CONTINUUM SUPPLEMENT

Vol 4, No 3 September/October 1996

THE ISOLATION OF HIV: HAS IT REALLY BEEN ACHIEVED?

The Case Against

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"Listening to both sides of a story will convince you that there is more to a story than both sides" *Frank Tyger*

The definite existence of any virus, including a retrovirus, can be proven only by isolating it. For nearly half a century retroviruses have been isolated by banding in density gradients. It is accepted that the procedures incorporated into this method, which is by no means perfect, have not been followed by the researchers who claim isolation of the human immunodeficiency virus, HIV-1. Nonetheless, it is said that at present, there is ample evidence that HIV has been isolated and shown to be a unique exogenous retrovirus.[1]

In this critique we have analysed the relevant data that purport to prove that HIV has been isolated. To simplify the presentation for readers of this article, the major arguments for HIV isolation (as presented by Peter Duesberg in Vol 4, No 2 of Continuum[1]) are used as the headings in the discussion. Since the topic is both complex and controversial it is necessary to present substantial original data and sometimes to repeat it in order to critically assess the basis for the view that HIV has been isolated.

1. "In 1983 Montagnier et al isolated a retrovirus".

In the 1983 Montagnier et al study there is no proof of virus isolation by "the most rigorous method available to date". Nor did they follow the "traditional...Pasteur rules". How then did they isolate a retrovirus? Even if Montagnier and his colleagues or others had followed the "Pasteur rules", since "viral and cellular proteins, and cellular contaminants...copurify with virus purified by conventional density gradients",[1] there is no reason to accept any claim of HIV isolation by any research group who did not use "the most rigorous method available to date, i.e. molecular cloning of infectious HIV DNA". However, to prove that HIV "has been isolated" by "the most rigorous method available to date", virus cloning, one must start with HIV RNA (DNA). Since the propriety of naming an RNA "HIV RNA" is contingent upon prior isolation of a particle proven to be a retrovirus, on this basis alone, "the most rigorous method available to date, i.e. molecular cloning of infectious HIV DNA", cannot prove HIV isolation.

2. "reverse transcriptase associated with such particles".

There is not one single study which proves that the enzyme present in the "growth medium" or even in the material which in sucrose density gradients bands at 1.16 gm/ml, (the density which defines retroviral particles), and which catalyses the transcription of RNA into DNA, is a constituent of particles of any kind, much less of retroviral-like particles or a unique retrovirus. The only association between "particles" and "reverse transcriptase" (RT) arises from experiments which show that some cultures/cocultures with tissues from AIDS patients exhibit both particles, many of which are not even retroviral-like, and transcription of the synthetic RNA template-primer A(n).dT_{15}. However, this does not constitute proof of the existence of RT or RT as a constituent of a retroviral particle. Furthermore, since:

(a) the presence of reverse transcriptase (RT) is proven indirectly, that is, by demonstrating transcription of the RNA template-primer A(n).dT_{15};

(b) the template-primer A(n).dT_{15} can be transcribed not only by RT but by other cellular DNA polymerases. All the cellular DNA polymerases, a, b and g, can copy A(n).dT_{15} [2]. In fact, in 1975, an International Conference on Eukaryotic DNA polymerases, which included Baltimore and Gallo[3] defined DNA polymerase g, "a component of normal cells"[4], "found to be widespread in occurrence"[2], whose activity can be increased by many factors including PHA stimulation[5], as the enzyme which "copies A(n).dT_{15} with high efficiency but does not copy DNA well";[3] it is impossible to say whether the polymerase in the "growth medium" or in the material banding at 1.16 gm/ml which catalyses reverse transcription of A(n).dT_{15} is RT or one of a number of other cellular DNA polymerases.

3. "...indeed, each of these criteria could reflect another retrovirus, and some of these criteria, eg, particles and proteins, could reflect non-viral material altogether".

Although the HIV/AIDS experts, including Montagnier, Gallo and Barré-Sinoussi claim that RT is "unique to retroviruses" and "the hallmark of a retrovirus",[6-8] this is not the case, a fact accepted by some of the best known scientists.[9] "Reverse transcriptase (RT) was first discovered as an essential catalyst in the biological cycle of retro-

viruses. 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...the possibility that reverse transcription first took place in the early Archean" is supported by a number of facts and "the hypothesis that RNA preceded DNA as cellular genetic material".[10] 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".[11] "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" (pol=polymerase).[12] "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.[13]. 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" [14]. Lymphocyte stimulation in general and PHA stimulation in particular is associated with production of hepatitis B virus from peripheral blood lymphocytes in patients infected with HBV including "viral replication in chronic hepatitis B infection of childhood".[15, 16] According to Doolittle et al, "...there are many reverse transcriptase-bearing entities other than retroviruses, including mobile elements found in a wide variety of eukaryocytes, some plant and animal DNA viruses, and even some introns"[17]. In one of his most recent publications, one of the best known retrovirologists, Robin Weiss from the Institute of Cancer Research, London, UK, wrote, "Now we know that a broader group of genetic elements than retroviruses utilise reverse transcription at some stage of replication; these include hepadnaviruses (including hepatitis B virus), cauliflower mosaic virus and retrotransposons of eukaryotes and prokaryotes. Indeed lamivudine may find a place in the treatment of hepatitis B infections as well as HIV".[18] In other words, RT does not seem to be more specific to retroviruses than ATPase, an enzyme now known to be ubiquitous but which, before the discovery of RT, was used to both detect and quantify retroviruses.[19] Since in all the HIV literature, by HIV isolation is meant nothing more than the detection of "HIV particles", proteins and RT (and frequently only one of them), and since any or all of these phenomena "could reflect non-viral material altogether", does it not therefore follow that HIV could reflect non-viral material altogether?

