AZT treated patients (children). This may lead to the erroneous interpretation that AZT has an inhibitory effect on MCT.

In 1981 Gallo accepted the evidence that the antibodies which reacted with "retroviral" glycoproteins[356,357] were directed "against the carbohydrate moieties on the molecule that are introduced by the host cell as a post-transcriptional event, and which are therefore cell-specific and not virus-specific".[358]

Since,

(a) The vast majority of the criteria used to define a positive WB test (including Africa) require a glycoprotein band (gp41, gp140, gp160);

(b) AZT "inhibits glycosylation and dramatically alters glycosphingolipid synthesis in whole cells at clinically relevant concentrations";[349]

it follows that in patients (children) exposed to AZT the frequency of the gp41, gp120, gp160 bands would be lower compared to non-exposed individuals. This may be interpreted that AZT decreases MCT even when such an effect is non-existent.

Although AZT is not efficiently triphosphorylated it is very efficiently mono-phosphorylated. The mono-phosphorylation of AZT could act as an inhibitor of phosphorylation of cellular constituents including cellular nucleotides as Furman *et al* have showed. Exposure of cells to 50μM of AZT for 72 hours led to an approximately 95% decrease in dTTP and dCTP and approximately 63% decrease in dGTP. In the presence of such a profound, global reduction in the concentrations of the naturally occurring nucleotides one would expect a decrease in DNA synthesis and untoward effects on many tissues especially those with the most rapid cellular turnover including the gut and bone marrow. Indeed, "a characteristic feature of zidovudine therapy is an elevated MCV",[22] (mean corpuscular red cell volume) and "The antiviral agent zidovudine (AZT), used for treating the human immunodeficiency virus (HIV), often causes severe megaloblastic anemia", anaemia "caused by impaired DNA synthesis".[359] (This well known fact makes blinded studies of AZT therapy problematic since physicians cannot avoid their patients' blood test results). Since antibodies are proteins for whose production DNA (RNA) synthesis is an absolute requirement, the failure to produce antibodies which react in the "HIV" antibody tests may merely reflect impairment of protein synthesis rather than inhibition of viral replication.

It is significant that in 1998 in the "Public Health Task Force Recommendations for the Use of Antiretroviral Drugs in Pregnant Women Infected with HIV-1 for Maternal Health and for Reducing Perinatal HIV-1 Transmission in the United States", the CDC advised: "when considering treatment of pregnant women with HIV infection, antiretroviral monotherapy [AZT] is considered suboptimal for treatment; combination drug therapy is the current standard of care".[347] If AZT was "suboptimal for treatment" of pregnant USA women in 1998, since no data to the contrary have been published, it will also be suboptimal for any other women. Yet according to researchers from the USA and Africa,[26,360] in Africa, including South Africa, even a short course of AZT monotherapy will reduce MCT by 50% in non-breastfeeding mothers and by 37% in breastfeeding mothers. AZT treatment of pregnant women "would probably save at least 15 000 lives per year in South Africa".[360]

4.5 Nevirapine

Since all the HIV experts agree that AZT treatment may be too costly, nevirapine monotherapy is also recommended.[360] The use of nevirapine is based on a single study, the 1999 Uganda study[343] ("administration of a single 200 mg tablet given to the mother at the onset of labour, and a single oral dose of nevirapine suspension 2 mg/kg for the neonate, administered by study staff with a calibrated oral syringe at 72 h after birth or at discharge from hospital, which occurred first") which is claimed to result in a transmission rate of 8.2% at birth, 11.9% by age 6-8 weeks and 13.1% by age 14-16 weeks. The corresponding values reported for a regime of AZT, two 300mg tablets on onset of labour, followed by one 300mg tablet every 3 hours during labour and 4mg/kg twice daily for 7 days after birth to the infants, were 10.4%, 21.% and 25.1%.

Comments

1. The study was not a randomised, double-blind controlled study. In fact, no placebo was used.

2. The infant's "HIV-1 infection was defined as a positive qualitative HIV-1 RNA assay confirmed by quantitative HIV-1 RNA assay or HIV-1 culture on a second blood sample. If babies died after only one positive RNA assay on the sample we classified the baby as being infected".

3. Perinatally infected infants were diagnosed with the HIV RNA PCR although authorities including the CDC[81,253] and manufacturer Roche assert that HIV RNA PCR cannot be used either to diagnose HIV infection or as a screening test for "adults, adolescents, and children".

4. No mention is made as to what percentage of children were deemed infected based on mortality data and what percent on the basis of their virological test. (It is notable that nevirapine monotherapy does not have a significant effect on T4 cells and does not inhibit progression to AIDS).[361,362]

5. After an oral dose of 200 mg in HIV positive adults it takes an average of 4.6 hours to reach a maximum plasma concentration (T_{max}) with a plasma half-life of 20 hours. The maximum plasma concentration (C_{max}) reached after this dose of drug is 1.9μg/ml.[361] In a study published in 1998 entitled: "Pharmacokinetics of Nevirapine in Human Immunodeficiency Virus Type 1–Infected Pregnant Women and Their Neonates" from many institutions in the USA, the pharmacokinetics of nevirapine were evaluated "in 17 human immunodeficiency virus type-1-infected women in labour and their newborns". The women (13 Hispanic/Black and 4 White) were divided into two cohorts. In cohort 1 mothers received single oral dose of nevirapine during labour and their infants received no nevirapine. The median C_{max} in the plasma of women was "1663 ng/mL, (447-2639) for those receiving 200mg doses. Median T_{max} was 3 h (range, 1-8)". The women "delivered at a median of 6.7 h (range, 0.9 – 10.5) after dosing. Their median nevirapine concentration at delivery was 714ng/mL (range, 80–1678). The median cord blood concentration in the infants born to those mothers was 787 ng/mL (range, 64-2030), and the median ratio of cord blood nevirapine concentration to the maternal nevirapine concentration at the time of delivery was 99.7% (range, 80.0% - 122.8%)…The median ratio of the concentration of nevirapine in breast milk to that in maternal serum was 76% with a range of 54% to 104%". Median C_{max} in the plasma of these infants was 925 ng/mL (range, 64 – 2030) with a half-life of 45.4 h. In cohort 2 mothers received a single dose of 200mg when in labour and the infants received "a single 2-mg/Kg dose between 48 and 72 h after birth" ("median age of 53.3 h, (range, 48 – 77.6)"). In these infants, "median nevirapine concentration before dosing was 541 ng/mL (range, 141 – 768)...Median pharmacokinetic parameters for the infants were as follows: C_{max}, 1355 ng/mL (range, 644-1607; T_{max}, 12 h after dosing (range, 2-24)...Median calculated nevirapine concentration at 168 h (7 days) of life was 215 ng/mL (range, 112-275).[363] The fact that the women deliver at 0.9 –10.5 hours after dosing and that it takes between 1-8 hours to reach maximum plasma nevirapine concentration means that an unknown number of women deliver before a maximum concentration of the drug is reached. Most importantly, the maximum nevirapine concentration in both mothers and infants is always significantly lower than the steady state concentration required to obtain any virological response ("4.7μg/mL [17.7 μM]; range, 3.4 - 8μg/mL").[362]

6. A single dose of 200mg nevirapine has no effect on HIV RNA. Even daily doses of 200mg lead to a maximum decline of 2.2 $\log_{10}$ from the baseline value. However, this is unsustained and after a nadir at 2 weeks the HIV RNA levels increase exponentially with a doubling time ranging from 1.80 to 5.73 days.[364,365] In a study published in 1997 twenty patients were "treated with nevirapine at a single daily dose of 400mg after a 2-week period of treatment with a daily dose of 200 mg...Four patients discontinued treatment prematurely because of adverse events and 1 patient was lost to follow-up at day 141...Changes in HIV-1 RNA load were measured in 18 subjects. A mean decline of 0.46 ± 0.47 log RNA copy numbers was observed after 4 weeks of treatment, with a return to baseline values within 12 weeks of treatment".[366] As

far back as 1995 "The nonnucleoside RT inhibitors held great promise for clinical application in the treatment of HIV-infected persons" but "the predicted loss of antiviral activity" soon led "to diminished enthusiasm for this class of compounds".[362]

7. The pharmacological mode of action of nevirapine can only prevent infection of cells not already infected. Thus, when given to the mother, it could prevent transmission only if the child is not already infected.

8. Since nevirapine like AZT is capable only of preventing infection of new cells and is unable to inhibit the expression of HIV within already infected cells or eradicate the virus, when the drug is given to neonates, especially 3 days post partum, it will have no effect on MCT *in utero* or during labour and delivery. Under these circumstances nevirapine may prevent transmission via breast feeding and then only for a very short period of time (days). However, since,

    (a) a single dose of 200mg administered to the mother leads to a drug concentration in milk much lower than the concentration necessary to have an anti-retroviral effect;

    (b) the concentration reached in the infant after a single dose of 200mg to the mother and 2mg/Kg to the infant is much lower than that necessary to induce an anti-HIV effect;

    such a regime of the drug cannot inhibit MCT via breast milk even for a very short period of time.

Given the pharmacological action of the drug and its pharmacokinetics, one wonders how anyone can propose a protocol like that used in the Uganda study and expect an effect on MCT?

In recommending nevirapine as mono or combination therapy it is important to consider that not only is its anti-viral effect even when administered for lengthy periods very limited and of extremely short duration, but that it confers resistance to the treatment with other anti-retrovirals.[361,365,367] It is also significant that[367] the European Agency for the Evaluation of Medicinal Products recommends the use of nevirapine only for combination therapy and only for "infected patients with advanced or progressive immunodeficiency".[368]

4.6   Conclusion

At present, no proof exists that children become infected by their mothers either *in utero* or *post partum* with a unique human retrovirus, HIV or this can be prevented by AZT or nevirapine.

PART V
ALTERNATIVE PREVENTION OF THE PUTATIVE MOTHER TO CHILD TRANSMISSION OF HIV

5.1   Introduction

It makes no sense, indeed it is contrary to the Hippocratic Oath, to administer a drug that is toxic and devoid of therapeutic effects. While there is no proof that neither AZT nor nevirapine possess antiretroviral effects, (indeed, given their pharmacological properties and dosing schedules, it is not possible for these agents to have such effects, see Part IV) there is ample evidence that both drugs are toxic to adults and, in the case of AZT, to the children of mothers administered the drug during pregnancy.

The claim of a beneficial effect of AZT and nevirapine on MCT is based on their effects on test parameters. These parameters are

   (i)   antibody/antigen reactions, that is, the reaction of antigens present in the antibody test kits with antibodies in patient sera—the antibody tests;
   (ii) the reaction of antibodies to p24 with antigens present in test cultures, "HIV isolation";
   (iii) the PCR test.

To date there is no evidence that these drugs favourably effect these parameters. Even if there were such proof, their benefits in patients can be judged only by effects on disease progression or mortality rates. The current vogue for the use of surrogate end points to document benefit and harm from particular treatments requires surrogates which reliably predict overall clinical outcomes. For a variety of reasons, "In practice this frequently fails…The validity of a surrogate end point has rarely been rigorously established". For example, "Predictions having an accuracy of approximately 50%, such as the accuracy seen with the CD4 count in the HIV setting, are as informative as a toss of a coin…Proper validation of surrogates also requires an in-depth understanding of the causal pathways of the disease process as well as the intervention's intended mechanisms of action. Such insights are rarely achievable…In definitive phase 3 trials, except for rare circumstances in which the validity of the surrogate end point has already been rigorously established, the primary end point should be the true clinical outcome".[369] In this regard the presently available evidence indicates that AZT may worsen rather than improve end points. (No similar data are presently available for nevirapine but the drug is toxic to adults and may be even more toxic than AZT.[370-373] Indeed in April 2000 "severe and life-threatening cutaneous and hepatic reactions" caused by nevirapine prompted the European Agency for the Evaluation of Medicinal Products to issue a public warning on the EMEA website.[374] Thus one would also expect this drug to be also toxic to infants).

5.2   The safety of AZT

Children studied in the Pediatric AIDS Clinical Trial Group 076 who received either AZT or placebo, were enrolled in "a long term observational protocol", Protocol 219. The aim was, "To evaluate the long-term effects of in utero exposure to zidovudine [AZT] vs placebo" in uninfected children. The authors concluded:

   (a) "No significant short-term toxic effects were observed in PACTG 076 for those mothers and infants who received zidovudine";

   (b) "No adverse effects were observed in HIV-uninfected children with in-utero and neonatal exposure to zidovudine followed up for as long as 5-6 years".

However, neither the authors of the PACTG 076 protocol[331] nor those of the PACT 219 identified their placebo. In the original 1994 publication from the PACTG 076 study the authors reported that 22 children stopped therapy "because of toxic effects (11 in each group)". Most importantly, in the 219 study, instead of following blindly all the children from the 076 study as is required in this type of epidemiological research, the authors selected only 234 (122 in the AZT group and 112 in the placebo), without giving any reason(s). At the end of the study, when the analysis was performed only "86% of the uninfected children enrolled in PACTG 219 were still participating in the study; 26 children were lost to follow-up or their caregivers refused further contact". No data are provided on the classification of the children enrolled in the 219 but lost to follow-up. Although in the PACTG 219 protocol "Echocardiograms and ophthalmology examinations (including visual acuity assessment and funduscopic examination) were required for all children by 36 months of age", only:

(a) "One hundred eighty-six uninfected children (80%) had at least 1 echocardiogram result recorded in the database";

(b) "One hundred thirty-seven uninfected children (59%) had at least one ophthalmologic examination (including funduscopic results) recorded in the database".

By designing, executing and analysing data using the methods adopted by the authors of the PACTG 219 study one may certainly find that AZT has no long term toxic effects, as the authors of those studies reported, even if the drug is very toxic. Equally as certain is the fact that such findings cannot be considered scientific proof. The authors themselves stated: "There are caveats to the data presented. Only two thirds of the children enrolled in the original PACTG 076 protocol are currently being followed up in this late-effects protocol…With a sample size of 120 per arm, there is limited power to detect very rare adverse events".[375]

In another study[376] the records of "HIV-exposed infants with known ZDV exposure (in utero and/or neonatal) were reviewed for reports of the development of tumours. Participants in this review were HIV-exposed infants with known antiretroviral exposure (in utero or neonatal ZDV) and participating in one of two national, multicentre studies: PACTG 076/219 or the Women and Infants Transmission Study (WITS). Of the 188 PACTG 076 participants who met the first two criteria [were taking part in PACTG 076 or WITS and were exposed to AZT either "in utero and/or neonatal"], 115 (61%) co-enrolled in PACTG 219 and could be included in this analysis. WITS is an ongoing observational natural history study of factors that produce an impact on perinatal HIV transmission and disease progression in adult women and their HIV-exposed children. Prospective enrolment has occurred at 6 U.S. sites since 1989. In total 612 infants in the WITS with in utero or neonatal ZDV exposure were assessed. Infants co-enrolled into both PACTG 076/219 and WITS were reported and analysed in the WITS cohort alone". The authors of this study reported that: "Race/ethnicity distribution in these cohorts is representative of national demographic patterns of HIV infection exposure in infants with black and Hispanic infants disproportionately represented. Mean infant follow-up was longer for PACTG 076/219 participants at 38.3 months (366.9 person-years follow-up) and reflects closure of the PACTG 076 study in 1994. The WITS study has had continued prospective enrolment to date and has a shorter mean follow-up of 14.5 months with 743.7 person years follow-up. The range of infant follow-up was as short as the first month of life and as long as 6 years". "…no tumours of any nature were reported in these 727 HIV and ZDV-exposed infants…These data are reassuring regarding the short-term lack of tumours for ZDV-exposed infants observed to date". Their "reassuring" can be questioned on several grounds, suffice to mention those acknowledged by the authors themselves: "Limitations of this analysis are acknowledged. First, tumour surveillance in the clinical studies PACTG 076, 219, and WITS is relatively passive and under-reporting is possible. Assessment of the reproductive tract is included only in PACTG 219 at age appropriate intervals, as instanced, adolescence and young adulthood, but not performed in the young cohort reported in this paper. Second, follow-up for our patient cohort is relatively short, which leads to a wide confidence interval for the relative risk. The longest reported follow-up for infants was just over 6 years with median follow-up by cohort at almost 1 year for WITS and just over 3 years for PACTG 076/219. In the cited rodent study, mice with in utero ZDV exposure were serially sacrificed and examined histologically at the human equivalent of early childhood and again at the second to third decade. Tumours were documented only in mice sacrificed at or after the human equivalent of the second decade…This analysis clearly does not completely define the potential for carcinogenicity of ZDV in the child with foetal/infant exposure for HIV-1 transmission risk reduction". As far as exposure to AZT for "HIV-1 transmission risk reduction" is concerned, it is of interest that the summary starts with the sentence: "Zidovudine (ZDV) therapy during pregnancy and to the neonate reduced perinatal HIV transmission by nearly 70% in Paediatric AID Clinical Trials Group (PACTG) protocol 076". However, in the paper published in 1996 with data from WITS, with two of the co-authors as co-authors, one reads: "ZDV was not associated with a significant decrease in transmission in this overall cohort (18 versus 20% transmission in ZDV users versus non-users".[43]

In yet another study[376] the infants from the PACTG 076 "were followed through 18 months and the clinical and laboratory data of the uninfected children who were exposed to AZT during pregnancy and for 6 weeks after delivery were compared with children who were similarly exposed to placebo". Reading their conclusion: "There were no identified problems that would alter current recommendation for the routine use of ZDV for prevention of mother-to-child HIV-1 transmission", and the title of the paper, "Safety of the Maternal-Infant Zidovudine regimen utilisation in the Pediatric AIDS Clinical Trial Group 076 Study", one may get the impression that the authors proved that no toxicity is associated with AZT treatment. It is true, that by looking at the two tables where they present their data, the outcome in both the AZT and the placebo groups may appear similar. However, this does not mean that AZT is not toxic. From the tables it is obvious that the uninfected children in the placebo group had high levels of clinical and laboratory abnormalities. An example of this is seen in the following data extracted from table 2 of the study.[376]

Table 5.1

	n(%)	
	Zidovudine	**Placebo**
Severe anaemia[†]	1 (0.5)	3 (2)
Severe neutropenia[†]	45 (21)	55 (27)
Other severe haematologic toxicity[†]	2 (1)	2 (1)
Severe chemistry toxicity[†]	27 (13)	32 (16)

"[†]Includes protocol-defined severe or life-threatening toxicities"

This means that either both AZT and the placebo used were toxic or there was an unknown underlying abnormality in either the mothers or the children in the PACTG 076 study. Notwithstanding, from this study one cannot conclude that AZT is non toxic.

Most importantly, the claim that AZT has no adverse clinical effects on children exposed either in utero or post partum is not supported by the data published in other studies. In the follow-up study of MCT from the ACTG 076 study published in 1999, the authors reported that the rates of rapid disease progression for the zidovudine group were 6/14 (43%) and for the placebo group 16/43 (37%).[258]

Italian researchers followed up, for the first three years of life, HIV seropositive children born to mothers who either did or did not receive AZT treatment. The two groups of children were similar in regard to all variables taken into account (year of birth, maternal clinical condition, birth weight and treatments) apart from age at the beginning of PCP chemoprophylaxis, which was undertaken earlier in these children who were born to mothers give AZT. They found that the children born to the mothers given AZT "had a higher probability of developing severe disease" (57.3% versus 37.2%) or severe immune suppression (53.9% versus 37.5%) and a lower survival (72.2% versus 81%).[377]

Table 5.2 HIV-infected children—adverse outcome probabilities (%) within three years

Mothers	Severe Disease	Severe Immune Depression	Death
Took AZT	57%	54%	28%
Did not take AZT	37%	38%	19%

In the French National Epidemiological Network for studying mother-to-child transmission between 1986-1998, 1754 mother-child pairs were exposed to AZT. The diagnosis of two children with serious diseases resulting in death (visual impairment, refractory epilepsy, abnormal growth and deterioration of cognitive and psychomotor abilities) suggestive of mitochondrial dysfunction, led the authors to investigate "mitochondrial toxic effects in children exposed to zidovudine" (AZT). Eight children, all HIV negative born to HIV positive mothers (5 of whom were of African origin), "had mitochondrial dysfunction. Five, two of whom died, presented with delayed neurological symptoms and three were symptom-free but had severe biological or neurological abnormalities". It was reported that "A continuing study of the incidence of neurological mitochondrial diseases in the UK that correspond to those seen in patients one, two, four and five, has identified only 21 cases in 20 months in about 12 million children younger than 16 years. Even if this UK study had underestimated the number, the observation of several cases in a population of about 1700 exposed children in our network strongly suggests an acquired mitochondrial dysfunction in these non-HIV-1 infected children born to infected mothers". In discussing their findings the authors pointed out that "Antiretroviral nucleoside analogues are toxic to mitochondria in HIV-1-infected adults and children…The symptoms in the children in our study were not specific, and may therefore have not been identified as toxic effects of treatment. In symptom-free children who had only persistent biological abnormalities, the persistent lactic acidosis and the anomalies of myelinisation and electroretinographic findings–the long term progression of which is unknown–were detected by specific diagnostic procedures. Prospective studies designed to investigate this effect are essential".[378]

The medical records of 403 infants and their HIV-1 seropositive mothers attending the Special Immunology Perinatal Clinic at the University of Miami/Jackson Memorial Hospital between January 1, 1990 and December 31, 1994 were retrospectively reviewed. Of 291 mother-infant pairs included in the analysis 152 mothers received no AZT. The other 139 mothers were treated with AZT for a minimum of 7 days prior to delivery.

"Mother-infant pairs in the ZDV group received one of the following combinations: prenatal ZDV only; prenatal and intrapartum ZDV; prenatal and postnatal ZDV; or prenatal, intrapartum and 6 weeks of postnatal ZDV…HIV-1 infection was defined according to U.S. Centers for Disease Control and Prevention (CDC) criteria" (The 1994 CDC definition for children[8], Appendix III). The authors defined rapid progression of HIV-1 disease (RPD) as: "occurrence of a CDC class C clinical event or AIDS related death by 18 months of age", and nonrapid progression of HIV-1 disease (NRPD) as: "absence of any RPD endpoint by 18 months of age". The authors reported that "Among treated mothers 12.2% (17 of 139) transmitted the virus to their infants, whereas 22.4% (34 of 152) of the untreated mothers transmitted the virus to their infants", (54% reduction). The "risk of RPD was clearly related to maternal treatment: the proportion of infants with RPD was 29.4% (10 of 34) in the no ZDV group compared with 70.6% (12 of 17) in the ZDV group...the time to development of RPD in perinatally infected children was consistently shorter for infants in the ZDV compared with those in the no ZDV group (log-rank p value = .004). RPD was three times more likely in preterm versus term infants (risk ratio [RR], 2.7, p = .032); and twice as likely for infants born to mothers with versus without clinical symptoms". Discussing their findings the authors wrote: "Our results suggest that maternal treatment with ZDV may influence the course of disease among perinatally infected infants. In this retrospective study, the risk of RPD was five to six times higher among infants born to treated compared with untreated mothers. This relationship remained after controlling for other maternal characteristics (maternal CD4$^+$ count, gestational age, duration of rupture of membranes, and maternal clinical symptoms of HIV infection)".[379]

In a study published in the Journal of Infectious Diseases, 2000, researchers from the USA including the CDC, "presented data from a large multicenter, cohort study to compare the clinical course and early viral diagnosis of HIV-infected children exposed and unexposed to ZDV during prenatal and perinatal periods". They reported that, of 196 children born during or after 1992, "The probability of AIDS or death in the first year was 30% (95% CI, 9-51) among 20 infected children whose mothers used ZDV for > 6 months during the current pregnancy or before, and 32% (95% C1, 20-44) among 50 infected children whose mothers used ZDV for shorter periods". In comparison, 25 of 117 (21%) of non-exposed children progressed to AIDS or died.[492]

In another study published in the same year the authors pointed out that "the U.S Public Health Service Task Force currently recommends combination therapy including the use of protease inhibitors (PIs) in pregnant women. However, at the Twelfth World Congress on AIDS in Geneva, 1998, Lorenzi reported that PIs may contribute to premature birth. This resulted in a temporary moratorium on the use of PIs in ACTG clinical trials involving pregnant women. Since low birth weight is the major source of newborn morbidity and mortality, and since premature birth is thought to be a risk factor for perinatal transmission, identifying an association with HIV therapy is critically important". In their study "The concern about the use of PIs in pregnant women based on Lorenzi's data was confirmed" and they also showed that antepartum treatment with zidovudine alone had similar effects.[380]

Comments

AZT and nevirapine are not given to children merely to decrease the number of times a particular laboratory test is positive. The ultimate reason for the use of antiretroviral treatment is to improve childrens' health and save childrens' lives. Thus the effects of the drugs on parameters claimed to prove HIV infection are entirely subordinate to their principal goal. However, the presently available data indicate that treatment with AZT increases, not decreases, morbidity and mortality in children. To date no clinical end point data exist on the effects of nevirapine in children. However, since the drug is equally as toxic (if not more so) in adults as AZT, one would expect the same to be true for children exposed to this agent.[370-373] Furthermore,

1. Since,

    (a) The toxic effects, including those resulting in death, in non-HIV infected children who are exposed to AZT are non-specific;

    (b) The symptoms and diseases which constitute AIDS in children are also non-specific;

    how is it possible to claim that AZT causes only rapid disease progression and not disease and death? Especially when there are data that suggest AZT causes PCP in infants who are not HIV-infected.[381]

2. Since,

(a) AZT treatment leads to an increase in the rate of death and AIDS in the first year of life;

(b) In many, if not all the studies of MCT, the diagnosis of AIDS or death in the first year of life is considered proof for HIV infection and thus for MCT of HIV;

how is it possible to simultaneously assert an increase in death and AIDS in the first year of life and a decrease of MCT of HIV?

3. Since AZT is toxic to the child, would not it then be advisable to use other non-toxic modalities to reduce MCT if indeed such transmission takes place? Especially as there are ample data that such modalities exist?

5.3 Parameters associated with "MCT" and their modulation by means other than antiretroviral drugs

5.3.1 Viral load data

The maternal viral load is said to be the main risk factor for MCT. However, while there is proof that AZT and nevirapine do not decrease viral load, there are means by which the viral load can be significantly reduced. In 1993 some of the best experts on HIV/AIDS reported that, in patients with signs and symptoms of primary infection with HIV, "Virion-associated HIV-1 RNA levels peaked between 8 and 23 days after the onset of symptoms, reaching values between 3.55×10^5 and 2.18×10^7 copies per milliliter (corresponding to 1.78×10^5 to 1.09×10^7 virions per milliliter)...Within the first 100 days after onset of symptoms, plasma RNA levels fell by between 20 and 235-fold", even without antiretroviral therapy.[382]

William O'Brien and his associates from several institutions in the USA studied the effect of an influenza vaccine on the HIV DNA and RNA. "Study subjects were self-assigned to the vaccinated (*n* = 20) or nonvaccinated control group (*n* = 14)...Patients were excluded if there was clinical or laboratory evidence of acute viral hepatitis, active herpes simplex virus infection, pneumonia or other acute respiratory infection, psychosis, or transfusion within the last 2 months...Patient histories and examinations at 1- to 2- week intervals during the 2 month study period did not show any side effects from vaccination, nor were there any symptoms of acute infections in the study population. Therefore, we do not believe overt infections with bacteria or with heterologous viruses were important confounders during the course of observation". Although all but two of the study patients were receiving AZT, "Over the study period there was little change in levels of proviral DNA in peripheral blood mononuclear cells…In contrast with what was observed for viral DNA, there was a significant relative increase in postvaccination HIV-1 RNA levels in PBMC from the 20 patients receiving influenza vaccination (11.6±5.0-fold increase, median 2.7, *p* < 0.002)…The peak HIV-1 RNA levels typically occurred at 1 or 2 weeks postvaccination (in 9/10 patients showing greater than fourfold increase), and returned to baseline at later time points...Equivalent increases in peak PBMC RNA levels during the same time frame were not seen in the 14 nonvaccinated controls". Discussing their results, the authors wrote: "Our results suggest that continued immunologic (antigenic) stimulation may result in increased virus load *in vivo*...a recent study suggests that recurrent herpes simplex virus infection can also lead to marked increases in HIV-1 expression. Furthermore, actual influenza virus infection may lead to a greater level of HIV-1 expression than the transient nature of the increase in viral expression observed here, because of the prolonged nature of the infection. This relationship may hold true for other vaccine-versus disease-combinations".[383]

Researchers from the Gladstone Institute of Virology and Immunology, and a number of other institutions from the USA, noted that "It is generally recommended that HIV-1 infected individuals be vaccinated against several important pathogens, including influenza viruses, *Streptococcus pneumonia, Haemophilus influenza*, and hepatitis B. In addition, it is recommended that HIV-1 infected infants be vaccinated against diphtheria, tetanus, measles, mumps, rubella, polio, and pertussis. Although the efficacy of these vaccines in immunocompetent individuals has been established, the protective value of vaccination in the context of HIV-1 infection has not been demonstrated. It has been reported that many HIV-1 infected individuals do not make a significant antibody response to vaccine antigens, suggesting that routine vaccination of HIV-1 seropositive patients may be of little benefit". To clarify some of these problems, they vaccinated 32 adults "with HIV-1 infection" and 10 seronegative controls with "a standard dose (0.5 ml) of the 1993-1994 formulation of trivalent influenza vaccine…The majority of HIV-1 infected participants were receiving antiretroviral therapy (with the nucleoside analogues zidovudine, zalcitabine, didanosine, or stavudine monotherapy or various combinations thereof) before and during the study period. None of the study participants had evidence of active opportunistic infections at the time of study entry. There were no adverse clinical reactions to the influenza vaccine and there were no reports of an influenza-like illness". Considering a 3-fold [0.5 log] change in the level of plasma HIV-RNA to be significant, they found that "the majority (83%) of vaccinated individuals experienced a significant increase in plasma HIV-1 RNA levels within 1-2 wk of immunization and returned to their prevaccination levels within 4 weeks after immunization…Among all HIV-1 infected participants, peak plasma HIV-1 RNA levels seen after vaccination ranged from 1-to-369-fold above baseline values (median 7.3)...*Patients on antiretroviral*

therapy were not noticeably different from those not on therapy with regard to increases in plasma viremia. In a few patients, plasma viremia did not return to baseline or showed a second increase during the study. None of these study participants were given a second vaccination during the study period, but these few subjects did have evidence of an intercurrent infection, such as the development of CMV retinitis or *Pneumocystis carinii* pneumonia, which may have caused the second wave of viremia". Commenting on their findings the authors wrote: "given the significant activation of virus production that follows a discrete vaccine-induced antigenic exposure, it is likely that the immune activation associated with an actual opportunistic infection may cause even more dramatic stimulation of virus production. These issues must be considered in determining the advisability of particular vaccines. Our additional anecdotal experience suggests that acute *M. tuberculosis* and *P. carinii* infections can cause increased viral load (Staprans S, and M.B. Feinberg, unpublished observations). Indeed, intercurrent infections occurred in a few of the influenza-vaccinated patients whose plasma viremia levels either did not return of baseline values or who manifested a second, later peak in plasma viremia"[384] (italics ours).

In a study published in 1997, researchers from the ARL-AIDS Research Center, VA Palo Alto Health Care System, California, evaluated the effect of herpes simplex virus (HSV) infection on HIV viral load. They reported that plasma viral load increased a median of 3.4-fold during the acute outbreak (range 0 to 10-fold; P=.002) "while post-outbreak levels (30-45 days after the appearance of lesions and treatment with acyclovir) "remained above pre-outbreak baseline levels in some subjects". They concluded; "If antigenic stimulation from immunisation or concomitant infection results in latently infected T cell activation in vivo, then suppression of these events may be clinically beneficial. Further studies are necessary to determine whether active HSV infection and/or antiviral intervention against HSV affects the pathogenesis of HIV disease".[385]

Because,

1. "Several intercurrent infections, including tuberculosis herpes simplex and bacterial pneumonia result in increased plasma concentrations of HIV-1. In each case, the appropriate treatment of the concurrent infection significantly reduced these high viral loads".

2. "*Plasmodium falciparum* malaria and HIV-1 are among the most prevalent infectious agents in sub-Saharan Africa and are major causes of morbidity and mortality. Co-infection probably occurs frequently; an estimated 14 million individuals in sub-Saharan Africa are infected with HIV and at least 500 million Africans suffer from malaria each year. In many parts of sub-Saharan Africa more than 30% of urban, sexually active adults are infected with HIV, and in some populations almost all individuals are infected or re-infected with *P. falciparum* malaria each year".

researchers from Malawi and the USA investigated "the association between malaria and HIV concentration in blood plasma, and prospectively to monitor viral concentrations after antimalarial therapy". They reported that in their study of 89 HIV infected individuals (47 with malaria; 42 without malaria), "At enrolment blood plasma HIV-1 RNA concentrations were approximately sevenfold higher in patients with malaria than in blood donors (medians 15.1×10^4 and 2.24×10^4 copies/ml, respectively, P = 0.0001). No significant changes in median HIV-1 concentrations occurred in the 21 blood donors followed to week 4 (P = 0.68). In the 27 subjects successfully treated for malaria who were followed to week 4, a reduction in plasma HIV-1 RNA was observed from a median of 19.1×10^4 RNA copies/ml at enrolment, to 12.0×10^4 copies/ml at week 4, (P = 0.02). Plasma HIV-1 concentrations remained higher in malaria patients than controls (median 12.0×10^4 compared with 4.17×10^4 copies/ml, P = 0.086)". Discussing their findings they wrote: "These data show that subjects with malaria illness have a higher HIV-1 RNA burden than a control group without malaria, and in some patients a significant reduction in viral load can be achieved 4 weeks after antimalarial therapy…A fuller evaluation of the effects of malaria on HIV-1 replication will require a prospective cohort study that captures HIV-infected patients before, during and months after acute malaria infection".[386]

USA researchers studied the HIV RNA changes in 13 patients who "had a diagnosis of bacterial pneumonia; had serum samples collected appropriately before diagnosis, during the acute phase, and after the pneumonia had resolved; had been continuously on antiretroviral therapy and had no other opportunistic infections during the interval". They reported that: "The serum HIV RNA levels increased with the onset of bacterial pneumonia in all 13 of patients (p = 0.001). The median increase in absolute amount of serum HIV RNA was 179,000 copies per ml…There was a decline in the level of HIV RNA recovery from pneumonia in 12 of 13 patients (p = 0.001). The difference between pre-pneumonia RNA levels and post-pneumonia RNA levels is not significant (p = 0.4)…Because viral load information appears to be especially useful in therapeutic decision making in this patient population, it may be particularly important to consider the existence of concurrent opportunistic infections when interpreting changes in HIV RNA in patients with higher CD4 cell counts…Finally, the potential pathogenic consequences of repeated bouts of opportunistic infections in HIV-infected patients needs to be considered. In this study, the change between HIV RNA levels before and after pneumonia was not

statistically significant, although the post-pneumonia levels were a median of 37% higher and 3,000 copies per ml more than the pre-pneumonia levels".[387]

In another study by the same USA researchers the changes in the HIV RNA during AIDS-associated opportunistic diseases were analysed in 14 individuals, 10 blacks and 4 whites, who "had an AIDS-associated opportunistic disease [CMV enteritis, PCP, bacterial pneumonia, disseminated *Mycobacterium avian* complex, *Candida* esophagitis, KS] had appropriately collected blood samples, and had been maintained on stable antiretroviral therapy through the sampling period". They reported that: "Prior to opportunistic disease, the median level was 21,000 RNA copies/mL. There was increase in the level with the onset of an opportunistic disease…The median increase in plasma HIV RNA was 360%…there was a decline in the plasma level of RNA after recovery from disease. The median level after the AIDS-associated opportunistic disease events was 29,700 RNA copies/mL". Discussing their findings they wrote: "Last, the pathogenic and clinical significances of these observations need further work. It is possible that multiple bursts in HIV production caused by events that stimulate the immune system may enhance HIV disease progression and thereby exacerbate immune decline. If this is true, it is feasible that suppression or avoidance of these bursts could have an important clinical impact on the health of HIV-infected patients and the rate of disease progression…Since all patients in this study received uninterrupted antiretroviral therapy, it is not possible to assess the impact of their treatment on plasma HIV RNA levels. However, it is worth emphasizing that increased amounts of plasma HIV RNA occurred despite antiretroviral therapy".[388]

In a paper published in 1996 by researchers from Italy and the USA, including Anthony Fauci, the authors point out that "*Mycobacterium tuberculosis* (MTB) disease is an extremely important life-threatening bacterial disease with 8 million new cases and 3 million deaths reported world-wide each year to the World Health Organization; the vast majority of these cases are in developing countries…The World Health Organization has estimated that 5.6 million worldwide and 80,000 people in the United States are co-infected with HIV and MTB". In light of these, and other observations, including "the findings of enhanced viral replication following immune stimulation", they conducted a study in which the "Plasma viral load [HIV RNA] was measured in HIV-infected individuals before, during, and after the development of MTB disease". They reported "a 5-to-160-fold increase" in HIV RNA "during the acute phase of MTB disease and decreases after successful therapy…No decrease in viral load was observed in patients who failed anti-MTB therapy". Discussing their findings they wrote: "The observed higher rate of HIV disease progression as well as susceptibility to infection upon exposure to HIV in individuals in sub-Saharan Africa might be due, at least in part, to the chronic and persistent immune activation with ongoing immune responses to parasites and other infections. In this regard, it is interesting to note that MTB disease is one of the most common infections in African patients who die from AIDS, suggesting *a possible role for MTB in the pathogenesis of AIDS* in this population…These findings highlight the importance of prophylactic therapy against MTB in HIV-infected individuals, which may help to control not only the spread of tuberculosis, but also the enhanced replication of HIV that is associated with MTB disease"[118] (italics ours).

Comments
1. If, as Fauci *et al* conclude, MTB has a role in the pathogenesis of AIDS, what is the role of HIV in this syndrome?

2. If,
    (a) MTB disease is one of the most common infections in African patients who die from AIDS;

    (b) MTB plays a role in the pathogenesis of AIDS;

    then given that MTB disease has been endemic in Africa long before the AIDS era, why is African AIDS claimed to be caused by HIV? Why, to prevent and treat AIDS do HIV/AIDS experts advocate antiretroviral rather than anti-bacterial drugs?