4. "HIV antigens or proteins associated with such particles".
To date nobody has presented evidence that the "HIV antigens or proteins" are constituents of retrovirus particle or even a retrovirus-like particle let alone a unique retrovirus, HIV.

5. "Antibodies against Montagnier's HIV strain – the global standard of all "HIV tests"".
5.1 In the 1983 paper entitled "Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS)",[20] where Montagnier and his colleagues reported the "isolation" of their "HIV" strain, cells from a lymph node biopsy of a gay man with lymphadenopathy (lymphadenopathy syndrome [LAS]) were put in culture with PHA, IL-2 and antiserum to human interferon. (The latter had previously been shown in mice to lead to "increased retrovirus production by a factor of 10 to 50"). After 15 days RT activity was detected using the synthetic primer-template A(n).dT_{15}. The reverse transcription of A(n).dT_{15} was considered proof that a retrovirus was present in the lymph node cells. The finding of the same activity in the supernatant of a co-culture of the same cells with lymphocytes from a healthy individual was considered proof that the retrovirus could be transmitted. In another experiment, polybrene and supernatant from the co-cultures were added to two, three day old umbilical cord lymphocyte cultures. After seven days "a relatively high titer" of A(n).dT_{15} transcription was detected. This was considered proof not only of transmission but isolation as well. "That this new isolate was a retrovirus was further indicated by its density in a sucrose density gradient, which was 1.16, and by its labelling with [^{3}H] uridine (fig. 1)". In figure 1 evidence was presented that A(n).dT_{12-18} could be transcribed by the material from the supernatant of the umbilical cell cultures which, in sucrose density gradients, banded at 1.16 gm/ml.

The "infected" umbilical cord lymphocytes as well as "HTLV-producing" cells were lysed. The proteins from a "cell extract" obtained from the lysates were reacted with the sera from the patient with lymphadenopathy, another patient with "multiple adenopathies", a healthy individual, a normal goat and goat antiserum "to HTLV-I p24". Many proteins from both cell types but especially from the "infected" umbilical cords, reacted with ALL sera. However, the "infected" umbilical cord cells did not react with the antiserum to "HTLV-I p24". The proteins from the culture supernatant which banded at 1.16 gm/ml were also reacted with the sera but instead of the goat anti-p25 antiserum they used sera from another healthy donor. In the published strips it is difficult if not impossible to distinguish any reactive bands with any serum. In the text it is stated "three major proteins could be seen: the p25 protein and proteins with molecular weights of 80,000 and 45,000" in the strip with the serum from the patient with LAS. Montagnier et al also reported that "Electron microscopy of the infected umbilical cord lymphocytes showed characteristic immature particles with dense crescent (C-type) budding at the plasma membrane". They gave no electron microscopic (EM) data on the material banding at 1.16 gm/ml but concluded "A retrovirus belonging to the family of recently discovered human T-cell leukemia 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"[20]. (When it was realised that individuals who have antibodies which react with this "virus strain" did not rapidly progress to AIDS, without proof, the taxonomically distinct "typical type-C" retrovirus became a taxonomically distinct, typical Lentivirus).

5.2 THE WORD "ISOLATION" IS DERIVED FROM THE LATIN "INSULATUS" MEANING "MADE INTO AN ISLAND". IT REFERS TO THE ACT OF SEPARATING AN OBJECT FROM ALL EXTRANEOUS MATTER THAT IS NOT THAT OBJECT. The object of interest is not a protein, nor a fragment of RNA (DNA) but a unique exogenous retrovirus, HIV. Nothing more and nothing less. No such evidence was presented by Montagnier et al. Obviously, at the very best, the finding of phenomena such as virus-like particles in cell cultures, antibody/antigen reactions and evidence for reverse transcription of A(n).dT_{15} can be considered proof only for detection of a retrovirus and then if and only if each are shown to be specific to the retrovirus. This cannot be done unless the retrovirus is first isolated. Thus it comes as no surprise that Popovic, Gallo and their colleagues did not consider Montagnier et al's data as proof of "true isolation".[21] [In their 1984 papers Gallo and his colleagues defined isolation as detection of "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); intracellular expression of virus-related antigens detected with antibodies from seropositive donors or with rabbit antiserum to HTLV-III; or transmission of particles". (By transmission of particles was meant detection of RT or particles in cultures of human umbilical cord blood, bone marrow or peripheral blood T lymphocytes, cultured with supernatants from the "infected" cultures). Since this is no different from the experiments that Montagnier and his colleagues performed, it follows that Gallo and his colleagues did not prove "true isolation" either. In fact, Gallo et al's definition of isolation raises additional questions including: How was it possible to obtain rabbit antiserum "to HTLV-III" before the virus was isolated and how was it possible, before the virus was isolated, to ascertain that both the rabbit antiserum and the patient sera used to test material from the cultures interacted specifically with the virus? According to their definition, one can isolate HIV even if no RT is detected. How is this possible since RT is the "hallmark" of HIV?[22]]

It is also significant that in his and his colleagues' 1986 patent application "Improvements relating to viral isolates and their use", Robin Weiss referred to Montagnier's "HIV strain" as "the material". "A so-called Aids virus isolate was first reported in 1983 by Montagnier and his colleagues in France who named the material "Lymphadenopathy Associated Virus One"".[23] Furthermore, isolation of a retrovirus from the umbilical cord cultures is not proof that the retrovirus was introduced from the outside, that is, that it originated from the patient with lymphadenopathy. All cells contain endogenous retroviruses (see 6.3.2). In fact sperm, ova, placenta, foetal and embryonic tissues, and to a lesser extent, umbilical cord lymphocytes, were extensively studied because retroviruses were said to be transmitted vertically (in the germ cell line) and because they were thought to play a significant role in differentiation. By the beginning of the AIDS era one or more of the following phenomena were reported from experiments with such cells: retrovirus-like particles, reverse

transcriptase activity and retroviral antigens.[24-26] Thus such findings cannot be proof for the existence of HIV.