3. If MTB plays a role in AIDS why not also the "parasites and other infections" present in the African population as well as opportunistic agents such as *Pneumocystis carinii*, CMV, *Candida*, and *Mycobacterium avium* and antigenic stimulation with foreign agents such as factor VIII, blood transfusions and drugs to which individuals belonging to other AIDS risk groups are subjected?

4. Since,

    (a) the specificity of the "HIV RNA" has never been determined and in fact in not one of the studies which determine the changes of RNA induced by antigenic stimulation are controls used, that is, individuals who suffer from the same diseases accepted to be the result of factors other than HIV;

(b) many diseases raise the level of "HIV RNA" including "*Herpes simplex* virus, *Pneumocystis carinii* pneumonia, *Mycobacterium avium* complex and Giardia lamblia";[118]

how can one claim that the many discrepant "HIV RNAs" (sequence variability up to 40%) is the RNA of a unique retrovirus and not a collection of "novel" cellular RNAs resulting from repeated antigenic stimulation, in the same manner as the "novel" RNAs observed in Gulf War veterans?

5. If,

    (a) the "HIV RNA" is a retroviral RNA, then
       (i)   given that to date no retroviral therapy has resulted in a sustained decrease of this RNA;
       (ii)   the increased viral load induced by many and varied factors including immunisation, MTB, *Herpes simplex* virus, *Pneumocystis carinii* pneumonia, *Mycobacterium avium* complex, Giardia lamblia" and also sexually transmitted diseases[389] is not affected by prior and current use of antiretroviral drugs;[384,388]
       (iii) since one of the leading experts on anti-retroviral treatment of AIDS, Paul Volberding, recently admitted that at present there are no answers to the question: "If [patients are] left untreated [with anti-retroviral drugs], will HIV disease progress? Will deferring therapy worsen prognosis?"

    (b) by using drugs other than anti-retrovirals one can treat the signs, symptoms and infections which constitute AIDS and also decrease the HIV RNA by up to 350-fold;

why should one use expensive and toxic drugs to treat the clinical syndrome and decrease the viral load?

6. Is the decrease in HIV RNA induced by anti-retroviral drugs due to their effect on HIV or a result of some effect they may have on the causative agents of the infectious diseases? Especially if one considers the evidence that AZT can "inhibit or prevent bacterial infection in immunodepressed hosts".[390] AZT has "potent bacterial activity against many members of the Enterobacteriaceae".[391] "The antibacterial effect of zidovudine (AZT) has been demonstrated both *in vitro* and *in vivo* with experimental models of gram-negative bacterial infections".[392] In addition, there is *in vivo* evidence that AZT, as well as another nucleoside analogue (ddG), "can exert a potent antiviral activity against HBV [hepatitis B virus]", as judged by suppression of the replication of hepatitis B virus in "hep g2-derived hepatoblastoma cells".[393] According to its manufacturers, AZT had "an ID_{50} of 1.4 to 2.7 microgram/ml against the Epstein-Barr virus, the clinical significance of which is now known at this time".[394]

7. It is a scientific fact that cause precedes effect. If HIV is the cause of AIDS, then "HIV replication" (increase in HIV RNA) should precede the appearance of AIDS not follow it, as the presently available data show.[388]

8. If as the researchers from the CDC and Thailand claim, "about 80% of the treatment effect" of AZT on MCT can be "explained by lowered maternal viral concentration at delivery" then given:

    (a) Neither AZT nor nevirapine had any effect on the level of HIV RNA at delivery and in fact given their pharmacokinetics and mode of administration it is not possible for them to have such an effect;
    (b) The resolution of the signs, symptoms and infections which constitute AIDS leads to a decrease of up to 350-fold in the HIV RNA;

why, for the prevention of MCT of HIV, advocate two drugs which have no effect on HIV RNA especially where one of which has been shown to lead to an increased death rate? Would it not be better to prevent and treat the signs, symptoms and infections with means other than anti-retroviral drugs which will both prevent deaths from these diseases and also decrease MCT?

5.3.2 *Malarial infection of the placenta*

In a paper published in 1995 researchers from the CDC and Malawi pointed out that: "HIV is one of sub-Saharan Africa's newest and fastest spreading infectious diseases; *Plasmodium falciparum* malaria is one of the oldest and most prevalent infectious diseases. Separately, they represent two of the largest health threats, especially to pregnant women and children. The World Health Organisation has estimated that, as of the beginning of 1990, over 2.5 million African women were infected with HIV and that by the year 2000, as many of 4-5 million will be infected. Studies conducted from various locations in Africa have found HIV-1 infection

rates as high as ≥ 20% among pregnant women. *P. falciparum* is estimated to cause between 0.5 and 2 million deaths per year in Africa alone, excluding those due to malaria-related low birthweight or anaemia. In sub-Saharan Africa, *P. falciparum*, because of its affinity for human placenta, is one of the leading causes of low birthweight and one of the leading risk factors for paediatric mortality in malaria-endemic areas".

"To examine the relationship between maternal HIV infection, placental malaria infection, and infant mortality", these authors tested 2608 women for HIV infection using ELISA and WB and, at delivery, the placenta for malarial infection. Of 2608 women tested, 138 (5.3%) were reported HIV positive. Significantly more women with placental malaria infection (23.9% v 11.8%) were found to be HIV positive. "Infants born into this study were visited every 2 months for the first 2-3 years of life. Deaths were investigated using standardized 'verbal autopsy' interview". (No mention is made if the children were tested for HIV infection and, if tested, what were the results. Or if not tested, why not?). They reported that "The neonatal mortality rates were 44 and 49 per 1000 live births (P = 0.3), whereas the post-neonatal mortality rates were 100 and 186 per 1000 live births (P < 0.001) for infants born to HIV-seronegative and HIV-seropositive women, respectively. Causes of death among children born to HIV-seropositive mothers did not differ from those among children born to HIV-seronegative mothers during the neonatal period. During the post-neonatal period, however, children born to HIV-seropositive women had a higher risk of death associated with diarrhoea or gastrointestinal disease than children born to seronegative women". Although the children "were monitored approximately once every 2 months for 2-3 years", for unknown reason(s) the reported post-neonatal death rates are only for children between 29 and 365 days after birth. "In a multivariate model, the odds of dying during the post-neonatal period for an infant born to a mother with both placental malaria and HIV infection was 4.5 times greater than an infant born to a mother with only placental malaria, and between 2.7 and 7.7 times greater (depending on birthweight) than an infant born to a mother with only HIV infection". Although 2608 women were found positive for this analysis, data from only 1347 women (51.5%) was used. Discussing their findings the researchers from the CDC and Malawi wrote: "Confirmation of placental malaria infections associated with an increased risk of vertical transmission of HIV-1, independent of the mother's immune status, would have enormous programmatic implications in sub-Saharan Africa. Malaria chemoprophylaxis during pregnancy would then offer an intervention that would not only decrease the burden of malaria infection during pregnancy, malaria-associated low birthweight, and their subsequent impact on child survival, but also decrease the likelihood of transmission of HIV from mother to infant: no small achievement in malaria-endemic areas where HIV seropositivity rates can reach 20% or more".[395]

Indeed, given the fact that in the majority of studies, an infant born to an HIV positive mother and who dies before one year of age, by definition:

(a)   is infected with HIV and the mode of acquisition is by MCT;

(b)   the cause of death is AIDS;

then malaria prevention and even treatment during pregnancy will lead to a significantly greater decrease in both, death from AIDS and MCT than that resulting from anti-retroviral treatment.

5.3.3   *Malnutrition*
In 1986, children treated at a centre specialising in protein-energy malnutrition (PEM) in Bangui, Central African Republic, were tested for HIV (ELISA and WB) by researchers from the Centre and the Institut Pasteur. Of 175 malnourished children 21 (12.3%) were positive compared to 3 out of 96 (3.1%) of healthy children. None of the children with PEM "was positive before 9 months of age". 25 (24.8%) of their mothers were positive. The authors "have collected some preliminary data on the 21 mothers whose children had antibodies, 2 had died; of the other 19, 3 were widows, 5 were married, and 11 lived alone or lived with men (2 being polygamous). The mean age was 24 years, the mean number of pregnancies was 5.3 (4.2 normal deliveries and 1.1 abortion). 1 mother had pulmonary tuberculosis and 8 had three or more sexually transmitted diseases such as gonorrhoea or syphilis within the previous 5 years. 47% of all the mothers of malnourished children (including mothers without HIV antibodies) had financial difficulties and lived in households with average annual income of $40 per head". They wrote: "Factors usually recognised as being responsible for PEM in children in developing countries are weaning, diet (low energy intake and protein and vitamin deficiency), infections (eg, intestinal parasites, measles, and malaria), and socioeconomic factors. Perhaps HIV, already known as a cause of diarrhoea and malabsorption in adults and of immunodeficiency syndromes in children, should be included among the agents responsible for PEM in infants in Central Africa".[396]

A paper[397] entitled "Severe malnutrition and pediatric AIDS: a diagnostic problem in rural Africa" was published in 1988. The authors from the Ivory Coast and France noticed that "The clinical case definition for AIDS, as developed in 1985 by the WHO workshop in Bangui, suggested that cases of severe malnutrition

should be eliminated", and tested (ELISA and WB) 94 children reported as having AIDS. "90% of the children tested suffered from severe malnutrition (weight less than 60% of the expected weight for their age)". 32% (30/94) of the children were found seropositive and 68% (64/94) seronegative. The "symptoms according to the Bangui criteria" in the two groups of children are listed in the following table:

Table 5.3   Morbidity in HIV positive and HIV negative malnourished children

Disease	HIV positive (30/94)	HIV negative (64/94)
Chronic diarrhoea	26 (87%)	43 (67%)
Generalised lymphadenopathy	22 (73%)	34 (53%)
Oropharyngeal candidiasis	20 (67%)	34 (53%)
Prolonged fever	19 (63%)	21 (33%)
Persistent cough	12 (40%)	13 (20%)
Generalised dermatitis	4 (13%)	6 (9%)

"In order to further evaluate the relationship between severe malnutrition and HIV infection", 100 consecutive children, aged 0-4 years of age suffering from severe malnutrition were considered. Three children died prior to blood sampling. Out of the 97 children remaining, "When looking at types of severe malnutrition, out of 40 cases of marasmus five were HIV-positive; out of 25 suffering from kwashiorkor only one was HIV-positive and out of the 12 cases with marasmic kwashiorkor, three were HIV-positive" They wrote that "The problems of diagnosing between severe malnutrition and pediatric AIDS, already noticed at the Mama Yemo Hospital and elsewhere demonstrate a need to review the Bangui clinical criteria for diagnosis of pediatric AIDS in rural Africa…In the meantime, HIV antibody testing should be compulsory in cases of severe malnutrition in young children in rural Equatorial Africa, since seropositivity would modify both the treatment and the evaluation of the cases".[397]

In a study published in 1993 by researchers from Rwanda, Germany and the USA, the authors wrote: "In Rwanda, as well as in most of the Third World, protein-energy malnutrition (PEM) is one of the principal public health problems among young children. In fact, 137 (23.2%) out of a total of 590 children hospitalised at the National University Hospital in Butare between 20 February and 31 July 1989 showed signs of PEM. A similar percentage was found during earlier periods at the same Center (unpublished manuscript: Gatsinzi T, 1984). On the other hand, the crisis presented by HIV infection and AIDS has emerged as a new and pressing public health problem in Central Africa, as well as in many other regions of the world". To investigate the relationship between PEM and HIV infection, 101 children with PEM admitted to the National University Hospital were tested for HIV (ELISA and WB). "Fourteen per cent of malnourished children were HIV-1 seropositive…Among children above 15 months of age, HIV-1 seropositivity was more common among marasmic children than among malnourished children presenting with oedema at admission to the hospital. Also, HIV-1 infection was found more frequently among chronically malnourished children (low height for age and weight for age) than among acutely malnourished children (low weight for height). Mortality during the 2-year follow-up was 75% among HIV-1 seropositive children and 23% among HIV-1 seronegatives". Discussing their results they wrote: "In pediatric AIDS, protracted diarrhoea, generalised lymphadenopathy, oral thrush, hepatosplenomegaly, and weight loss are commonly observed. Of these symptoms, weight loss or failure to thrive has been found most consistently in HIV infected African children. A recent evaluation of the WHO clinical definition of pediatric AIDS at the University Hospital of Butare also showed that 'slimming' occupies a preponderant place in the inventory of clinical manifestations of pediatric AIDS (72 per cent, $n = 39$). However, these data have to be cautiously interpreted since the prevalence of protein energy malnutrition (PEM) has been high in Rwandan children even before AIDS appeared…To assist in the differential diagnosis between severe, chronic malnutrition, and pediatric AIDS, HIV antibody testing should, whenever possible, be carried out because HIV infection will modify both the treatment and the prognosis of the cases". They also added: "Aggressive nutritional support may help to prolong the survival of HIV-1 infected children and nutritional counselling should begin as soon as HIV-1 infection is diagnosed, emphasizing in particular the prevention of PEM. Unfortunately, this may often be an unattainable goal as a result of poverty and premature death of the parents".[398]

In yet another paper published in 1993, researchers from Burkina Faso and France conducted a study at the National Hospital in Bobo Dioulasso. In this hospital in 1988, 12% of all paediatric admissions were for severe malnutrition. This was not a local phenomenon since "Malnutrition is widespread in tropical and subtropical regions. An estimated 80-100 million children are malnourished throughout the world. Of these, 40,000 die

each day". 428 children with PEM were tested (ELISA and WB) for HIV. "Of the 349 children aged over 12 months, 48 (13.8%) were HIV-positive...Marasmus was the form of malnutrition most frequently associated with [HIV] seropositivity". The Clinical symptoms in the PEM children were:

Table 5.4

Symptoms	No. (%)	
	HIV +	HIV -
Chronic diarrhoea	41 (85.4)	241 (80.6)
Pulmonary infection	22 (45.8)	130 (43.1)
Otitis	2 (4.1)	7 (2.3)
Fever > 1 month's duration	38 (79.1)	203 (68.1)
Anaemia	6 (12.5)	54 (17.9)
Adenopathy	12 (25.0)	13 (4.3)
Oral candidiasis	32 (66.6)	137 (45.5)
Hepatomegaly	22 (45.8)	94 (32.0)
Splenomegaly	4 (8.3)	28 (29.7)
Skin disorders	11 (22.9)	28 (9.3)
Physical incapacities	17 (35.4)	157 (52.1)
Neurological disorders	3 (6.2)	8 (2.6)

"For evaluable children, the case fatality rate was at least 38.4% in seropositive children, versus 32.1% in seronegative children". In their discussion the authors wrote: "We found that all the clinical symptoms studied were particularly frequent in the population studied. This is related to the multiple causes (bacterial, viral and parasitic) of symptoms such as diarrhoea, anaemia and pulmonary infections in children in tropical regions…However, the logistic model determined a highly specific clinical profile, including marasmus, adenopathy and oral candidiasis, for identifying HIV infection in the malnourished children studied".[399]

To evaluate the nature and magnitude of the effect of congenitally or perinatally acquired human immunodeficiency virus (HIV) infection on somatic growths from birth through 18 months of age, anthropometry was performed prospectively in a multicenter cohort study of infants born to HIV-infected women in five WITS sites in the mainland, United States and Puerto Rico. The authors compared the weight-for-age, length-for-age, weight-for-length and head circumference-for-age between infected and uninfected infants and the covariates "infant gender; maternal education; prenatal alcohol; tobacco and/or illicit drug exposure and mean prenatal CD4+ lymphocytes count. A separate repeated measures model was used to assess the effect of infant zidovudine treatment on growth". They reported that: "Infants infected with HIV were an estimated average 0.28 kg lighter and 1.64 cm shorter than uninfected infants at birth, were 0.71 kg lighter and 2.25 cm shorter by 18 months of age, and had a sustained estimated average decrement of 0.70 to 0.75 cm in head circumference". Prenatal drug exposure had a decremental effect on weight-for-age. Alcohol and maternal education attainment were "significant predictors" for a decremental effect, respectively, for length-for-age. In the abstract they wrote: "Zidovudine treatment was not associated with improved growth". In fact they have found that AZT treatment had a decremental effect on both height-for-age and head circumference. Discussing their findings they wrote: "Birth weight reflects maternal health status during pregnancy and is a strong predictor of later childhood growth. Weight-for-age is a composite index of length-for-age and weight-for-length. Low length-for-age (shortness or stunting) can be a result of poor nutrition, increased frequency of infections, or both. Low weight-for-length (thinness or wasting) is often associated with recent severe disease, and generally indicates acute malnutrition, mainly because of starvation, persistent diarrhoea, or both…The finding of significant influence by covariates other than HIV infection on growth emphasises the importance of controlling for these factors to assess accurately the magnitude of the HIV effect on growth. Prenatal exposure to toxic effects of drugs or alcohol was associated with lower weight-for-age and length-for-age values, similar to findings in other populations…In contrast with anecdotal clinical observations and other studies indicating that zidovudine favourably influences weight growth rates, our analysis suggests the opposite".[400]

In Zimbabwe, "In 1973 severe malnutrition was the leading cause of death in children at Harare Central Hospital. A national nutritional survey conducted in 1992 showed that the situation and the level of malnutrition had improved compared with 1973 but the overall prevalence had remained high. In this survey 32% of children between the age of 24 and 59 months had moderate and severe stunting and 2% of children between 12 to 23 months had evidence of moderate and severe wasting. In recent years, the recurrent drought seasons experienced in Zimbabwe (1983/84, 1986/87, 1991/92) and the human immunodeficiency virus (HIV) epidemic have contributed to increasing prevalence of malnutrition. Weight loss or failure to thrive has been found to be a consistent feature of HIV infected children. The World Health Organization (WHO) clinical case definitions of acquired immunodeficiency syndrome (AIDS) in children has included weight loss or failure to thrive as one of the three major criteria". Since the characteristics of HIV infection in the children admitted to the Harare hospital were unknown, researchers from the hospital and the University of Zimbabwe undertook "A descriptive study", to compare the pattern of socio-demographic features, nutritional profile and clinical features of HIV infected and non infected children with malnutrition. Between December 1993 and February 1994 "177 malnourished children aged 15 months and above were admitted. Twenty five (14%) died soon after admission before adequate information could be obtained and, therefore, were excluded from further analysis. Consent for HIV testing was refused by the parents in 12 children (6.8%)." The socio-demographic profile, presenting clinical features, the nutritional profile, the HIV status (determined by ELISA with WB confirmation when the ELISA result was doubtful) and its relationship to malnutrition were as follows:

Table 5.5   Sociodemographic features

	HIV positive Total = 68 No. (%)	HIV negative Total = 72 No. (%)
Area of residence		
Rural	27 (39.7)	31 (42.1)
Urban	41 (60.3)	41 (56.9)
Caretaker		
Mother	49 (72.1)	54 (75.0)
Grandmother	15 (22.1)	17 (23.6)
Other	4 (5.9)	1 (1.4)
Mother		
Died	8 (11.8)	8 (11.1)
Married	44 (64.7)	50 (69.4)
Remarried	4 (5.9)	4 (5.6)
Single/widow	12 (17.6)	10 (13.9)
Education		
None	11 (16.2)	12 (1.7)
Some	57 (83.8)	60 (83.3)
Breast feeding stopped < 15 months		
Yes	14 (20.6)	19 (26.4)
No	54 (79.4)	53 (73.6)

Table 5.6   Presenting features of 140 children with malnutrition

	HIV positive Total = 68 No. (%)	HIV negative Total = 72 No. (%)
Diarrhoea	59 (86.8)	63 (87.5)
Persistent diarrhoea	27 (39.7)	28 (28.9)
Vomiting	39 (57.3)	21 (29.2)
Oedema	16 (23.5)	41 (56.9)
Lympadenopathy	36 (52.9)	16 (22.2)
Hepatomegaly	49 (72.1)	47 (65.2)
Splenomegaly	8 (11.8)	4 (5.6)
Pneumonia	31 (45.6)	17 (23.6)
Discharging ears	26 (17.7)	5 (6.9)
Oral thrush	41 (63.4)	21 (29.1)
Parotid swelling	7 (10.3)	4 (5.6)

Table 5.7   Pattern of malnutrition

	HIV positive Total = 68 No. (%)	HIV negative Total = 72 No. (%)
Weight for height		
<80%	54 (79.)	0 (55.6)
≥80%	14 (20.6)	32 (44.4)
Height for age		
<90%	40 (58.8)	29 (40.3)
≥90%	28 (41.2)	43 (59.7)
Weight for age with oedema		
<60%	24 (47.1)	16 (25.4)
≥60%	27 (52.9)	47 (74.6)
Weight for age with no oedema		
<60%	12	2
>60%	5	7

Discussing their findings they wrote: "On logistic regression modelling, marasmus, lymphadenopathy, discharging ears and oral thrush were found to be predictive of HIV infection in children with malnutrition. However, the model was not adequate because about 35% of the children would be wrongly classified…These findings indicate the difficulty in identifying a clinical profile with high sensitivity and specificity in HIV infected malnourished children…A total of 32 children in the study population died during the course of their hospital stay, giving an overall case fatality rate of 22.8%", with "a case fatality rate in HIV positive (16.7%) and HIV negative 29.4%". Nonetheless they concluded: "The high prevalence of HIV infection [48%] among the malnourished children emphasises the impact of the HIV epidemic on childhood nutritional morbidity".[401]

One of the latest studies in regard to the relationship between malnutrition and HIV infection was published by researchers the United Kingdom and South Africa including David Wilkinson. They pointed out that, "Available data, although limited, suggest that the range of disease experienced by infected children is broadly similar to that experienced by all children in Africa, and includes diarrhoea, malnutrition, acute respiratory infections (ARI) and malaria. There is some evidence that disease among the HIV-infected is more often recurrent, persistent and fatal. However, in a study among 132 rural Zambian children with acute lower respiratory tract infection most of the 14 who were HIV infected responded well to conventional treatment. In Dar Es Salaam, HIV infection was 17 times more frequent among 200 severely malnourished children than their

matched controls".   To obtain more data regarding the prevalence of HIV infection, its relationship to malnutrition, the diseases in infected children "and outcome of HIV disease among children admitted to this pediatric medical service", of a total of 368 children admitted to the Hlabisa hospital in Kwazulu-Natal, 281 (76%) were tested. "HIV infection was defined as two positive ELISAs in those aged >12 months; a positive ELISA plus a positive IgG3 in those aged 6-12 months; and a positive ELISA plus positive p24 antigens or PCR in those aged 0-5 months…In all, 72 children were HIV infected (26 per cent)…Of the 72 HIV infected children, 37 (52 per cent) had severe malnutrition compared with 36 of 206 HIV uninfected children (17 per cent; p<0.0001)…Although some children presented with multiple problems (e.g. acute respiratory infection plus severe malnutrition), we report here the primary (syndromic) diagnosis. This seems reasonable as the most frequent secondary diagnosis is malnutrition and these data are captured through the measure of nutritional status made in all children...While diarrhoeal disease (51 per cent vs 32 per cent) and malnutrition (18 pre cent vs 11 per cent) caused proportionately more disease among HIV-infected children, ARI was more frequent among the uninfected (23 per cent vs 13 per cent). Prevalence of HIV infection among children with diarrhoea was 34 per cent, among those with ARI it was 15 per cent and among those with malnutrition it was 36 per cent. Satisfactory response to initial antibiotic therapy was substantially less frequent among the HIV-infected (56 per cent) than the uninfected (73 per cent; p=0.02)". Discussing their findings they wrote: "We observed a marked difference in nutritional status between HIV infected and uninfected children, but did not observe the lower HIV prevalence recorded in other studies amongst those with kwashiorkor compared with marasmus. In infants aged over 15 months in Rwanda, prevalence of HIV infection among those with marasmus was three times higher than that among those with kwashiorkor; and a similar difference was recorded in a study in Dar Es Salaam. The reasons for much worse nutritional status among HIV-infected children warrants further study. Studies elsewhere in Africa have demonstrated high rates of diarrhoeal disease among HIV-infected children and associated high case fatality rates. There is little evidence that the aetiology of diarrhoea among HIV-infected children is any different from that among uninfected children. Likewise, other studies have also reported relatively low HIV prevalence among children with ARI, as well as a similar case fatality rate in the HIV-infected and uninfected". Wilkinson and his colleagues concluded: "HIV-infected children present with disease syndromes common to this setting, but do so more frequently and with worse outcome than their uninfected counterparts".[402]

Note
Since:

1.   The specificities of the tests used to prove HIV infection have never been determined. In fact, the tests used in this particular study have been accepted as being non-valid even by HIV experts.

2.   The sicker the children the higher the probability of treatment failure leading to worse outcomes.

Wilkinson *et al's* findings including "HIV infection" may indicate nothing more than the type, multiplicity and the degree of morbidity in their study population.

Comments
The evidence from the studies in which the relationship between PEM, HIV infection and the clinical syndrome is examined shows a high prevalence of HIV-infection in the children with PEM. The question that arises is whether HIV-infection is the cause of PEM as is implied if not stated in most of these studies or is PEM the cause of the parameters interpreted as HIV-infection? In *Harrisons* text book of Internal Medicine one reads that in developing nations PEM "may be present in endemic form, and under famine conditions the prevalence may approach 25%. The primary disorder occurs when socioeconomic factors limit the quantity and quality of food; it is a particular problem when vegetable proteins of low biologic value are major components of the diet and when the incidence of infectious diseases is high. The problem is accentuated when energy intake is insufficient so that dietary proteins are oxidised as fuel rather than utilised for the synthesis of body protein. In children of developing nations two syndromes of PEM have been distinguished: (1) *marasmus*, manifested by stunted growth, loss of adipose tissue, generalized wasting of protein mass, and no edema, is thought to be due to the combined effects of protein and energy malnutrition and (2) *kwashiorkor*, manifested by growth failure, edema, hypoalbuminemia, fatty liver and preservation of subcutaneous fat, is thought to be due to selective protein malnutrition". "In developed nations PEM occurs commonly as a secondary disorder in people who were previously well nourished who develop the disorder in association with subacute or chronic illness. Predisposing features include anorexia, hypermetabolism, malabsorption, and drug and alcohol abuse, and, in the aged, depression, isolation, and low income may play a role. As many as half of the hospitalised elderly are malnourished at admission or develop nutritional deficits during hospitalization. Synergism between primary and secondary causes is common in that those individuals with scanty reserves of protein and energy develop clinical PEM more rapidly than well-nourished subjects when challenged by hypermetabolism, the anorexia of infection, or other catabolic illness". In PEM "cell-mediated immunity is impaired, as indicated by all standard

tests, whereas antibody responses are generally intact".[403] It is also known that "Protein-energy malnutrition is generally associated with an increased frequency and severity of infection. Both children and adults with the syndrome of kwashiorkor and marasmus show atrophy of lymphoid organs, particularly the thymus, and the pattern of microorganisms isolated from malnourished subjects resembles the spectrum of pathogens found in patients with primary immunodeficiency disorders". PEM also causes "A marked reduction in the number of T4 inducer cells" which are "reversed by nutritional supplements given for a period of 4-8 weeks".[404]

For HIV to be the cause of PEM in the infected children born to African women, to drug abusing, Black and Hispanic women in the USA as well as Asian women:

1. All the causes of PEM which existed prior to the AIDS era must have abruptly disappeared coincident with the discovery of HIV or;

2. HIV discriminates between children suffering from PEM and well nourished children.

No evidence exists to support either of these possibilities. However,

1. Children with PEM reported HIV-infected are exposed to many antigenic stimulants including infectious agents other than HIV. In fact in these children:

    (a) "Precipitating illnesses tend to be more severe and difficult to manage, and it may not be possible to restore nitrogen balance until infection and fever are brought under control";

    (b) "The degree of malnutrition itself may hamper recovery from associated life-threatening illness so that early intervention with assisted enteral feeding or parenteral nutrition may be urgently required";

    (c) "Introduction of nutrients in the gastrointestinal tract may itself cause diarrhoea because of the atrophy of the intestinal mucosa and decrease in intestinal and pancreatic enzymes, and total parenteral nutrition may be necessary".[403]

    Since "antibody responses are generally intact" in these children, then the plethora of antibodies present in their blood may cross-react with the proteins present in the ELISA and WB, resulting in a positive test even if these children have never been infected by such a virus.

2. Evidence reported in a study conducted in adults over a 24 month period shows that nutritional status, assessed by loss of body weight, is "a significant predictor of eventual HIV seroconversion. Subsequent seroconvertors lost an average of 1.5 kg during the six months of the study compared with 1.0 kg gain (p = 0.001) for non-convertors. Nine of 27 (33%) seroconvertors compared with one of 44 (2%) controls, lost at least 5 kg in the 6-month period beginning 1 year before their seroconversion. In addition to those findings for measured weight loss during follow-up, reported weight loss before enrolment was also a risk factor for subsequent seroconversion".[250]

    That is, significant weight loss precedes HIV seroconversion by many months or even years. In other words, whilst no evidence exists that the usual causes of PEM are not operating in HIV infected children or that HIV can differentiate between sick and healthy children, and infects only the latter, there is evidence which suggest that:

    (a) A positive HIV test in children with PEM does not prove infection;

    (b) If the children are infected, the infection is the consequence not the cause of PEM.

    Since,

    (a) From the beginning of the AIDS era some of the best HIV/AIDS acknowledged that "recognition of paediatric AIDS is particularly difficult in Kinshasha [that is, Africa], since many children have severe infant and childhood diseases with similar manifestations (i.e. weight loss, chronic diarrhoea"),[405] "malnutrition and general lack of medical services contributed to diarrhoea, tuberculosis and other common African diseases that signify AIDS" (Myron Essex, New Scientist, 18th February 1988);

(b) The vast majority of cases of MCT of HIV are born to African women;

(c) In the vast majority of the studies of MCT a child born to a HIV positive woman who dies before 12 months or who is considered to have AIDS is, by definition, HIV infected and acquired the virus from the mother;

then a significant decrease in MCT may be achieved just by improving the nutritional status of African children.

5.3.4   Vitamin A

Management of PEM is complicated for several reasons including the fact that in these patients associated deficiencies, including vitamin-deficiencies, are common and "Many types of immune responses are variably impaired in deficiency of proteins, energy, vitamins and trace elements".[404]   In a study conducted at the University of Natal, Durban, the authors, Coutsoudis, Coovadia *et al*, determined the nutritional status of 190 children in "a typical urban shack settlement north of Durban". They also determined the levels of: vitamins A and E, calcium, magnesium, phosphorus, albumin, haemoglobin, serum iron and ferritin and percentage transferrin saturation. They reported that malnutrition "was evident in 13% of children who were underweight…and in 27% who were stunted…Concentrations of albumin, calcium, magnesium, phosphorus and vitamin E were close to normal, with no more than 10% of the sample having values outside the normal range. However, 44% of the children had low serum retinol levels (<20 micrograms/d1) and 21% of the children had anaemia (haemoglobin <11 micrograms/d1). Significant positive correlations were found between serum retinol and all biochemical indicators of iron status except serum ferritin". They concluded: "This study highlights the fact that nutrient deficiencies are interrelated, particularly protein energy malnutrition and poor vitamin A and iron status. A broad multifaceted comprehensive health intervention programme is therefore required".[406]

In a paper published in 1997, researchers from New Jersey, USA, published the results of a study conducted "to examine the association of prenatal multivitamin/mineral supplement use during the first and second trimesters of pregnancy by low income, urban women in the Camden Study", a study which "examines the effects of maternal nutrition and growth during pregnancy in one of the poorest cities in the continental United States". Prenatal vitamins were routinely prescribed for all women in this study at the time of the first prenatal visit. Although a range of supplements was available, the product usually prescribed contained vitamins, minerals, and trace elements including folic acid (1 mg), zinc (25 mg), calcium (200 mg), and iron (65 mg). In the published analysis "data were restricted to women who entered prenatal care during the first and second trimesters and who had singleton pregnancies". They reported that: "After controlling for potential confounding variables, risk of very preterm delivery (<33 weeks' gestation) was reduced more than fourfold for first trimester users and approximately twofold when use dated from the second trimester. Infant low birth weight and very low birth weight (<1,500 g) risks were also reduced. Risk of low birth weight was reduced approximately twofold with supplement use during the first and second trimester. Diminution in risk was greater for very low birth weight infants, amounting to a sevenfold reduction in risk of very low birth weight with first trimester supplementation and a greater than sixfold reduction when supplement use started in the second trimester". They concluded "Thus, in low income, urban women, use of prenatal multivitamin/mineral supplements may have the potential to diminish infant morbidity and mortality…In urban areas like Camden, an ounce of prevention may be worth a pound of cure".[407]   Since pre-term delivery is a risk factor for transmission (in the ECS the transmission rate in children born before 34 weeks was 33%, compared to 14% rate in children born thereafter;[234] and in the 1999 Bangkok study "High maternal viral load at delivery and low birth-weight (<2500 g) were independently associated with *in utero* transmission…Pre-term infants (<37 weeks gestational age, by modified Ballard) had a 25% risk for *in utero* transmission compared to 5% for full-term infants (OR, 6.7; 95% CI, 0.8-35.6")[244]), that is, maturity at birth leads to a reduction in mother to child transmission at least as good as that obtained with antiretroviral drugs_it follows that vitamin/mineral supplementation would result in a decrease in the transmission rate.

Researchers from the USA and Tanzania, conducted a study to examine the effects of vitamin supplements on pregnancy outcome and T cell counts in HIV-1 infected women in Tanzania. "Women were assigned in a two-by-two factorial design. 1075 women received a daily oral dose of: vitamin A (30 mg β-carotene plus 5000 IU preformed vitamin A, n=269); multivitamins excluding vitamin A (20 mg B1, 20 mg B2, 25 mg B6, 100 mg niacin, 50 µg B12, 500 mg C, 30 mg E, and 0.8 mg folic acid, n=269); multivitamins including A (n=270), all formulated in two tablets; or two tablets of placebo (n=267)…At delivery all women taking vitamin A were to receive an additional oral dose of vitamin A of 200,000 IU and the others an extra dose of a placebo". The authors of this study reported that: 30 foetal deaths occurred among women assigned multivitamins compared with 49 among those not on multivitamins. Multivitamin supplementation decreased the risk of low birthweight by 39%, and small size for gestational age at birth by 43%. Vitamin A supplementation had no significant effect

on these variables. Multivitamins but not vitamin A resulted in a significant increase in CD4, CD8 and CD3 counts. They concluded: "Multivitamin supplementation provides a low-cost approach for substantial decreasing adverse pregnancy outcomes among HIV-infected women in developing countries".[408]

Their conclusion that Vitamin A supplementation had no benefits on pregnancy outcomes and on T cells was questioned by researchers from France, for a number of reasons including the following: "First, the sample size was calculated for an expected reduction of the HIV-1 mother-to-child transmission rate of 30%, with a baseline risk of 30%. This sample was likely to be insufficient to identify vitamin A benefits on outcomes with a frequency as low as 3% for very-low-birthweight or 14% for small-for-gestational-age babies. Second, the comparability of the two groups during pregnancy is not described, and possible differences might have occurred despite randomisation, especially in a factorial design. Third, the reported compliance of 91% is very high for a trial with 4 months of treatment in an African population, but no objective information was presented about a possible difference of compliance between the group of women who received multivitamins and the group who did not".[409] That the compliance was not the 91% reported by the authors is suggested by the fact that at baseline equal proportions of women (34%) had vitamin A deficiency. Although a decrease was noted in the rate of deficiency after vitamin A supplementation at delivery, 22% of the women who received the vitamin remained deficient.

The first study that examined the relationships between vitamin A concentration in the mother's serum and MCT of HIV, determined by testing the children for HIV antibodies (ELISA and WB at 12 months), was published in 1994 by Semba and his associates from the USA and Malawi. Of 567 HIV infected mothers who delivered their infants at Queen Elizabeth Central Hospital in Blantyre, Malawi, "146 mothers had infants who died before 12 months. 40 infants were lost to follow-up or had mothers who did not agree to blood testing of their infants at 1 year. 381 infants were tested for HIV-1 infection at 1 year: 283 infants were HIV-1 seronegative, 84 infants were HIV-1 seropositive, and 14 infants had indeterminate western blots. Of 381 HIV-infected mothers, 84 (21.9%), had transmitted HIV infection to their infants. The overall transmission rate was calculated by a standard method that notes excess perinatal and infant mortality and in our cohort was 35.1%". In other words, by considering children infected because they died before one year of age, the transmission rate increased to 35.1%, a 60% increase over the 21.9% rate as determined by antibody testing. The mean vitamin A concentration in transmitting mothers was 0.86% vs 1.07 µmol/L in mothers who did not transmit. The authors reported a direct relationship between vitamin A deficiency and MCT. The women were divided in 4 groups "those with vitamin A concentrations of less than 0.70, between 0.70 and 1.05, between 1.05 and 1.40, and greater than or equal to 1.40 µmol/L. The mother-to-child transmission rates for each group were 32.4%, 26.2%, 16.0% and 7.2% respectively (p < 0.001)". Discussing their findings they wrote: "Our study shows that vitamin A deficiency is common among pregnant women who are infected with HIV in Africa…HIV infected women who transmitted HIV to their infants had vitamin A concentrations that were about half those of healthy, noninfected pregnant women in the US". Summarising their data the authors of this study wrote: "Vitamin A deficiency during pregnancy was associated with a three-fold to four-fold increased risk of mother-to-child transmission of HIV. The relation between low vitamin A and the estimates of attributable risk suggest that vitamin A or other closely correlated nutritional factors might be directly related to mother-to-infant transmission of HIV...The temporal relation we find between low vitamin A in the second and third trimesters of pregnancy and increased mother-to-child transmission is important, because it suggests that improving vitamin A during pregnancy may lower vertical transmission rates of HIV…Nutritional intervention may be a practical, inexpensive, and widely applicable option among several strategies that have been proposed to reduce mother-to-child transmission".[410]

In 1997 researchers from several institutions in the USA published a paper entitled "Maternal Serum Vitamin A Levels Are Not Associated with Mother-to-Child Transmission of HIV-1 in the United States". The study was conducted "To assess the possible relationship between serum vitamin A levels during the third trimester of pregnancy and the occurrence of vertical HIV-1 transmission". For this they tested (culture, PCR) the infants of 95 HIV positive women, 30 living in New York and 65 in Los Angeles. "Sixteen of the 95 women transmitted HIV-1 to their infants. Statistical analysis of the data indicated that low maternal serum retinol levels during the third trimester of pregnancy were not associated with mother-to-child transmission of HIV-1". However, in this study only 4 women had retinol levels of between 10 and 20 µg/dL (16.5; 18.3; 12.5 and 19.1 µg/dL), levels which, according to the author of this study, the World Health Organisation considers "as indicating marginal vitamin A nutritional status". All the other women had normal levels of vitamin A and none had levels below 10µg retinol/dl which "should be considered indicative of true vitamin A deficiency". It is also of interest to note that in this study, for unknown reason(s), the mother to child transmission rate in New York was 26.6% (8/30) infants versus 12.3% (8/65 infants) in Los Angeles,[411] which is not significantly different from the difference between the placebo and the AZT group in the ACTG076 study.