Neither is the presence of antibodies in the AIDS patients, but not in the healthy controls, which react with the proteins which band at 1.16 gm/ml, proof that such individuals are infected with an exogenous retrovirus, HIV. For example, in a study published this year, one of the best known retrovirologists, Reinhard Kurth, from the Paul-Ehrlich Institute in Germany, and his colleagues, reported that 70% of "HIV-positive patients", compared to only 3% of blood donors, had antibodies which reacted with the retrovirus HTDV/HERV-K. However, HTDV/HERV-K is not a retrovirus which is present only in AIDS patients, that is, an exogenous retrovirus as HIV is said to be, but HTDV/HERV-K is an endogenous retrovirus or, as Kurth put it, a retrovirus present "in all of us". How is it possible then to say, based just on an antibody test, that "Montagnier's strain", if one assumes Montagnier did isolate such a virus, is not another endogenous retrovirus generated by the conditions present in these patients? (see 6.3.2).

5.3 Apparently Montagnier's group found reactions between patient sera and three proteins, p25 (p24), p45 (p41) and p80 in banded material but only p24 was considered to be an HIV protein.

However, in 1984, Gallo's group reported that "No antigen from the uninfected clones reacted with the sera, with the exception of a protein with a molecular weight of 80,000 in H17 which bound antibodies from all of the human serum samples tested [including normal serum] but not from rabbit or goat serum". Because of this the p80 protein was considered to be non-specific. "Antigens newly expressed [reactive with sera in the cell extracts] after viral infection and recognized by the human serum used for this analysis included p65, p55, p41, p39, p32 and p24. A large protein with a molecular weight of approximately 130,000 and a protein of 48,000 were also detected". Unlike Montagnier, Gallo's group also reported that, "With normal human serum, none of the antigens was detected (not shown)", and concluded, "These results show clearly that the antigens detected after virus infection are either virus-coded proteins or cellular antigens specifically induced by the infection".[27] Gallo and his colleagues also reported that of the proteins from the supernatant of the "infected" cultures which in sucrose density gradients banded at 1.16 gm/ml, only two proteins, p41 and p24, reacted with patient sera and concluded that "these molecules are the major components of the virus preparation. p24 and p41 may therefore be considered the viral structural proteins".

In the two years following their discovery of HIV, although Montagnier's group apparently made repeated attempts, unlike Gallo's group, they could not detect a "high molecular weight" protein which reacted with different sera but which "was not present in the supernatant of uninfected control cells". In experiments reported in 1985, instead of using umbilical cord lymphocytes, they used "infected" H9 and CEM cells, two leukaemic cell lines, and cultured (labelled) them with radioactive cysteine, ^{35}S cysteine, (an essential amino acid constituent of human proteins). They reported that in the supernatant "a protein of approximately 110-120K could be specifically immunoprecipitated by sera from pre-AIDS or AIDS patients, in addition to core proteins, and not by sera from normal, healthy blood donors or of laboratory workers. The protein was absent in supernatants of uninfected T lymphocytes, T- or B- cell lines" . They also showed that the 110K protein was a glycoprotein (gp110). For reasons not stated, they thought that the 110K protein had a cellular precursor. To demonstrate this, instead of using the CEM or the H9 cell lines, they formed "A cellular hybrid, between normal T4 lymphocytes and the MOLT-4 cell line", which was then "infected" with LAV and cultured with radioactive cysteine. The resulting syncytia were lysed and the proteins were reacted "with LAV-positive serum". "After 3 hr labelling, a band of 150K was detected. Upon longer labelling, (12 hr) another band of 135K appeared". Curiously, this was interpreted as "suggesting that it [135] was derived from the 150K precursor" and that "either in the cytoplasm or at the cell membrane, the gp150 is converted into the gp135 form...During virus morphogenesis, the gp135 is converted into gp110-120 by partial enzymatic removal of carbohydrates, without proteolytic cleavage. The virus-associated [Not one single piece of their data was derived even from a viral-like particle or material which banded at 1.16 gm/ml. All was either from "infected" cells or culture supernatant] gp110 may itself be further processed during virus aging...besides the main 110-120K band seen after labelling of the virus, three other thin bands of 70K, 40K and 34K respectively, could be specially immunoprecipitated by patients' sera. Since some of these sera did not precipitate any gag protein, it may be assumed that these proteins are antigenically related to gp110 and are cleavage products of the latter".[28] This conclusion can be questioned on several grounds. Suffice it to mention only two:

(a) The culture supernatant and the cells cannot be considered synonymous with a retrovirus.

(b) Although Montagnier et al did not comment, their data shows that many proteins, including a p40 found in the supernatant of both "non-infected" CEM and H9 cells react with sera from the patients with lymphadenopathy.

Somehow, without proof that they are coded by "HIV DNA", or that they belong to a retrovirus-like particle, the following proteins, gp160/150, gp 120, gp45/40, p34/32, p24, p18/17 found either in cells, supernatants, or banding at 1.16 gm/ml in sucrose density gradients became known as the HIV proteins. In other words, contrary to all scientific reasoning, it was postulated that AIDS sera contain specific HIV antibodies and the proteins with which these antibodies react were defined HIV specific proteins.

5.4 The "HIV glycoproteins", gp160, gp120 and gp41.

(a) In 1983,[20] and again in 1984 Montagnier and his colleagues[29] claimed that although p45/41 reacted with patient sera, this protein was not viral but the ubiquitous cellular protein, actin. It is interesting that even this year, the criteria used by Montagnier to define a positive HIV Western blot is: "the presence of antibodies against products of the env gene (gp160, gp120) and reactivity at least with one gag gene product (WHO criteria)"[30]. However to date, no other criteria, not even the WHO criteria, exclude p41. The WHO criteria is "2 env bands (precursor, external gp, or transmembrane gp)" with or without any other bands (transmembrane=gp41).[31] Unlike Montagnier, Gallo considers gp41 the most specific HIV protein.

In 1985, Gallo and his colleagues, comparing the fourth open reading frame (ORF) of the "HIV DNA", which they called env-lor with the env genes of other retroviruses, reported that, "The predicted product of the fourth reading frame env-lor shares many features in common with the envelope gene precursors of other retroviruses, the most striking of which is a hydrophobic region near the middle of the protein...The amino-terminal domain of the translation product of the fourth open reading frame also resembles the env protein precursors of other retroviruses...we believe that the fourth open reading frame encodes an env precursor...In its mature form it is probably cleaved into a large heavily glycosylated exterior membrane protein about 481 amino acids long and a transmembrane protein, 345 amino acids long which may be glycosylated. The size of these predicted products agrees with the detection of a large glycosylated protein of M_r 120-160K in HTLV-III-infected cells which is probably the glycosylated env gene precursor and a smaller, virion-associated gp41 which is probably the membrane protein".[32] However, in a study published in 1987 by Gallo and his colleagues, where they performed a "Computer-assisted analysis" of "the amino acid sequences of the envelope protein complexes derived from the nucleotide sequences of seven AIDS virus isolates", it was reported that, "Although the overall sizes and structures of the seven surface proteins are rather similar, the deduced amino acid sequences differ substantially. On the average, only 66% of the amino acids are conserved in the exterior part of the protein...gp41, the transmembrane part of the envelope protein complex, shows more than 80% conserved amino acids", but "gp41 should be about 52,000 to 54,000 daltons by calculation".[33] Even if the molecular weight of the glycoprotein predicted from the length of the "HIV" fourth ORF was found to be identical to that of the protein present in the Western blot (41,000), the claim by Gallo that the interaction of gp41 with antibodies found in AIDS patient sera is proof that gp41 is coded by the "HIV genome", and that both gp41 and the antibodies are specific to a retrovirus, is at odds with what Gallo was saying in 1981.

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;[34]

(b) published an electron micrograph of virus-like particles banding at a sucrose density of 1.16 gm/ml.[35]

Following the discovery of HL23V, some researchers attempted to determine its prevalence utilising antibody tests[36] while others were interested to determine the specificity of the antibody reactions. The former included two of the best known HIV experts Reinhard Kurth and Robin Weiss, and their colleagues who, for this purpose used "the simian sarcoma-associated helper virus (SSAV) and the M7 strain of baboon endogenous virus (BEV) to survey human sera for specific antibodies. Also included is a virus (HL23V-1) originally isolated from cultured peripheral blood leukocytes of a patient with acute myelogenous leukemia. HL23V-1 was shown to comprise a mixture of two viruses, one closely related to SSAV, the other to BEV" and found that "A survey of human sera from healthy individuals revealed the presence of naturally occurring antibodies that react in radioim-

munoprecipitation assays with proteins of mammalian type-C viruses" including the internal (gag) and envelope (env) proteins of HL23V, SSAV and BEV and concluded, "The serological studies presented here and by others provide indirect evidence that the infectious mode of transmission remains a real possibility in humans, and suggests that infection with an oncornavirus [retrovirus] may be extremely widespread".[37] Three years later, in 1980, two research groups,[38, 39] one from the Laboratory of Cellular and Molecular Biology, National Cancer Institute and the other from the Laboratory of Viral Oncology, Memorial Sloan-Kettering Cancer Center, using the "viral glycoproteins", 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". In 1981 Gallo accepted the evidence that the antibodies which reacted with proteins of HL23V were directed not against the proteins "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".[40] This discovery was of such significance that today nobody, not even Gallo, considers HL23V as being the first human retrovirus, or even a retrovirus. In fact, in 1981 when Gallo and his colleagues reported the presence in humans of antibodies to what he now calls the first human retrovirus, HTLV-I, (according to Weiss, "The first 'human' retrovirus to be isolated in 1971 was human foamy virus (HFV) from a nasopharyngeal carcinoma line",[18]) the title of the paper was, "Antibodies in human sera reactive against an internal structural protein of human T-cell lymphoma virus".[40] In this paper Gallo and his colleagues described the finding of antibodies to a "major internal structural protein (p24) of $HTLV_{CR}$" and claimed that such antibodies were "specifically directed at $HTLV_{CR}$ proteins and not at cell-specific determinants – in other words, the immunological reactions are not those reported in human sera against animal virus glycoproteins which, lacking virus specificity, are directed against the carbohydrate residues of the glycoprotein".

(b) By 1989, researchers from New York showed that in Western blot analyses, "the components visualized in the 120-160 kDa region do not correspond to gp120 or its precursor but rather represent oligomers of gp41". 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".[41, 42] (Little if any notice was taken of this report!). Indeed, if, as it is claimed, HIV Western blots are prepared from lysates of purified HIV virions, then it would be impossible for p160 and p120 to be found in WB strips since:

(i) All HIV researchers agree with Montagnier and Gallo that gp160 is a precursor to gp120 and gp41 and unlike the latter two proteins, is only found in infected cells and not in mature particles;
(ii) Although many EM have been published of virus-like particles in non-banded material nobody,[43, 44] not even the CDC,[45] or Hans Gelderblom and his colleagues who have most thoroughly studied these particles, have proven the existence in the cultures of cell-free particles possessing knobs (spikes). In one of his latest publications Gelderblom and his colleagues have estimated that immediately after being released, "HIV particles" possess an average of 0.5 knobs per particle but also pointed out that "it was possible that structures resembling knobs might be observed even when there was no gp120 present, i.e., false positives".[46] It is accepted that gp120 is present only in the knobs (spikes). Since there is no evidence for the presence of knobs in the cell-free particles, even immediately after release from the cell, it is not possible for the gp120 to be present in the Western blot.