In the same year, 1997, another group of researchers from the USA, including the CDC, published the results of a study which was also designed to study the relationship between third trimester maternal vitamin A levels and MCT. In this study, conducted in two centres in the USA, the University of Maryland, Baltimore and the Montefiore Medical Center, New York, the serum vitamin A levels were determined in 44 women who transmitted HIV to their infants and in 89 who did not. In this study "A child was considered infected if one of the following applied: (i) two separate blood specimens were positive for HIV by PCR; (ii) presence of HIV antibodies persisting after 15 months of age; or (iii) the 1987 CDC criteria were met for class P2 with either an AIDS-defining illness or HIV-related death and one positive PCR. A child was considered uninfected if no AIDS-defining illness had been diagnosed and HIV serology was negative after 15 months of age; or no AIDS-defining illness and at least two samples were negative by PCR and no samples tested positive...Serum vitamin A levels were divided into three groups as per convention: severe deficiency, <0.70 μmol/1; mild to moderate deficiency, 0.70 – 1.04 μmol/1; normal, ≥1.05 μmol/1". The authors reported that: "Increased risk of maternal-infant transmission was associated with severe vitamin A deficiency among non-breastfeeding women in these cohorts from the United States...Maternal-infant transmission was also associated with prematurity <37 weeks gestation (P = 0.02), and Cesarean section delivery (P = 0.04), CD4 percentage (P = 0.03) and marginally associated with duration of membrane rupture of ≥4 h (P = 0.06) by univariate analysis. In a multivariate logistic regression model, severe vitamin A deficiency [adjusted odds ratio (AOR), 5.05; 95% confidence interval (CI), 1.20-21.24], Cesarean section delivery (AOR, 3.75; 95% CI, 1.10-12.78), and prematurity (AOR, 2.25; 95% CI, 1.22-4.13) were associated with transmission after adjusting for CD4+ percentage, and duration of membrane rupture". Under discussion they wrote: "Although vitamin A deficiency is generally regarded as infrequent in the United States, this study suggests that vitamin A deficiency is relatively common (30%) among inner-city HIV-infected pregnant women. Furthermore, HIV-infected women who were severely vitamin A-deficient during pregnancy had four-to-five-fold increased risk of transmitting HIV infection to their infants…Recently, it has been demonstrated that vitamin A deficiency is common among pregnant women of low socioeconomic status and among minority groups. Our study population in Baltimore and New York City consisted primarily of black and Hispanic women of low socioeconomic status, many former or current injecting drug users".[412]

In yet another study published from Durban, South Africa researchers noted that "No African country has yet provided antiretroviral agents for the prevention of mother-to-child transmission of HIV-1 on a nation-wide basis. The reasons for this are mostly, although not exclusively, related to questions of cost; even the cheapest course of these drugs (4 weeks of zidovudine as used in the Centres for Disease Control and Prevention trial in Thailand at $80, is beyond the entire per capita health expenditure of many African countries. It is therefore urgent that alternative strategies more relevant to poorer populations be evaluated. One such alternative is vitamin A. Vitamin A is cheap, easily provided through existing health services, and has been shown to be capable of reducing child mortality by approximately 30%. Given the affordability of this intervention, even substantially lesser degrees of benefit than those achieved for child morbidity and mortality may be acceptable to developing countries". In their study published in 1999, they tested the effect of vitamin A supplementation (5000 IU retinyl palmitate and 30 mg β-carotene daily, commencing between 28 and 32 weeks gestation and 200,000 IU of retinyl palmitate at delivery on MCT. In this study "children with at least one positive HIV RNA assay were considered infected". Six hundred and thirty two children born to 661 women (335 women received vitamin A and 326 placebo) were analysed. The authors reported that:

1. "the estimated transmission probability" was 20.3% in the vitamin A group and 22.3% in the placebo group";

2. "In the subgroup of 80 preterm deliveries, those assigned to the vitamin A group had a lower probability of infection by 3 months of age, than those assigned to the placebo group" 17.9% vs 33.8%;

3. "Women with low serum retinol levels at baseline (<30μg/dl) had a higher transmission rate than those with higher serum retinol levels";

4. "The incidence of preterm deliveries was reduced from 17.4% in the mothers on placebo to 11.4% in the treated group".

They concluded that "Vitamin A supplementation to a population of HIV-infected pregnant women, many of whom had low vitamin A levels, was associated with a decreased number of preterm births and with reduced mother-to-child transmission of HIV in preterm babies, but was not associated with a reduction in HIV transmission overall. The results of this study need further investigation as there is potential for a substantial impact, given that the rate of preterm deliveries in HIV-infected women is known to be high; in a recent dissertation reviewing available data on preterm births in HIV-1-infected women the rates of preterm deliveries ranged from 15% in Europe to 33% in the USA and 42% in Rwanda…Vitamin A may even be useful in

industrial countries to counter the effects of antiretroviral therapy which appears to increase the risk of preterm deliveries".[413]

The results of a study conducted in Abidjan (Côte d'Ivoire) and Bodo-Dioulasso (Burkina Faso) were published in 2000. In this study, "Paediatric HIV infection was defined by a positive polymerase chain reaction test at day 180 after birth or earlier. However, for some unknown reason, in their analysis of MCT the authors considered only "the cases of paediatric infection up to 90 days after birth". Of 234 children born to 241 women whose serum samples could be analysed for vitamin A, 54 children (23%) were infected. They reported that 48.1% of the transmitting mothers and 50.0% of the non-transmitting mothers had vitamin A levels of <30 μg/dL, which they consider as indicating "moderate deficit. A concentration of <20μg/dL, which they considered to represent "severe deficit" was present in 22.2% of the transmitting mothers and 29.4% in the non-transmitting.

However,

1. in the WHO (as stated by Burger *et al*[411]) "guidelines, serum retinol levels below 10μg retinol/dl (0.35 μmol/L) should be considered indicative of true vitamin A deficiency, whereas serum retinol levels ranging between 10 to 20μg retinol/dl (0.35 to 0.7 μmol/L) should be interpreted as indicating marginal vitamin A nutritional status". Since the authors do not mention what percentage, if any, of the women had vitamin A levels <10 μg/dL, is not possible to know if any of the women had "true vitamin A deficiency" according to the WHO definition;

2. the women in this study were the same women who took part in the DITRAME study conducted in Côte d'Ivoire and Burkina Faso to evaluate the effect of AZT on MCT. Given that, at least, half of the mothers of the 234 children were treated with AZT, the possibility cannot be excluded that the effect of vitamin A, if any, may have been masked by the AZT treatment.[414]

Comments
In some of the studies an association has been found between the vitamin A status of the mother and the MCT rates. The exceptions may be due to:

(a) "the lack of a satisfactory biomarker for assessing the vitamin A status of individuals or population except in the case of extreme hypovitaminosis A";

(b) low bioavailability.[415]

"Circulating retinol values in mature newborns are always 50% lower than those in the mother". This means that if the mother is vitamin A deficient, there is a high probability that the infant will also be deficient. "The situation is more critical for premature deliveries, because both serum and hepatic vitamin A concentrations can be very low and may pose a direct threat to the child's health. Although vitamin A supplementation can be used, its ability to prevent and reduce lung injury such as bronchopulmonary dysplasia is still controversial. The foetus starts to accumulate vitamin A during the third trimester of pregnancy, and needs several months of sufficient intake after birth to build up an adequate hepatic store. In many countries, babies are breast-fed, in which case the vitamin A content of the breast milk is of primary importance. The composition of breast milk is influenced by the vitamin A status and serum concentrations of the mother during the last trimester of pregnancy. Colostrum and early milk are extremely rich in vitamin A, and even the milk of a mildly undernourished woman may meet the physiologic needs of the newborn during the first weeks. After this time, however, a rapidly-growing infant may exhibit negative vitamin A balance, with severe consequences for health. Young children who are vitamin A deficient are at greater risk of morbidity and mortality than vitamin A-sufficient children. Diarrhoea, respiratory infections, and measles are the diseases most frequently associated with a deficient vitamin A status". Thus an adequate vitamin A intake may not only decrease the MCT of HIV but will also have many additional health benefits in children, including the elimination of xerophthalmia which at present affects 2.8 million children. However, since doses of vitamin A higher than 10,000 IU may be toxic, it may be better to treat vitamin A deficiency "with the provitamin β-carotene, which has never been associated with any teratogenic risk".[415] In fact the best way to treat vitamin A deficiency would be to supplement the mothers and the children's diet with fruit and vegetables rich in β-carotene such as apricots, oranges, peaches and carrots, which would also correct other vitamin deficiencies as well as mineral deficiencies.

In a very recent study from Zimbabwe[469] the authors noted "Vitamin A deficiency is common in women of reproductive age and young children in developing countries is an important determinant of morbidity and

mortality". In their study, the authors documented an inverse relationship between serum beta-carotene and retinol levels in pregnant women and HIV seropositivity.

"In an equally recent study, "In a nested case-control study in individuals attending two sexually transmitted disease (STD) clinics in Pune, India, serum micronutrient levels were measured in 44 cases with documented HIV seroconversion (11 women and 33 men) and in STD patients matched for gender and length of follow-up with no subsequent HIV seroconversion (controls). STD patients in Pune had low vitamin A and carotenoid levels, and low serum beta-carotene levels were independently associated with an increased risk of subsequent HIV seroconversion. STD patients with beta-carotene levels less than 0.075 micromol/L were 21 times more likely to acquire HIV infection than those with higher levels".[470] Thus an adequate dietary vitamin A intake by women of reproductive age will decrease the number of "infected" children not only directly (by decreasing MCT) but also indirectly by decreasing the number of infected women.

5.3.5   Anti-oxidants

With the exception of vitamin A, to date no studies have been conducted to evaluate the effect of anti-oxidants (reducing agents) on MCT. However, there is evidence that suggests that MCT may be preventable by anti-oxidants. In 1983 Luc Montagnier and in 1984 Robert Gallo and their colleagues stimulated cell cultures from tissues of AIDS patients with numerous chemical agents and observed a number of phenomena which, long before the AIDS era, were known to be non-specific. In their rush to discover the cause of AIDS, Montagnier and Gallo interpreted these phenomena as proof of isolation of a new and unique human retrovirus, HIV. By 1986 Montagnier and Gallo acknowledged that the "HIV" phenomena cannot be detected unless the cells are stimulated[96,416] but apparently they failed to appreciate that oxidation is implicated in cellular stimulation and that their laboratory agents were oxidising.[28,94,417] Subsequently, many researchers including Anthony Fauci showed that when the cell cultures are treated with reducing agents, that is, anti-oxidants, the phenomena claimed as "HIV" cannot be detected.[418-421]

From the beginning of the AIDS era there has been evidence that individuals belonging to the AIDS risk groups (gay men, haemophiliacs, drug users) are exposed to oxidising agents.[28,94] There are also data that positive PCRs and antibody tests revert from positive to negative when exposure to oxidising agents is discontinued.[423-425] In a 1998 study researchers from Canada reported that "Supplementation of Vitamin E and C reduce oxidative stress in HIV and produce a trend towards a reduction in viral load. [After 3 months of supplementation the viral load was -0.45 ± 0.39 in the vitamins group versus $+0.50 \pm 0.40 \log_{10}$ copies/ml in the placebo group. Significantly, after a high dose of nevirapine (2 weeks of 200mg daily followed by 400mg daily), the viral load returns to baseline values within 2 months[491])]. Linear regression analysis used to evaluate the association between viral load at baseline and the change after 3 months was significant ($P = 0.026$) indicating that the change after 3 months depended on the initial score". Discussing their results the Canadian researchers wrote: "This study is the first randomised controlled trial to demonstrate that, in an HIV-positive population, daily supplementation of 800 IU vitamin E and 1000mg vitamin C significantly decreases oxidative stress and produces a trend towards a reduction in HIV viral load suggesting that there may be some clinical benefit worthy of larger clinical trials. Since combination antiretroviral therapies containing protease inhibitors are limited for economic reasons to only about 10% of HIV-infected individuals in the world, consideration of the potential for this antioxidant therapy remains important for the developing world. It could have great benefit, perhaps similar to the effect of vitamin A supplementation on childhood mortality in developing countries".[471]

As long ago as 1983, one of us (EPE) proposed that oxidative mechanisms are of fundamental significance in the genesis of AIDS. The claim of the discovery of HIV resulted in a broadening of this hypothesis in that it considered oxidation as a principle mechanism in both the development of AIDS and the phenomena that are known as "HIV".[28,94] The prediction of this theory included the following:

(a) AIDS patients and those at risk will be oxidised;

(b) the mechanisms responsible for AIDS could be prevented and reversed by administration of reducing agents especially those containing sulphydryl groups;

(c) "HIV" phenomenology may be induced by exposure to oxidising agents and eradicated by treatment with reducing agents.

Unlike the predictions of the HIV theory of AIDS, most if not all predictions of the oxidative theory including the above have been proven correct.[426-430]

Poverty and malnutrition are not only associated with vitamin A deficiency but also with deficits of other anti-oxidants. Evidence exists which shows that malnutrition decreases tissue levels of reduced glutathione, one of the most important anti-oxidants.[431] In a study published in 1995 entitled "Glutathione and Associated Antioxidant Systems in Protein Energy Malnutrition: Results of a Study in Nigeria", the authors from Germany and Nigeria pointed out that: "Hypotheses like hypoproteinemia or aflatoxin poisoning could not adequately explain the pathophysiologic changes observed in kwashiorkor. One supposition, namely the involvement of reactive oxygen species (ROS), has attracted interest over the last years and appears to account for a number of clinical and biochemical characteristics of the syndrome. Kwashiorkor was found to be associated with the following alterations of antioxidant parameters: (1) Low concentrations of vitamin E, carotene, Zn^{2+}, selenium, and glutathione; (2) a reduced NADPH/NADP$^+$ ratio; (3) low glutathione peroxidase activity and low inducible glutathione-S-transferase activity. Furthermore, high concentrations of circulating ferritin and hepatic iron as sources of oxidative stress have been reported…Systematic studies on the role of oxidative stress in PEM are rare. Further evidence for an imbalance in the equilibrium of pro- and antioxidants – representing a causative factor or a secondary phenomenon – could have therapeutical consequences for patients. We, therefore, measured and correlated parameters of antioxidant capacity in malnourished and control children in Nigeria".

In their study "changes were observed for erythrocyte glutathione and corresponding for non-protein thiols in whole blood (0.72 ± 0.29mM thiols in controls, 0.50 ± 0.22mM in marasmus, 0.35 ± 0.23mM in severe marasmus, and 0.22 ± 0.13mM in kwashiorkor)…Retinol-binding protein, a typical housekeeping serum protein, was found to be normal in patients and controls. In contrast, the concentrations of albumin and total serum protein as well as the albumin/total protein ratio were drastically decreased in kwashiorkor…Total serum protein and albumin were also decreased in patients suffering from marasmus, particularly in the clinically severe cases…these findings were statistically significant; they corroborate the notion that hypoalbuminemia is not a dominating pathogenic factor of edema in kwashiorkor". The authors of the Nigerian study concluded: "New strategies for improving the clinical situation of patients with severe protein-energy malnutrition are urgently required. Taking the results of our study and the above-discussed aspects into account, a therapy directed to the restoration of the antioxidant capacity – for instance, the administration of glutathione – might be beneficial for children with malnutrition, especially kwashiorkor. However, new therapeutic approaches should be considered with greatest caution because the metabolic equilibrium of severely malnourished children is highly fragile.[472]

In a study published two years later by researchers from the Netherlands and Kenya, it was reported that the: "Concentrations of interleukin 6 (IL-6), C-reactive protein, and the soluble receptors of tumour necrosis factor α (sTNFR-p55 and sTNFR-p75) are greater in children with PEM, particularly in those with kwashiorkor…Antioxidant status, as determined by plasma concentrations of glutathione and vitamin E, is significantly reduced in kwashiorkor patients…Changes in the other inflammatory indexes also support the notion that there was an inflammatory reaction in the PEM group. Both sTNFRs have been found to be elevated in human immunodeficiency virus (HIV)-infected patients but only indeterminate serologic results for HIV were found in five PEM patients (data not shown)".[473]

In a study entitled "Antioxidant status in children with protein-energy malnutrition (PEM) living in Cairo, Egypt", among other indices of the redox studies, "A select group of vitamins known to have antioxidant activities were measured", by researchers from Canada and Egypt. "The mean plasma levels of these vitamins [A, E, C] were significantly lower in children with either KWO (kwashiorkor) or MAR (marasmus) than those of the non-malnourished children…The mean plasma proteins including albumin, RBP, and copper-containing ceruloplasmin were all significantly lower in both KWO and MAR than those in their control counterparts. The albumin/globulin ratio was also markedly reduced in the presence of malnutrition". According to the authors of this study the decreased anti-oxidant level "in PEM children suggests that these children are potentially susceptible to high oxidative stress. An elevated plasma Fe concentration, especially with KWO may augment the harmful effect of free radicals with a clinical consequence of oedema".[474]

According to a study published last year by researchers from the USA and Jamaica, "Despite several reports of the compromised GSH status of children with edematous PEM, the in vivo kinetic mechanism(s) responsible for GSH deficiency has not been determined. One obvious mechanism in the severely malnourished state is suppressed synthesis secondary to a shortage of amino acids. Such a mechanism is supported by the studies of Grimble et al and Hunter and Grimbe, which show that the slower hepatic and pulmonary GSH synthesis and lower GSH concentrations of rats fed protein-deficient diets or with low food intakes return to control values when the deficient diets are adequately supplemented with either methionine or cysteine plus glycine".

To elucidate the mechanism for the decreased glutathione concentration in PEM children, "in this study a recently developed technique was used to determine the rates of synthesis of GSH in the erythrocytes of children with edematous and nonedematous PEM. The data reported demonstrate that erythrocyte GSH concentration

and rate of synthesis were considerably lower in children with edematous PEM than in children with nonedematous PEM both shortly after admission when they were infected and malnourished and 7-10 days later when they were still malnourished but were free of infection. At both times, the slower GSH synthesis rates were associated with lower erythrocyte and plasma free cysteine concentrations. These findings strongly suggest that the GSH deficiency of patients with edematous PEM is due to impaired synthesis secondary to a shortage in the supply of cysteine".[475] (The study had no controls).

In a presentation entitled "Malnutrition, morbidity and mortality in children and their mothers", to the Summer Meeting of the Nutrition Society held at the University of Glasgow, 1999, Andrew Tomkins from the Centre for International Child Health, Institute of Child Health, London, stressed that "It is important to reflect on why nutrition has not been taken more seriously by national governments and international agencies who seek to reduce childhood mortality…there are several hurdles to overcome before nutrition interventions are taken more seriously". One among the five discussed in some detail is HIV/AIDS. He stated: "An additional novel hypothesis has been put forward during studies of breast-milk immunology among women in Bangladesh and South Africa. It was noted that approximately 20% of women in these countries have subclinical mastitis, as assessed by high levels of Na:K and interleukin 8 levels in breast milk. This condition is especially important within the HIV context because of the association between high numbers of HIV particles [viral load] and subclinical mastitis. The veterinary literature has recognised subclinical mastitis for several decades. It is known to be associated with a high load of a range of bacteria, and is especially common among cattle being fed on antioxidant-deficient pastures. Subclinical mastitis has been noted to be associated with poor milk volume and growth faltering in farm animals, and these factors were also present in the study of infant growth in relation to subclinical mastitis in Bangladesh. A recent study in South Africa shows that there are certain patterns of occurrence of subclinical mastitis. Bilateral subclinical mastitis is of a typical mild form with low Na:K values, whereas unilateral subclinical mastitis is more common and is often more severe with high Na:K values and elevated levels of interleukin 8; there are higher viral loads in samples from women with subclinical mastitis in Durban, South Africa. The association between subclinical mastitis, viral load and MTCT has been demonstrated in Malawi, although no information on the pattern of subclinical mastitis was provided. The demonstration that HIV viral load is increased among women with subclinical mastitis has enormous implications for the transmission of HIV in the breast milk. It puts great emphasis on the reduction of the prevalence and severity of subclinical mastitis by whatever means possible. A recent study among women in Tanzania shows that the prevalence of subclinical mastitis is lower among women who received dietary supplements with sunflower-seed oil during pregnancy and lactation. Sunflower-seed oil has a high level of vitamin E; the potential antioxidant capacity of this oil may be extremely relevant to the decrease in levels of mastitis".[476]

Last year researchers from the Harvard School of Public Health and the Harvard Institute of International Development, published a paper entitled "Tomato Intake in Relation to Mortality and Morbidity among Sudanese Children". In this study the authors "examined the relationship between the consumption of tomatoes, a rich source of antioxidants, and mortality and diarrhoeal and respiratory morbidity rates among 28,753 children who were 6-60 mo old and enrolled in a longitudinal study in the Sudan. Children in each household were visited every 6 mo for a maximum of four visits. At each round, mothers recalled whether a child had consumed tomatoes in the previous 24 h. Events (death or morbidity) reported at each round were prospectively allocated according to the number of days of tomato intake". They reported that: "Intake of tomatoes for 2 or 3 d compared with none was associated, respectively, with 48% (relative risk, 0.53; 95% confidence interval, 0.30-0.91) and 83% (0.17; 0.04-0.72) reductions in mortality rates (P for trend = 0.002). The association between tomato use and death remained statistically significant (P for trend = 0.004), even after further adjustment for total vitamin A intake. Tomato intake was also associated with a reduced risk of death associated with diarrhoea in the week preceding death (P for trend = 0.009) or fever (P for trend = 0.04). Intake of tomatoes was also inversely and significantly associated with the risks of diarrhoeal and respiratory infections".

Discussing their findings and those of others, the American researchers wrote: "The inverse relationships between tomato consumption and the risks of cancer and cardiovascular disease have been attributed to its high content of antioxidant substances including carotenoids, mainly lycopene. Oxidative stress may also contribute to the occurrence of diarrhoeal disease and malnutrition among children and antioxidants, including lycopene, may be beneficial in reducing the risk of these adverse conditions. Lycopene may be associated with enhanced immunity and reduced risks of infectious disease. In a study among healthy men, 2 wk of ingestion of tomato juice resulted in a significant increase in lycopene levels, as well an increase in T-lymphocyte function. Plasma lycopene levels were also significantly low in malnourished children from Morocco and Nigeria and among human immunodeficiency virus-infected children. In animal studies, intraperitoneal or intravenous injection of lycopene prolonged the survival of bacterially infected mice…To our knowledge, this is the first study that examined the relationship of tomato intake and infectious disease and mortality rates in childhood. Additional data on this subject are needed. Nonetheless, our data emphasise the importance of considering food-based approaches to the prevention of micronutrient malnutrition and reduction in child morbidity and mortality rates in developing countries".[477]

According to researchers from Germany and Ghana "Severe malnutrition represents one of the most severe socioeconomic and health problems in the world. Clinically, protein-energy malnutrition (PEM) can be divided into three major forms: (1) marasmus, which is characterised by severe deficit of body mass; (2) kwashiorkor, an edematous form of malnutrition; and (3) marasmic kwashiorkor…The clinical management of kwashiorkor is still highly insufficient with a mortality ranging between 10 and 50%. During the last 40 y, this situation has not significantly improved. This worrying constellation is based on the fact that our knowledge on pathophysiology and therapeutical interventions is highly inadequate…As initially postulated by Golden and Ramdath, an imbalance between reactive oxygen species generated and the available antioxidant capacity may play a significant role in the pathophysiology of kwashiorkor. Over the last years, the syndrome was indeed shown to be associated with drastically decreased concentrations of glutathione, with low concentrations of vitamin E, carotene, and polyunsaturated fatty acids, with a reduced $NADPH/NADP^+$ ratio, and with low glutathione peroxidase and elevated glutathione-S-transferase activity. Furthermore, high concentrations of circulating ferritin and hepatic iron have been reported as signs of liver injury".

To "further elucidate the role of oxidative stress in kwashiorkor" they conducted a longitudinal study in which reducing and oxidative factors in kwashiorkor children were compared with those in a control group consisting of healthy children. "As a diet the control children received the local mixed food consisting mainly of yam, tomatoes, rice and maize. The kwashiorkor patients were treated for infections…Patients received 150mL of a rehabilitation formula (5g/L skimmed milk powder, 30g/L sugar and 30g/L oil) three times daily…the patients also received small amounts of local food". "All kwashiorkor patients (K) were followed up for 20 d".

"The erythrocyte glutathione concentrations were drastically reduced in kwashiorkor patients and were clearly related to the clinical outcome. After 2 wk, the concentrations reached normal levels in the patients who survived and dropped or were constantly low in the patients with lethal outcome. This phenomenon has been described in previous studies". The total antioxidant status (TAOS), which was "measured by the ABTs [2,2′-azino-di(3-ethylbenzothiazoline sulphonate] assay, which is based on the capacity of plasma to scavenge the $ABTs^+$ radical...in children suffering from kwashiorkor is reduced to less than 50% of local control values. When comparing the TAOS of our patients with healthy European term babies at the age of 5 d [TAOS = 1.50 + 0.14 mM], only 27% of this antioxidant status is reached. This result underlines the importance of ROS in the malnutrition syndrome". The oxidative agents "nitrite and nitrate were found to be increased by a factor of 2 in kwashiorkor". The authors concluded: "Our study strongly supports the hypothesis that oxidative and nitrosative stress play a role in the pathophysiology of the malnutrition syndrome kwashiorkor…In kwashiorkor, prophylactic and therapeutic strategies should aim at treating the infections, controlling the hydration state, and carefully correcting the reduced antioxidant capacity and, in direct connection, the protein deficiency. The great challenge in developing an effective therapy that is able to break the underlying vicious cycle is the fact that kwashiorkor patients suffer from severe liver dysfunction, thus reacting very sensitively to the administration of proteins and various antioxidant compounds".[478]

Consuming a quantitatively adequate diet is no guarantee of a balanced redox state. In Africa, Professor Lodewyk Kock and his associates from the Free State University in Bloemfontain have shown that South Africans, "especially the poor communities in South Africa are continuously exposed to large quantities of potent oxidising agents through the consumption of highly oxidised fats. Fats in use containing more than 60% free radical-induced polymerised triglycerides, called by us Super Oxidised Soups (SOS). Of concern is the fact that most of these unstable SOS are continuously channelled to the poor communities where it is further oxidised in frying practices. It is estimated that more than 100,000 tons of unstable SOS are consumed by these communities each year. This is in sharp contrast to the high quality frying fats used in first world countries. The latter fats rarely contain more than 16% polymerised triglycerides. It is also not allowed in these countries to sell fats for further use in food preparation"[479-481] (and J Kock, personal communication).

In a study conducted in Nigeria repeatedly thermoxidised palm oil, simulating local culinary practice, was fed to male and female rats for eight weeks at 15% of a balanced basal diet. The authors concluded that "The results of these studies when extrapolated to the Nigerian populace imply that the average Nigerian may be in grave danger of hitherto unsuspected sources of reproductive toxicity. Both males and females could be at risk. Marginal malnutrition is suffered in endemic proportions in this country and the coupled consumption of thermoxidised dietary oils may contribute to poor reproductive capacities, infertilities, low birth weights and thus, reduced life expectancy".[432]

An individual does not have to live in a poor country or community to be malnourished. In fact, wealthy individuals, eating an unbalanced diet, may be malnourished as well as oxidised. Cigarette smoking is recognised as being an important oxidising factor to which humans are exposed. It is also accepted that cigarette smoking affects the well-being of both mother and foetus. In 1994 in France "The effects of alcohol consumption on plasma concentrations of antioxidant vitamins (alpha-tocopherol and ascorbic acid), selenium,

and markers of oxidative stress, especially malondialdehyde (MDA) and autoantibodies directed to MDA adducts to proteins (Ig-NH2-MDA) were investigated in a large population of 417 supposedly healthy men who consumed only low or moderate amounts of alcohol as compared with 102 alcoholic patients without severe liver disease, who were studied both before and after 21 d of withdrawal treatment. Plasma concentrations of alpha-tocopherol, ascorbic acid, and selenium were lower in alcoholics than in men who drank low amounts of alcohol ($P <$ or $= 0.001$), whereas MDA and Ig-NH2-MDA were higher ($P <$ or $= 0.001$). Plasma concentrations of alpha-tocopherol and selenium remained unchanged after the withdrawal period, whereas ascorbic acid ($P <$ or $= 0.01$), MDA, and Ig-NH2-MDA concentrations decreased ($P <$ or $= 0.001$". The authors concluded: "Adjustment of data for circulating lipids and nutritional intake suggests a specific effect of alcohol on antioxidant vitamins, independent of nutritional status".[433]

One must not forget that oxidation is either the cause or is associated with of many if not all diseases including infectious diseases such as malaria[434-438] and tuberculosis.[28,92-94,439-444]

As far as AIDS is concerned, if one reads Montagnier's latest writings[428] and interviews[445] one would be excused for concluding that Montagnier is an apologist for the oxidative and not the HIV theory of AIDS. More than a decade after the oxidative theory of AIDS and "HIV" was proposed, Montagnier wrote: "A large body of data on in vitro human immunodeficiency virus (HIV) infection and biochemical clinical studies suggests that oxidative stress plays a role in AIDS pathogenesis. Recent reports have implicated intracellular excess of reactive oxygen species (ROS) in the induction of HIV expression and in the initiation of apoptotic cell death. Studies showing a decrease in glutathione in peripheral blood mononuclear cells from symptom-free persons offer further evidence of a metabolic alteration leading to the decreased ability to counteract oxidative stress. . . . In AIDS pathogenesis, oxidative stress *is proposed* as a metabolic alteration that favours disease progression by inducing both viral replication and apoptotic death. . . . Indeed, evidence that oxidative stress induces, while antioxidants inhibit, HIV replication and apoptosis suggests the use of these molecules as an antiretroviral therapy to reduce cell death in AIDS patients"[446] (italics ours). However Montagnier does not indicate what difference exists between his proposal of the role oxidation plays and our oxidative theory of AIDS and "HIV". Furthermore, he does not indicate what is the cause(s) of the oxidation in these patients nor does he comment on the causes we have been proposing. In an interview Gallo gave to *Positive Nation* in March 2000 he was asked "It's been sixteen years since HIV was discovered. Yet not only alternative health fans, but scientists with pretty distinguished backgrounds like Peter Duesberg and Eleni Eleopulos maintain that HIV doesn't exist or doesn't cause AIDS. How come?" Gallo replied, "I think the reasons Duesberg sticks to his theories are purely personal. As soon as he is forced to drop one argument, he just picks up another one. And Eleopulos's theories are just off the wall. I cannot think of any disease-remember I was a physician before I was a researcher - where we know more about the cause than with Aids. Short of seeing someone run over by a car, you'll never be more certain as to the cause of a medical problem". Yet, when he was asked "How much truth is there in this [oxidative] idea?" He replied, "Oxidative stress is a real phenomenon. Many of my patients take anti-oxidants like n-Acetyl Cysteine (NAC). They can help; but they are not a cure".[447]

It is true that currently there is no proof that anti-oxidants "are a cure" for AIDS. Nowadays there are ample data to prove that HAART is "not a cure" for AIDS, as Gallo admitted in the same interview. However, while the conclusion regarding HAART is based on extensive and exhaustive clinical studies nobody has applied the resources and efforts devoted to antiretroviral compounds into the evaluation of reducing agents.

Comments
Although the oxidative theory of HIV and AIDS proposed at the beginning of the AIDS era is not acknowledged by the HIV experts, some of the strongest supporters of the HIV theory of AIDS lately have accepted that the redox is a "key factor" in both the expression of the HIV phenomena and the development of AIDS.[445] Adopting this view means that the primary cause(s) for the oxidation in AIDS patients cannot be either HIV or any of the supposed HIV effects.[482] That is, agreement exists that:

(a) oxidation is necessary for the detection of the phenomena interpreted as proof of HIV infection and reducing agents inhibit these phenomena;

(b) oxidation plays a role in AIDS development that can be prevented by anti-oxidants.

This implies that cessation of exposure to either oxidising agents or a diet deficient in anti-oxidants followed by supplementation with anti-oxidants would decrease MCT, both by a direct effect on the HIV phenomena and by preventing the development of AIDS, which by itself is considered proof for MCT. However, since any substance in high concentration may be toxic, it may be better to obtain the reducing agents from an anti-

oxidant-rich diet. Such a diet would correct not only the redox but also other dietary deficiencies, especially in poor countries.

5.3.6   *Breast feeding*

By 1992 some experts were claiming that the rate of HIV transmission via breast-feeding was 14%.[310]   Although evidence also existed which showed lower "infection rate among breastfed children" and that breastfeeding may delay progression to AIDS in HIV-infected children,[292,310,448] the claim of the high rate of transmission has been accepted by nearly everybody. Even in 1999 researchers from the USA and Malawi were claiming to have proven, that "an uninfected infant, breastfed by an HIV-positive mother for 23 months had at least a 10.3% risk of becoming infected".[325]

There are some exceptions. In 1995 Phillipe Van de Perre, a researcher who has done his utmost to prove MCT of HIV, accepted that "Assessments of the risk of mother-to-child transmission of HIV-1 by breast-feeding have many drawbacks. They have been drawn from observational studies. To date no intervention trials, randomised or not, have been reported on this subject. Also, many of these estimates have been based on studies with a limited sample size". He added, the "meta-analytic approach is probably weakened by the observational and retrospective nature of the pooled studies and by the fact that studies with negative results may have been potentially omitted".[292]   In a paper published in the British Medical Journal, 17 June, 2000, Michael Latham, a professor of international nutrition, Cornell University, USA and Elizabeth Preble, international health consultant, Santa Fe, USA, wrote: "The role of breast feeding in the vertical transmission of HIV has been exaggerated…It is estimated that in countries with a low seroprevalence of HIV (5% of women infected) fewer than 1% of all infants are likely to become infected through breast feeding, whereas in those with high prevalence (25% of women infected) fewer than 4% of infants will be affected through healthy breast feeding…Exclusive breast feeding reduces HIV transmission. Promoting infant formula feeding to prevent HIV infection might increase infant morbidity, malnutrition, and mortality".[449]

As mentioned the best evidence that infants HIV infection was significantly lower in those exclusively breast fed comparing to those receiving mixed feeding has been published by researchers from Durban, South Africa. In the same study it was also found that children who were exclusively formula fed, had consistently higher infection than those exclusively breast fed. In a recent commentary by one of the principle authors of the Durban study, Anna Coutsoudis, she said that "Some preliminary evidence from Kenya confirms" their findings. Arguing in favour of exclusively breast feeding she pointed out that: "among poor disadvantaged populations…the benefits of exclusive breast feeding are vital for child survival…Promotion of exclusive breast feeding entails good management of lactation, which encourages proper attachment of the infant to the nipple and frequent emptying of the breast, both of which are important for preventing cracked nipples, engorgement, and mastitis, which have been suggested to be risk factors for transmission of HIV-1…Exclusive breast feeding is less likely than mixed breast feeding to be associated with diarrhoea and respiratory illness. Even replacing colostrum with prelacteal feeds has been shown to be a risk for death; a study in the Gambia showed that prelacteal fluids were associated with an odds ratio of 3.4 for neonatal mortality".[320]

In a *Lancet* editorial the beneficial effects of breast feeding were summarised as follows: "Breast feeding helps to limit fertility and prevent ovarian and premenopausal breast cancer. It helps to prevent sepsis in newborn babies, and gut, chest, ear, and urinary tract infections in all young children, and is valuable in the management of both acute and persistent diarrhoea. In countries with a moderate or high infant mortality rate, artificially fed infants are at least 14 times more likely to die from diarrhoea than are breast fed children, and 4 times more likely to die from pneumonia. Even in countries where infant mortality is low, artificially fed infants require hospital treatment up to 5 times more often than those who are fully or partly breast fed. In France, the cost of these extra admissions is conservatively estimated to be over 71 million francs (about US$12 million, £8 million), with the cost of outpatient and other treatments making a total of 1116 million francs (US$199 million). In the UK, hospital costs are said to be as much or more".[450]

In a study published in 1990 researchers compared the benefits of breast feeding with the risks associated with HIV transmission by breast feeding. The authors of this study, from the Family Health International, Research Triangle Park Branch, Durban, USA, although of the opinion that "The evidence that breast feeding constitutes a mode of HIV transmission is incomplete", for the purpose of their analysis "assumed that transmission of HIV by breastmilk is possible" and made the following assumptions:

(a) theoretical probabilities of 1%, 5%, 10% and 20% of HIV transmission by breastmilk;

(b) relative risks 3, 4, 5 and 6 times of death due to diseases of infancy for bottlefed babies compared to breastfed babies;

(c) an extremely high mortality of HIV infected babies, 95% dying before their first birthday.

They showed that "The breastmilk transmission rate must be about 20% before the expected number of deaths among breastfed babies approaches that associated with bottlefeeding" and concluded "The estimated number of infant deaths associated with bottlefeeding by an HIV + mother appears to greatly outweigh the comparable number associated with breastfeeding, especially in settings with high baseline levels of infant mortality, such as those in developing countries. (This may also be true in developed countries where HIV infection most often affects women in population groups with higher than average rates of infant mortality"[451]). Others agree.[452,453]

Comments
Since,
1. The death of a child born to an HIV positive woman at the age of less than 12 months is considered proof that the child died from AIDS or "HIV-related disease".