5.5 The "HIV pol protein", p31/34.
In 1987 Henderson et al 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 histocompatibility DR proteins, they concluded that "the DR alpha and beta chains appeared to be identical to the p34-36 and p30-32 proteins respectively".[47]

5.6 The "HIV gag protein", p24
As far as Montagnier is concerned, p24 is THE HIV protein, and for at least three years after the introduction of the "HIV" antibody test, a p24 band found in the WB was considered by most laboratories, including the CDC, as proof for HIV infection. 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 p24 band in the WB is considered proof of HIV infection then about 30% of individuals who are transfused with HIV negative blood become infected as a result.[48] Since, according to the AIDS vaccine Clinical Trials Group,[49] "The presence of p24 band was common among low-risk, uninfected volunteers and complicated the interpretation of the Western blot test results", HIV infection should be common among healthy at 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, as proof of HIV infection. Yet, when the same reaction takes place between an antibody to the p24 of the WB and a patient serum, it is considered proof of viraemia, and when between an antibody to p24 and material present in a cell culture, the same reaction is considered proof of HIV isolation!

Obviously, the detection of a protein, even if known to be virus specific, in sera or even culture, does not constitute proof for isolation or viraemia. That such a finding is non-specific can be best illustrated by a few examples. In 1992, Jorg Shupbach, the principle author of one of the first four 1984 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".[50] 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. In 1989, David Ho and his colleagues used p24 measurements in serum and in cultures of non-infected cells cultured with plasma from "infected" patients, to estimate active virus, "infectious HIV-1", viraemia, viral load. The serum from 14/53 patients whose plasma cultures were positive, was negative for p24. They concluded, "Thus, plasma culture was more sensitive than serum p24 antigen measurement in detecting the presence of cell-free HIV-1 in blood". They also reported that treatment with AZT for four weeks induced "a 94 percent reduction in the load of cell-free virus".[51] Even Jackson et al who claim an overall 98.3% "HIV isolation" rate, can detect p24 in serum of 42% of AIDS patients, 37% of ARC patients and 17% of asymptomatic seropositive individuals[52] which is a much lower rate 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 bacteriological, 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 neutralizable 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".[53] Using two kits, the Abbot and Diagnostic Pasteur, 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 characterization of this 25-30kD protein may represent an important contribution to the detection of HIV-1-related endogenous retroviruses".[54]

The disagreement between Montagnier and Gallo about which proteins were actually "HIV" proteins was not limited to gp41 but included p24. Montagnier always mentioned that "no cross-reactivity

existed between HIV p24 and other antibodies including antibodies to HTLV-I, II". Until 1985 he also maintained that there was "a very close homology between LAV and HTLV-III but an absence of homology with HTLV-I and -II".[28] However, in 1985 he wrote, "We have also compared the deduced amino-acid sequences of LAV proteins with those of HTLV-I and other retroviruses and find no significant homology, except for domains pol and gag which are generally conserved among retroviruses".[55]

Gallo always maintained that homology exists between the HTLV-I, II and HIV gag genes[56] and the many features shared by all "human retroviruses" include "a small (p24/p25) major capsid protein; p24 cross-reactive antigenic determinant detected with either heterologous (rabbit) antisera or human monoclonal antibodies".[57] Indeed, gag stands for group specific antigens. As far back as 1974 Gelderblom and his colleagues wrote, "While the virus envelope antigens are primarily virus-strain specific, the bulk of internal proteins of the virion with molecular weight (mw) between 10,000 d and 30,000 d are group-specific (gs) for viruses originating in a given animal species (gs-spec. antigens). The major protein constituent of mammalian C-type oncornaviruses [retroviruses] with a molecular weight in the range of 30,000 d was found to possess, besides gs spec. antigen, an antigenic determinant that is shared by C-type viruses of many mammalian species including monkeys and was thus termed gs interspecies (gs-interspec.) antigen".[58] In 1989 William Blattner, a well known HIV/AIDS expert stated: "It may be feasible to use viral antigen probes to look for cross-reactive antibodies, since certain viral proteins, particularly the polymerase and gag proteins may be highly conserved between subtypes of virus".[59] Thus, even if p24 were to be specific to retroviruses, it cannot be HIV specific.

If p24 detected in culture supernatants is a component of similar particles, viral or non-viral, then in density gradients all the p24 should be found at least in one band (fraction), even if not at a density of 1.16 gm/ml. That this is not the case has been demonstrated by Montagnier himself. In one experiment Montagnier and his colleagues divided the density gradient into sixteen fractions. The RT peak was found in fraction five and six, while the p24 and gp110 were present in all but three (1, 2, 3) fractions.[28]

5.7 The role of actin and myosin in particle budding.

There is no scientific reason to define a protein present in a cell, culture supernatant, or even in material banding at 1.16 gm/ml in sucrose density gradients as being retroviral on the basis that it reacts with antibodies in AIDS patient sera, as Montagnier and Gallo's groups did. According to Gelderblom, AIDS patient sera are "polyspecific"[60, 61] and at present there is ample evidence that these sera react with a plethora of self and non-self antigens including proteins of "non-infected" lymphocytes. Why then should they not also react with the "HIV proteins", even if such proteins are cellular proteins, or with a variety of recombinant or synthetic antigens? If the proteins in the cultures/co-cultures of tissues derived from AIDS patients and which react with AIDS patient sera are indeed retroviral, then what are the proteins in the "non-infected" cells and supernatants which Montagnier repeatedly reported to also react with AIDS patient sera? On the basis of reactivity with AIDS patient sera, only 20% of the proteins which band at 1.16 gm/ml can be considered "HIV proteins" and, as the HIV/AIDS experts claim, without proof, can be considered coded by "HIV DNA".[47, 62] Even if there was proof that pure (isolated) "HIV" particles are present at 1.16 gm/ml, then all the proteins banding at 1.16 gm/ml should be embodied in such particles. However, since only 20% of these proteins are "HIV" proteins, the question then arises, what is the origin and role of the remaining 80% of the proteins in such particles and by what genes are they coded? Why are only 20% of the proteins viral and non-cellular? Why not all of them and vice versa?

If the gp41 protein present in the Western blot band and which reacts with AIDS patient sera could be the ubiquitous protein actin, then why should not one consider the p24 protein as being one of the light chains of myosin, another equally ubiquitous protein especially given that:

(a) Matsiota, Montagnier and their colleagues at the Pasteur Institute have shown that AIDS patients and those at risk have high levels of antibodies to this protein;[63]

(b) at present there is ample evidence that the plethora of cellular proteins (b2 microglobulin, the a and b chains of human lymphocyte antigen (HLA) DR, CD71, CD63, CD43, CD8, "the major leukocyte adhesion receptors LFA-1 (CD11A/CD18) and CD44) which are present in the "HIV particles", include actin and myosin.[64-68]

Indeed, in the last few years researchers from a number of institutions expressed the view that actin polymerisation (or actin/myosin interaction) "mediates HIV budding" and release. Researchers from New York and Philadelphia found that colchicine treatment of "MOLT4/HIV-1_{IIIB}" cells, "induced lymphocyte polarization, redistribution of F-actin into a pseudopod, and secretion of HIV from the pseudopod", and that the particles were "observed exclusively on the tip of the pseudopod".[65] Two of the studies which examined the role of actin and myosin in "HIV particle" budding and release are by researchers from Japan. In one publication the authors concluded, "Since F-actin is essential for maintaining cell-shape and cellular function, polarization of F-actin might change the cell membrane configuration or cell fragility, which may be essential for HIV release".[67]

In the other study, the authors "demonstrated that myosin and actin are colocalised at the budding site of viral particles. In particular, myosin was concentrated on the same area of the plasma membrane as the dense spots of the viral particles. In contrast, actin was widely distributed on the plasma membrane and was always found in areas where viral particles were present". They concluded, "actin might participate with myosin in an active process leading to the release of viral particles from the membrane". Because these researchers, like most others, are of the opinion that "the initiation of a myosin-actin interaction requires an increase in free intracellular calcium", they have performed a preliminary experiment using two calcium chelators, one, BAPTA which they consider chelates only intracellular free calcium and the other, EGTA, which in their view chelates only the free calcium on the outer side of the cell. They found that "HIV-1 release was suppressed most pronouncably when both" the inner and the outer free calcium was chelated, and that inhibition was stronger with the outer chelater than the inner. "From these results, we suggest that $[Ca^{2+}]_o$ might enter the cell by the stimulation of viral budding itself at the budding site...it may be difficult to detect an increase of $[Ca^{2+}]_i$...because the budding mechanism is going on continuously and slowly in a very narrow region without any synchronization".[64]

At present evidence also exists that:

(a) there is an association between the redistribution of polymerised actin, myosin and other cellular proteins (glycoproteins) and many cellular processes including budding unrelated to HIV release;[69-73]

(b) polymerisation of actin, actin-myosin interaction and cross-linking of polymers in general is regulated by the redox state, oxidation leading to interaction;[74-76]

(c) 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).[77] Ten years ago Montagnier wrote, "Indeed, LAV infection of resting T4 cells does not lead to viral replication or to expression of viral antigen on the cell surface, while stimulation by lectins or antigens of the same cells results in the production of viral particles, antigenic expression and the cytopathic effect".[78]

(d) in the presence of antioxidants no "HIV" phenomena can be observed.[77, 79, 80] In a study presented at this year's International AIDS Conference, researchers from Rome reported, "The results obtained using 3-ABA, NAC [antioxidants] and a combined treatment 3-ABA/NAC given together seem to confirm the role of intracellular redox balance in the modulation of the HIV expression. In fact, a significant reduction in the number of viral particles was observed in cultures which have received the combined treatment with NAC/ABA".[81]

Given the above data, may one be tempted to speculate that the "HIV" particles and proteins are nothing more than "non-viral material altogether", induced by the agents to which the AIDS patients and cultures are exposed?

CONCLUSION—The statement "antibodies against Montagnier's HIV strain-the global standard of all "HIV tests"", presumes proof of:

(a) the existence of more than one "HIV strain", including one of Montagnier's. Such evidence can be obtained only by isolating the retrovirus. However, Montagnier's evidence does not prove the isolation of a retrovirus;

(b) the existence of "HIV" specific immunogenic proteins. Again, such proof can be obtained only by isolating the retrovirus;

(c) antibodies specifically induced by HIV infection. It is true that for detection of such antibodies one does not need to use HIV or the HIV immunogenic proteins. For example, serological tests for both infectious mononucleosis and syphilis employ antigens derived from horse red blood cells and ox heart respectively but nonetheless predict infection with Epstein-Barr virus and Treponema pallidum. However, the only way to prove that "HIV antibodies" are directed against "HIV", that is, the only way to use the antibody test to prove HIV infection, is to present evidence which proves that the antibodies are HIV specific. Such proof can be obtained only by using HIV isolation as a gold standard. Since this has not been done it is not possible to say that "the global standard of all "HIV tests"" proves HIV

infection.