2. Breast feeding decreases the death rate of infants and children.

then breast feeding will decrease the death rates in general and from AIDS in particular.

Since,
1. As Coutsoudis, Coovadia and their colleagues have shown, breastfeeding decreased MCT, as determinate by virological tests.

2. Death of a child born to a positive woman, before the age of 12 months, is considered proof that the child is infected with HIV and it acquired the virus from the mother.

then breastfeeding will decrease MCT of HIV.

In other words, breastfeeding will decrease the incidences of AIDS and of MCT of HIV. It is difficult to comprehend why some researchers (especially from developing countries) such as Zwi and her colleagues from South Africa, consider breastfeeding of "secondary importance" in preventing vertical transmission of HIV. "There is a danger that this issue may muddy the waters in respect of methods of reducing mother to child transmission which are already largely resolved–that is, the use of antiretroviral drugs in the perinatal period...data from South Africa suggest that their impact in reducing paediatric HIV infection could be major, regardless of the mode of feeding. Antiretroviral treatments save lives and money and seem to remain cost effective across a wide range of infant mortality, healthcare spending, and screening uptake rates so long as seroprevalence rates are high".[454] In fact the reference cited in support of this argument is a paper by the same authors in which they "attempted to develop a generally applicable simulation model for assessing the effectiveness and cost effectiveness of interventions to prevent mother to child transmission of HIV".[455] However, although they were "confident that administration of a low cost antiretroviral regimen or advocating formula feeding for infants of HIV infected women, or both, would save lives and, in many cases, save money, their "model" was based on many unsubstantiated assumptions as have already been pointed out by others[456] including researchers from South Africa.[457]

Understandably, the best person to comment on the importance of breastfeeding, is Anna Coutsoudis, who with her colleagues from Durban, South Africa, has conducted the best study to date: "As several of you have mentioned it is disconcerting that people are not keen to accept our results but very quick to accept the Ugandan results based on 1 study – I mentioned this at the Montreal conference that exclusive brf [breast feeding] reduces the risk of transmission relative to mixed in the same magnitude that nevirapine did (48%) and the comment was that ours wasn't a randomised controlled trial (RCT) [neither was the nevirapine trial] and that's why we can't put too much store by it. The Nduati study has highlighted very clearly for us that in the field of infant feeding practices unfortunately a RCT is not the study design one can use because as Nduati's study showed you cannot randomise behaviour which is as innate as brf – you can randomise treatment regimens but not brf behaviour. I still feel it is unethical to randomise women to formula in countries where infant mortality from infectious diseases is relatively high. In fact the Nduati study showed this so clearly as well – where what was gained in the formula group by reducing HIV transmission was quickly lost because of increased mortality due to infectious diseases. The mortality in the formula group of 20% was very high for a group who had a large amount of support and help with formula feeding, clean water, free formula etc and theoretically the high contact with health workers should have made the infants more likely to survive. I would hate to think what the mortality would have been if these were not available as would presumably occur if women are simply encouraged to formula feed" (Professor Anna Coutsoudis, Department of Paediatrics and Child Health, University of Natal, Durban, addressing subscribers to *AnotherLook*, a private Internet discussion group devoted

to an examination of breastfeeding and HIV/AIDS, 3/7/2000. Reproduced with permission of Professor Coutsoudis).

5.3.7   Other Factors Reported to Affect MCT
5.3.7.1   Drugs
Maternal drug, smoking and alcohol use, especially during pregnancy have been shown to be associated with MCT.[43,44,214,260]   Considering that:

1. drugs cross the placenta;[285]

2. women who abuse drugs and alcohol are malnourished;[403]

3. perinatal exposure to drugs, smoke and alcohol is associated with lower weight-for-age and length-for-age values and prematurity;[260,282,285]

4. the clinical syndrome of the children born to drug using HIV negative mothers is similar to the clinical syndrome of the children born to HIV positive mothers;[260]

one would expect drugs and alcohol to be significantly associated with MCT.

5.3.7.2   Antenatal obstetric factors
In a few studies in which it was evaluated sexual contact during pregnancy was significantly associated with transmission.[122,214,458]   Chorioamnionitis has been reported in one study[69] and in another study a similar association has been reported between MCT and pneumonia, TB, anaemia, smoking and low birth weight.[44] Because "neonatal and maternal postpartum morbidity and mortality due to bacterial infections are substantial in developing countries", researchers from the USA and Malawi conducted a large trial (6965 women and their 7160 babies) to examine the effect of cleansing the birth canal and the infants with a 0.25% solution of the antiseptic chlorhexidine. Cleansing resulted in "significantly fewer neonatal admissions, fewer cases of neonatal and maternal postpartum sepsis, lower early neonatal mortality rates, and shorter duration of hospital stay...There was an abrupt 25% reduction in early neonatal mortality with introduction of the intervention, and mortality increased when the intervention was stopped…The cleansing procedure was easily administered and took almost no extra time, and the cost of the chlorhexidine solution and cotton was less than US$10 per patient. None of the patients had complaints or complications related to the intervention". The authors of this study concluded: "The beneficial effects, safety, simplicity, and low cost of the intervention encourage its adoption to reduce maternal and neonatal morbidity and mortality".

Since,
>   (a) "coitus during pregnancy markedly increases both the frequency of bacterial infections of amniotic fluid and the mortality due to them. The effects are greatest at mid-pregnancy and gradually decrease to term";[127]
>
>   (b) thirty per cent of the women in this study were found to be HIV positive;
>
>   (c) children of seropositive women who die at <1 year of age are said to have died from AIDS, that is, from infection with HIV acquired from their mothers;

this simple and low cost intervention would result in a significant decrease in MCT.[459]

5.4   Discussion
While there is no proof that AZT and nevirapine inhibit the putative MCT, there is evidence that AZT is toxic to the children. So far, no such evidence exists for nevirapine. Given that nevirapine is toxic to adults, it is most likely toxic to children. Furthermore, since so many factors have a significant effect on MCT, then

1. in studies in which MCT is compared in groups of women who are treated with anti-retroviral drugs (AZT, nevirapine) with those who receive placebo, one must control for all these factors, otherwise no meaningful conclusion can be drawn no matter what the findings;

2. given the fact that all mothers who are said to transmit HIV to their infants are associated with at least one of the most important factors (drugs, PEM, vitamin deficiency, antigenic stimulation, including infectious

agents), then the possibility cannot be excluded that the evidence which is claimed to prove MCT of HIV may represent nothing more than effects caused by the above factors and not HIV.

5.5   Conclusion

Many factors affect the putative MCT. An approach to MCT taking into consideration these factors will lead not only to a decrease in MCT but also to general improvement in well-being of both mothers and children.

PART VI
GENERAL DISCUSSION

6.1   HIV Tests

In 1983 Montagnier and his colleagues claimed to have proven the existence of a new, unique retrovirus and of its constituent proteins and RNA by isolating (purifying) retroviral particles. However, no proof of purification was presented. In July 1997 Montagnier admitted that, despite their 1983 claims, he and his colleagues did not purify HIV and in fact the material which they called "purified" virus did not contain even particles "with the morphology typical of retroviruses". Also in 1997 the first electron micrographs of "purified HIV" were published establishing that "purified HIV" is particulate material consisting overwhelmingly of cellular fragments in which are interspersed a small number of particles whose morphology more resembles that of retrovirus particles than the predominant particles but none of which have all the structural features of retrovirus particles. The fact that particles similar to the latter are observed in material from "non-infected" cultures obtained in the same manner as the "purified HIV", and that this material consists of the same proteins (quantitative differences aside) as the "purified" virus, demonstrates beyond reasonable doubt that "purified HIV" is devoid of retroviral particles, proteins and RNA. Yet from this material proteins and RNA have been selected and employed as antigens in antibody tests and primers and probes in PCR and hybridisation experiments to prove humans infected with a lethal retrovirus.

If the RNA that has been arbitrarily selected from the 1.16 gm/ml band is the genome of an exogenous retrovirus, then:

(i)   there must be evidence to prove the existence of a unique molecular entity "HIV RNA", and a corresponding fragment of DNA ("HIV DNA") which has a unique length and unique nucleic acid sequences;

(ii)   when the full length of "HIV RNA" or "HIV DNA" is used for hybridisation studies all infected people should give a positive result. That is, if the "HIV RNA" is the genome of an exogenous virus which infects individuals who have AIDS or those at risk then the full length of this RNA (cDNA) should be present in fresh uncultured tissue from all these individuals and in nobody else.

This is not the case. From the beginning of the HIV era it became obvious that no two HIV genomes are identical, not even from the same individual.[483-487]   While the genomes of the most variable RNA viruses do not differ by more than 1%[488] and the difference between the human and the chimpanzee genomes is no more than 2%, there is up to 40% variation between "HIV" genomes.[489]

Gallo and his colleagues were the first to report hybridisation studies using fresh lymphocytes from AIDS patients and those at risk. Summarising their finding they wrote: "We have previously been able to isolate HTLV-III from peripheral blood or lymph node tissue from most patients with AIDS or ARC" (they "isolated" it from approximately 50% of patients). "However, as shown herein, HTLV-III DNA is usually not detected by standard Southern Blotting hybridisation of these same tissues and, when it is, the bands are often faint…the lymph node enlargement commonly found in ARC and AIDS patients cannot be due directly to the proliferation of HTLV-III-infected cells…the absence of detectable HTLV-III sequences is Kaposi's sarcoma tissue of AIDS patients suggests that this tumor is not directly induced by infection of each tumor cell with HTLV-III…the observation that HTLV-III sequences are found rarely, if at all, in peripheral blood mononuclear cells, bone marrow, and spleen provides the first direct evidence that these tissues are not heavily or widely infected with HTLV-III in either AIDS or ARC".[490]   These findings were confirmed by many other researchers. The finding that when the results were positive the hybridisation bands were "faint", "low signal" was interpreted as proof that HIV seropositive individuals contain HIV DNA in small numbers of cells and at low copy numbers, an interpretation which became generally accepted although Gallo and his colleagues had an alternative explanation. "Theoretically, this low signal intensity could also be explained by the presence of virus distantly homologous to HTLV-III in these cells".[490]   By 1994 Gallo admitted "We have never found HIV DNA in the tumor cells of KS…In fact we have never found HIV DNA in T-cells".[280]

To improve detection PCR was introduced. However:

(a)   "a striking feature of the results obtained" with this method, as with the standard hybridisation technique, "is the scarcity or apparent absence of viral DNA in a proportion of patients";[484]

(b)   there is no proof that the PCR amplifies the "HIV RNA" (DNA).

113

In other words, even at present there is no proof for the existence of the HIV genome and thus of HIV.

Although practically everybody accepts that the existence of HIV proteins and nucleic acids has been proven, the usefulness of every laboratory test presently employed to prove HIV infection has been questioned by the HIV experts themselves. Yet these tests have been used in the epidemiological studies in which it is claimed that a unique retrovirus, HIV, is transmitted from the mother to the child.

6.2   Epidemiological Evidence
In the epidemiological evidence of MCT the most important features are the following:

6.2.1   Racial distribution
With negligible exception the children who are said to have been infected by their mothers are Black and to a lesser extent Hispanic, that is, these are children born to poor women. According to Dr. Helene Gayle Director, National Center for HIV, STD and TB Prevention Centers for Disease Control and Prevention, over 95% of the total estimated HIV/AIDS cases are in the developing world. "Sub-Sahara Africa also has more children infected with HIV than any other region of the world, accounting for 87% of the cumulative total".[4] In the United States, of all the AIDS cases reported in children through June 2000, 17% were white, 58.6% black, 22.9% Hispanic and the rest other minorities. In other words AIDS cases in children appear limited to poor mothers.

6.2.2   Low number of Paediatric AIDS cases
Given that,

(a) as far as the HIV experts are concerned, HIV and thus AIDS originated in Africa. According to Gallo, in 1973 67% of Ugandan children were infected with HIV;[58]

(b) no drug has ever been found to eradicate HIV and in most of the world including sub-Saharan Africa, even today no anti-retroviral drugs are used;

(c) safe sex education was introduced only in the second half of the 1980s;

(d) "Prior to HAART, in the most developed nations about 60% of HIV-infected adults were expected to progress to AIDS within 12-13 years after becoming infected. There is less information about the natural history of HIV in less developed countries but it is possible that progress from HIV infection to AIDS is more rapid in those countries and could be related to: poverty; lack of access to health care, including limited resources for prevention and care; poor nutrition; poor underlying health status; or characteristics of immune function, among other factors";[4]

by now no sub-Saharan African child should be living. Yet, the number of AIDS cases in children, in general, and sub-Saharan Africa are still relatively low.

According to the WHO a minimum of 330,000 and a maximum of 670,000 children in the world died from AIDS in 1999. The reported WHO estimated number for Australia and New Zealand is <100 (in Australia the cumulative total till September 30th 2000 is 32 deaths from AIDS in children under the age of 13 years). No estimated deaths in children are reported from Eastern Europe and Central Asia, Western Europe, and with the exception of Cyprus (<100) and Israel (<100) from North Africa and Middle East. The reported estimated 1999 deaths in Canada were <100 and in the United States a minimum of 250 and a maximum of 380. (In fact the cumulative children deaths from AIDS in the USA till June 2000, is 8804[460]). The estimates for Ethiopia were 35,000–91,000, for Nigeria 41,000-64,000, South Africa 36,000-74,000 and for Uganda 18,000-32,000. Although the estimated deaths in Africa, especially in sub-Saharan Africa are high, they are only estimates and furthermore they are insignificant when compared with the deaths from other causes. For example, about 8 million people develop tuberculosis each year with a resulting 3 million deaths. The vast majority of these cases occur in developing countries.[118]

According to the WHO there are 300-500 million clinical cases of malaria each year and more than 90% of all cases occur in sub-Saharan Africa.[461] "Most of the 1-3 million who die from malaria are children, mainly in Africa, which is hyperendemic for malaria…The mortality rate is highest during the first two years of life".[462] "Diarrhoea is a leading cause of illness and death among children in developing countries, where an estimated 1.3 thousand million episodes and 4 million deaths occur each year in under fives. Worldwide, these children

experience an average of 3.3 episodes each year, but in some areas the average exceeds nine episodes each year. Where episodes are frequent, young children may spend more than 15% of their days with diarrhoea. About 80% of deaths due to diarrhoea occur in the first two years of life".[463] Malnutrition is widespread in tropical and subtropical regions. An estimated 80-1100 million children are malnourished throughout the world. Of these, 40,000 die each day".

6.2.3   Causes of death in HIV infected children

Most, if not all, experts in MCT of HIV agree that the diseases of which HIV infected children die are the same diseases of which HIV non-infected children die. In particular, African HIV infected children die from the "common" causes in that continent; namely, TB, diarrhoea, pneumonia and malnutrition. Indeed, the signs and symptoms which are considered to signify death from AIDS in the Bangui definition are signs and symptoms of TB, malaria, gastrointestinal, parasitic infections and malnutrition. In other words and as African physicians attest, clinically it is not possible to distinguish between death from AIDS or other "common" African diseases.

Significantly,

1. the vast majority of childhood deaths from non-AIDS diseases are in the first few years;

2. if AIDS was caused by HIV then the death rate in infected children, at least in the absence of antiretroviral treatment, should increase or at least remain constant until all the children have succumbed. Yet in children who survive the first year of life "the disease progresses more slowly and most children remain stable or even improve during the second year"[233] or improve their health status instead of deteriorating even without antiretroviral treatment.

If every child who dies and satisfies the Bangui or even the latest, 1994, AIDS definition for children then the vast majority, if not all children who die in the developing world will die from AIDS and no other disease, including TB, malnutrition, malaria, pneumonia and diarrhoea. To assist in the differential diagnosis some researchers advocate the use of antibody testing to confirm deaths from AIDS. However,

1. the specificity of the HIV antibody test has never been determined. Even if proof existed that the tests are highly specific in some groups of individuals, this cannot be the case in children who are exposed to a plethora of antigens. At least some, if not all the antibodies directed against these antigens, will react with the proteins in the HIV test kits. Furthermore, since what the HIV experts call, "HIV isolation" (culture results) is, like the antibody test, an antigen antibody reaction, the same applies to the culture results.

2. Neither is it possible to use the PCR test results to differentiate between deaths for "common" diseases and AIDS. This is because:

    (a) There is no proof that the primers and probes used in this test originated from retroviral ("HIV") particles. In fact it is highly likely that they are cellular RNA (cDNA);[11,12,31]

    (b) Even if the primers and probes are accepted to be HIV, there is no proof that the PCR specifically amplifies the HIV RNA (DNA).

Furthermore, the presently available data show that a positive PCR result, at least as far as a quantitative PCR test is concerned, is the result of antigenic stimulation. In other words the positive PCR and increased expression of "HIV" RNA (DNA) ("HIV activation"), is the result not the cause of the "common" diseases.

The only way to differentiate between the common diseases and AIDS is to isolate/purify HIV from the patients deemed to have died from AIDS. However, to date nobody not even Luc Montagnier and his associates, who are credited to have been the first group to have achieved HIV isolation/purification, has managed to do so.[10-12] Even if a retrovirus were to be isolated from some children, one would have to prove that the retrovirus is pathogenic and not a harmless endogenous retrovirus present "in all of us"[72] including those present in and "purified" from the human placenta.[221,222,224-226]

If in the AIDS era the are two reasons for the "common" diseases and deaths of children in the developing countries—poverty and HIV, and if the effects of HIV are as devastating as it is claimed, then at least proof must exist that the death rates in the AIDS era are substantially higher than the baseline mortality, that is, mortality before the AIDS era.

A paper entitled "The impact of HIV-1 infection on mortality in children under 5 years of age in sub-Saharan Africa: a demographic and epidemiologic analysis" was published in 1994 by researchers from the Communicable Disease Surveillance Centre, Public Health Laboratory Service and London School of Hygiene and Tropical Medicine, the Centre for Population Studies, London School of Hygiene and Tropical Medicine, London, the United Nations Children Fund (UNICEF), Iringa, the Designated District Hospital, Sumve and the Department of Epidemiology and Biostatics, Muhimili University College of Health Sciences, Muhimbili, Tanzania. Discussing their findings the authors of this study wrote: "It is important to appreciate that even if the highest [30%] current prevalences of HIV-1 in Africa were found among all women of childbearing age, HIV would still only account for a minority of child deaths and rank some way behind mortality associated with respiratory tract infections and diarrhoeal diseases. Similarly, HIV is not the only prevalent lethal congenital infection. Syphilis is a massive source of fetal wastage and infant death in Africa. Our calculations suggest that the HIV-1 epidemic is unlikely to overwhelm most existing differentials between African countries in the level of child mortality. Countries with relatively low child mortality in the 1980s are likely to remain so in the future…There are recent reports of increased child mortality in a number of African countries severely affected by HIV-1. However, these are open to different interpretations and more detailed studies are needed".[1]

In 1998, a more detailed study was conducted by one of the authors of the 1994 paper, Ian Timæus, from the Centre for Population Studies, London School of Hygiene and Tropical Medicine. In this study entitled "Impact of the HIV epidemic on mortality in sub-Saharan Africa: evidence from national surveys and censuses" and supported, in part, by The World Bank and the United Kingdom Department of International Development, Timæus pointed out:

1. "By now one would expect all-cause mortality to be rising across a large part of the continent".

2. "The only way to obtain such data is to measure mortality and, in particular, the mortality of adults".

3. "Estimates of mortality in the 1990s are very difficult to interpret in countries where earlier data are lacking: high mortality may reflect the enduring impact of underdevelopment, not the spread of HIV. HIV infection is not the only reason why mortality decline in Africa may have slowed or been reversed in recent years. Other infectious diseases, such as chloroquine-resistant malaria, may also be a factor. Moreover, economic difficulties have afflicted most African countries since the 1970s. Output per head in the region as a whole fell between the end of the 1970s and mid-1990s. Only a handful of countries, notably Botswana, experienced significant growth. Economic decline and adjustment programmes designed to reduce inflation and budgetary deficits have had an adverse impact on social development programmes. The health sector has been affected particularly severely. Government expenditure on health services in many African countries has not grown since the beginning of the 1980s, reducing the resources per head of the population devoted to health".

In this study mortality trends were assessed in three ways:

"(i) by comparing of data collected in the 1990s, with those from the 1980s;

(ii) using retrospective reports of the survival of women's children and siblings collected by Demographic and Health Survey [DHS] enquiries;

(iii) by comparing the latter estimates with estimates from data on orphanhood".

As far as mortality trends in children are concerned Timaeus reported: "The overall impression is that under-five mortality continued to decline in Africa during the 1980s. After stagnating in the early 1980s, under-five mortality in Ghana began to fall again in the second half of the decade. Moreover, mortality may have fallen particularly rapidly in some of the higher mortality countries. Nevertheless, quite widespread evidence exists of a slowdown in the rate of mortality decline in recent years. First, improvements in child survival seem to have tapered off at some point during the 1980s in a number of the lower mortality countries including Botswana, the Central African Republic, Côte d'Ivoire, Kenya, Togo and Zimbabwe. The rate of decline in mortality was also slow in several high mortality countries, namely Malawi, Liberia and Niger. In Nigeria, no improvement occurred in under-five mortality between the late 1970s and the late 1980s. The most worrying development, however, is in Zambia where both DHS and census data suggest that infant and child mortality rose substantially during the 1980s.

Can such adverse trends in the under-five mortality rate be attributed to the HIV epidemic or are other factors also important? One can attempt to assess this because estimation of the impact of the HIV epidemic on infant and child mortality is simpler than estimation of its impact on adult mortality. First, data on the prevalence of HIV infection collected at antenatal clinics directly measure the proportion of births at risk of vertical

transmission of the virus. Second, most African infants who acquire HIV infection from their mothers die in the first 5 years of their life. Thus, the mortality impact of a rise in the prevalence of HIV infection is lagged by only a couple of years".

Timæus, then makes general assumptions regarding the prevalence of HIV in pregnant women and the rate of MCT. "If about one-third of infected women giving birth transmit HIV to their child, one would expect each 1% rise in seroprevalence among women attending antenatal clinics to raise the under-five mortality rate by about 3 per 1000. More sophisticated modelling of the impact of the HIV epidemic on infant and child mortality suggests that this is a robust approximation. Thus, in countries where the seroprevalence rate among pregnant women is 6.5%, the under-five mortality rate could rise by about 20 per 1000. If the seroprevalence rate rises to 20%, an increase in under-five mortality of nearly 60 per 1000 is likely to follow". And although he points out that: "HIV seroprevalence data have done much to reduce uncertainty about the impact on mortality of HIV in Africa. Modelling alone, however, cannot determine how many Africans are dying of AIDS. The epidemiological data on how rapidly the epidemic has spread, on who has become infected, and on their survival after infection are too limited to produce useful estimates for particular countries of how much mortality has risen, at what ages, and who has been affected most". Using his assumption he calculates by how much mortality could have risen in some African countries. "Such calculations suggest that the HIV epidemic may be responsible for the slowdown or reversal of the decline in infant and child mortality in some African countries. In Malawi, Rwanda, Uganda, Zambia, and Zimbabwe, HIV may have become sufficiently prevalent by the mid-1980s to account for rises in the under-five mortality rate of 15-20 per 1000 by the late 1980s. By the early 1990s, paediatric AIDS mortality in these five countries could have risen to 25-30 per 1000 births. HIV could also have driven up the most recent mortality estimates available for Burkina Faso, the Central African Republic, Côte d'Ivoire, and Kenya by 10-20 per 1000. In all these countries, a proportion of these additional deaths will not be reflected in the birth history data because the children's mothers have also died of HIV-related disease.

Thus, the impact of HIV could account for the adverse trends in under-five mortality in Kenya and Zimbabwe during the early 1990s revealed by the most recent DHS surveys. Moreover, the HIV epidemic might also account for the slow decline in early-age mortality in Malawi in the 1980s and the large rise in mortality in Zambia. In Zambia at least, however, it seems likely that the overall under-five mortality rate rose by so much only because other factors were not exerting a strong downward influence on mortality. Infant and child mortality probably would not have improved much in Zambia during the 1980s and early 1990s even without the HIV epidemic.

Elsewhere, it is unlikely that the HIV epidemic is responsible for the adverse trend in the under-five mortality rate and the explanation of it must be sought elsewhere. For example, AIDS cannot explain the stagnation or rise in early-age mortality in Nigeria since the 1970s. Similarly, it is unlikely that the slowdown in the early 1980s of mortality decline in Botswana, Côte d'Ivoire and the Central African Republic is related to HIV. However, the epidemic might be implicated in the continuing lack of improvement during the early 1990s in child survival in the latter two countries.

It is worth emphasising that infant and child mortality fell in Uganda in the early 1990s despite the severity of the HIV epidemic in this country. Presumably developments acting to improve child health have outweighed the impact on mortality of the spread of HIV. Thus, these estimates suggest that the HIV epidemic exerts an important but not decisive influence on trends in infant and child mortality. Without additional data, one cannot separate the impact of AIDS on infant and child mortality from that of other factors".[464]

In other words, in Africa no proof exists of an increased mortality in children above that reflected by the "enduring impact of under-development" resulting from HIV infection, not even in Uganda, where no less an authority on HIV and AIDS than Robert Gallo reported that as far back as 1973, 50/75 (67%) of a sample of 75 children were infected with HIV.[58] This means that a similar proportion of mothers and presumably fathers in Uganda would have also been infected in 1973. If the HIV antibody tests do prove HIV infection and if HIV is the cause of AIDS one should have witnessed an inexorable decline in the Ugandan population over the past twenty years. Instead "The population in Uganda has increased from the 4.9 million enumerated in the 1948 census to 6.5 million in 1959; 9.5 million by 1969; 12.6 million by 1980; and 16.7 million were enumerated at the 1991 census. Uganda's population is growing at a rate of 2.5 per cent which leads to an estimated population of 21 million people by 1998. It is estimated that 47 per cent of the population is under age 15, while only 3 per cent are above 65 years. Thus the population is young and has in-built potential to grow (momentum) as the large proportion of children become parents".[465]

These data amply justify scientific scrutiny by both individuals and governments as to what relationship, if any, exists between the development of illness and the "virus that never was".[466]

6.3   Conclusion

The one necessary and sufficient measure to decrease childhood mortality in the developing world, including deaths from "AIDS", as well as the phenomena claimed to prove HIV infection and thus the putative mother-to-child transmission of "HIV", is to eliminate poverty.

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APPENDIX I
The WHO Bangui Definition 1986

Source: World Health Organisation, 1986. Acquired Immunodeficiency Syndrome (AIDS) WHO/CDC case definition for AIDS. Weekly Epidemiology Record 61:69-76.

A *clinical* case definition is needed in countries where diagnostic resources are limited. A provisional case definition was developed at a WHO workshop on AIDS held in Bangui, Central African Republic, 22-24 October 1985. This definition was reviewed and slightly adapted at the Second Meeting of the WHO Collaborating Centres on AIDS as follows:

Adults
>AIDS in an adult is defined by the existence of at least 2 of the major signs associated with at least 1 minor sign, in the absence of known causes of immunosuppression such as cancer or severe malnutrition or other recognized etiologies.

1. Major signs
>(a) weight loss ≥ 10% of body weight;
>(b) chronic diarrhoea > 1 month;
>(c) prolonged fever > 1 month (intermittent or constant);

2. Minor signs
>(a) persistent cough for > 1 month;
>(b) generalised pruritic dermatitis;
>(c) recurrent herpes zoster;
>(d) oro-pharyngeal candidiasis;
>(e) chronic progressive and disseminated herpes simplex infection;
>(f) generalised lymphadenopathy.

The presence of generalised Kaposi's sarcoma or cryptococcal meningitis are sufficient by themselves for the diagnosis of AIDS.

Children
>Paediatric AIDS is suspected in an infant or child presenting with at least 2 of the following major signs associated with at least 2 of the following minor signs in the absence of known cases of immunosuppression such as cancer of severe malnutrition or other recognized etiologies.

1. Major signs
>(a) weight loss or abnormally slow growth;
>(b) oro-pharyngeal candidiasis;
>(c) repeated common infections (otitis, pharyngitis, etc.);
>(d) persistent cough;
>(e) generalised dermatitis;
>(f) confirmed maternal LAV/HTLV-III infection.

(Note: Under the Bangui definition tests for HIV antibodies or tests of immunological function are not required).

The Ghent Definitions of Paediatric AIDS 1992

Source: Dabis F, Msellati P, Dunn D, et al. (1993). Estimating the rate of mother-to-child transmission of HIV. Report of a workshop on methodological issues Ghent (Belgium), 17-20 February 1992. The Working Group on Mother-to-Child Transmission of HIV. *AIDS* 7:1139-48.

Table 1. World Health Organization case definition for paediatric AIDS* used for the Ghent classification of paediatric HIV infection, 1992

WHO clinical case definition Paediatric AIDS	The Modified WHO clinical case definition for paediatric AIDS
Major signs	**Major signs**
Weight loss or failure to thrive	Weight loss or failure to thrive
Chronic diarrhoea (>1 month)	Chronic diarrhoea (> 1 month)
Prolonged fever (> 1 month)	Prolonged fever (> 1 month)
	Severe or repeated pneumonia
Minor signs	**Minor signs**
Generalised lymphadenopathy	Generalised lymphadenopathy
Oro-pharyngeal candidiasis	Oro-pharyngeal candidiasis
Repeated common infections	Repeated common infections
Persistent cough	Persistent cough
Generalised dermatitis	Generalised pruritic dermatitis
Confirmed maternal HIV infection	Confirmed maternal HIV infection

*With both definitions, paediatric AIDS is suspected in a child presenting with a least two major signs and two minor signs in the absence of known causes of immunosuppression.

Table 2. HIV-related signs and symptoms in children born to HIV-seropositive mothers used for the Ghent classification of paediatric HIV infection, 1992.

Signs
Persistent diarrhoea (≥ 15 days)
Oral candidiasis (beyond the neonatal period)
Generalized lymphadenopathy (enlarged lymph nodes in at least two independent anatomic sites)
Failure to thrive (no weight gain for a period of 3 months or crossing two percentiles lines in the growth chart)
Chronic parotitis (> 1 month)
Herpes zoster infection ('shingles')
Recurrent pneumonia (two or more episodes)

Table 3. Definition for children born to HIV-seropositive mothers who died before infection status could be determined by serology. Ghent classification, 1992.

Probable HIV-related death	Probable non-HIV-related death	Death with indeterminate relation to HIV infection
Either AIDS*	No HIV-related sign/symptom** when last seen	All deaths occurring within the first 4 weeks of life
Or	And	Or
At least one HIV-related signs/symptom when last seen	Dying from cause other than severe infection[†] or persistent diarrhoea after the first 4 weeks of life	Beyond this period, all the deaths not classified above
And		
Dying from severe infection or persistent[†] diarrhoea beyond the first 4 weeks of life[‡]		

* See definition in table 1. ** See definition in Table 2. [†] It may be necessary to establish a list of severe paediatric infections for this purpose. [‡]Children who died from persistent diarrhoea but do not present any other HIV-related sign/symptom should be considered as a death with indeterminate relation to HIV infection.

Table 4. Ghent classification* of children born to HIV-seropositive mothers according to their probable HIV infection status, 1992.

HIV-infected	HIV-non-infected	Indeterminate HIV infection status
HIV WB-antibody positive at 15 months	HIV WB-serum-antibody-negative at 15 months	Death before 15 months with indeterminate relation to HIV infection (see definition in Table 3)
or	Or	or
HIV-related death (see definition in Table 3)	HIV WB-negative ≥ 9 months in a child lost to follow-up without AIDS	Child died of probable not HIV-related cause while WB-positive or indeterminate before 15 months or WB-negative < 9 months (when last seen)
or	Or	or
AIDS (see definition in Table 1)	HIV WB negative ≥ 9 months in a child who died from probable non-HIV-related cause (see definition in Table 3)	Child lost to follow-up while WB-positive or indeterminate before 15 months or WB-negative < 9 months (when last seen)
		or
		Child with indeterminate WB and alive at 15 months

This classification is designed to be used in the estimation of the rate of mother-to-child transmission of HIV and the comparisons between studies and not in the management of children born to HIV-seropositive mothers. The use of this classification must be restricted to a cohort of children born to HIV-seropositive mothers, all of whom were born more than 15 months before analysis. All children of the cohort should be accounted for using the above classification. In those studies where other methods of diagnosis such as viral culture are used, the results of these methods should be described if modifying the classification, but should be ignored for the purpose of comparisons. WB, Western Blot.

CDC 1987 Classification System for HIV Infection in Children under 13 years of age

Source: Classification system for human immunodeficiency virus (HIV) infection in children under 13 years of age. *Morbidity and Mortality Weekly Reports* 1987;36**:**225-30, 235-6.

Infants and children under 15 months of age with perinatal infection – infection in infants and children up to 15 months of age who were exposed to infected mothers in the perinatal period may be defined by one or more of the following:

1) the identification of the virus in blood or tissues,
2) the presence of HIV antibody as indicated by a repeatedly reactive screening test (e.g., enzyme immunoassay) plus a positive confirmatory test (e.g., Western blot, immunofluorescence assay) in an infant or child who has abnormal immunologic test results indicating both humoral and cellular immunodeficiency (increased immunoglobulin levels, depressed T4 (T-helper) absolute cell count, absolute lymphopenia, decreased T4/T8 ratio) and who meets the requirements of one or more of the subclasses listed under class P-2 (described below), or
3) the confirmation that a child's symptoms meet the previously published CDC case definition for pediatric AIDS (1,2)

Classification System

Children fulfilling the definition of HIV infection discussed above may be classified into one of two mutually exclusive classes based on the presence or absence of clinical signs and symptoms. Class Pediatric-1 (P-1) is further subcategorized on the basis of the presence or absence of immunologic abnormalities, whereas Class P-2 is subdivided by specific disease patterns. Once a child has signs and symptoms and is therefore classified in P-2, he or she should not be reassigned to class P-1 if signs and symptoms resolve.

Perinatally exposed infants and children whose infection status is indeterminate are classified into class P-0.

Class P-0. Indeterminate infection. Includes perinatally exposed infants and children up to 15 months of age who cannot be classified as definitely infected according to the above definition but who have antibody to HIV, indicating exposure to a mother who is infected.

Class P-1. Asymptomatic infection. Includes patients who meet one of the above definitions for HIV infection but who have had no previous signs or symptoms that would have led to classification in Class P-2.

Subclass A – Normal immune function. Includes children with no immune abnormalities associated with HIV infection.

Subclass B – Abnormal immune function. Includes children with one or more of the commonly observed immune abnormalities associated with HIV infection, such as hypergammaglobulinemia, T-helper (T4) lymphopenia, decreased T-helper/T-suppressor (T4/T8) ratio, and absolute lymphopenia.
Other causes of these abnormalities must be excluded.

Subclass C – Not tested. Includes children for whom no or incomplete (see above) immunologic testing has been done.

Class P-2. Symptomatic infection. Includes patients meeting the above definitions for HIV infection and having signs and symptoms of infection. Other causes of these signs and symptoms should be excluded. Subclasses are defined based on the type of signs and symptoms that are present. Patients may be classified in more than one subclass.

Subclass A – Nonspecific findings. Includes children with one or more of the unexplained nonspecific findings persisting for more than 2 months, including fever, failure-to-thrive or weight loss of more than 10% of baseline, hepatomegaly, splenomegaly, generalized lymphadenopathy (lymph nodes measuring at least 0.5 cm present in two or more sites, with bilateral lymph nodes counting as one site), parotitis, and diarrhea (three or more loose stools per day) that is either persistent or recurrent (defined as two or more episodes of diarrhea accompanied by dehydration within a 2-month period).

Subclass B – Progressive neurologic disease. Includes children with one or more of the following progressive findings: 1) loss of developmental milestones or intellectual ability, 2) impaired brain growth (acquired microcephaly and/or brain atrophy demonstrated on computerized tomographic scan or magnetic resonance

imaging scan), or 3) progressive symmetrical motor deficits manifested by two or more of these findings: paresis, abnormal tone, pathologic reflexes, ataxia, or gait disturbance.

Subclass C – Lymphoid interstitial pneumonitis.   Includes children with a histologically confirmed pneumonitis characterized by diffuse interstitial and peribronchiolar infiltration of lymphocytes and plasma cells and without identifiable pathogens, or, in the absence of a histologic diagnosis, a chronic pneumonitis – characterized by bilateral reticulonodular interstitial infiltrates with or without hilar lymphadenopathy – present on chest X-ray for a period of at least 2 months and unresponsive to appropriate antimicrobial therapy.   Other causes of interstitial infiltrates should be excluded, such as tuberculosis, Pneumocystis carinii pneumonia, cytomegalovirus infection, or other viral or parasitic infections.

Subclass D – Secondary Infectious diseases.   Includes children with a diagnosis of an infectious disease that occurs as a result of immune deficiency caused by infection with HIV.

Category D-1. Includes patients with secondary infectious disease due to one of the specified infectious diseases listed in the CDC surveillance definition for AIDS: Pneumocystis carinii pneumonia; chronic cryptosporidiosis; disseminated toxoplasmoisis with onset after 1 month of age; extra-intestinal strongyloidiasis; chronic isosporiasis; candidiasis (esophageal, bronchial, or pulmonary); extrapulmonary cryptococcosis; disseminated histoplasmosis; noncutaneous, extrapulmonary, or disseminated mycobacterial infection (any species other than leprae); cytomegalovirus infection with onset after 1 month of age; chronic mucocutaneous or disseminated herpes simplex virus infection with onset after 1 month of age; extrapulmonary or disseminated coccidiodomycosis; nocardiosis, and progressive multifocal leukoencephalopathy.

Category D-2.   Includes patients with unexplained, recurrent, serious bacterial infections (two or more within a 2-year period) including sepsis, meningitis, pneumonia, abscess of an internal organ and bone/joint infections.