6. "HIV DNA"
In debating the proof for the existence of a unique, exogenous retroviral agent one cannot adopt as an initial premise ("Full-length HIV-1 and HIV-2 DNAs...") that is contingent upon proof of the argument ("ergo...HIV exists and has been isolated"). The a priori designation of a particular fragment of DNA as "HIV DNA" merely begs the question under consideration.

6.1 MINIMUM EVIDENCE REQUIRED TO PROVE THE EXISTENCE OF HIV DNA
If "HIV DNA" is the genome of a unique retroviral particle then the most basic requirement is proof for the existence of a unique molecular entity "HIV DNA", that is, unique fragments of 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 a particle with the morphological, physical and replicative characteristics of a retroviral particle. Proof of these properties can only by obtained by isolating the putative viral particles, that is, by obtaining them separated from everything else, extracting the nucleic acids and demonstrating that such particles are identical (their constituents including their nucleic acids are identical) and infectious. The correct procedures, now having been used for over half a century to achieve this proof, require demonstration that:
1. In "infected" cell cultures (cocultures) there are particles with a diameter of 100-120nM containing "condensed inner bodies (cores)" and surfaces "studded with projections (spikes, knobs)"; [82]
2. In sucrose density gradients the particles band at a density of 1.16 gm/ml;
3. At the density of 1.16 gm/ml there is nothing else but particles with the morphological characteristics of retroviral particles;
4. The particles contain only RNA and not DNA and that the RNA consistently has the same length (number of bases) and composition no matter how many times the experiment is repeated;
5. When the particles are introduced into secondary cultures, but mindful of the critical caveat discussed below:
(a) the particles are taken up by the cells;
(b) the entire RNA is reverse transcribed into cDNA;
(c) the entire cDNA is inserted into the cellular DNA;
(d) the DNA is transcribed into RNA which is translated into proteins;
6. As a result of 5 the cells in the secondary cultures release particles into the culture medium;
7. The particles released in the secondary cultures have exactly the same characteristics as the original particles, that is, they must have identical morphology, band at 1.16 gm/ml and contain the same RNA and proteins.

The caveat is that while the introduction of the majority of infectious particles into cell cultures and subsequent release of similar particles is proof that such particles are indeed infectious, this is not the sufficient case for retroviruses. The basis of this exception is the fact that "one of the most striking features that distinguishes retroviruses from all other animal viruses is the presence in the chromosomes of normal uninfected cells, of genomes closely related to, or identical with, those of infectious viruses".[83] In fact, a cell may contain the genome of many retroviruses. As far back as 1976 retrovirologists recognised that "the failure to isolate endogenous viruses from certain species may reflect the limitations of in vitro cocultivation techniques".[84] In other words, the finding of a retrovirus in both the primary and secondary "infected" cultures/cocultures is not proof that the cells have been infected with an exogenous retrovirus.

One way which will suggest but will not prove that the cells acquired virus from the outside (exogenously acquired retrovirus, infectious retrovirus) and have not assembled a retrovirus HIV from information already existing in normal cells (endogenous retrovirus) is to conduct experiments that use controls, that is, to run in parallel with test cultures/cocultures control cultures/cocultures. The only difference between the test and control cultures should be the introduction of particles into the test cultures. In other words, apart from the introduction of particles, 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 those of AIDS patients;

(b) because the mere act of co-cultivation alone may lead to release of endogenous retroviral particles, if test cells are cocultured, so should the cells used in control experiments;[85]

(c) extracts, even from normal unstimulated cells when added to the cultures may increase endogenous retroviral expression.[86] Because of this, when cells are cultured with "HIV" (supernatant or material which bands at 1.16 gm/ml), the controls must be cultured with similar material from cell cultures originating from sick individuals with illnesses similar to AIDS, that is, matched individuals who are immunosuppressed;

(d) the appearance of endogenous retrovirus can be accelerated and the yield increased a million fold by stimulating the cultures with mitogens,[87] mutagens, chemical carcinogens and radiation.[88, 89] If test cultures are exposed to or employ such agents so should the controls;

(e) since AIDS patients and those at risk of developing the syndrome are exposed to strong oxidising agents,[79, 90] the control cells should also originate from such patients;

(g) to avoid observer bias and in the best interests of science, blind examination of test and control cultures/cocultures should be performed.

6.2 EVIDENCE FOR THE EXISTENCE OF "HIV DNA"
6.2.1 In 1984, in the first of two papers, Montagnier and his colleagues described the following experiment: "Because LAV can induce T-cell fusion and because EBV [Epstein Barr virus] is known to have fusion activity in B cells, we performed co-infection experiments of unfractionated lymphocytes (B and T) with both viruses. It was hoped that stable hybrids of LAV-infected T cells and of EBV-transformed B cells would be formed and that such hybrids would be able to continuously produce LAV. Several regimens were tried. The one that gave rise to continuous productive infection of LAV was the following. Whole lymphocytes of F. R. were first stimulated for 24 hours with Protein A and then infected with and EBV strain, M81, derived from a nasopharyngeal carcinoma. Five days later, half of this culture was infected with LAV as described (1) and then divided in two subcultures: one was cultured in medium lacking T-cell growth factor (TCGF: interleukin-2), the other in medium containing TCGF. As expected, the TCGF-fed culture produced LAV as detected by a peak of RT activity appearing between day 12 (day 6 after LAV infection) and day 21 in the supernatant. In contrast, the cells cultured in the absence of TCGF did not yield any detectable RT...On day 19, at the time of decline of LAV production, a subculture of the TCGF-fed cells received fresh T cells from the same donor: these T cells had been activated for three days with phytohemagglutinin (PHA)...Six days later (day 25), a new peak of RT appeared, but contrary to the first infection, it was not transient...At the time of the second LAV infection, large round cells transformed by EBV could be readily seen in this culture, as well as in the control culture not infected with LAV, indicating that immortalization of the B cells by EBV had already occurred. The immortalized B-cell line was termed RF8".[29] [Reference 1 to which Montagnier refers is the 1983 paper in which Montagnier et al described the first "isolation" of HIV (see 5)].