Category D-3.   Includes patients with other infectious diseases, including oral candidiasis persisting for 2 months or more, two or more episodes of herpes stomatitis within a year, or multidermatomal or disseminated herpes zoster infection.

Subclass E – Secondary Cancers.   Includes children with any cancer described below in categories E-1 and E-2.
Category E-1. Includes patients with the diagnosis of one or more kinds of cancer known to be associated with HIV infection as listed in the surveillance definition of AIDS and indicative of a defect in cell-mediated immunity: Kaposi's sarcoma, B-cell non-Hodgkin's lymphoma, or primary lymphoma of the brain.

Category E-2. Includes patients with the diagnosis of other malignancies possibly associated with HIV infection.

Subclass F – Other diseases.   Includes children with other conditions possibly due to HIV infection not listed in the above subclasses, such as hepatitis, cardiopathy, nephropathy, hematologic disorders (anemia, thrombocytopenia), and dermatologic diseases.

CDC 1994 Revised Classification System for HIV Infection in Children under 13 years of age

Source: 1994 Revised Classification System for Human Immunodeficiency Virus Infection in Children Less Than 13 Years of Age. *Mortality and Morbidity Weekly Reports* 1994;43 (RR-12)**:**1-10.

Summary
This revised classification system for human immunodeficiency virus (HIV) infection in children replaces the pediatric HIV classification system published in 1987 (1). This revision was prompted by additional knowledge about the progression of HIV disease among children. In the new system, infected children are classified into mutually exclusive categories according to three parameters: a) infection status, b) clinical status, and c) immunologic status. The revised classification system reflects the stage of the child's disease, establishes mutually exclusive classification categories, and balances simplicity and medical accuracy in the classification process. This document also describes revised pediatric definitions for two acquired immunodeficiency syndrome-defining conditions.

INTRODUCTION

Following the initial report in 1982 of acquired immunodeficiency syndrome (AIDS) in children (2), it became evident that the clinical characteristics of AIDS in children were different from those in adults. In 1987, CDC published a classification system for children infected with human immunodeficiency virus (HIV) (1), the causative agent of AIDS. This classification system categorized clinical manifestations of HIV infection in children based on the limited data available early in the epidemic. New knowledge about the progression of HIV disease among children warranted revision of the 1987 classification system to better reflect the disease process.

In 1991, CDC convened a working group of Public Health Service and other consult-ants to discuss revision of the pediatric HIV classification system. The 1994 revised classification system was developed through ongoing collaborations with the consult-ants following the 1991 meeting. The goal of the working group was to construct a revised system that would:

reflect the stage of disease for an HIV-infected child (i.e., the child's placement in the classification should have prognostic significance);

establish mutually exclusive classification categories; and

balance simplicity and medical accuracy in the classification process.

In the new system (Table 1), HIV-infected children are classified into mutually exclusive categories according to three parameters: a) infection status, b) clinical status, and c) immunologic status. Once classified, an HIV-infected child cannot be reclassified in a less severe category even if the child's clinical or immunologic status improves.

DIAGNOSING HIV INFECTION IN CHILDREN

Diagnosis of HIV infection in children born to HIV-infected mothers (Box 1) is complicated by the presence of maternal anti-HIV IgG antibody, which crosses the placenta to the fetus. Virtually all these children are HIV-antibody positive at birth, although only 15%–30% are actually infected. In uninfected children, this antibody usually becomes undetectable by 9 months of age but occasionally remains detect-able until 18 months of age. Therefore, standard anti-HIV IgG antibody tests cannot be used to indicate reliably a child's infection status before 18 months of age (3). Polymerase chain reaction (PCR) and virus culture are probably the most sensitive and specific assays for detecting HIV infection in children born to infected mothers (4–6). Use of these assays can identify approximately 30%–50% of infected infants at birth and nearly 100% of infected infants by 3–6 months of age (7).

The standard p24-antigen assay is less sensitive than either virus culture or PCR, especially when anti-HIV antibody levels are high, because it fails to detect immune-complexed p24 antigen (8). However, modification of the p24-antigen assay to dissociate immune complexes has increased its sensitivity in diagnosing HIV infection among children exposed to HIV (9).

Other laboratory assays (e.g., anti-HIV IgA and ELISPOT/in vitro antibody production [IVAP]) have not been included in the algorithm for determining infection status because they are not commonly used. In addition, they are less sensitive than both PCR or virus culture. However, clinicians who determine a child's antiretroviral therapy on the basis of such assays may use them to classify the child as being infected.

Some children develop severe clinical conditions resulting from HIV infection before their infection status has been sufficiently established. For the purposes of classification, a child meeting the criteria for AIDS in the 1987 case definition (10) should be considered HIV-infected—even in the absence of definitive laboratory assays.

Children born to mothers with HIV infection are defined as seroreverters (SRs) and are considered uninfected with HIV if they a) become HIV-antibody negative after 6 months of age, b) have no other laboratory evidence of HIV infection, and c) have not met the AIDS surveillance case definition criteria (Box 1). Sufficient data are not available to conclusively define a child who is uninfected on the

TABLE 1. Pediatric human immunodeficiency virus (HIV) classification*

Immunologic Categories	Clinical categories			
	N: No signs/ symptoms	A: Mild signs/ symptoms	B: † Moderate Signs/symptoms	C: † Severe signs/symptoms
1: No evidence of suppression	N1	A1	B1	C1
2: Evidence of moderate suppression	N2	A2	B2	C2
3: Severe suppression	N3	A3	B3	C3

*Children whose HIV infection status is not confirmed are classified by using the above grid with a letter E (for perinatally exposed) placed before the appropriate classification code (e.g., EN2).
† Both Category C and lymphoid interstitial pneumonitis in Category B are reportable to state and local health departments as acquired immunodeficiency syndrome.

BOX 1. Diagnosis of human immunodeficiency virus (HIV) infection in children*

DIAGNOSIS: HIV INFECTED
a) A child <18 months of age who is known to be HIV seropositive or born to an HIV-infected mother **and:**
    has positive results on two separate determinations (excluding cord blood) from one or more of the following HIV detection tests:
    — HIV culture,
    — HIV polymerase chain reaction,
    — HIV antigen (p24),
                             or
   meets criteria for acquired immunodeficiency syndrome (AIDS) diagnosis based on the
   1987 AIDS surveillance case definition (10).

   b) A child ≥ 18 months of age born to an HIV-infected mother or any child infected by
   blood, blood products, or other known modes of transmission (e.g., sexual contact) who:
   is HIV-antibody positive by repeatedly reactive enzyme immunoassay (EIA) and
   confirmatory test (e.g., Western blot or immunofluorescence assay [IFA]);
   or
   meets any of the criteria in a) above.

DIAGNOSIS: PERINATALLY EXPOSED (PREFIX E)
   A child who does not meet the criteria above who:
   is HIV seropositive by EIA and confirmatory test (e.g., Western blot or IFA) and is <18
   months of age at the time of test;
   or
   has unknown antibody status, but was born to a mother known to be infected with HIV.

DIAGNOSIS: SEROREVERTER (SR)
  A child who is born to an HIV-infected mother and who:
  has been documented as HIV-antibody negative (i.e., two or more negative EIA tests
  performed at 6–18 months of age or one negative EIA test after 18 months of age);
  and
  has had no other laboratory evidence of infection (has not had two positive viral detection tests,
  if performed);
  and
  has not had an AIDS-defining condition.

*This definition of HIV infection replaces the definition published in the 1987 AIDS surveillance case definition (10).

basis of viral detection tests. However, in certain situations (e.g., clinical trials), negative viral detection tests may be used presumptively to exclude infection.

IMMUNOLOGIC CATEGORIES

The three immunologic categories (Table 2) were established to categorize children by the severity of immunosuppression attributable to HIV infection. CD4+ T-lymphocyte depletion is a major consequence of HIV infection and is responsible for many of the severe manifestations of HIV infection in adults. For this reason, CD4+ counts are used in the adult HIV classification system (11). However, several findings complicate the use of CD4+ counts for assessing immunosuppression resulting from HIV infection in children. Normal CD4+ counts are higher in infants and young children than in adults and decline over the first few years of life (12–16). In addition, children may develop opportunistic infections at higher CD4+ levels than adults (17–19). Although insufficient data exist to correlate CD4+ levels with disease progression at all age groups, low age-specific CD4+ counts appear to correlate with conditions associated with immunosuppression in children (12,17,20,21). Therefore, despite these complications, classification based on age-specific CD4+ levels appears to be useful for describing the immunologic status of HIV-infected children.

Fewer data are available on age-specific values for CD4+ T-lymphocyte percent of total lymphocytes than for absolute counts. However, the CD4+ T-lymphocyte percent has less measurement variability than the absolute count (22). To establish the age-specific values of CD4+ percent that correlate with the CD4+ count thresholds, CDC compiled data from selected clinical projects in the United States and Europe. The data included >9,000 CD4+ counts, with the corresponding CD4+ percent determinations, from both HIV-infected and uninfected children <13 years of age. Nonparametric regression modelling was used to establish the CD4+ percent boundaries that best correlated with the CD4+ count boundaries in the classification system.

The immunologic category classification (Table 2) is based on either the CD4+ T-lymphocyte count or the CD4+ percent of total lymphocytes. If both the CD4+ count and the CD4+ percent indicate different classification categories, the child should be classified into the more severe category. Repeated or follow-up CD4+ values that result in a change in classification should be confirmed by a second determination. Values thought to be in error should not be used. A child should not be reclassified to a less severe category regardless of subsequent CD4+ determinations.

TABLE 2. Immunologic categories based on age-specific CD4+ T-lymphocyte counts and percent of total lymphocytes

Immunologic category	Age of child					
	<12 mos		1–5 yrs		6–12 yrs	
	μL	(%)	μL	(%)	μL	(%)
1: No evidence of suppression	≥1,500	(≥25)	≥1,000	(≥25)	≥500	(≥25)
2: Evidence of moderate suppression	750–1,499	(15–24)	500–999	(15–24)	200–499	(15–24)
3: Severe suppression	<750	(<15)	<500	(<15)	<200	(<15)

CLINICAL CATEGORIES

Children infected with HIV or perinatally exposed to HIV may be classified into one of four mutually exclusive clinical categories based on signs, symptoms, or diagnoses related to HIV infection (Box 2). As with the immunologic categories, the clinical categories have been defined to provide a staging classification (e.g., the prognosis for children in the second category would be less favorable than for those in the first category).

Category N, **not symptomatic**, includes children with no signs or symptoms considered to be the result of HIV infection or with only one of the conditions listed in Category A, mildly symptomatic. Category N was separated from Category A partly because of the substantial amount of time that can elapse before a child manifests the signs or symptoms defined in Category B, moderately symptomatic. Also, more staging information can be obtained during this early stage of disease by separating Categories N and A. In addition, for children who have uncertain HIV-infection status (prefix E), Categories N and A may help to distinguish those children who are more likely to be infected with HIV (23) (i.e., children in Category EA may be more likely to be infected than children in Category EN).

Category B includes all children with signs and symptoms thought to be caused by HIV infection but not

specifically outlined under Category A or Category C, severely symptomatic. The conditions listed in Box 2 are examples only; any other HIV-related condition not included in Category A or C should be included in Category B. Anemia, thrombocytopenia, and lymphopenia have defined thresholds in the new classification system (23).

Category C includes all AIDS-defining conditions except lymphoid interstitial pneumonitis (LIP) (Box 3). Several reports indicate that the prognosis for children with LIP is substantially better than that for children who have other AIDS-defining conditions (21,24,25). Thus, LIP has been separated from the other AIDS-defining conditions in Category C and placed in Category B.

Signs and symptoms related to causes other than HIV infection (e.g., inflammatory or drug-related causes) should not be used to classify children. For example, a child with drug-related hepatitis or anemia should not be classified in Category B solely because these conditions may be associated with HIV infection. In contrast, a child with anemia or hepatitis should be classified in Category B when the condition is thought to be related to HIV infection. The criteria for diagnosing some conditions and determining whether a child's signs, symptoms, or diagnoses are related to HIV infection may not be clear in all cases, and therefore may require judgment of the clinicians and researchers using the classification system.

Categories in the 1987 pediatric HIV classification system can be translated into categories in the 1994 system in most cases (Box 4). Class P0 is now designated by the prefix "E," and Class P1 is now Class N. Children previously classified as P2A are now classified in more than one category, reflecting the different prognoses for children with different conditions included in the P2A category (e.g., children who have wasting syndrome have a worse prognosis than those who have lymphadenopathy).

BOX 2. Clinical categories for children with human immunodeficiency virus (HIV) infection

CATEGORY N: NOT SYMPTOMATIC
Children who have no signs or symptoms considered to be the result of HIV infection or who have only one of the conditions listed in Category A.

CATEGORY A: MILDLY SYMPTOMATIC
Children with two or more of the conditions listed below but none of the conditions listed in Categories B and C.
Lymphadenopathy ≥0.5 cm at more than two sites; bilateral = one site)
      Hepatomegaly
      Splenomegaly
      Dermatitis
      Parotitis
      Recurrent or persistent upper respiratory infection, sinusitis, or otitis media

CATEGORY B: MODERATELY SYMPTOMATIC
Children who have symptomatic conditions other than those listed for Category A or C that are attributed to HIV infection. Examples of conditions in clinical Category B include but are not limited to:
      Anemia (<8 gm/dL), neutropenia (<1,000/mm 3), or thrombocytopenia (<100,000/mm 3)
      persisting ≥30 days
      Bacterial meningitis, pneumonia, or sepsis (single episode)
      Candidiasis, oropharyngeal (thrush), persisting (>2 months) in children >6 months of age
      Cardiomyopathy
      Cytomegalovirus infection, with onset before 1 month of age
      Diarrhea, recurrent or chronic
      Hepatitis
      Herpes simplex virus (HSV) stomatitis, recurrent (more than two episodes within 1 year)
      HSV bronchitis, pneumonitis, or esophagitis with onset before 1 month of age
      Herpes zoster (shingles) involving at least two distinct episodes or more than one
      dermatome
      Leiomyosarcoma
      Lymphoid interstitial pneumonia (LIP) or pulmonary lymphoid hyperplasia complex
      Nephropathy
      Nocardiosis
      Persistent fever (lasting >1 month)
      Toxoplasmosis, onset before 1 month of age
      Varicella, disseminated (complicated chickenpox)

EFFECT ON THE AIDS SURVEILLANCE CASE DEFINITION
FOR CHILDREN

Because the classification system is used in conjunction with the AIDS case definition, the 1994 revision provided an opportunity to update certain features of the 1987 AIDS surveillance case definition for children <13 years of age (10). Although LIP is in Category B under the new pediatric HIV classification system, it will continue to be reportable to state and local health departments (along with the conditions in Category C) as an AIDS-defining condition in children. Two changes in the definitions for other conditions are summarized in the following bulletted text:

The new definitions for HIV encephalopathy and HIV wasting syndrome reflect increased knowledge of these conditions in children and replace the definitions published in the 1987 AIDS surveillance case definition for children. The definition of HIV encephalopathy follows the recommendations of the American Academy of Neurology AIDS Task Force (26). Because this condition is complex, diagnosis may require neurologic consultation.

The new definition of HIV infection (Box 1) replaces the definition for laboratory evidence of HIV infection in children used in the 1987 pediatric AIDS case definition. For children with an AIDS-defining condition that requires laboratory evidence of HIV infection, a single positive HIV-detection test (i.e., HIV culture, HIV PCR, or HIV antigen [p24]) is sufficient for a reportable AIDS diagnosis if the diagnosis is confirmed by a clinician.

BOX 3. Conditions included in clinical Category C for children infected with human immunodeficiency virus (HIV)

CATEGORY C: SEVERELY SYMPTOMATIC*

Serious bacterial infections, multiple or recurrent (i.e., any combination of at least two culture-confirmed infections within a 2-year period), of the following types: septicemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ or body cavity (excluding otitis media, superficial skin or mucosal abscesses, and indwelling catheter-related infections)
Candidiasis, esophageal or pulmonary (bronchi, trachea, lungs)
Coccidioidomycosis, disseminated (at site other than or in addition to lungs or cervical or hilar lymph nodes)
Cryptococcosis, extrapulmonary
Cryptosporidiosis or isosporiasis with diarrhea persisting >1 month
Cytomegalovirus disease with onset of symptoms at age >1 month (at a site other than liver, spleen, or lymph nodes)
Encephalopathy (at least one of the following progressive findings present for at least 2 months in the absence of a concurrent illness other than HIV infection that could explain the findings): a) failure to attain or loss of developmental milestones or loss of intellectual ability, verified by standard developmental scale or neuropsychological tests; b) impaired brain growth or acquired microcephaly demonstrated by head circumference measurements or brain atrophy demonstrated by computerized tomography or magnetic resonance imaging (serial imaging is required for children <2 years of age); c) acquired symmetric motor deficit manifested by two or more of the following: paresis, pathologic reflexes, ataxia, or gait disturbance
Herpes simplex virus infection causing a mucocutaneous ulcer that persists for >1 month; or bronchitis, pneumonitis, or esophagitis for any duration affecting a child >1 month of age
Histoplasmosis, disseminated (at a site other than or in addition to lungs or cervical or hilar lymph nodes)
Kaposi's sarcoma
Lymphoma, primary, in brain
Lymphoma, small, noncleaved cell (Burkitt's), or immunoblastic or large cell lymphoma of B-cell or unknown immunologic phenotype
Mycobacterium tuberculosis, disseminated or extrapulmonary
Mycobacterium, other species or unidentified species, disseminated (at a site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
Mycobacterium avium complex or Mycobacterium kansasii, disseminated (at site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
Pneumocystis carinii pneumonia
Progressive multifocal leukoencephalopathy
Salmonella (nontyphoid) septicemia, recurrent

Toxoplasmosis of the brain with onset at >1 month of age

Wasting syndrome in the absence of a concurrent illness other than HIV infection that could explain the following findings: a) persistent weight loss >10% of baseline OR b) downward crossing of at least two of the following percentile lines on the weight-for-age chart (e.g., 95th, 75th, 50th, 25th, 5th) in a child ≥1 year of age OR c) <5th percentile on weight-for-height chart on two consecutive measurements, ≥30 days apart PLUS a) chronic diarrhea (i.e., at least two loose stools per day (for ≥30 days) OR b) documented fever (for ≥30 days, intermittent or constant)

*See the 1987 AIDS surveillance case definition (10) for diagnosis criteria.

BOX 4. Comparison of the 1987 and 1994 pediatric human immunodeficiency virus classification systems

1987 Classification	1994 Classification
P-0	Prefix "E"
P-1	N
P-2A	A, B, and C
P-2B	C
P-2C	B
P-2D1	C
P-2D2	C
P-2D3	B
P-2E1	C
P-2E2	B
P-2F	B

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APPENDIX IV
CDC 2000 Revised AIDS Surveillance Definition

Source: Centers for Disease Control and Prevention. *Mortality and Morbidity Weekly Reports* 1999;48 (RR-13):1-27, 29-31.

Revised Surveillance Case Definition for HIV Infection*

This revised definition of HIV infection, which applies to any HIV (e.g., HIV-1 or HIV-2), is intended for public health surveillance only. It incorporates the reporting criteria for HIV infection and AIDS into a single case definition. The revised criteria for HIV infection update the definition of HIV infection implemented in 1993 (18); the revised HIV criteria apply to AIDS-defining conditions for adults (18) and children (17,19), which require laboratory evidence of HIV. This definition is **not** presented as a guide to clinical diagnosis or for other uses.

I.   **In adults, adolescents, or children aged ≥ 18 months † , a reportable case of HIV infection must meet at least one of the following criteria:**

Laboratory Criteria
   Positive result on a screening test for HIV antibody (e.g., repeatedly reactive enzyme immunoassay), followed by a positive result on a confirmatory (sensitive and more specific) test for HIV antibody (e.g., Western blot or immunofluorescence antibody test)

<div align="center">or</div>

   Positive result or report of a detectable quantity on any of the following HIV virologic (nonantibody) tests:
   - HIV nucleic acid (DNA or RNA) detection (e.g., DNA polymerase chain reaction [PCR] or plasma HIV-1 RNA) §
   - HIV p24 antigen test, including neutralization assay
   - HIV isolation (viral culture)

   OR

Clinical or Other Criteria (if the above laboratory criteria are not met)
   Diagnosis of HIV infection, based on the laboratory criteria above, that is documented in a medical record by a physician

<div align="center">or</div>

Conditions that meet criteria included in the case definition for AIDS

II.   **In a child aged <18 months, a reportable case of HIV infection must meet at least one of the following criteria:**

Laboratory Criteria
<u>Definitive</u>
   Positive results on two separate specimens (excluding cord blood) using one or more of the following HIV virologic (nonantibody) tests:
   - HIV nucleic acid (DNA or RNA) detection
   - HIV p24 antigen test, including neutralization assay, in a child ≥ 1 month of age
   - HIV isolation (viral culture)

<div align="center">or</div>

immunoassay). In addition, a negative (i.e., undetectable) plasma HIV-1 RNA test result does not rule out the diagnosis of HIV infection.

Presumptive
A child who does not meet the criteria for definitive HIV infection but who has:
Positive results on only one specimen (excluding cord blood) using the above HIV virologic tests and no subsequent negative HIV virologic or negative HIV antibody tests

OR

Clinical or Other Criteria (if the above definitive or presumptive laboratory criteria are not met)
Diagnosis of HIV infection, based on the laboratory criteria above, that is documented in a medical record by a physician
or
Conditions that meet criteria included in the 1987 pediatric surveillance case definition for AIDS

III.   **A child aged <18 months born to an HIV-infected mother will be categorized for surveillance purposes as "not infected with HIV" if the child does not meet the criteria for HIV infection but meets the following criteria:**

Laboratory Criteria
Definitive
At least two negative HIV antibody tests from separate specimens obtained at ≥ 6 months of age
or

At least two negative HIV virologic tests* from separate specimens, both of which were performed at ≥1 month of age and one of which was performed at ≥4 months of age

AND

No other laboratory or clinical evidence of HIV infection (i.e., has not had any positive virologic tests, if performed, and has not had an AIDS-defining condition)
Or

Presumptive
A child who does not meet the above criteria for definitive "not infected" status but who has:
One negative EIA HIV antibody test performed at ≥6 months of age and NO positive HIV virologic tests, if performed
Or

One negative HIV virologic test* performed at ≥4 months of age and NO positive HIV virologic tests, if performed
Or

One positive HIV virologic test with at least two subsequent negative virologic tests*, at least one of which is at ≥4 months of age; or negative HIV antibody test results, at least one of which is at ≥6 months of age
AND

No other laboratory or clinical evidence of HIV infection (i.e., has not had any positive virologic tests, if performed, and has not had an AIDS-defining condition).

OR

Clinical or Other Criteria (if the above definitive or presumptive laboratory criteria are not met)
Determined by a physician to be "not infected", and a physician has noted the results of the preceding HIV diagnostic tests in the medical record
AND
NO other laboratory or clinical evidence of HIV infection (i.e., has not had any positive virologic tests, if performed, and has not had an AIDS-defining condition)

**IV.	A child aged <18 months born to an HIV-infected mother will be categorized as having perinatal exposure to HIV infection if the child does not meet the criteria for HIV infection (II) or the criteria for "not infected with HIV" (III).**

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*HIV nucleic acid (DNA or RNA) detection tests are the virologic methods of choice to exclude infection in children aged <18 months. Although HIV culture can be used for this purpose, it is more complex and expensive to perform and is less well standardized than nucleic acid detection tests. The use of p24 antigen testing to exclude infection in children aged <18 months is not recommended because of its lack of sensitivity.

**Reported Literature Measuring AZT triphosphorylation in Humans**

In none of these studies does AZTTP reach the concentration estimated ideally *in vitro* of 0.7 μM

Year	Peak Concentration of Triphosphorylated AZT Reported	Reference
1991	0.5 pmol/10^6 cells	Kuster H, et al. J Infect Dis; 164: 773–776
1991	56 pmol/10^7 cells (5.6 pmol/10^6 cells)	Toyoshima T, et al. Analytical Bioch; 196: 302–307
1992	0.14 pmol/10^6 cells	Slusher JT, et al. Antimic Agents & Chemoth; 36: 2473–2477
1994	326 fmol/10^6 cells (0.326 pmol/10^6 cells)	Robbins BL, et al. Antimicrob Agents Chemother; 38: 115–121
1994	0.06 pmol/10^6 cells	Barry MG, et al. AIDS; 8: F1–F5
1996	95 fmol/10^6 cells (0.095 pmol/10^6 cells)	Rodman JH, et al. J Infec Dis; 174: 490-499
1996	0.069 pmol/10^6 cells	Peter K, et al. J Pharm & Biomed Anal; 14: 491–499
1996	0.042 pmol/10^6 cells (average)	Peter K and Gambertoglio JC. Clin Pharmacol Ther; 60: 168–176
1996	0.07 pmol/10^6 cells	Barry MG, et al. AIDS; 10: 1361–1367
1998	0.046 pmol/10^6 cells, in mononuclear cells from lymph nodes. 0.085 pmol/10^6 cells in PBMC	Peter K et al. AIDS; 12: 1729–1731[a]
1998	0.07 pmol/10^6 cells	Robbins BL, et al. Antimicrob Agents Chemother; 42: 2656-2660
1998	160 fmol/10^6 cells (average) (0.16 pmol/10^6 cells)	Fletcher CV, et al. Clin Pharmacol Ther 64: 331–338
1999	329 fmol/10^6 cells (0.329 pmol/10^6 cells)	Rodman JH et al. J Infec Dis; 180:1844-50
1999	193 fmol/10^6 cells (0.193 pmol/10^6 cells)	Font E, et al. Antimicrob Agents Chemother; 43: 2964-8
2000	0.32 pmol/10^6 cells	Wattanagoon Y, et al. Antimicrob Agents Chemother; 44: 1986-1989

1μmol = 10^{-6} mole; 1 pmol = 10^{-12} mole; 1 fmol = 10^{-15} mole; 1 pmol/10^6 cells ≈ 1 μM
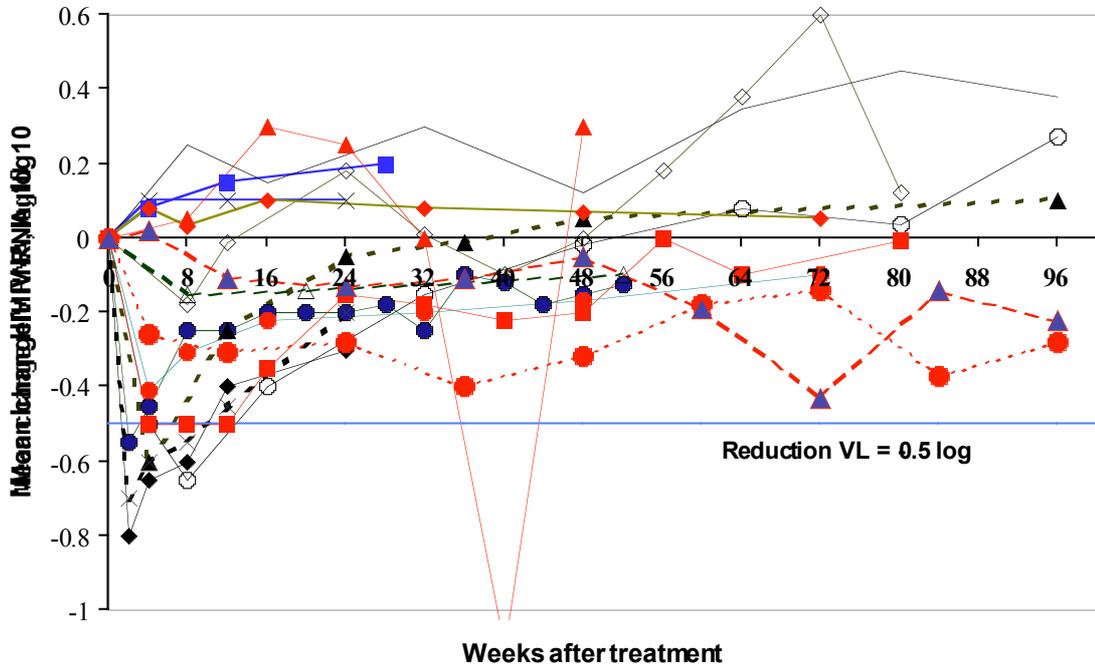
a)	Eron JJ et al. NEJM 1995;333:1662-9	●———●
b)	De Jong MD, et al. PNAS 1996;93:5501-6	▲– – –▲
c)	Katlama C, et al. JAMA 1996;276:118-25	✕– – –✕
d)	Katlama C, et al. JAMA 1996;276:118-25	◆———◆
e)	Staszewski S et al. JAMA 1996;276:111-7	✕———✕
f)	Carr A, AIDS 1996;10:635-41	■———■
g)	O'Brien WA, et al. NEJM 1996;334;426-31	○———○
h)	O'Brien WA, et al. NEJM 1996;334;426-31	□———□
i)	Katzenstein D, et al. NEJM 1996:1091-8	△– – –△
j)	Bakshi SS, et al. J Infect Dis 1997;175:1039-50	◇———◇
k)	Bruisten SM et al. AIDS Res & Hum Retr 1998;12:1053-8	■———■
l)	Delta Committee. AIDS, 1999:57-65	●———●
m)	Delta Committee. AIDS, 1999:57-65	◆———◆
n)	Lillo FB, et al. AIDS 1999;13:791-6	▲———▲
o)	Arch Dis Child 2001: 84: 230-60	●············●
p)	Arch Dis Child 2001: 84: 230-60	▲– – –▲

APPENDIX VII
AIDS Reporting form for South Africa

Department of Health, Pretoria.

GW 8/87

DEPARTMENT OF HEALTH

ANONYMOUS AIDS NOTIFICATION

All information on this report is strictly confidential. Kindly forward the completed form to the appropriate Provincial Health Department

BASIC PATIENT INFORMATION

Date of birth — Day / Month / Year: d d m m c c y y

Date of diagnosis — Day / Month / Year: d d m m c c y y

Age at diagnosis of AIDS — Years

Current status — Alive / Dead

Sex — Female / Male

Population group (in which group would the patient say that she/he belongs?): Asian, Black, Coloured, White, Other

Place of residence — Town/Township/Suburb

City/town or magisterial district

PROBABLE MODE OF TRANSMISSION

(Please tick all relevant categories)

- Sexual activity
- Child of HIV positive mother
- IV drug user
- Blood transfusion
  - Date of transfusion — Day / Month / Year: d d m m c c y y
- Unknown
- Other specify _____

FACILITY OF DIAGNOSIS

- Private hospital
- Public Hospital or care institution
- Other specify _____

REPORT SOURCE

Name of institution _____

Completed by _____

Were any of the following notified (Y/N):

Care giver ☐   Family member ☐

Date: ___/___/___

AIDS INDICATOR DISEASE (ADULTS)

(Please tick all that apply)

Major
- Weight loss greater than 10% of body weight
- Chronic diarrhoea for more than one month
- Fever for more than one month

Minor
- Persistent cough for more than one month
- Generalised pruritic dermatitis
- Recurrent herpes zoster (shingles)
- Candidiasis (oral or pharyngeal)
- Chronic or persistent herpes simplex

Confirmatory
- Cryptococcal meningitis
- Kaposi's sarcoma

AIDS INDICATOR DISEASE (CHILDREN)

(Please tick all that apply)

Major
- Weight loss or failure to thrive
- Chronic diarrhoea for more than one month
- Fever for more than one month
- Recurrent or severe pneumonia

Minor
- Generalised dermatitis
- Repeated common infections (otitis, pharyngitis)
- Candidiasis (oral or pharyngeal)
- General lympadenopathy
- Confirmed maternal HIV infection

LABORATORY DATA

HIV Tested Yes ☐ No ☐ if Yes, result: ☐☐

Laboratory _____ Lab no: _____

PLEASE SEE THE BACK OF THIS FORM FOR THE WORLD HEALTH ORGANISATION CLINICAL AIDS CASE DEFINITIONS

159

APPENDIX VIII
AIDS Reporting form for Uganda

MINISTRY OF HEALTH/ACP   Rev.09/91
P.O.BOX 8, ENTEBBE
TEL: 20353, 20534

UGANDA MINISTRY OF HEALTH
ADULT (12 years and above) AIDS REPORTING FORM

Instructions:
Please fill out this form for every patient diagnosed with AIDS
at the initial time of diagnosis.
Diagnosis will be based on the Uganda WHO modified
clinical case definition

FORM NO:

HOSPITAL_____

HOSPITAL REGISTER NO:_____ AGE_____ SEX_____

DISTRICT OF RESIDENCE_____ OCCUPATION_____

DOES THE PATIENT HAVE THE FOLLOWING?

SYMPTOMS/PHYSICAL FINDINGS. (PLEASE TICK)

☐ DISSEMINATED KAPOSI SARCOMA

☐ CRYPTOCOCCAL MENINGITIS

MAJOR SIGNS

☐ WEIGHT LOSS AT LEAST 10%

☐ DIARRHOEA AT LEAST 1 MONTH

☐ FEVER AT LEAST 1 MONTH

MINOR SIGNS

☐ ORO-PHARYNGEAL CANDIASIS

☐ PRURITIC SKIN RASH

☐ HERPES ZOSTER

☐ GENERALISED LYMPHADENOPATHY

☐ COUGH AT LEAST 1 MONTH
        (WITHOUT TB)

☐ CHRONIC ULCERATED HERPES
        SIMPLEX

☐ TUBERCULOSIS

☐ OTHERS......................

DATE____/____/____ NAME OF REPORTING OFFICER....................

PLEASE RETURN THE COMPLETED FORMS TO ACP/MINISTRY OF HEALTH

161

APPENDIX IX
Calculation of the probability of HIV transmission between sexual partners

Livio Mina. Mathematician and Statistician, Department of Medical Physics, Royal Perth Hospital, Perth, Western Australia

Suppose that for each contact episode there is a constant probability, p of being infected which is independent of any previous contact history.

The number of contacts needed to first contract the disease follows the geometric distribution with probability function

$$P(n) = p\,(1-p)^{n-1}$$

where P(n) is the probability of first contracting the disease at the nth contact.

If we are interested in knowing the probability of having caught the disease after a given number of contacts (say n) we must sum all the probabilities of first catching the disease at the first, second, third, etc. contact up to n. This is somewhat tedious, and for this question we can turn instead to the Binomial distribution which gives us the distribution of the number of times we would catch the disease (at least notionally) in n contact episodes.

The probability function here is

$$P(x) = n!/[x!(n-x)!]\ \ p^{x}\,(1-p)^{n-x}$$

where P(x) is the probability of being infected x times (sic) in n contacts.

The idea of multiple infection may not make a great deal of sense biologically but we can legitimately ask what is the probability that there be no infection at all (ie. x = 0) after n contacts. From the formula we see that this will be $(1-p)^{n}$ so that the probability of contracting the disease (regardless of the notion of multiple infections) is $1 - (1-p)^{n}$.

Email Correspondence with the CDC re Scientific Basis of HIV Testing in Children and Adults

March 14th 2001 V. Turner to Helene Gayle

Dear Helene,

I would be most grateful if you could explain the scientific basis for the following:

According to the latest CDC AIDS surveillance definition,

"In adults, adolescents, and children infected by other than perinatal exposure, plasma viral RNA nucleic acid tests should **NOT** be used in lieu of licensed HIV screening tests (e.g., repeatedly reactive enzyme immunoassay)".

Is not the basis of these tests the existence and recognition of nucleic acid sequences unique to HIV? Is it not true that by this means HIV specific primers/probes detect and count nucleic acid molecules which are HIV and none other? If these tests respond to HIV specific sequences how can they fail to diagnose HIV infection? Is the CDC suggesting that these tests should "**NOT**" be used because they also detect and measure nucleic acid sequences which are not HIV? Regardless, if the tests are not *bona fide* in adults, why are they permitted in infants < 18 months of age?

On the other hand, if the nucleic acid tests can be used to diagnose humans <18 months of age, then why not humans >18 months of age? Certainly the antibody tests are problematic before 18 months of age but that does not provide an answer to the question.

Best wishes,

Val Turner MD

March 22nd 2001   Helene Gayle declines to answer but refers V Turner's email to Dr. ML Lindegren.

Dr. Turner,

I am following up on an email requesting information on the CDC HIV case definition from Dr. Gayle. I would love to be able to help, can you forward to me your questions on the use of the RNA assays for children,

Best,

Mary Lou Lindegren

March 22nd 2001

Reply to Dr. Lindegren

Dear Dr. Lindergren,

Thank you very much for your kind offer to answer some questions in relation to the CDC 2000 Revision AIDS definition. Please let me first explain that we are researching matters arising out of the Presidential AIDS Panel meeting which met last July in Johannesberg and which Helene attended.

According to the CDC 2000 AIDS surveillance definition,

"In adults, adolescents, and children infected by other than perinatal exposure, plasma viral RNA nucleic acid tests should **NOT** be used in lieu of licensed HIV screening tests (e.g., repeatedly reactive enzyme immunoassay)".

Given that the basis of these tests is the existence and recognition of nucleic acid sequences unique to HIV, and thus the use of HIV specific primers/probes to detect and count nucleic acid molecules:

1. How can these tests not diagnose HIV infection?   Is there an implication of circumstances where the tests may detect and measure nucleic acid sequences which are not HIV?

2. If yes, are perinatally exposed children an exception to these circumstances?

3. Why are the tests not to be used in adults, neither for diagnosis nor screening, yet are permitted in perinatally exposed infants < 18 months of age for diagnosis and screening?

3. One can also ask Q2 the other way around. If the RNA tests can be used to diagnose perinatally exposed infants <18 months of age, why not also to diagnose and screen humans >18 months of age?

4. If the tests are able to diagnose perinatally exposed children, why are they not permitted in the same children for the same purpose when, for example, HIV infection is suspected following a blood transfusion?

Yours sincerely,

Val Turner MD

28th March Further question from V Turner to Dr. Lindegren

Dear Dr. Lindegren,

I wonder if I could pose an additional question re HIV testing?
In the 2000 definition it states:

"In adults, adolescents, or children aged greater than or equal to 18 months**, a reportable case of HIV infection must meet at least one of the following criteria:

Laboratory Criteria

> Positive result on a screening test for HIV antibody (e.g., repeatedly reactive enzyme immunoassay), followed by a positive result on a confirmatory (sensitive and more specific) test for HIV antibody (e.g., Western blot or immunofluorescence antibody test)".

Is two ELISAs ("repeatedly reactive" rather than one) now a "screening test for HIV antibody"?

Is there an explanation as to why in England two ELISAs are considered proof of HIV infection but apparently not in the US? Does the CDC or WHO make allowance for this fact when compiling numbers of individuals infected?

Also, would you be able to point me to any data which prove that children lose their mother's IgG by a certain age?

Kind regards,

Val Turner MD

5th April 2001 No reply from Dr. Lindegren. Further request sent

Dear Dr. Lindegren,

You may recall I emailed you on March 22nd some questions in relation to HIV diagnosis in adults and children. I also sent and additional two questions on the 28th of March which I have also included in this email. I would be most grateful if you could provide me with a response.

Best wishes,

Val Turner

7th April 2001 Reply from Dr. Lindegren

Thanks, I have been working with Pat Fleming and the appropriate groups here at CDC to give you the best references. We have not forgotten you!!

Best wishes,

Mary Lou

May 3rd 2001 Further request for answers to Dr. Lindegren

Dear Dr. Lindegren,

Several weeks ago you responded that you "would love to be able to help" with some answers to questions I had put to Dr. Gayle.

I need the answers in relation to matters arising out of the July 2000 Presidential Panel meeting where I was fortunate to meet Dr. Gayle.

I believe the questions fair and reasonable: On what ground does the CDC recommend a test to diagnose perinatal acquired HIV infection whereas the same test cannot be used even as a screening test in all other situations? Especially when the test is predicated on recognition of specific genetic sequences unique to HIV.

I am greatly puzzled by your apparent reluctance to respond.

Kind regards,

Val Turner MD

16th May 2001 Dr. Lindegren responds

Hi, we have asked the appropriate group at CDC to respond, which turns out is not me. Sorry for the delay, we will follow up,

Best,

ML

May 31st 2001 Further request for an answer from Dr. Lindegren

Dear Dr. Lindegren,

It's now two more weeks since you emailed me again with your willingness to answer the questions I put to Dr. Gayle months ago. Surely I can't be the first person in the world to point out the inconsistencies of the CDC recommendations for HIV diagnosis in adults, children and neonates. Whoever or whatever committee met to formulate these recommendations must have discussed the matter between themselves. Perhaps the answer is more to do with what is achievable than strict scientific rigor although this is no more than conjecture on my part. I would appreciate an honest answer as to whether you ever intend to enlighten me. If not, please say so. As I said earlier, it won't be the end of the world and we can cease exchanging these rather fruitless emails.

Best wishes,

Val Turner

June 5th Response from Dr. Lindegren

Hi, I hope that you will get a response on this. Patricia Fleming is the surveillance branch chief and we passed the response on to the appropriate people, so sorry, I will keep trying to follow up also, ML

June 29th 2001 V Turner to Dr. Lindegren

Dear Mary Lou,

I'm still waiting for a response from the lady you referred my questions to, now, as you know, several months old. Since these are basic questions, which must have arisen when the recommendations were being developed, I can only assume the person responsible either does not regard the answers warrant priority, or there are no answers.

I wonder why, since Helene Gayle referred me to you, you can't offer an answer?   Surely Dr. Gayle must know the right person to approach?

Perhaps you can imagine a patient asking you the same things? What would you say?

Best wishes,

Val Turner MD

June 29th 2001 Response from Dr. Lindegren

Mr. Turner,

Again, I am sorry you have not received a response. I am now working in a different Division at CDC, not HIV, and I referred your question on to the appropriate persons. I know you will get your response and I again apologize for the very long delay.

Best,

Mary Lou

July 28th V Turner to Dr. Lindegren

Dear Dr. Lindegren,

Still no word from anyone at the CDC on my questions. Earlier you said you weren't the right person to ask. Yet the other day I was looking up the 2000 Revised Definition and found this on page 29. I assume you are the M L Lindegren mentioned in the footnote [1]--an outside, pediatric* expert consultant?

Is there some reason for the several months delay in answering the apparent inconsistency between diagnosis in infants and adults? As highlighted in the last footnote? Surely this has come up in your discussions. Is the explanation arcane?

Kind regards,

Val Turner MD

1. Draft revised surveillance criteria for HIV infection were approved and recommended by the membership of the Council of State and Territorial Epidemiologists (CSTE) at the 1998 annual meeting (11). Draft versions of these criteria were previously reviewed by state HIV/AIDS surveillance staffs, CDC, CSTE, and laboratory experts. In addition, the pediatric criteria were reviewed by an expert panel of consultants. [External Pediatric Consultants: C. Hanson, M. Kaiser, S. Paul, G. Scott, and P. Thomas. CDC staff: J. Bertolli, K. Dominguez, M. Kalish, M.L. Lindegren, M. Rogers, C. Schable, R.J. Simonds, and J. Ward]

*Dr. Lindegren is a member of the CDC staff.

August 15th 2001 V Turner to Dr. Lindegren

Dear Dr. Lindegren,

I am attaching a summary of our emails re the questions I originally sent to Dr. Gayle in March this year. In one of the emails I intimated that I was seeking a response from the CDC in relation to matters arising out of the July 2000 Presidential AIDS Panel meeting. This matter is now complete and I intend to include this summary as an Appendix to the report. If you would like to add any further comments, (and that includes answering the questions I asked five months ago), I will certainly consider adding them to the report.

Yours sincerely,

VF Turner MD

August 22nd Dr. Lindegren to V Turner

Dr. Turner, as I mentioned to you several months ago and included on multiple emails, I am not the correct CDC contact and you need to direct your questions to Dr. Fleming who will make sure that CDC responds to your questions.

Sincerely, Mary Lou

August 23rd V Turner to Dr. P. Fleming

Dear Dr. Fleming,

Dr. Lindegren has requested I email you directly re questions first put to Dr. Helene Gayle in March this year. I attach the questions in a WORD 97 file.

I am an Emergency Physician and need to know the scientific basis for the CDC recommendations re diagnostic HIV testing in adults and children.

My original email was referred to Dr. Lindegren from Dr. Gayle for answering. Dr. Lindegren responded she would "love to be able to help" and following her kind offer sent my questions to you, then apparently was working with you ("I have been working with Pat Fleming and the appropriate groups here at CDC to give you the best references. We have not forgotten you!! Best wishes, Mary Lou"), was then transferred to another section of the CDC, then could not explain why you had not answered and has now virtually directed me to email you myself. This I gather can only mean the Dr. Lindegren would no longer "love to be able to help". It is puzzling why a CDC staffer whose name appeared on an "expert panel of consultants" in regard to this matter cannot answer these questions within a few days straight off the top of her head. Also, in the unlikely event that no one at the CDC has pondered these matters for themselves, why has Dr. Lindegren and indeed yourself, not been proactive in providing an explanation? I hope you realise the questions could have just as easily been asked by an informed patient. I wonder what response he or she would receive from the CDC?

Quite frankly this whole affair is "a riddle wrapped in a mystery inside an enigma". And, as far as I am concerned, a six month runaround. If you are willing to send me the scientific rationale for your recommendations in regard to HIV testing then please do so. I would appreciate this as soon as possible.

Yours sincerely,

VF Turner

Department of Emergency Medicine
Royal Perth Hospital
Perth
Western Australia

Response from Drs. Fleming/Klevens/Schulte of the CDC. October 1st 2001

(404) 639-2050
October 1, 2001

Dr. Val Turner
vturner@cyllene.uwa.edu.au <mailto:vturner@cyllene.uwa.edu.au>
Department of Emergency Medicine
Royal Perth Hospital
GPO Box X2213
Perth, Western Australia 6001

Dear Dr. Turner,

We are responding to your email of August 23rd, in which you compile questions posed in previous emails. We apologize for the delay in responding, and will do our best to answer your questions.

In response to your question of 5/3/2001: "On what ground does the CDC recommend a test to diagnose perinatal acquired HIV infection whereas the same test cannot be used even as a screening test in all other situations. Especially when the test is predicated on recognition of specific genetic sequences unique to HIV".

This CDC recommendation for HIV testing applies to the HIV surveillance case definition, and is not offered as a guide to clinical diagnosis. For surveillance purposes, a positive result on any of the following: HIV DNA or RNA nucleic acid detection or HIV p24 antigen or viral culture of HIV is
acceptable evidence to report a case of HIV among children ≥18 months of age, adolescents, or adults. HIV and AIDS case surveillance efforts should result in collection of data from all private and public sources of HIV-related testing and care services. Laboratory-initiated surveillance
methods should identify all cases that meet the laboratory reporting criteria for HIV infection and/or AIDS. However, these methods require follow-up with the health care provider to verify the infection status or clinical stage of disease and to obtain complete demographic and exposure
risk data.

The issues for using viral load tests in infants are different than those for adults. In the case of infants, a positive result for any one of the above listed tests is acceptable, if a separate specimen is also positive (a confirmatory test). This is because passive transfer of maternal antibody will cause an infant to appear seropositive for as long as 18 months, and thus is not an indicator of the infant's infection status. IgG begins to cross the placenta at about 12 weeks gestation and the quantity increases steadily. At birth, cord serum concentrations of IgG are comparable to that of maternal serum. Infants with perinatally acquired HIV/AIDS pose a unique
challenge because IgG antibody is passed transplacentally to virtually all infants born to HIV-seropositive women. Before widespread use of antiretroviral therapy, studies found that 20-30% of infants born to infected women become HIV-infected themselves. However, almost 100% of such infants tested positive by enzyme immunoassay (EIA) or Western blot assay during the first 28 days of life; maternal antibody can persist for up to 18 months of age. (The median age at seroreversion is about 10 months of age). The standard EIA and Western Blot assays most efficiently detect IgG and thus cannot distinguish between maternal antibody that crossed the placenta and IgG made by the infant.

In a patient care setting, clinicians may order viral load tests to try to diagnose early HIV infection in persons who experienced a high risk exposure and are seronegative. However, the specificity of commercially available viral load tests is about 97-98%, and therefore 2-3% of positive results would be false positives, and the tests are not licensed for diagnostic purposes.

Your questions of 3/28/01 were "Is two ELISAs now a screening test for HIV antibody"? "Would you be able to point me to any data which prove that children lose their mother's IgG by a certain age?"

The surveillance recommendation for criteria for reporting an HIV case in persons ≥ 18 months of age allows for two positive antibody tests, where one can be a screening test such as the enzyme immunoassay, and the second to be a more sensitive and specific confirmatory test.

Standard reference pediatrics texts and multiple studies describe the presence of maternal antibodies for HIV in infants through ≥18 months of age. A few useful sources of information on this topic follow:

* Nelson Textbook of Pediatrics, 15th edition, p 563

* Schochetman G and George JR. AIDS Testing: A comprehensive guide to technical, medical, social, legal and management issues. Second edition. P 270-271.
* Quinn TC, Kline RL, Halsey N, et al. Early diagnosis of perinatal HIV infection by detection of viral-specific IgA antibodies. JAMA 1991;266:3439-3442.
* Landesman S, Weiblen B, Mendez H, et al. Clinical utility of HIV-IgA immunoblot assay in the early diagnosis of perinatal HIV infection. JAMA 1991;266:3443-3446.
* Weiblen BJ et al. Natural history and serologic diagnosis of infants born to human immunodeficiency virus-infected women. Am J Dis Child 1989;143:1147-1153.
* Ryder RW, Hassig SE. The epidemiology of perinatal transmission of HIV. AIDS 1988;2:583-589.
* Italian Multicentre Study. Epidemiology, clinical features, and prognostic factors of pediatric HIV infection. Lancet 1988;2:1043-1045.
* Aiuti F, Luzi G, Mezzaroma I, Scano G, Papetti C. Delayed appearance of HIV infection in children. Lancet 1987;2:858.

Your questions of 3/22/01 were related to the footnote on page 29 that states "In adults, adolescents, and children infected by other than perinatal exposure, plasma viral RNA nucleic acid tests should NOT be used in lieu of licensed HIV screening tests (e.g., repeatedly reactive enzyme immunoassay).

1. How can these tests not diagnose HIV infection? Is there an implication of circumstances where the tests may detect and measure nucleic acid sequences which are not HIV?

2. If yes, are perinatally exposed children an exception to these circumstances?

3. Why are the tests not to be used in adults, neither for diagnosis nor screening, yet are permitted in perinatally exposed infants < 18 months of age for diagnosis and screening?

4. One can also ask Q2 the other way around. If the RNA tests can be used to diagnose perinatally exposed infants <18 months of age, why not also to diagnose and screen humans >18 months of age?

5. If the tests are able to diagnose perinatally exposed children, why are they not permitted in the same children for the same purpose when, for example, HIV infection is suspected following a blood transfusion?"

The issue of testing for diagnosis of HIV infection is a clinical question. The Food and Drug Administration is responsible for setting standards for diagnostic tests for HIV infection. CDC recommendations are only for surveillance purposes. The footnote you mention refers to the surveillance and public health application of screening tests. In general, before recommending any test for screening in the population, issues of risk, efficacy (sensitivity, specificity, reliability), cost, burden, and availability of effective prevention are taken into account. We are unaware
of evaluations that demonstrate the benefit of using plasma viral RNA nucleic acid tests for screening in the population. Also, tests for detection of plasma RNA may result in false positive results, and the Food and Drug Administration has not licensed these tests for the diagnosis of
HIV infection. Including these tests in the surveillance case definition for HIV case reporting enables health departments to identify potential cases for follow-up.

The tests you state CDC does not recommend for reporting a case of HIV infection in adults, in fact, are accepted laboratory criteria (CDC). Guidelines for national human immunodeficiency virus case surveillance, including monitoring for human immunodeficiency virus infection and acquired immunodeficiency syndrome. MMWR 1999;48(No. RR-13):29). We recommend that
the non-antibody tests not be used instead of the antibody tests because HIV infected persons who are receiving effective antiretroviral therapy can have undetected quantities on the non-antibody tests, yet still remain positive and should be reported to surveillance as the local law or regulation may require.

In clinical settings, the first choice for diagnosis of HIV infection for persons ≥18 months of age is an antibody test. However, in the case of perinatally exposed infants, the antibody test cannot be used, since the infant has antibodies transferred across the placenta from the mother.
Because current clinical guidelines call for initiating PCP prophylaxis while infection status is determined and early treatment with antiretroviral therapy if the infant is HIV positive, the algorithm for defining infection status in infants is complex. It includes results of serial tests,
including viral detection. For infants <18 months of age, viral detection methods may be used as an alternative.

We hope your concerns have been addressed. Dr. Lindegren accepted a new assignment and may have assumed that the Branch had already responded to you; we apologize. If you have additional questions, please feel free to

contact Dr. Fleming in writing at the address listed below. In addition to this letter by email, we are also sending this letter by post. Know that we receive many emails and correspondence, most of which require follow-up, and we respond as resources allow.

Sincerely,

Patricia L. Fleming, PhD
Chief, Surveillance Branch
DHAP/NCHSTP
Mailstop E-47
1600 Clifton Road
Atlanta, GA 30333

R. Monina Klevens, DDS, MPH
Chief, Research and Dissemination Section
Surveillance Branch
DHAP/NCHSTP

Joann Schulte, DO, MPH
Medical Epidemiologist, Reporting, Analysis,
Surveillance Branch
DHAP/NCHSTP

Comments on reply from the CDC

1. This reply by Drs. Fleming/Klevens/Schulte is obfuscatory and falls well short of any scientific response.
2. Drs. Fleming/Klevens/Schulte assert that the CDC recommendations in regard to HIV testing apply only to "surveillance" and are "not offered as a guide for clinical diagnosis…The issue of testing for diagnosis of HIV infection is a clinical question". This implies that determining HIV infection is a separate process performed by clinicians using history and physical examination with or without the results of HIV testing. If HIV can be diagnosed on clinical grounds, why does one need tests for HIV and how is it possible to diagnose HIV infection when all the symptoms and diseases indicative of HIV infection are non-specific? Especially in Africa.
3. If HIV is a clinical diagnosis how it is possible to diagnose HIV infection in individuals not belonging to a risk group who have no abnormal clinical symptoms or signs?
4. Drs. Fleming/Klevens/Schulte assert that "The issues for using viral load tests in infants are different than those in adults". They affirm that nucleic acid tests are "acceptable" in infants and, in contradistinction to their own recommendations, state that "The tests you state CDC does not recommend for a case of HIV infection in adults, in fact, are accepted laboratory criteria". (How can there be "accepted laboratory criteria" yet the "laboratory criteria" recommended by the CDC are merely for "surveillance"?) The authors' justification for the use of nucleic acid tests in perinatally infected children is that "the infant has antibodies transferred across the placenta from the mother". However, this does not answer the question including those asked in March 2001:

> "4. One can also ask Q2 the other way around. If the RNA tests can be used to diagnose perinatally exposed infants <18 months of age, why not also to diagnose and screen humans >18 months of age?
>
> 5. If the tests are able to diagnose perinatally exposed children, why are they not permitted in the same children for the same purpose when, for example, HIV infection is suspected following a blood transfusion?"

5. The authors have also recast three questions of March 28th:

> "Is two ELISAs ("repeatedly reactive" rather than one) now a "screening test for HIV antibody"?
>
> Is there an explanation as to why in England two ELISAs are considered proof of HIV infection but apparently not in the US? Does the CDC or WHO make allowance for this fact when compiling numbers of individuals infected?
>
> Also, would you be able to point me to any data which prove that children lose their mother's IgG by a certain age?"

as:

> "Your questions of 3/28/01 were "Is two ELISAs now a screening test for HIV antibody"? "Would you be able to point me to any data which prove that children lose their mother's IgG by a certain age?"

The first two questions are ignored and the authors either confuse or ignore the crucial question re children losing maternal antibodies by a particular date. This question clearly refers to loss of the general class of antibodies known as IgG. (These are the only antibody class that cross the placenta and include the mother's "HIV" antibodies). Their answer refers to loss of "HIV" where for all intents and purposes they answer the question by repeating their recommendation. In fact, according to the data presented in reference 256 (Part II, page 45), maternal IgG disappears from the child by 9 months of age. Unless the authors have basic scientific data to the contrary (and this was the question they were asked), and if, as the authors assert, "the median age of seroreversion is about 10 months", then all children who serorevert beyond "about 10 months" cannot do so because of loss of maternal antibodies. This can only be interpreted as children clearing HIV infection or the tests being non-specific.

6. The authors offer no scientific reason for the CDC recommendation that tests involving nucleic acid sequences unique to HIV cannot diagnose HIV infection in adults but do so in perinatally infected children, or why, despite the former, such tests can measure the HIV "viral load". How is it possible to measure HIV if the laboratory technique employed cannot determine that the object being measured is HIV? How does one count apples if one cannot distinguish between apples and every other object in the Universe? Even if we accept there exists proof (there is no such proof) that the specificity is "97-98%, and therefore 2-3% of positive results would be false positives", on what grounds are individual newborns deemed "infected" with HIV?

From reading this response one concludes that the authors and the CDC base their rationale for HIV testing recommendations on the notion that individuals identified by the HIV surveillance definition are not necessarily "clinically infected" with HIV. If this is the case, how does the CDC know many HIV infected persons there are? And why does the CDC repeatedly present surveillance data knowing full well it will be interpreted as the "true" numbers of HIV infected persons? And how does the CDC regard other statistics such as those collected by the WHO? Are these "surveillance infections" or "clinical infections"?

APPENDIX XI
A Critical Examination of the Evidence for the Existence of HIV

INTRODUCTION

Following the appearance of AIDS in 1981 many aetiological factors were proposed. In May 1983 Montagnier announced the discovery of a retrovirus, now known as HIV, from lymphatic tissue of a gay man with lymphadenopathy. One year later Gallo reported data which "suggests that HTLV-III [HIV] is the primary cause of AIDS". By 1986 the scientific community accepted the Gallo assertion that the same data was "clearcut evidence" that HIV is the causative agent of the clinical syndrome. Even today the five *Science* papers published by these French and American groups are still widely regarded as proving beyond all reasonable doubt that HIV exists and is the cause of AIDS.

However, not all scientists accepted these findings. In 1987 Peter Duesberg published an invited paper in *Cancer Research* on retroviruses and cancer in which he also questioned the role of HIV in AIDS.[1] At the same time one of us (EPE) also challenged the theory[2] including the data claimed to prove the existence of HIV. Papadopulos-Eleopulos also proposed an alternative, non-infectious aetiology and treatments based on this hypothesis. Since then our group has published papers addressing every facet of the HIV theory[2-18] including a detailed examination of HIV isolation and the HIV genome.[9,19] Here we confine ourselves largely to addressing the data published by Montagnier and Gallo in their 1983/84 *Science* papers. Genomic data are only briefly discussed because the existence of HIV and the HIV theory of AIDS were universally accepted before such data were available. For a comprehensive discussion on genomic data the reader is referred to reference 19.

RETROVIRUSES AND THEIR IDENTIFICATION

A virus possesses two characteristic properties. The first is anatomical, that is, being a microscopic particle of individual morphology, the second, the ability to generate identical progeny by synthetic processes obligatorily occurring within living cells. It is the latter attribute which defines a particle with the appearances of a virus, that is, a viral-like particle, as infectious and thus a virus. The three subfamilies (*Oncovirinae*, *Lentivirinae* and *Spumavirinae*) of *Retroviridae* (Retroviruses) are "enveloped viruses with a diameter of 100-120 nm budding at cellular membranes. Cell released virions contain condensed inner bodies (cores) and are studded with projections (spikes, knobs)".[20] The retroviral particles contain RNA and the enzyme reverse transcriptase (RT), an RNA dependent DNA polymerase which catalyses the synthesis of DNA contrary to the central dogma of biology, that is, in a direction "reverse" from DNA to RNA. According to retrovirologists, such DNA is then integrated into existing cellular DNA as a "provirus". Retroviral particles share the property of concentrating (banding) at a density of 1.16 gm/ml when centrifuged at high speeds in sucrose density gradients, a fact long used in their purification.[21,22]

All retrovirologists agree that to prove the existence of a new retrovirus one must isolate it. However, the term "virus isolation" is beset with semantic difficulties and ambiguities. The dictionary meaning of "isolation" derives from the Latin *insulatus* (made into an island) and refers to the act of separating an object from all other matter that is not that object. "Purification" means to obtain something free from impurities. In this context isolation is the same as purification. Because virus particles are small it is not possible to obtain a single, isolated particle. The next best thing is to obtain a mass of particles separate from everything else. Until the early 1980s, for the isolation of animal retroviruses as well as the "first" human retrovirus HL23V, by isolation retrovirologists meant purification. On the other hand, nowadays both basic and specialised texts rarely define "isolation" and when they do such attempts are non-illuminating. For example, Levy defines isolation as a "sample of a virus from a defined source",[23] and White as the ability to "identify a totally unforeseen virus, or even discover an entirely new agent".[24] Encompassed as "virus isolation" are listed methods of culturing specimens in tissue and chick embryo cells, as well as live animals, following by documentation therein of cytopathic and pathological effects, haemoabsorption, immunofluorescence, antigen/antibody reactions and "characterisation of the viral genome".[24-27] HIV experts, including Luc Montagnier and Robin Weiss define "virus isolation" as "propagating them [viruses] in cells in culture".[28,29] However, if "virus isolation" is to "take a sample of a virus from a defined source", or "propagating them in cells in culture", then first one must have prior proof that a virus exists in "a defined source" or "in cells in culture". One cannot know that a virus exists or define its constituents without purification (isolation) of the putative viral particles.

There are several reasons why this is mandatory:

To prove that the retrovirus-like particles are infectious, that is, they are a virus

The finding of particles with the appearances of retroviruses, is not proof that such particles are retroviruses and even less proof a particle is a particular retrovirus. Particles bearing the morphological characteristics of retroviruses are ubiquitous. In the 1970s such particles were frequently observed in human leukaemia tissues,[30] cultures of embryonic tissues and "in the majority if not all, human placentas".[31] Type-C retroviral particles are present in "fish, snakes, worms, pheasant, quail, partridge, turkey, tree-mouse and agouti"[32] as well as in "tapeworms, insects...and mammals".[33] Gallo was well aware of this problem as far back as 1976 when he wrote: "Release of virus-like particles morphologically and biochemically resembling type-C virus but apparently lacking the ability to replicate have been frequently observed from leukaemic tissue".[30] In other words, it is not possible to claim a particle is a retrovirus merely by appearances. To prove that retrovirus-like particles observed in a culture are a virus one must isolate the particles, characterise their proteins and RNA and introduce the particles into a secondary culture. If any particles are released in the secondary culture they too must be isolated and proven that their proteins and RNA are the same as those from the primary culture. In such experiments one must not ignore the use of legitimate controls and in doing so take in consideration an important difference between retroviral and other infectious agents.

When one finds an infectious agent, for example a virus or a bacterium, either *in vitro* or *in vivo*, one may be assured that the agent has been introduced into the culture or animal from outside. Retroviruses are the exception. This is because normal human and animal genomes contain information which, under the appropriate conditions, leads to the synthesis of retroviral RNA and proteins, or even to the assembly of retroviral particles, that is, to the expression of endogenous retroviruses. And although as late as 1994 both Gallo and Fauci taught "there are no known human endogenous retroviruses",[34] it is known that at least 1% of the human DNA is retroviral DNA and that endogenous retroviruses are present "in all of us".[35,36] Furthermore, new endogenous retroviral genomes may arise from rearrangements of existing retroviral genomes, cellular DNA or both, caused by many factors, including pathogenic processes.[37,38] The expression of endogenous retroviral genomes may arise spontaneously[39] and may be significantly accelerated and the yield increased by conditions which induce cellular activation.[40-42] According to the eminent retrovirologist George Todaro, "the failure to isolate endogenous viruses from certain species may reflect the limitations of in-vitro cocultivation techniques".[43] Endogenously produced retroviruses are morphologically and biochemically indistinguishable from exogenous retroviruses. Because of this, the finding of identical retrovirus in serially "infected" cultures/cocultures is not proof that the cells are infected with exogenous retrovirus. One method which may assist resolve but will not prove whether cells acquire virus from the outside (exogenously acquired retrovirus, infectious retrovirus) and have not assembled a retrovirus from information already existing in normal cells (endogenous retrovirus), is to conduct control cultures/cocultures in parallel with test cultures/cocultures. The only difference between test and control cultures should be the introduction of tissue assumed infected into the test cultures. In every other respect control cultures must be dealt with identically. For example:

(a) because detection of RT and retroviral genetic sequences, and release of retroviral particles depends on the metabolic state of the cells, the physiological state of the cells used in the control cultures should be as close as possible to the test culture;

(b) because the mere act of co-cocultivation may lead to release of endogenous retroviral particles, if test cells are cocultured, so should the controls;

(c) extracts even from normal, unstimulated cells when added to the cultures may increase endogenous retroviral expression. Because of this, when host cells are cultured with supernatant or material which bands at 1.16 gm/ml from cultures thought to be infected, the controls must be cultured with similar material from noninfected cultures;

(d) since the appearance of endogenous retrovirus can be accelerated and the yield increased a million fold by stimulating the cultures with mitogens, mutagens, chemical carcinogens and radiation, if test cultures are exposed to or employ such agents so should the controls;

(e) to avoid observer bias and in the best interests of science, blind examination of test and control cultures/cocultures should be performed.

To determine their biological effects

Without recourse to pure particles it is impossible to determine whether effects are due to virus particles or contaminants including "chemical stimulants", a fact stressed as far back as 1911 by the Peyton Rous, the father of retrovirology.[44]

In 1911 Rous induced malignancy in chickens by injections of cell-free filtrates obtained from a muscle tumour. Similar experiments were repeated by many researchers and the tumour inducing filtrates became known as filterable agents, filterable viruses, Rous agents, Rous virus and ultimately retroviruses. However, Rous himself expressed doubts that the agents which caused the tumours were infectious in nature. Indeed he warned, "The first tendency will be to regard the self-perpetuating agent active in this sarcoma of the fowl as a minute parasitic organism. Analogy with several infectious diseases of man and the lower animals, caused by ultramicroscopic organisms, gives support to this view of the findings, and at present work is being directed to its experimental verification. But an agency of another sort is not out of the question. It is conceivable that a chemical stimulant, elaborated by the neoplastic cells, might cause the tumour in another host and bring about in consequence a further production of the same stimulant".[44]

To characterise the viral proteins
The only way to prove that a protein is a constituent of an object is to obtain it from that object, or when the object is very small as is the case of viruses, from material consisting of purified virus particles. If the material contains impurities which are proteins or contain proteins, it is not possible to determine which are viral and which are not. Yet only after the viral proteins are characterised is it possible to employ them as antigens in antibody tests.

To characterise the viral genome
As for viral proteins the only way to prove that a stretch of RNA is viral it is to obtain it from material which contains nothing else but virus particles. If the material contains impurities the impurities must not include RNA. Then and only then can the RNA and its complementary DNA (cDNA) be used as probes and primers for genomic hybridisation and PCR studies.

To act as a gold standard for the antibody tests
The reaction of a virus or viral protein with an antibody present in a patient's serum does not prove that the antibody is induced by or directed against the virus or a viral protein. That is, that the reaction is specific. This is because there are significant obstacles which hinder the interpretation of antibody/antigen reactivity including non-specific stimulation,[45-47] cross-reactivity[48-55] or both. Cross reactivity results from antibody molecules, even monoclonal antibodies, interacting not only with the inducing antigen but also with other antigens. Indeed, there are instances where "cross-reactive antibodies may have higher affinity with antigens other than the inducing antigen. Even antigens that differ for most of their structure can share one determinant, and a monoclonal antibody recognising this site would then give a 100% cross-reaction. An example is the reaction of autoantibodies in lupus with both DNA and cardiolipin...It should be emphasised that sharing a "determinant" does not mean that the antigens contain identical chemical structures, but rather that they bear a chemical resemblance that may not be well understood, for example, a distribution of surface charges".[55] Since polyclonal antibodies are composites of monoclonal antibodies these facts apply equally, if not more so, to polyclonal antibodies. These facts have been extensively exploited in clinical medicine for the diagnosis of diseases such as syphilis and infectious mononucleosis. In these diseases, *T. pallidum* and Epstein-Barr virus cause the appearance of antibodies reactive with ox-heart proteins and sheep and horse red blood cells. However, this does not mean that patients are "infected" with ox-heart, or horse red blood cells and the diseases are induced by these agents. The only way to determine the specificity of an antibody/antigen reaction is to use an independent method, a gold standard to prove the presence or absence of the antigen. The only possible gold standard for a test to prove a virus infection is the virus in question. That is, virus isolation/purification.

METHODS FOR THE ISOLATION/PURIFICATION OF RETROVIRUS-LIKE PARTICLES
Up till the 1950s retroviruses were isolated/purified by filtration although this method was less than satisfactory. With the development of the electron microscope, apparently, for some retrovirologists, the detection of retrovirus-like particles was deemed sufficient to prove the existence of a retrovirus. However, other scientists including the well-known retrovirologist, JW Beard, recognised that cells, including uninfected cells, under various conditions, were responsible for the generation of a heterogenous array of particles some with the appearances of retroviruses. Beard stressed: "identification, characterisation, and analysis are subject to well-known disciplines established by intensive investigations, and the possibilities have by no means been exhausted. Strangely enough, it is in this field that the most frequent shortcomings are seen. These are related at times to evasion of disciplines or to their application to unsuitable materials. As was foreseen, much of the interest in the more tedious aspects of particle isolation and analysis has been diverted by the simpler and undoubtedly informative processes of electron microscopy. While much can be learned quickly with the instrument, *it is nevertheless clear that the results obtained with it can never replace, and all too often may*

obscure, the need for the critical fundamental analyses that are dependent on access to homogenous materials"[56] (italics ours).

By the 1970s there was general agreement that "Virions of RTV [retroviruses] have a characteristic buoyant density, and centrifugation to equilibrium in density gradients is the preferred technique for purification of RTV".[57] The method of banding in density gradients is not ideal either. Substances other than retroviruses may band at the same density. This is why at a meeting held at the Pasteur Institute in 1972, Francoise Barre-Sinoussi and Jean-Claude Chermann stressed that to claim purification of retrovirus-like particles using sucrose density gradients it is absolutely necessary to prove, using the electron microscope, that the 1.16 gm/ml band contains nothing else but particles with "no apparent differences in physical appearances".[21,22]

THE PHENOMENA CLAIMED TO PROVE THE EXISTENCE OF HIV
In May 1983 Luc Montagnier, Francoise Barre-Sinoussi, Jean-Claude Chermann and colleagues published a paper in *Science* entitled, "Isolation of a T-Lymphotropic retrovirus from a patient at risk for Acquired Immune Deficiency Syndrome (AIDS)".[58] This is the paper which, since the resolution of the polemics between Montagnier and Gallo concerning allegations of misappropriation by the latter of the French virus sent to the US by the Pasteur Institute, is accepted as being the study which proved the existence of HIV. There it was shown that mitogen stimulated lymph node cell cultures from a gay man (BRU) with lymphadenopathy were able to transcribe the synthetic RNA primer-template An.dT$_{15}$. From this data Montagnier and his colleagues concluded that BRU's lymph node cells were infected with a retrovirus. The finding of the same activity in the supernatant of a coculture consisting of the same cells with stimulated lymphocytes from a healthy individual was considered proof for virus transmission as well as isolation. In another experiment supernatants from the cocultures were added to two, three day old, stimulated umbilical cord lymphocytes cultures. "Electron microscopy of the infected umbilical cord lymphocytes showed characteristic immature particles with dense crescent (C-type) budding at the plasma membrane". Supernatant from the culture was banded in sucrose density gradient and the 1.16 gm/ml band was shown to transcribe An.dT$_{15}$. The proteins in the 1.16 gm/ml band as well as the proteins of a cellular extract were separated according to their molecular weight using "denaturing buffer and electrophoresed on 12.5 percent polyacrylamide-SDS slab gel". When the strips were incubated with human sera many proteins from the cellular extracts were found to react with serum from BRU, another gay man as well as a "healthy donor". In the strips containing the proteins from the 1.16 gm/ml band three proteins including a p25 ('p' for protein, 25 for its molecular weight in thousands) were found to react. They also reported that the p25 did not react with antibodies to HTLV-I. The material banding at 1.16 gm/ml was claimed to be "purified, labelled, virus" although no electron microscopic data were presented. The authors concluded: "A retrovirus belonging to the family of recently discovered human T-cell leukaemia viruses (HTLV), but clearly distinct from each previous isolate, has been isolated from a Caucasian patient with signs and symptoms that often precede the acquired immune deficiency syndrome (AIDS). This virus is a typical type-C RNA tumor virus, buds from the cell membrane, prefers magnesium for reverse transcriptase activity, and has an internal antigen (p25) similar to HTLV p24".[58]

Robert Gallo and his associates did not consider the Montagnier group data as proving "true isolation".[59] As late as 1997, in a book published by one of the best known HIV experts, Jaap Goudsmit, one reads: "The BRU lymph node was first cultured in early January 1983 and, on January 15, it shed an enzyme absolutely unique to the lentivirus group. [The enzyme is not even specific to retroviruses much less to *Lentiviruses* (see below)]...The BRU virus grew slowly and with difficulty, but its identity and activity were reported in the May 20, 1983 issue of *Science*...The Pasteur Group was widely acclaimed but very worried. In the world of virology, finding a new virus is not enough: You must propagate and isolate the organism for analysis by other virologists. The French had not yet isolated their new lentivirus".[60]

Why then did (a) Gallo, who reviewed the Montagnier manuscript, recommend its publication? (b) all the HIV experts including Gallo and Goudsmit (on page 24 one reads: "BRU was the first strain to be isolated") accept that the first isolation of HIV and thus of its existence was proven in the May 1983 *Science* paper?

A year later, in May 1984, Gallo, Popovic and their colleagues published four papers in *Science* in which they claimed "isolation" of another retrovirus from AIDS patients.[59,61-63] However, in addition to the use of a leukaemic cell line, the only difference between the Montagnier and Gallo groups' data were quantitative. In the first paper entitled "Detection, Isolation and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and pre-AIDS", (HTLV-III=HIV), experiments were described in which "concentrated culture fluids harvested from short-term [mitogenically stimulated] cultures of T-cells" from patients with AIDS or pre-AIDS were cultured with a mitogenically stimulated leukaemic cell line HT and highly selected clones obtained by culturing HT with irradiated cells of a healthy donor. The data presented as proof of isolation of

HIV were (a) RT activity in cell free supernatants and the 1.16 gm/ml band; (b) reaction in the cultures with "Rabbit antiserum to HTLV-III" and "Patient serum (E.T.)"; (c) EM showing the presence of retroviral-like particles in the cultures.

An enquiry conducted by the National Institutes of Health Office of Scientific Integrity found that the HT cell line was cultured not with concentrated fluids (supernatant) originating from individual AIDS patients, but with concentrated fluids pooled initially from individual cultures of three patients and ultimately from the individual cultures of ten patients.[64] In evidence given to this enquiry the reason given was because none of the supernatants "individually was producing high concentrations of reverse transcriptase". In other words, Gallo and his colleagues did not regard the levels of RT from individual cultures as proof that individual specimens contained a retrovirus. The Gallo investigation found the pooling of specimens to be "of dubious scientific rigor". One scientist described the procedure as "really crazy".[65] Most importantly how could Gallo use the reaction with rabbit antiserum to prove HIV isolation (purification) when, to obtain rabbit antiserum rabbits must first be injected with pure HIV?[66] It is inexplicable how Gallo and his colleagues could already possess "Rabbit serum to HTLV-III" before they had proved the existence of a novel virus. However, if the antiserum was manufactured "from rabbits infected repeatedly with disrupted HTLV-III", that is, the material banding at 1.16 gm/ml, one would expect to obtain antibodies to all the proteins constituting this material even if the proteins were cellular and not viral.

In the second paper entitled "Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from patients with AIDS and at risk of AIDS", Gallo and his colleagues claimed to have "isolated" HTLV-III (HIV) from 26/72 (36%) of AIDS patients. In this paper isolation was defined as "more than one of the following": "repeated detection of a Mg^{2+}-dependent reverse transcriptase activity in supernatant fluids; virus observed by electron microscopy (EM) [retrovirus-like particles in the culture]; intracellular expression of virus-related antigens detected with antibodies from seropositive donors or with rabbit antiserum to HTLV-III; or transmission of particles". Transmission of particles was defined as "detected by RT assays or by electron microscopic observation, to fresh human [umbilical] cord blood, bone marrow, or peripheral blood T lymphocytes", cultured with supernatants from the "infected" cultures.[61] (It can be seen that the Gallo group method permitted "isolation" of a retrovirus without evidence for either particles or RT).

In the third paper, proteins from the 1.16 gm/ml band which they claimed was "purified" HIV, as well as the proteins from the "infected" cells, were "lysed and fractionated by electrophoresis on a 12% polyacrylamide slab gel in the presence of SDS. The protein bands were electrophoretically transferred to a nitrocellulose sheet" and reacted with different sera. In other words Gallo used a technique which is known as Western blot (WB) antibody test. Many proteins from the cellular extract were found to react with sera from both patients and healthy individuals. They also reported that two proteins from the 1.16 gm/ml band, p24 and p41, reacted with patient sera. For this and no other reason it was claimed that "these molecules are the major components of the virus preparation. p24 and p41 may therefore be considered the viral structural proteins". As far as morphology is concerned, the Gallo group reported that the HIV particle "is produced in high numbers from infected cells by budding from the plasma membrane. A possible unique feature of this virus is the cylindrical shaped core observed in many mature virions...HTLV-III is a true member of the HTLV family". (HTLVs are type C retroviral particles and not *Lentiviruses* as HIV is claimed to be).

In the fourth paper, instead of separating the proteins which banded at 1.16 gm/ml and then incubating them with patient sera, the mixture of all proteins was used, that is, they performed a test known as enzyme-linked immunosorbent assay (ELISA). "To understand the molecular nature of the antigens recognised by ELISA", they also performed WB, with some of the sera. They reported that: "Serum samples from 88 percent of patients with AIDS and from 79 percent of homosexual men with signs and symptoms that frequently precede AIDS, but from less than 1 percent of heterosexual subjects, have antibodies reactive against antigens of HTLV-III. The major immune reactivity appears to be directed against p41, the presumed envelope antigen of the virus…The data presented here and in the accompanying reports suggest that HTLV-III is the primary cause of AIDS". Two years later Gallo wrote that "The results presented in our four papers provided *clearcut evidence that the aetiology of AIDS and ARC was the new lymphotropic retrovirus, HTLV-III*"[67] (italics ours).

COMMENTS

As mentioned, apart from the quantitative difference, the Gallo group experiments are no different from those performed by Montagnier and his colleagues. It follows then, that if Montagnier's group data do not prove "true isolation" neither did Gallo's nor anybody else's because to date everybody has repeated (for the vast majority only part therof) the same experiments as these two groups. It is of pivotal significance that neither group reported the use of valid controls (see above) nor did they prove they had obtained purified retrovirus-like particles. The question then is, do the data obtained by the Montagnier and Gallo group, that is RT, particles in culture and antigen/antibody reactions prove the isolation of a unique, or even a retrovirus, any retrovirus?

Without doubt, if by isolation one expects proof of purification, then the detection of an enzyme, or retrovirus-like particles in a culture, or proteins either in the cells or the 1.16 gm/ml band which react with antibodies present in human or animal sera, do not comply. To argue otherwise one must define the detection of cardiac or hepatic enzymes in the blood of patients suffering chest pain or jaundice as proof for isolation of the human heart or liver. Likewise, if an antibody/antigen reaction is proof for isolation of a virus then antibody reactivity to the protein _HCG is proof for isolation of the human placenta. The assertion that the detection of a retrovirus-like particle in a culture proves isolation is no different from asserting that the detection of a fish-like creature in the ocean is the same as having a definite fish in your frying pan. The detection of RT, retrovirus-like particles and antibody/antigen reactivity can only be considered proof for the detection of a retrovirus, and then if, and only if, there is prior knowledge that the three phenomena are specific to retroviruses.

To claim that the detection of this phenomena proves the existence of a new retrovirus one must have proof that at least one of the three phenomena is different from that observed in all other known retroviruses.

Reverse transcriptase
At present some of the leading HIV researchers consider RT as being the "*sine qua non*" of retroviruses and regard the detection of reverse transcription in lymphocyte cultures from AIDS patients not only as proof of the presence of such viruses but of HIV itself including HIV isolation[68-70] and quantification.[71] However, according to some of the best known retrovirologists including its discoverer, as well as the Nobel Laureate and former Director of the US National Institute of Health Harold Varmus, reverse transcriptases are present in all cells as well as bacteria and viruses.[72-74] "Reverse transcriptase (RT) was first discovered as an essential catalyst in the biological cycle of retroviruses. However, in the past years, evidence has accumulated showing that RTs are involved in a surprisingly large number of RNA-mediated transcriptional events that include both viral and nonviral genetic entities".[75] Even if RT were a property only of viruses it is not specific to retroviruses. According to Varmus, "Reverse transcription was assigned a central role in the replication of other viruses [hepatitis B and cauliflower mosaic viruses] and in the transposition and generation of other kinds of eukaryotic DNA".[76] "The hepatitis B viruses (HBVs) are small DNA viruses that produce persistent hepatic infections in a variety of animal hosts and replicate their DNA genomes via reverse transcription of an RNA intermediate. All members of this family contain an open reading frame (ORF), "P" (for *pol*), which is homologous to retroviral *pol* genes"[77] [*pol*=polymerase]. "Hepatitis B virus (HBV) resembles retroviruses, including HIV, in several respects. In particular, both viruses contain reverse transcriptase, and replicate through an RNA intermediate". Because of this, it has been suggested that hepatitis B infection should be treated with the same antiretroviral agents as HIV infection.[78,79]

At present, evidence exists which shows that although the major target organ for hepatitis B virus is the liver, cells other than hepatocytes "including peripheral blood lymphocytes and monocytes, may become infected with HBV".[80] Lymphocyte stimulation in general and PHA (an agent employed in the majority of cultures with tissues from AIDS patients), is associated with the production of hepatitis B virus from peripheral blood lymphocytes in patients infected with HBV including "viral replication in chronic hepatitis B infection of childhood".[81,82] Hepatitis B virus infection is widespread in the AIDS risk groups.

In the early 1970s Gallo proved that cultures of leukaemic cells transcribe the An.dT$_{15}$ template-primer as does material banding at 1.16 gm/ml originating from "PHA stimulated (but not unstimulated normal human blood lymphocytes".[83] Reading the Gallo 1984 *Science* papers the impression is gained that the leukaemic HT cell line and thus its clones, including H9 which Gallo used, was a new cell line developed in Gallo's laboratory. However it is now known that the HT cell line is the HUT78 cell line which originated from a patient with adult T cell leukaemia, a disease which Gallo claims is caused by the retrovirus, HTLV-I. A year earlier Gallo claimed to have proven that the HUT78 cells are infected with HTLV-I.[84] If this is the case, the Gallo group would have detected RT in their cultures even if the enzyme is specific to retroviruses and the cultures were not infected with HIV. Other researchers reported RT activity in normal non-infected spermatozoa.[85]

It must also be pointed out that the presence of HIV reverse transcriptase was detected by both Montagnier and Gallo, (and all HIV experts since) indirectly, that is, by detecting the transcription of the template-primer An.dT$_{15}$. However, at least in 1984 if not 1983, Montagnier and his colleagues knew that in the 1970s there was proof that "Among a number of template primers, $(rA)_n.(dT)_{12-18}$ has been most frequently employed since RT shows high activity with this template primer. However, the problem is that the cellular DNA polymerases (pol β and pol γ) also effectively utilize the same template primer".[86] In fact, in 1975, an International Conference on eukaryotic DNA polymerases defined DNA polymerase _ as the cellular enzyme which "copies An.dT$_{15}$ with

high efficiency but does not copy DNA well".[87] One year earlier Gallo wrote: "Under appropriate reaction conditions DNA polymerase _ can efficiently transcribe poly (A) primed by oligo (dT)".[88] Thus it is possible to detect transcription of $An.dT_{15}$ even when no RT, either viral or cellular, is present, especially under the conditions used to prove the existence of HIV. (Both Montagnier and Gallo accept that the phenomena detected in cultures which are said to prove HIV isolation cannot be detected unless the cells are chemically stimulated[89,90]). Nowadays the non-specificity of RT is broadcast even in the popular press to readers contemplating the purchase of shares in biotechnology companies.[91]

In conclusion, even if one accepts Montagnier and Gallo's groups' definition of isolation, given that RTs and reverse transcription are nonspecific to retroviruses, their detection even in an unlimited number of consecutive cultures/co-cultures cannot be considered proof for isolation and propagation of a retrovirus.

"HIV" particles

Montagnier and his colleagues reported HIV initially as a type C particle, then as a type D particle and then as a *Lentivirus*. In 1984 Gallo and his colleagues reported HIV as a type C particle. However, in 1985 Gallo wrote: "A possible unique feature of the virions is the cylindrical core observed in many presumably mature virions. Virions having this type of core have been frequently reported for certain type D retroviruses, and in some instances, for type C retroviruses". Jay Levy reported HIV as a type D particle.[92] Others at the University of California wrote that "AIDS virus isolated show morphologic characteristics of type C, type D and Lentiviruses".[93] According to Anthony Fauci and others" "T-cells and macrophages handle the virus very differently. In the T-cell, virus buds out of the external plasma membrane of the cell. In the monocyte/macrophage cultures it buds into membrane-bound vesicles inside the cells".[94] The latter is a description of a type A, retroviral particle.[33] Thus the leading HIV experts have described HIV as a member of two subfamilies and three genera of *Retroviridae*. These taxonomical differences imply that if HIV was a newly discovered mammal, it could have been either human, a gorilla or an orang-utan. By consensus at present HIV is regarded as a *Lentivirus*. This agreement was reached when it was realised that in "HIV" positive individuals AIDS did not appear soon after "infection", although the designation *Lentivirus* is a morphological description.[33]

"HIV infected cultures" contain in addition to the particles with the morphology attributed to HIV many other "viral particles". For example: Hockley and his colleagues from the Electron Microscopy and Photography Section and Division of Virology at the National Institute for Biological Standards and Control in the United Kingdom describe a profusion of particles which they divide broadly into three groups, mature, ring-like and small with spikes. The mature particles "were approximately spherical in shape and 100 to 150nm in diameter. The outer lipid membrane was frequently broken or absent in places, and there was no evidence of surface spikes...A few mature particles were found that were larger than average and appeared to contain a double nucleoid...in the preparation of HIV there were always many vesicles with granular contents in which it was not possible to recognise a distinct nucleoid". Also, "The ring-like particles had a more consistently spherical shape and were larger (140nm in diameter)" and the small particles "were unusually spherical but sometimes slightly angular in shape and 65 to 90nm in diameter" and had spike-like projections on their surface.[95] Hans Gelderblom who has done most of the EM studies in HIV/AIDS research reported that although *Lentiviruses* and thus HIV is considered to have a cone shaped core, he and his colleagues found centrosymmetric and tubular cores as well. The caption to one photograph reads: "Virions can be seen having either elongated, 'baton-like' tubular cores 30-35nm in diameter or containing more than one core. Tubular and regular cone-shaped cores can coexist within one virion". The text states: "Rarely, tubular core structures reminiscent of batons with a diameter of 30-35nm and a length of 150-250nm are observed".[20] Lekatsas and other virologists from Pretoria and Johannesburg reported: "We used the characteristic cylindrical structure in the core as an identifying characteristic for the virus to distinguish it from cellular debris and also noted that it may vary considerably in its dimensions and morphological features. We have found two basic virus particle sizes, 90nm and 120nm, both present in large numbers.   The larger particle bears no surface projections while the smaller particle is rarely 'naked' and usually bears projections".[96] The US CDC reported: HIV particles are "usually round and have a diameter of about 85-95nm...Virus with bar-shaped nucleoids and particles with a tear-drop shape are commonly seen in HTLV-III/LAV infected lymphocytes, sometimes ring-shaped particles without dense nucleoids are also seen".[97] The question then arises if the particles with the "unique" morphology considered to be HIV represent an exogenous retrovirus originating from tissues of AIDS patients or those at risk, then what is the origin and role of the many non-HIV particles and which, if any, of the "HIV" or non-HIV particles band at 1.16 gm/ml? That is, which have the density characteristic of retroviruses? Retrovirus-like particles have been found in non-HIV-infected cord blood lymphocytes cell cultures and in cells used for HIV "isolation" such as and H9 (HUT-78), CEM, C8166 and EBV transformed B-cells.[98] Retrovirus-like particles antigenically related to HIV have been found in cultures of salivary gland extracts from patients with Sjorgen's syndrome.[99] In the only EM study,

either *in vivo* or *in vitro* in which suitable controls were used and in which extensive blind examination of controls and test material was performed, particles indistinguishable from "HIV" were found in 18/20 (90%) of AIDS as well as in 13/15 (87%) of non-AIDS related lymph node enlargements. This led the authors to conclude: "The presence of such particles do not, by themselves indicate infection with HIV".[100]
It is of pivotal significance to note that:

1. As Hans Gelderblom and his colleagues pointed out in 1998, to date nobody has reported the presence of "infectious plasma HIV".[101]

2. In no "HIV-infected" cultures are there particles which display both principle morphological characteristics of retroviruses, that is, "a diameter of 100-120nm" AND surfaces which "are studded with projections (spikes, knobs)". The electron microscopist agree that the "HIV" particles are devoid of knobs[95-98] and thus of the "HIV" protein gp120 (see below) said to be the constituent protein of the knobs.

Hans Gelderblom and his colleagues have estimated that immediately after being released from the cell membrane "HIV particles" possess an average of 0.5 knobs per particle which are rapidly lost, but also pointed out that "it was possible that structures resembling knobs might be observed even when there was no gp120 [knobs] present, i.e. false positives".[102] Yet all HIV experts agree that the infectivity of the HIV particles is determined by the gp120 (knobs). Thus, according to Montagnier *et al*, "The gp120 is responsible for binding the CD4 receptor"[103] while for Matthews and Bolognesi, "First gp120 binds to the CD4 receptor on an uninfected cell; then gp41 becomes anchored in the adjoining membrane; next the two membranes begin to fuse, and the virus spills its contents into the cell".[104] Callebaut *et al* state, "The human immunodeficiency virus (HIV) infects lymphocytes, monocytes, and macrophages by binding to its principal receptor, the CD4 molecule, through the viral envelope glycoprotein gp120. The V3 loop of gp120 is critical for HIV infection".[105] Others are in complete agreement.[106-111]

The general agreement that gp120 (knobs) is absolutely necessary for HIV particles to be infectious and the fact that this protein (knobs) is not present in the cell free particles leads to one unavoidable conclusion, that is, the "HIV" particles are not infectious, they are not viral particles. Furthermore,

1. To infect umbilical cord lymphocytes and the HUT-78 cells Montagnier and Gallo used cell-free fluids (supernatants). Montagnier cultured umbilical cord lymphocytes with supernatant from the cocultures of lymphocytes from BRU cultured with lymphocytes from a healthy individual. Gallo cultured the HUT-78 cell line with supernatants from the cultures of ten "infected" individuals. Even if the supernatants contained particles, being cell free the particles would be devoid of knobs (gp120), that is, they would not have been infectious. This means that even if Montagnier and Gallo had proof that their umbilical and HUT-78 cloned cultures contained particles with all the morphological characteristics of retroviruses, that in sucrose density gradients the particles banded at the 1.16 gm/ml band and the band contained nothing else but particles, the particles could not have originated from their patients. Furthermore, since the only samples which the Pasteur Institute gave to the Gallo laboratory[67] were cell free, one must question the basis upon which Gallo was accused of misappropriation of the French virus.

2. It is generally accepted that haemophiliacs are infected with HIV by contaminated factor VIII. However, to date nobody has reported the presence of "HIV" particles in plasma. Even if such particles were present the particles would be devoid of gp120. Since gp120 is "crucial to HIV's ability to infect new cells" it is not possible for haemophiliacs to be infected with HIV through factor VIII administration. (There is another fundamental reason why it is impossible for haemophiliacs to be infected with "HIV" from "contaminated" factor VIII. According to a publication from the CDC,[213] "In order to obtain data on the survival of HIV, laboratory studies have required the use of artificially high concentrations of laboratory grown virus...the amount of virus studied is not found in human specimens or any place else in nature,...it does not spread or maintain infectiousness outside its host. Although these unnatural concentrations of HIV can be kept alive under precisely controlled and limited laboratory conditions, CDC studies have shown that drying of even these high concentrations of HIV reduces the number of infectious viruses by 90 to 99 percent within several hours. Since the HIV concentrations used in laboratory studies are much higher than those actually found in blood or other body specimens, drying of HIV-infected human blood or other body fluids reduces the theoretical risk of environmental transmission to that which has been observed--essentially zero". Given that factor VIII is dispensed as a freeze-dried powder which spends many weeks or months waiting use, it is incomprehensible that the CDC and others continue to regard patients with haemophilia at risk for HIV infection via contaminated factor VIII concentrates and enigmatic that another explanation for "HIV" and AIDS in haemophiliacs has not been sought.[4]

The only conclusion one can draw from the electron microscopy data is that the reported particles are non-HIV or even retroviral specific which means that the detection of such particles, be it in an unlimited number of consecutive cultures/cocultures, is not proof for isolation, no matter how isolation is defined.

Furthermore:

1. Even if reverse transcriptase activity and retrovirus-like particles are specific to retroviruses, they are not specific for a unique retrovirus. The only evidence both groups presented for the existence of a unique retrovirus, HIV, is the antigen/antibody reaction, as acknowledged by both Montagnier and Gallo.

2. It is accepted that the finding of a retrovirus in culture, especially under the conditions used by Montagnier and Gallo is not proof that the retrovirus is present *in vivo*. The only evidence which both Gallo and Montagnier's groups presented for the existence of HIV *in vivo* was the antigen/antibody reaction.

3. Even today the only evidence given as proof that HIV is the cause of AIDS is a "correlation" between the antibody test and the appearance of AIDS.

   It is obvious that:

   (a) the Montagnier and Gallo interpretation of the antigen/antibody reaction is crucial for the HIV hypothesis of AIDS and in fact for the existence of a unique retrovirus, HIV;

   (b) if the interpretations are not correct, one would have no choice but to question not only the HIV hypothesis of AIDS but also the existence of HIV.

It has already been mentioned that the mere interaction between an antigen such as a protein or a virus and an antibody does not prove any more than a chemical relationship. In all other aspects the reaction may be totally nonspecific. To prove the specificity of the reaction one must employ an alternative, independent method of proving the existence of the antigen, that is, one must use what is generally known as a gold standard. The only possible gold standard for the HIV antibody tests (the antigen/antibody reaction) is HIV itself, that is, HIV isolation. No matter how one defines isolation, obviously the definition cannot include the antibody/antigen reaction (the antibody test, neither the WB nor the ELISA) because, by doing so, not only does one not have an independent analysis, the test becomes its own gold standard and is thus rendered meaningless. Reading the 1984 Gallo *Science* papers it appears that Gallo always defined isolation as "more than one of the following: reverse transcriptase activity either in the supernatant or the 1.16 gm/ml band; retrovirus-like particles in the culture or reaction between proteins (either in the cultured cells or the 1.16 gm/ml band) with antibodies present in the patients sera or "antiserum to HTLV-III". However, in an interview Gallo gave to Huw Christie, the editor of the British magazine *Continuum*, at the 1998 Geneva AIDS Conference, Gallo said, "Sometimes we had Western Blot positive but we couldn't isolate the virus. So we got worried and felt we were getting false positives sometimes so we added the Western Blot. That's all I can tell you. It was an experimental tool when we added it and for us it worked well because we could isolate the virus when we did it".[112] In other words:

1. In 1984 Gallo knew that to prove the specificity of the antibody test one must use a gold standard.

2. The gold standard had to be HIV isolation.

3. Although it is not known how he and his colleagues initially defined isolation, the definition did not include the antibody/antigen reaction (the Western Blot). This means that Gallo and his colleagues were aware that an antibody/antigen reaction cannot be used as proof for isolation. Yet, by the time their *Science* papers were published, and in order to reconcile the low correlation between what they initially called isolation and the antibody/antigen reaction, arbitrarily, and against all scientific reasoning, they "added the Western Blot" to their definition of isolation. (It is interesting that even with their novel definition the correlation between "isolation" and antibody tests was still less than perfect. They "isolated" HIV from 26/72 (36%) of patients with AIDS while 88% of patients with AIDS were seropositive using an ELISA test Gallo considered highly specific[62]).

In an interview which Montagnier gave to the French Journalist Djamel Tahi in 1997, he stated: "analysis of the proteins of the virus demands mass production and purification. It is necessary to do that". Indeed, the only way to prove that a protein is a viral constituent is to obtain it from material which contains nothing else but viral particles. Instead Montagnier's and Gallo's groups incubated the proteins which banded at 1.16 gm/ml with sera from AIDS patients. The proteins which were found to react more often with the sera were said to be HIV proteins (although neither group published any evidence for the existence of retrovirus-like particles, pure or

impure, at the 1.16 gm/ml band);   and the antibodies to be HIV antibodies.   However, even if the antigen/antibody reaction is 100% specific, that is, antibodies react only with inducing antigens and with no other, from such a reaction it is impossible to determine the origin even of one reactant much less the origin of both the antigen and the antibody. Let us consider differing scenarios:

1. Both Gallo and Montagnier had proof that the 1.16 gm/ml band contained nothing else but retrovirus particles and that their antibodies reacted specifically with their proteins. According to Montagnier, in the cultures which contain cells originating from patients with adult T4 cell leukaemia, as did Gallo's HUT-78 cell line, "it is a real soup" of retroviruses. Indeed, even if the cultures do not harbour HIV they will harbour HTLV-I and, given the condition used by Gallo and the fact that the cells were leukaemic, they may also contain endogenous retroviruses.[35,113-115] According to one well known HIV expert, Myron Essex, 35% of AIDS patients possess antibodies to HTLV-I.[116] (In the same issue of *Science* in which Montagnier published his isolation of "HIV", Gallo published three papers claiming isolation of HTLV-I from AIDS patients suggesting this retrovirus was the cause of AIDS). According to another equally as well known HIV expert, Reinhard Kurth, from the Paul-Ehrich Institute in Germany, 70% of "HIV-positive patients" have antibodies which react with the retrovirus HTDV/HERV-K, an endogenous retrovirus, or, as Kurth put it, a retrovirus present "in all of us".[35] Thirty seven per cent of HIV positive individuals were also found to have antibodies to type D retroviruses whereas HIV is claimed to be a *Lentivirus*.[117] Although Montagnier's cultures may not have contained HTLV-I, the result may still have been "a real soup" of endogenous retroviruses especially if one considers their culture conditions and the cells used, umbilical cord lymphocytes, which have been shown to release retrovirus-like particles even when not infected with HIV.[98] Since we cannot obtain the proteins from each particle and characterise them, the next best thing is to take the mass of particles, disrupt their proteins and position them in an electrophoretic strip according to their molecular weights. Although we know that the proteins in the strip are retroviral, we have no way of determining which protein belongs to which retrovirus if more than one retrovirus is present at the 1.16 gm/ml band. When the proteins are incubated with sera, we may find some of the proteins react. From such a reaction we will be able to say that the antibodies and obviously the proteins are viral but we cannot determine which protein belongs to which virus and by which virus the antibodies were induced. We will be definitely wrong if we consider such a reaction proof that the 1.16 gm/ml band (a) contains only one retrovirus; (b) the retrovirus is a new exogenous retrovirus, HIV; (c) the proteins are the HIV proteins; (d) the antibodies are HIV antibodies; (e) these data are proof that the patient is infected with HIV even if the antibody antigen reactions are specific.

2. Both Montagnier and Gallo had proof that the vast majority of the 1.16 gm/ml band was composed of retrovirus particles but that the band also contained non-retroviral material. This material could have been of cellular origin (cellular constituents also band at 1.16 gm/ml[38,57,72]) and may be of bacterial, fungal and viral origin (constituents of the many infectious agents other than retroviruses, known to be present in the cultures and patients). It is a fact that in the USA, Europe and Australia individuals with AIDS as well as those at risk have antibodies to many infectious agents including viruses such as EBV, CMV and hepatitis B virus. Evidence also exists which shows that individuals with AIDS and those at risk have circulatory immune complexes, rheumatoid factor, anti-nuclear, anti-cellular, anti-platelet, anti-red cells, anti-actin, anti-tubulin, and anti-myosin antibodies.[118,119] Montagnier himself demonstrated that individuals with AIDS and those at risk have high levels of antibodies to the ubiquitous cellular protein actin whose molecular weight is 41,000, as well as to another ubiquitous protein, myosin, which has two sub-units of molecular weights, 18,000 and 25,000.[118] Anti-lymphocyte antibodies have been found in 87% of patients who have a positive "HIV" antibody test and their levels correlate with clinical status.[120] It is also acknowledged that Africans with AIDS and those at risk are infected with many agents other than HIV.

We know that it is not possible to take a protein from the 1.16 gm/ml mass and know from which component it originates. Let us then follow the same steps as Gallo and Montagnier. Take the mass of material banding at the 1.16 gm/ml, disrupt the proteins and electrophoretically position the proteins in a strip according to their molecular weights. Using this technique we are unable to state from which component of the 1.16 gm/ml band mass a protein on the strip derives. Next we incubate the proteins in the strip with patient sera and discover that some of the proteins react with antibodies present in the sera. Even if the antibody/antigen interaction is 100% specific such a reaction does permit us to define the origin of the proteins. Yet from such a reaction and without any proof that the antibodies specifically reacted, Montagnier and Gallo defined the origin of both the proteins and the antibodies as "HIV".

As mentioned, Montagnier found three proteins in the 1.16 gm/ml band which reacted with antibodies present in his patients' sera. These were p25, p80 and p45. Gallo found two proteins, p24 and p41. Montagnier concluded

that his patients were infected with a retrovirus which "contains a major p25 protein, similar in size to that of HTLV-I", but made no comment in regard to the p80 protein. Regarding p45 he wrote: "The 45K [p45] protein may be due to contamination of the virus by cellular actin".[58]

In 1997 Montagnier said that the protein he detected in 1983 had a molecular weight of 43,000 and was actin.[29] (The molecular weight of actin is neither 45,000 nor 43,000 but 41,000. However since Montagnier and Gallo determined the molecular weights of the proteins by their migration in an electrophoretic strip, and because the migration may be influenced by other factors, for example, by the protein's charge, it is possible that these slight differences in the molecular weight are simply the result of experimental variation).

When Gallo and his colleagues used the cellular proteins as antigens in the WB they reported: "The most prominent reactions were the antigens of the following molecular weights: 65,000, 60,000, 55,000, 41,000 and 24,000. Antigens with molecular weights of approximately 88,000, 80,000, 39,000, 32,000, 28,000 and 21,000 gave less prominent reactions…A large protein with a molecular weight of approximately 130,000 and a protein of 48,000 were also detected". In another experiment the "antigens from virus purified from the culture fluids", that is, the 1.16 gm/ml band, were incubated with different sera. They found an "Extensive" reaction of the AIDS patients sera with p24 and p41 and concluded: "these molecules are the major component of the virus-preparation. P24 and p41 may therefore be considered viral structural proteins…Furthermore, an antigen with a molecular weight of approximately 110,000 was detected in the virus preparation but was below limit of detection in the cells. Also, p39 was present in the virus preparation…Occasionally an additional set of antigens was recognised by a serum but their relation to the antigens described above is unclear".[63]

Between 1983-87 the detection of antibodies in patient sera which reacted with p24 or p41 (Montagnier considered reaction with p24 and Gallo with p41 HIV specific) was considered proof that the patient was infected with HIV. In the same period of time it became obvious that a significant number of individuals at no risk of AIDS had antibodies which reacted with these proteins. Since 1987 most of the proteins which Gallo found to react either in the cell extracts or the 1.16 gm/ml band are now considered HIV proteins[121] and laboratories require the presence of antibodies which react with more than one protein before the patient is considered infected with HIV. The number and identity of antibody/protein (Western blot) bands required vary from continent to continent, from country to country and even between and within laboratories in the same country. Thus it is possible for the same patient to be HIV seropositive in New York for example, but not in Africa or Australia (Figure 1.1 in Part I). However at present evidence exists which shows that the "HIV" proteins which react with antibodies present in patient sera are in fact cellular proteins.

The inescapable confusion is that the antibody/antigen reaction is not HIV specific and thus cannot be used to prove HIV isolation, no matter how isolation is defined.

ORIGIN OF THE "HIV" PROTEINS
The p41 protein

Although Montagnier and his colleagues found a protein p45 (p41) in their "purified" virus[58,122] and the protein reacted with antibodies present in the patients' sera, they concluded that the protein was not viral but the cellular protein actin. Since then many researchers reported the presence of actin in "HIV"[123-127] Indeed some of the best known HIV experts acknowledge that the proteins with molecular weight of approximately 41,000 present in "HIV" are in fact actin.[128]

The p24/p25 protein

At present there is ample evidence that antibodies which react with p24 are common in both human and animal sera, which can only be interpreted as that either p24, the antibodies, or both, are non-HIV-specific or a significant proportion of both humans and animals are infected with HIV. For example, if the interaction between p24 and the antibodies is considered proof for HIV infection then about 30% of individuals who are transfused with HIV negative blood become infected as a result.[129] Since, according to the AIDS vaccine Clinical Trials Group[130] "The presence of p24 was common among low-risk, uninfected volunteers and complicated the interpretation of the Western blot results", HIV infection should be common among healthy, no risk individuals. In fact, because of such evidence, since 1987, with perhaps only two exceptions, Montagnier and researchers conducting the Multicenter AIDS Cohort Study in the United States, no laboratory anywhere in the world considers a reaction between the p24 in the WB and antibodies present in sera proof of HIV infection. Yet, when the same reaction takes place between an antibody to p24 and a patient's serum, it is considered proof of viraemia, and when between an antibody and material present in a cell culture, the same reaction is considered proof of HIV isolation. In fact since 1987 this reaction has been the method of choice for "HIV" isolation by the vast majority of laboratories. However, the non-specificity of the p24 antigen test is obvious and is accepted by no

less an authority on HIV testing than Philip Mortimer and his colleagues from the UK Public Health Laboratory Service, "Experience has shown that neither HIV culture nor tests for p24 antigen are of much value in diagnostic testing. They may be insensitive and/or non-specific".[131]

The reaction of a protein, even if known to be HIV protein with antibodies is not proof for viral isolation, "viraemia" or "viral load". That such a finding is also non-specific can be best illustrated by a few examples. In 1992, Jorg Shupbach, the principle author of one of the first four 1984 *Science* papers published by Gallo's group on HIV isolation, reported that the whole blood cultures of 49/60 (82%) of "presumably uninfected but serologically indeterminate individuals and 5/5 seronegative blood donors were found positive for p24".[132]   If p24 is an HIV protein then it must be present in all AIDS patients if not all seropositive patients and not in persons not at risk of developing AIDS.   Yet Jackson *et al*, who claim an overall 98.3% "HIV isolation" rate, are unable to detect p24 in serum of 58% of AIDS patients, 63% of ARC patients and 83% of asymptomatic seropositive individuals.[133]   This rate of detection is much lower than in non-HIV-infected organ transplant recipients. "In one kidney recipient (the donor was negative for p24 antigen) who, three days following transplantation developed fever, weakness, myalgias, cough and diarrhoea, all bacterial, parasitological and virological samples remained negative [including HIV PCR]. The only positive result was antigenaemia p24, positive with Abbot antigen kits in very high titers of 1000pg/ml for polyclonal and 41pg/ml for monoclonal assays.   This antigenaemia was totally neutralised with Abbot antiserum anti-p24…2 months after transplantation, all assays for p24-antigen became negative, without appearance of antibodies against HIV. Five months after transplantation our patient remains asymptomatic, renal function is excellent, p24 antigenaemia still negative and HIV antibodies still negative.[134]   In one study, p24 was detected transiently in 12/14 kidney recipients. Peak titres ranged from 850 to 200 000 pg/ml 7-27 days post-transplantation. Two heart and 5/7 bone marrow recipients were also positive, although the titres were lower and ranged from 140-750 pg/ml. Disappearance of p24 took longer in kidney (approximately 6 months) than in bone-marrow (approximately 4-6 weeks) recipients. According to the authors: "This may be related to differences in immunosuppression therapy". Discussing their findings they wrote: "The observation of a 25-30kD protein binding to polyclonal anti-HIV human sera after immunoblots with reactive sera raises several questions. This protein could be related to a host immune response to grafts or transplants…Its early detection after transplantation might indicate the implications of immunosuppression therapy…the 25-30kD protein could therefore be compared with the p28 antigen recently described with human T-cell-related virus lymphotropic-endogenous sequence…The characterisation of this 25-30kD protein may represent an important contribution to the detection of HIV-1-related endogenous retroviruses".[135]

Ninety-seven percent of sera from homosexuals with immune thrombocytopenia (ITP) and 94% of sera from homosexuals with lymphadenopathy or AIDS contain an antibody that reacts with a 25kD membrane antigen found in platelets from healthy donors and AIDS patients, as well as a 25kD antigen found in green-monkey kidney cells, human skin fibroblasts, and herpes simplex cultured in monkey kidney cells. This reaction was absent in sera obtained from non-homosexual patients with ITP or non-immune thrombocytopenic purpura".[136]   Using monoclonal antibody p24 has also been found a constituent of the normal human placenta.[137]

As far as Montagnier is concerned, p24 is the crucial HIV protein. However, if p45 (p41) which also bands at 1.16 gm/ml and reacts with antibodies present in patient sera is the cellular protein actin, just because actin is ubiquitous, and has the same molecular weight, why should not p24 be the equally as ubiquitous protein, myosin, which is known to have a sub-unit of the same molecular weight? Especially if one considers the presently available evidence which shows that like actin, myosin is present in the "HIV particles"[123-127] and that Montagnier himself demonstrated that individuals with AIDS and those at risk have high levels of antibodies to this protein.[118]

The p32 protein
In 1987 Henderson isolated the p30-32 and p34-36 of "HIV purified by double banding" in sucrose density gradients. By comparing the amino-acid sequences of these proteins with Class II histocompatability DR proteins, they concluded that "the DR alpha and beta chains appeared to be identical to the p34-36 and p30-32 proteins respectively".[138]   That these proteins are cellular is acknowledged by other HIV experts.[128]

The p17/p18 protein
Sera from AIDS patients bind to a p18 protein mitogenically stimulated "HIV" infected T-cells but not to uninfected, unstimulated lymphocytes. However, when the lymphocytes are mitogenically stimulated but uninfected, the AIDS sera bind to a p18 protein in these uninfected lymphocytes.[139]   A monoclonal antibody to "HIV" p18 reacts with dendritic cells in the lymphatic tissues of a variety of patients with a number of non-AIDS related diseases[140] and the "same pattern reactivity was present in normal tissue taken from uninfected

individuals as in those taken from HIV positive subjects".[53]   It is of interest that one of myosin's two sub-units has a molecular weight of 18,000. The p17/18 protein is also present in the normal human placenta.[137]

The p160 and p120 proteins
The general agreement amongst the HIV experts is that p120 and p41 are cleavage products of p160, and that p160 is found only in the "infected" cells but not the "purified" virus, that is, the 1.16 gm/ml band. As mentioned, p120 (which in 1984 Gallo reported as p110), is said to be present only in the "HIV" particles knobs, spikes, and to date no electron microscopist could prove the existence of such knobs on cell-free "HIV" particles. Yet under certain experimental conditions, p120 and p160 are found in the electrophoretic strips prepared from proteins of the "purified" virus. According to Burke, "Most Western blot strips prepared in the United States before 1987 lacked appreciable bands corresponding to the high molecular weight viral envelope glycoproteins (gp120 and its precursor gp160). Underrepresentaion of these proteins was probably owing to several factors, especially by ultracentrifugation, and by denaturing of antigenic activity during electrophoresis and transblotting. Since early 1987 blot preparation methods have been modified to ensure that the high molecular weight envelope bands can be clearly identified with most patient sera".[121]   However, no amount of "blot preparation" modification are able to create what is not present. The explanation for the presence of the "high molecular weight envelope bands" was discovered in 1989 by researchers from New York who showed that in the Western blot strip, "the components visualised in the 120-160kDa region do not correspond to gp120 or its precursor but rather represent oligomers of gp41".[141]   It was also shown that the WB pattern obtained is dependent on many factors including temperature and the concentration of sodium dodecyl sulphate used to disrupt the "pure virus". "Confusion over the identification of these bands has resulted in incorrect conclusions in experimental studies. Similarly, some clinical specimens may have been identified erroneously as seropositive, on the assumption that these bands reflected specific reactivity against two distinct viral components and fulfilled a criterion for true or probable positivity. The correct identification of these bands will affect the standards to be established for Western blot positivity: it may necessitate the reinterpretation of published results".[142]   Little, if any notice was taken of these findings and recommendations. In fact, in Africa, the finding of antibody reactivity in an individual's serum with any two of p160, p120, p41, that is, antibodies to actin and its polymers, is considered proof that Africa is in the grip of an epidemic of HIV induced immunodeficiency. Also, as is the case of both p24 and p18, p120 is present in the normal human placenta.[137]

Even if proof exists that the "HIV" proteins do indeed belong to a unique exogenous retrovirus, it cannot be assumed that antibodies that react with them are diagnostic of HIV infection. To prove the specificity of the antibody tests it is mandatory to:

(a) test a large number of subjects with and without AIDS. The subjects without AIDS must not exclusively be healthy individuals (since they do not have high levels of antibodies, one would expect few if any reactions) but include the sick, such as patients with infectious diseases (other than those which are said to result from HIV infection), those receiving chemotherapy and those with auto-immune disorders;

(b) simultaneously (preferably on the same blood sample) perform tests for HIV isolation;

(c) compare the antibody test results with the results of HIV isolation, that is, use HIV as a gold standard for the antibody test.

To date nobody has published such studies using any definition of HIV isolation. However, the fact that such antibodies are present in sera obtained from humans accepted not be infected with HIV demonstrates that the antibodies are non-specific:

1. Although they did not comment (Montagnier commented only on p41), both Montagnier and Gallo found many proteins in the "infected" cells which reacted with antibodies present in both AIDS patients and "healthy donors". If the antibodies in the AIDS patients were HIV antibodies what were the antibodies in the healthy donors?

2. The first antibody tests in Africans were performed in 1984 by Montagnier and nineteen of his associates including researchers from the CDC.[143]   The sera were tested by ELISA and then by a radioimmunoprecipitation assay (a procedure similar to the Western blot). The latter was considered positive if a p24 band was present. The p41 band and also an 84-kDa band were not considered diagnostic because "The 43-kD [p41] band and the 84-kDa band are cellular contaminants that are immunoprecipitated in all the tested sera", from both patients and controls. (Yet today, in Africa, the p41 and its polymers, on its own is considered to represent a positive WB and thus proof of HIV infection). Thirty-two patients (88%) were positive by both tests. So were six out of 26 (23%) healthy controls.

3. Biggar and his colleagues were among the first to raise the possibility that, at least in Africans, the antibody tests may not be 100% specific, as was generally believed. They found that in healthy Africans the probability of finding a positive HIV antibody test increased significantly with increasing immune-complex levels. They concluded that "reactivity in both ELISA and Western blot analysis may be non-specific in Africans...the cause of the non-specificity needs to be clarified in order to determine how they might affect the seroepidemiology of retroviruses in areas other than Africa, such as the Caribbean and Japan...Serological studies from Africans would need to be re-evaluated with a more specific test before conclusions can be drawn".[144] In the same year, 1985, one of the best known HIV experts, Robin Weiss, and his colleagues, accepted that African sera "may give a false-positive result on direct binding assay systems, or on Western blots".[145] One year later some of the best known experts on HIV/AIDS in Africa expressed the view that in Africa "...serodiagnosis is complicated by the need for confirmatory tests because of the presence of possible cross-reacting antibodies".[146] However, experiments to determine the specificity of the "HIV" antibodies in Africans or anywhere else have never been reported. In fact, AIDS reporting in Africans is based on clinical grounds without the requirement for antibody testing or immunological function.[147]

4. In a study conducted in Africa[148] 83% of patients with suspected AIDS were HIV positive, but so were 44% with malaria, 97% with herpes zoster, 43% with pneumonia, 67% with amoebic dysentery and 41% with carcinoma. In another study 42% of women with recurrent abortions, 67% with vaginal ulcerations and 33% with haemorrhoids had a positive HIV antibody test.[149] In 2001 Ghosh reported that of 2/33 (6%) of patients with "Severe *P. falciparum* malaria" had a false positive HIV ELISA, considered to be caused by "intense, non-specific immune stimulation".[150]

5. In 1985 Gallo and his colleagues reported testing a number of sera collected in 1972/73 from the West Nile district of Uganda. These were obtained from healthy children randomly selected as controls for a study of Burkitt's lymphoma. Their mean age was 6.4 years and both ELISA and WB were performed . Fifty of the 75 children (67%) were found to be positive. According to HIV experts these positive results are explicable by virtue of mothers infecting their children. Thus Gallo and his colleagues expected to find at least an equal percentage of infected adults. Mortimer *et al* assert that "Very few HIV-infected children are surviving into adulthood in good health" and, given the fact that neither these children nor adults had treatment for HIV or AIDS, and the incubation period of AIDS in Africa is claimed to be four years and HIV heterosexually transmitted, then if the tests are HIV specific and if HIV causes AIDS, by now, few, if any Ugandans should be alive.[151]

6. One of the principal major signs of the Bangui definition of AIDS in Africa is loss of body weight. However, in a study of Rwandan women, over a 24 month period beginning in 1988, it was reported that nutritional status assessed by loss of body weight "was a significant predictor of eventual HIV seroconversion. Subsequent seroconvertors lost an average of 1.5 kg during the six months of the study compared with 1.0 kg gain (p = 0.001) for non-converters. Nine of 27 (33%) seroconvertors., compared with one of 44 (2%) controls, lost at least 5 kg in the 6 month period beginning one year before seroconversion...In addition to those findings for measured weight loss during follow-up, reported weight loss before enrolment was also a risk factor for subsequent seroconversion".[152] In other words, the effect (weight loss) has preceded the cause (HIV) by many months or even years.

7. In 1986, Jaffe *et al* tested 1129 serum samples from IV drug users and 89 controls from non-users. All samples were collected during 1971-1972 and tested by two commercial ELISAs and WB. Seventeen of the samples from the IV drug users, but not one of the controls was found positive. They concluded: "On the basis of our positive Western blot data, it appears that parenteral drug users may have been exposed to HTLV-III or a related virus as early as 1971. An alternative but equally viable explanation is that the HTLV-III seropositivity detected in these specimens represented false positive or non-specific reactions".[153]

8. In 1991, Elizabeth Dax and associates from the US National Institute on Drug Abuse HIV reanalysed 1985 Western blot strips of sera originally obtained from intravenous drug addicts in 1971-72. (Twenty years later the actual sera themselves were not available for retesting). Ten persons "with potentially positive WB patterns, when the more specific 1985 criteria were used", were traced. One patient had died from a motor vehicle accident and there were "no lymphoreticular changes at autopsy, and a thorough retrospective analysis provided no evidence of either current substance abuse of HIV infection". Of the nine living addicts, two could not be assessed clinically, seven were not chronically ill, (one was in prison but in good health, one had been successfully discharged from a methadone program, one was enrolled in a methadone

188

program, another sporadically consumed illicit drugs). "The two former patients whose 1971-72 WB results were most strongly reactive had current ELISA and WB assays that were negative. The immune function parameters were inconsistent with immune suppression". Their data led the authors to conclude, "it is possible that antibodies to a nonpathogenic virus would have disappeared during the 17 to 18 years...follow-up. Although this potential cannot be ruled out, it is more likely that the earlier results were false positives...definitive evidence of HIV infection in the United States' addict population as early as 1972 is still lacking".[154]

9. HIV is said to be transmitted by infected needles, yet a higher percentage of prostitutes who use oral drugs (84%) than intravenous (46%) have positive "HIV" antibody tests.[155]

10. According to the HIV experts, once infected with HIV, unless treated, always infected. Yet, in healthy individuals, partners of HIV positive individuals, organ transplant recipients and patients with systemic lupus erythematosus, a positive WB may revert to negative when exposure to semen, immunosuppressive therapy or clinical improvement occurs.[156,157]

11. Amazonian Indians who have no contact with individuals outside their tribes and have no AIDS have a 3.3-13.3% HIV WB seropositivity rate depending on the tribe studied.[158] In another study they found that 25%-41% of Venezuelan malaria patients had a positive WB, but no AIDS.[159] The above data means either that HIV is not causing AIDS "even in the presence of the severe immunoregulatory disturbances characteristic of acute malaria", as concluded, or the HIV antibody tests are non-specific.

12. Lundberg and his colleagues from the US Consortium for Retrovirus Serology Standardisation reported that 127/1306 (10%) of individuals at "low risk" for AIDS including "specimens from blood donor centres" had a positive HIV Western blot by the "most stringent" of the US criteria, that is, the presence of antibodies to p24, p32 and gp41 or gp120/160.[160]

13. p24 seroreactivity "was found in 27 (35%) of 77 patients with primary biliary cirrhosis, 14 (29%) of 48 patients with systemic lupus erythematosus, 14 (50%) of 28 patients with chronic viral hepatitis, and nine (39%) of 23 patients with either primary sclerosing cholangitis or biliary atresia, compared with only one (4%) of 24 patients with alcohol-related liver disease or alpha 1 - antitrypsin-deficiency liver disease, and only one (4%) of 25 healthy volunteers (p = 0.003)".[161]

14. An individual was given six 5 ml injections of donated Rh positive serum, administered at 4 day intervals. His "wife and child were seronegative on HIV ELISA". The donor serum "was shown to be negative on HIV antibody and antigen ELISA". "Blood taken after the first immunisation was shown to be negative on HIV antibody ELISA and immunoblot assay. After the second immunisation a weak signal on ELISA, slightly above the cut-off level, was monitored. After the third immunisation the signal was strong and immunoblot revealed distinct interaction with p17 and p55 proteins. An even stronger signal was monitored after the fifth immunisation. Interaction with p17, p31, gp41, p55 and some other proteins was evident.[162]

15. 11/208 (5%) of healthy blood donors and 10/50 (20%) of patients with measles, mumps, herpes simplex, dengue and other viral illnesses had either a p24 or p18 band on the HIV Western blot test.[163]

16. The "HIV proteins (p17, p24)" appear in the blood of patients (previously negative for all HIV markers) following "transfusions of HIV-negative blood and UV-irradiation of the autoblood".[164]

17. In 1991 Kion and Hoffman injected non-HIV -infected mice with T-lymphocytes from another strain of non-HIV-infected mice. The recipient mice developed antibodies to the HIV gp120 and p24 proteins.[165]

18. In 1991, Strandstrom and colleagues reported that 72/144 (50%) of dog blood samples "obtained from the Veterinary Medical Teaching Hospital, University of California, Davis" tested in commercial Western blot assays, "reacted with one or more HIV recombinant proteins [gp120--21.5%, gp41--23%, p31--22%, p24--43%].[166]

19. The Australian National HIV Reference Laboratory concedes that "False reactivity may be to one or more [HIV] protein bands and is common (20-25% of anti-HIV negative blood donors will exhibit one or more bands on a WB".[167]

20. Amongst 89,547 anonymously tested blood specimens from 26 US hospital patients at no risk of AIDS, between 0.7% to 21.7% of men and 0-7.8% of women aged 25-44 years were found to be HIV WB positive. It is important to note that this study not only excluded patients from the known AIDS risk groups but also those with even meagre HIV/AIDS risks including "gunshot and knife wounds, conditions which have been reported to be associated with a higher than expected rate of HIV-1 seroprevalence".[168]

21. In the USA, in a larger study of 1.2 million healthy military applicants, approximately 1% of all initial ELISAs were positive of which 50% were subsequently negative; 30-40% of first WB were positive and 96% of second WB were positive. In other words there were 6,000 individuals with an initially positive but subsequently negative ELISA, 4,000 individuals with two positive ELISAs followed by a negative WB, and 80 individuals with two positive ELISAs, an initially positive WB and a negative repeat WB. Thus several thousand healthy individuals were found to have antibodies that reacted with "HIV proteins" but who were ultimately deemed not to be HIV infected. Even in the best laboratories 80 of the healthy applicants would be diagnosed as HIV infected since only one WB is performed and it is considered 100% specific. The situation in Africa would be even worse since in Africans two positive ELISAs are considered 100% specific for HIV infection.

Anyone drawing a conclusion regarding the existence of HIV from an antibody/antigen reaction must not forget a lesson from history. In the mid-1970s, Gallo and his colleagues reported the isolation of the "first" human retrovirus, HL23V. In fact, the evidence for the "isolation" of HL23V surpassed that of HTLV-I and HIV in at least two aspects. Unlike HIV, Gallo's group: (a) reported the detection of reverse transcriptase activity in fresh, uncultured leucocytes, and (b) published an electron micrograph of virus-like particles banding at a sucrose density of 1.16 gm/ml, the density which defines retroviral particles. Following the discovery of HL23V, some researchers attempted to determine its prevalence utilising antibody tests while others were interested in determining the specificity of the antibody reactions. The latter included one group from the Laboratory of Cellular and Molecular Biology, National Cancer Institute, and another from the Laboratory of Viral Oncology, Memorial Sloan-Kettering Cancer Center. Using the "viral glycoproteins", these groups found that the antibodies present in human sera which reacted with these proteins were "directed against carbohydrate structures" and concluded that "The results are consistent with the idea that the antibodies in question are elicited as a result of exposure to many natural substances possessing widely cross-reacting antigens and are not a result of widespread infection of man with replication competent oncoviruses" (retroviruses).[169,170]   In 1981 Gallo accepted the evidence that the antibodies which reacted with the presumed viral proteins of HL23V were not so directed "but against the carbohydrate moieties on the molecule that are introduced by the host cell as a post-transcriptional event, and which are therefore cell-specific and not virus-specific".[171]   This discovery was of such significance that today nobody, not even Gallo, considers HL23V as being the first human retrovirus, or even a retrovirus.

There is compelling evidence that "HIV" antibodies also arise from exposure to "widely cross-reacting antigens" and are not a result of infection with a novel retrovirus.

1. "One half of the molecular weight of gp120 is represented by oligomannosidic oligosaccharides...Polyclonal antibodies to mannan from yeast also recognise the carbohydrate structure of gp120 of the AIDS virus.[172]

2. "The immunochemical determinants of the antigenic factors of *Candida albicans* display a high identity with the glycoprotein (gp) 120 of HIV-1: they contain _(1_2) and _(1_3)-linked mannose terminal residues".[173]

3. Antibodies to the mannans of *Candida albicans* "block infection of H9 cells by HIV-1" as well as the binding of lectins to gp120.[173]

4. Recognition of gp120 by antibodies to a synthetic peptide of the same antigen was "partially abolished if it was absorbed with the total polysaccharide fraction of *C. albicans*" while the antigen recognition by antibodies to "gp120 from human T-cell lymphotropic virus type IIIB...was totally blocked". From these data the authors concluded: "These results indicate that mannan residues of *C. albicans* can serve as antigens to raise neutralising antibodies against HIV infection".[173]

5. "Normal human serum contains antibodies capable of recognising the carbohydrate moiety of HIV envelope glycoproteins...from 100 ml of human serum approximately 200µg of MBIgG was recovered [MBIgG = mannan-binding IgG antibodies]...MBIgG bound to HIV envelope glycoproteins gp160, gp120 and gp41".[174]

6. Kashala, Essex and their colleagues have shown that antibodies to carbohydrate-containing antigens such as lipoarabinomannan and phenolic glycolipid that constitute the cell wall of *Mycobacterium leprae*, a bacterium which "shares several antigenic determinants with other mycobacterial species" cause "significant cross-reactivities with HIV-1 pol and gag [p32, p55, p68, p24, p18] proteins". This led the authors to warn that among leprosy patients and their contacts there is a "very high rate of HIV-1 false-positive ELISA and WB results", that "ELISA and WB results should be interpreted with caution when screening individuals infected with *M. tuberculosis* or other mycobacterial species", and furthermore that "ELISA and WB may not be sufficient for HIV diagnosis in AIDS-endemic areas of central Africa where the prevalence of mycobacterial diseases is quite high".[175]

7. Not only mycobacteria (*M. leprae, M. tuberculosis, M. avium-intracellulare*) but also the walls of all fungi (*Candida albicans, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum,* including *Pneumocystis carinii*), contain carbohydrate (mannans). One hundred per cent of AIDS patients (even those with "no *Candida* clinically") have *C. albicans* antibodies, leading researchers from St. Bartholomew's and St. Stephen's Hospitals to state: "It is possible that Candida may act as a cofactor in the development of overt AIDS in HIV-infected individuals".[176] It is significant that in gay men the only sexual act which is a risk factor for seroconversion is passive anal intercourse[177] (exposure to semen) and that mannose is present in both sperm and seminal plasma.[178]

8. Researchers from the University of Rome injected healthy mice with an *E. coli* lipopolysaccharide (LPS) and reacted their sera with two synthetic peptides, one encompassing gp120 V3 loop of "HIV-1 MN" and the other "representing a gp41 immunodominant epitope". The LPS-treated mice showed a "significant antibody reactivity" with the two peptides. (V. Colizzi *et al*, personal communication).

9. In the same study, the authors reported data from the sera of HIV-negative subjects with autoimmune disorders. Recombinant gp120 and a panel of synthetic peptides derived from the amino acid consensus sequences of the HIV gp120, gp41, p24 or several unrelated proteins were tested by specific ELISA. "The first set of experiments performed on four patients with Sjorgern syndrome (SjS) and four patients with systemic lupus erythematosus (SLE) revealed a significant anti-gp120 reactivity compared to healthy HIV-negative controls. Moreover, such binding could be almost completely inhibited by preincubation with free gp120. A significant anti-p24 reactivity was observed in 18 out of 29 [62%] sera from SjS patients and in 13 out of 25 [52%] from SLE patients, while anti-gp41 was observed only in 3 out of 14 [21%] SjS and in 2 out of 20 [10%] SLE affected patients. Similar analyses were performed in the murine model of autoimmunity, showing that sera from MRL/lpr mice were able to bind all HIV related peptides in age-dependent manner. The analysis of a panel of HIV unrelated peptides showed that SLE as MRL/lpr sera bind both HIV related and unrelated peptides while SjS sera failed to do so". In other words, sera which contain autoantibodies react with the principal "HIV" proteins gp120, gp41 and p24.[179]

10. The same authors also reported similar results from (i) experiments where "Two month old male CBA mice were immunised for 6 weeks with 50 x 10^6 allogenic lymphoid cells obtained from either BALB/c or B6 male mice"; (ii) "Sera from 62 polytransfused (at least 10 transfusions/year) patients with thalassemia".

Since antibodies to mannans react with the "HIV" proteins then, as Essex and his colleagues pointed out, for mycobacterial infection in Africa, one would expect the sera of all people infected with fungi and mycobacteria to cross-react with the "HIV-1 glycoproteins" as well as to cause "significant cross-reactivities with HIV-1 *pol* and *gag* proteins". Since humans and animals subjected to non-infected blood and blood products develop antibodies which react with one or more of the "HIV" proteins, one would expect that gay men, haemophiliacs, IV drug users, blood transfusion recipients who are repeatedly exposed to foreign blood and blood products will have a positive "HIV" antibody test even if not infected.

Given that:

1. Individuals with fungal and mycobacterial infections have antibodies which may react with "HIV proteins" in the absence of "HIV" and that *E. coli* is an intestinal commensal and a potential bacterium in all of us, how can one assert that:

   (a) reactions between antibodies in the sera of AIDS patients and proteins present in cultures derived from the tissues of AIDS patients is proof that the reacting proteins are constituents of a unique retrovirus HIV and the antibodies are specific to these proteins?

191

(b) PCP, candidiasis, cryptococcosis, coccidioidomycosis, histoplamosis, tuberculosis or *Mycobacterium avium-intracellular* disease, that is, the vast majority of the opportunistic infections (88% of AIDS cases diagnosed between 1988 and 1992 had one or more fungal or mycobacterial infections) which signify AIDS are caused by HIV on the basis of a positive antibody test?

(c) a positive antibody test in individuals with fungal and mycobacterial infections proves HIV infection?

2. Since:

(a) mice and patients with autoimmune diseases (SjS and SLE) and AIDS patients share many clinical and immunological (autoantibodies) manifestations;

(b) patients polytransfused with allogenic blood and mice injected with foreign cells and foreign proteins develop "HIV" antibodies but are not infected with HIV;

why should gay men, IV drug users and haemophiliacs, who are all exposed to foreign cells and/or foreign proteins, may not also develop "HIV" antibodies and even if not infected with HIV? The inevitable interpretation of the above data is that HIV antibodies have not been proven to exist and thus all seropositivity is the result of cross-reactivities or non-specific immune stimulation or both.

Indeed, given the pivotal importance of specific "HIV" antibodies not only in defining HIV infection but also in proving the existence of HIV proteins and HIV, it would seem crucial to test this interpretation in the same manner as twenty years ago when researchers questioned antibodies to the now defunct "first" human retrovirus HL23V. These two groups analysed the specificity of HL23V antibodies "in absorption tests with normal human serum proteins, assays of viral gp70 antigenicity after exposure to exo- and endoglycosidases or trypsin, and carbohydrate hapten inhibition studies". These data proved that antibodies to the HL23V glycoprotein were elicited non-specifically by "exposure to many natural substances possessing widely crossreacting antigens" and not by "widespread infection of man with replication-competent oncoviruses" (retroviruses).[169,170] We predict that in the AIDS era a significant number if not all of the "HIV positive" sera will have reactivity to the "HIV" proteins reduced or eliminated by absorbtion with fungal, mycobacterial and auto antigens.

RECENT DEVELOPMENTS

By 1997 some of the best known HIV experts[180,181] accepted that:

1. "Purified" HIV contains cellular proteins, "some are over-represented in comparison to the relative amount in the cell membrane, whereas others appear to be absent", and that these proteins "serve as protective immunogens in vaccination experiments".

2. HIV "used for biochemical and serological analyses or as immunogens is frequently prepared by centrifugation through sucrose gradients", but in none of the studies "the purity of the virus preparation has been verified". In other words, up till 1997 nobody has published electron micrographs of the 1.16 gm/ml band to prove that the "purified virus" contained nothing else but virus particles.

In that year, in *Virology*, two studies were published, one by a USA team, principal author Julian Bess, and the other by a Franco-German group, principal author Pablo Gluschankof, with the first electron micrographs of "purified HIV". While in the Gluschankof *et al* studies the EMs were from the 1.16 gm/ml band, in the Bess *et al* band they were from pooled bands. The authors of both studies claimed that their "purified" material contained some particles with the appearances of retroviruses and in fact were HIV particles. But they admitted that their material predominantly contained particles which were not retroviruses but "budding membrane particles frequently called microvesicles" or "mock virus". Indeed the caption to the Gluschankof *et al* electron micrograph reads, "Purified vesicles from infected H9 cells (a) and activated PBMC (b) supernatants", not purified HIV. In further experiments the supernatant from non-infected cultures was also banded in sucrose gradients. Both groups claimed that the banded material from these cultures contained only microvesicles, "mock virus" particles, but no HIV. Both the "HIV" particles and the mock-virus particles possessed membranes. In the USA study the "HIV-1 particles" were differentiated from the microvesicles "by the electron dense cores", whereas in the other study the "HIV" particles were "identified by the relatively homogenous diameter of about 110 nm, the dense cone-shaped core, and the "lateral bodies". However, in the arrowed particles which are said to be HIV it is difficult if not impossible to locate any which have cone-shaped cores or bilateral, "lateral bodies". In fact no particle in any study has the principle morphological characteristic of

retrovirus, a diameter of 100-120 nM and surface spikes, knobs. In the Franco-German study the average "HIV" particle diameter is 136 nM and no particle had a diameter less than 120 nM. In the USA study the corresponding dimensions are 236 nM and 160 nM. In other words, the American "HIV" is twice the diameter of the European "HIV", and all other "HIV" particles. The US authors did not note or explain this discrepancy and "were much more focused on showing the mixture of particles in the preparations as opposed to their actual diameters". The diameter of the microvesicles "range in size from about 50 to 500 nm".[180] Both the "HIV" and the "mock" virus particles contained RNA. The RNA of the latter had contained mRNA which is known to be rich in adenine and which, according to Gallo is specific to retroviral RNA.[182,183] According to Gluschankof *et al*, "The vesicles contain large amounts of protein and nucleic acids which are unstructured and thus are transparent by electron microscopy", that is why many, but not all, appear "empty" by electron microscopy, while the nucleic acids in the "HIV" particles are structured and for this reason they appear to have an "electron dense cores". However, according to a leading retrovirologist, John Bader, the core density can be changed by external conditions, that is, the culture conditions.[57] It is well known that a structural virus particle or virus-like particle can become "unstructured" in the presence of reducing agents. The possibility cannot be excluded then, that the apparent morphological differences between the two types of particles may be due to nothing more than the difference in the redox of the microenvironment in which they are assembled, released or both. It is significant there is evidence that actin polymerisation (or actin/myosin interaction) "mediates HIV budding" and release.[123,124,126,127] Evidence also exists that:

1. As shown by Bess *et al*, uninfected cells exhibit buds which are no different from those in infected cells.

2. There is an association between the redistribution of polymerised actin, myosin and other cellular proteins (glycoproteins) and many cellular processes including budding unrelated to release of retrovirus.[184-188]

3. Polymerisation of actin, actin-myosin interaction and cross-linking of polymers in general is regulated by the redox state, oxidation leading to interaction.[189-191]

4. Both AIDS patients and cultures derived from AIDS patients are subjected to oxidising agents. In fact, for the detection of "HIV" proteins and particles the cell cultures must be stimulated (treated with oxidising agents).

5. In the presence of antioxidants no "HIV" phenomena can be observed.[2,3,7,192]

The minimum absolutely necessary but not sufficient condition to claim that what are called "HIV-1 particles" are a retrovirus and not cellular microvesicles is to show that the sucrose density fractions obtained from the "infected" cells contain proteins which are not present in the same fractions obtained from non-infected cells. However, Bess *et al* have shown this is not the case. The only difference one can see in their SDS-polyacrylamide gel electrophoresis strips of "purified virus" and "mock virus" is quantitative, not qualitative. This quantitative difference may be due to many reasons including the fact that there were significant differences in the history and the mode of preparation of the non-infected and "infected" H9 cell cultures, in addition to the "infection".

A similar finding was reported by the same authors a few years earlier. However, while in both studies the proteins of molecular weight "near 42 kDa" (42,000) are labelled as "Actin" and "in the 30- to 40-kDa range" as "HLA DR", all the proteins with molecular weight higher than approximately 42,000 and lower than approximately 30,000 are left unlabelled in the earlier paper. In the 1997 study, three proteins of molecular weight lower than 30,000 are labelled as p24^{CA}, p17^{MA}, and p6/p7^{NC} and are said to represent "major bands of viral proteins". However, also according to the authors, "these labels were added when one of the reviewers asked for them. He felt it would help orient readers when looking at the figure - the reviewer is correct. We did not determine the identities of the bands in this particular gel".

In their earlier study the researchers from the USA presented further evidence that the "viral proteins" were nothing more than cellular proteins. In their efforts to make an HIV vaccine they immunised macaques with, amongst other antigens, "mock virus", that is, sucrose density banded material from the supernatants of non-infected H9 cell cultures. After the initial immunisation the monkeys were given boosters at 4, 8 and 12 weeks. The animals were then challenged with "SIV" propagated either in H9 cells or macaque cells. When the WBs obtained after immunisation but before "SIV" challenge were compared with the WBs post-challenge, it was found that challenge with "SIV" propagated in macaque cells had some additional bands. However, the WBs obtained after the challenge with SIV propagated in H9 cells were identical with the WBs obtained after immunisation but before challenge. In other words, the protein immunogens in the "virus" were identical with the immunogens in the "mock virus". Since both the "mock virus" and "purified" virus contain the same

proteins, then all the particles seen in the banded materials including what the authors of the 1997 *Virology* papers call "HIV" particles must be cellular vesicles. Since there is no proof that the banded, "purified virus", material contains retrovirus proteins and thus retrovirus particles then there can be no proof that any of the banded RNA is retroviral RNA. When such RNA (or its cDNA) is used as probes and primers for hybridisation and PCR studies, no matter what results are obtained, they cannot be considered proof for infection with a retrovirus, any retrovirus.

In the interview which Montagnier gave to Djamel Tahi he was asked why they did not publish an electron micrograph of the 1.16 gm/ml band to prove that the band represented "purified" virus, as they claimed. He replied that the reason for this was that even after "Roman effort" in their "purified" virus they could not see any particles with the "morphology typical of retroviruses. They were very different. Relatively different". When Montagnier was asked if Gallo isolated HIV he replied: "I do not believe so".[29] If there were no retrovirus-like particles in Montagnier's "purified" virus, then obviously Montagnier and his colleagues could not claim to have isolated a specific exogenous retrovirus, HIV. If HIV does not exist then the cause or causes of AIDS must be urgently reappraised and alternative hypotheses heeded.

THE "HIV GENOME"

To claim that the stretch of RNA (cDNA) is the genome of a unique retroviral particle, HIV, the most basic requirement is proof for the existence of a unique molecular entity "HIV RNA" ("HIV DNA") that is, a unique fragment of RNA (DNA) identical in both composition and length in all infected individuals. The claim that a stretch of RNA (cDNA) is a unique molecular entity which constitutes the genome of a unique retrovirus can be accepted if and only if it is shown that the RNA belongs to particles with the morphological, physical and replicative characteristic of retroviral particles. Proof of this can only be obtained isolating the particles, that is, by obtaining them separate from everything else (purifying them). In 1984 both Gallo's and Montagnier's groups reported finding polyadenylated (adenine rich) RNA (poly(A)-RNA) in the 1.16 gm/ml band material obtained from "infected" cultures. The RNA was claimed to be HIV RNA that is, the HIV genome, and its complementary DNA, the HIV provirus. However,

(a) as mentioned, although Montagnier claimed his 1.16 gm/ml band contained particles, neither his band nor that of Gallo's contained any particles with "morphology typical of retroviruses";

(b) poly(A)-RNA is not specific to retroviruses. It can be found in all cells and even at the 1.16 gm/ml band obtained from "non-infected" cells;[180]

(c) there is no proof for the existence of a unique molecular entity, "HIV-RNA" or "HIV-DNA", while the genomes of the most variable RNA viruses do not differ by more than 1%.[193] The difference between the human and the chimpanzee genomes is no more than 2% while there is up to 40% variation between "HIV" genomes;[194]

(d) in hybridization studies using the "HIV RNA" or cDNA, Gallo and since then many other researchers have been unable to prove the existence of the HIV genome in fresh lymphocytes from AIDS patients.[195] In 1994 Gallo stated "We have never found HIV DNA in the tumour cells of KS…In fact we have never found HIV DNA in T-cells".[196,197]

(e) All the claims of the existence of HIV in humans are based on polymerase chain reaction (PCR) studies using small fragments of the "HIV" genome as primers. However even researchers who believe that there is proof that the HIV primers and probes used in these studies are HIV accept that the specificity of this assay, using the antibody as a gold standard, varies between zero and 100 per cent.[198] Even with the PCR nobody has reported the existence of the full "HIV" genome in the fresh lymphocytes of even a single AIDS patient. Nonetheless it is generally believed:

(i) there is proof for the existence of a unique molecular entity RNA (cDNA) which is the genome of a unique retrovirus, HIV;

(ii) HIV RNA (HIV DNA) can be found only in infected individuals.

Furthermore, despite the ample evidence to the contrary, for most retrovirologists the finding of a novel nucleic acid in a cell can be due to nothing else but an infectious agent. Half a century has passed since the Nobel Laureate Barbara McClintock discovered the phenomenon of transposition which can lead to the appearance of new genotypes and phenotypes. According to McClintock, the genome can be restructured not only by transposition but also by other means as well. In her Nobel lecture of 1983, she said, "rapid reorganisation of genomes may underline some species formation. Our present knowledge would suggest that these reorganizations originate from some "shock" that forced the genome to restructure itself in order to overcome a

threat to its survival...Major genomic restructuring most certainly accompanied formation of new species". The "genomic shock" which leads to the origin of new species may be "either produced by accidents occurring within the cell itself, *or imposed from without such as virus infections, species crosses, poisons of various sorts, or even altered surroundings such as those imposed by tissue culture*. We are aware of some of the mishaps affecting DNA and also of their repair mechanisms, but many others could be difficult to recognize. Homeostatic adjustments to various accidents would be required if these accidents occur frequently. Many such mishaps and their adjustments would not be detected unless some event or observation directed attention to them...Unquestionably, we will emerge from this revolutionary period with modified views of components of cells and how they operate, but only however, to await the emergence of the next revolutionary phase that again will bring startling changes in concepts".[199] (It is worthy adding that although McClintock's ideas concerning the generation of novel nucleic acids and new species represent a milestone in their own right, one cannot fail to commend the prescience of Peyton Rous writing seventy three years earlier).

As we have mentioned elsewhere an exogenous retrovirus is only one possible explanation for the finding of novel nucleic acids in AIDS patients. Other explanations are:

1. The genome of an endogenous retrovirus, that is, a stretch of RNA with a corresponding proviral DNA present in the cellular DNA of uninfected animals and which is passed from generation to generation vertically (from parents to offspring via the germ cell line) and which under certain conditions can be expressed and incorporated into retroviral particles.

2. The genome of a retrovirus *de novo* assembled by genetic recombination and deletion of: (a) endogenous retroviral sequences or (b) retroviral and cellular sequences or (c) non-retroviral cellular genes.

3. An RNA obtained by transposition, that is, by certain replicating DNA sequences (transposons) becoming inserted elsewhere in the genome, or by retroposition, that is, by particular RNA (retrotransposons) first being transcribed into DNA and then similarly being inserted into the genome. Retroposition can "use cellular mechanisms for passive retroposition, as well as retroelements containing reverse transcriptase". The retroelements may be retrovirus-like elements or nonviral elements.[200,201] Not only can retroposition "shape and reshape the eukaryocytic genome in many different ways"[200] but the nonviral retroelements may be similar to the retroviral elements.

A basic principle of molecular biology is that the primary sequence of RNA faithfully reflects the primary sequence of the DNA from which it is transcribed. However, in the 1980s RNA editing, "broadly defined as a process that changes the nucleotide sequences of an RNA molecule from that of the DNA template encoding it", was discovered. In the process a non-functional transcript can be re-tailored, producing a translatable mRNA, or modify an already functioning mRNA so that it generates a protein of altered amino acid sequences. Sometimes editing is so extensive that the majority of sequences in a mRNA are not genomically encoded but are generated post-transcriptionally producing the "paradoxical situation of a transcript that lacks sufficient complementarity to hybridize to its own gene!".[202-204] According to Nancy Maizels and Alan Weiner from the Department of Molecular Biophysics and Biochemistry at Yale University, "the central dogma has survived hard times. The discovery of reverse transcriptase amended but did not violate the central dogma of how genes make proteins; introns qualified the conclusion that genes are necessarily collinear with the proteins they encode; somatic rearrangement of lymphocyte DNA called stability of eukaryotic genomes into doubt...and catalytic RNA challenged the pre-eminence of proteins and breathed new life into the ancient RNA world". However, the discovery of RNA editing "could come close to dealing it a mortal blow".[205]

Thus the finding of novel RNAs in human cells, especially those of AIDS patients and those at risk, can no longer be regarded as incontrovertible proof that the RNA has been exogenously introduced by a putative HIV or any other infectious agents.[9,19] That this may be the case has of late been accepted by Luc Montagnier. In a written testimony dated February 2nd 2000, to the US House of Representatives Committee on Government Reform, Subcommittee on National Security, Veterans Affairs and International Relations, in support of the work of his colleague, Howard B Urnovitz, (Montagnier is on the scientific advisory board of a publically traded biomedical company whose director is Urnovitz), Montagnier wrote: "I have reviewed Dr Urnovitz's published research and the testimony prepared for presentation to this Committee and strongly advise that future research on Gulf War Syndrome should include the study of the detected genetic material".[206]

Urnovitz and his colleagues presented evidence of the existence, in Persian Gulf War veterans, of "novel", "nonviral" RNAs, "possibly induced by exposure to environmental genotoxins". They concluded: "The patterns of the occurrence of RPAs [polyribonucleotides] in the sera of GWVs [Gulf War Veterans] and healthy controls are sufficiently distinct to suggest possible future diagnostic applications…Our studies of patients with active

multiple myeloma suggest that patients with individual chronic multifactorial diseases may have unique RPAs in their sera. Validated tests for such putative surrogate markers may aid in the diagnosis of such diseases or in the evaluation of responses to therapeutic modalities".[207]

It is also highly significant that in his "STATEMENT FOR THE DURBAN AIDS CONFERENCE",[208] which begins "What is 'HIV'?", Urnovitz offers no explanation for his parenthetic use of the terms "'HIV'", "'HIV" genome" and "'HIV' biomarker". In the same document it is also implied that the HIV genome may result from the "reshuffle" of cellular retroelements. That is, Urnovitz agrees with one of several possible explanations summarised above and earlier put forward by our group to account for the presence of novel RNAs in the cells of AIDS patients but which may not be present in the cells of healthy individuals.[9,19] Urnovitz also agrees with us that "Missing from the landmark 1983 analysis of "HIV" was an understanding of the role "poikilogenic" agents play in the laboratory protocol that is used to study human retroviruses. The term "poikilogenic" is derived from the Greek "poikilo" which means *diversity* and "gen" which stands for *generate.* Poikilogenic agents are those entities–chemical, physical, or biological–that create genetic diversity via genetic recombinatorial events. These events may include the inductive expression of retroelements and the resulting byproducts of newly reshuffled genetic material. One such poikilogenic agent was reported in the 1983 discovery of "HIV". The agent is phytohemagglutinin (PHA). "Using an HERV-H LTR probe, 6 and 4.5 kb transcripts were detected by Northern blot analysis which were induced in normal peripheral T cells after treatment with phytohaemagglutin"[209] (HERV≡known endogenous retrovirus). PHA has been and continues to be used as a laboratory agent not only by Montagnier but by virtually every retrovirologist who claims proof for HIV isolation.

Since Montagnier agrees with Urnovitz that novel, nonviral RNAs appear in the Gulf War Veterans, then why should the existence of novel RNAs:

1. In AIDS patients and those at risk be the genome of a retrovirus HIV and not the result of the many toxins including genotoxins to which they are exposed?[2,7,19,191,210]

2. In cultures containing tissues from AIDS patients be interpreted as HIV RNAs rather than the result of the many toxins including genotoxins[2,7,8] to which both the patients and the cultures are exposed? Especially when both Montagnier and Gallo accept that HIV cannot be detected in cultures which are not treated with such toxins (oxidant agents) including PHA?[89,207,208,211,212] When hard pressed all the HIV experts will ultimately accept the non-specificity of retroviral-like particles, reverse transcription and antibody/antigen reactions.

CONCLUSION
In 1983 Luc Montagnier and his colleagues and in 1984 Robert Gallo and his colleagues claimed to have proven the existence of HIV by purifying retroviral particles, that is, by obtaining a mass of particles isolated from everything else and showing that the particles are infectious. A critical analysis of their evidence shows that neither group presented proof of isolation of a novel retrovirus from AIDS patients. The phenomena they interpreted as HIV are all non-specific and were known to be so long before the AIDS era. In fact, given the origin of the cells and the culture conditions, one would expect to find all these phenomena even if the cultures are not infected with a retrovirus. In 1997 Montagnier himself stressed that to prove the existence of a unique retrovirus purification is absolutely necessary and admitted that he had not presented such proof, and in his view, neither had Gallo. Recognition of these facts may prove the first step in solving the problem of AIDS.

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