In the second study, 200 ml of supernatant from the "HIV infected" FR8 cells were banded in sucrose gradients, "Virus containing fractions were pooled" and centrifuged. (It is not stated how they determined the existence of "virus", in which band(s) (fraction(s)) "virus" was found, how many bands if any were found to have particles, or why there were more bands than one (1.16 gm/ml) containing the "virus"). The pellet was incubated with several substances, dATP, dGTP, dTPP, dCTP including ^{32}dCTP and an oligo(dT) primer. From the cDNAs thus obtained, three clones "pLAV13, 75 and 82, carrying inserts of 2.5, 0.6 and 0.8 kilobases (kb), respectively, were characterized further. All three inserts have a common restriction pattern at one end, indicative of a common priming site". "The 50-base pair (bp) common HindIII-P_{st}I fragment was sequenced and shown to contain an oligo(dA) stretch preceding the cloning dC tail. The clones are thus copies of the 3' end of a poly(A) RNA. The specificity of pLAV13 was determined in a series of filter hybridization experiments using nick-translated pLAV13 insert as a probe". Firstly, "using an adapted spot-blot technique" they tested the pellet obtained from the supernatant of "LAV-infected" normal lymphocytes and CEM cells as well as non-infected lymphocytes. The "infected" pellets were positive and the non-infected negative. "Second, the probe detected DNA in the Southern blots of LAV-infected T lymphocytes and CEM cells. No hybridization was detected in DNA from uninfected lymphocytes or from normal liver". No details are given regarding the method used to produce "infection", but it would appear that the normal cells and the CEM cells were cultured with supernatant from the FR8 cells, that is, the same supernatant they used to obtain the probe! They concluded: "Together, these data show that LAV pLAV13 DNA is exogenous to the human genome and detects both RNA and integrated DNA forms, derived from LAV-infected cells. Thus, pLAV13 is LAV specific".[91]

6.2.2 In May 1984, Gallo and his colleagues published four papers. To "isolate" HIV they used a leukaemic cell line which they called HT. It is impossible to know with what tissues from AIDS patients this cell line was cultured. Reading the May 1984 papers one gets the impression that the HT cell line was cultured with concentrated (supernatant) fluids originating from individual, AIDS patient, stimulated T-cell cultures. Subsequently, the Gallo investigation found the HT cell line was cultured with concentrated fluids pooled initially from individual cultures of three patients and ultimately from the individual cultures of ten patients.[92] The Gallo investigation found this procedure to be "of dubious scientific rigor". One scientist described the procedure as "really crazy".[93] In 1985, Gallo and his colleagues wrote, "The H9/HTLV-IIIB cell line was derived from the human T-cell line HT, following co-culture with T lymphocytes obtained from several AIDS patients, and contains many different HTLV-III forms".[94]

The detection of reverse transcription of A(n).dT_{15} in the supernatant, was considered proof the HT cells were infected with a retrovirus, HIV, which originated from the patients' tissues. A clone, H9 of the HT cell line was obtained "using irradiated blood of a healthy donor as a feeder".[21] The H9 cells were cultured with supernatant from the "HIV" infected HT cells. The H9 supernatant was banded in sucrose density gradients and the material which banded at 1.16 gm/ml which, without proof, Gallo and his colleagues considered to be synonymous with retroviral particles, was "lysed with sodium dodecyl sulfate (SDS), digested with proteinase K, and directly chromatographed on an oligo(dT) cellulose column. The resulting polyadenylate [poly(A)]-containing RNA was used as template to synthesize ^{32}P-labelled complementary DNA (cDNA) in the presence of oligo(dT) primers. The size of the resultant cDNA ranged from 0.1 to 10 kb. When these labelled cDNAs were hybridised to poly(A)-containing RNA purified from infected [that is, cells cultured with the same supernatants as those from which the probe was obtained] and uninfected H9 cells as well as other uninfected human cell lines, only the infected H9 cells contained homologous RNA sequences as evidenced by discrete RNA bands after Northern hybridisation. Figure 1 shows that cDNA preparations from HTLV-III_B and HTLV-III_Z gave identical patterns, detecting species of about 9.0, 4.2, and 2.0 kb...These bands are similar in size to those corresponding to genomic size messenger RNA (mRNA) and spliced mRNAs of env and pX sequences previously observed in cells infected with HTLV-I, consistent with the anticipated relatedness of these viruses. Furthermore, viral mRNA bands of HTLV-II-infected cells were detected with an HTLV-III cDNA probe and again the sizes of the mRNA were like those with HTLV-I"![56]

In another study by Gallo and colleagues, extrachromosomal DNA of "infected" H9 cells was extracted and "assayed for its content of unintegrated viral DNA" using the ^{32}P-labelled cDNA as a viral probe. "Unintegrated linear viral DNA was first detected after 10 hr [of "infection"] and was also present at the subsequent time points. Figure 1 shows a Southern blot of the 15-hr sampling. A band of 10 kilobases (kb) in the undigested DNA represents the linear form of unintegrated HTLV-III".[95] In yet another study Gallo and his colleagues reported that, "Since the HTLV-III provirus was found to lack Xba I restriction sites, a genomic library was constructed by using Xba I-digested H9/HTLV-III DNA, and this was screened with an HTLV-III cDNA probe to obtain molecular clones of full length integrated provirus with flanking cellular sequences. Fourteen such clones were obtained from an enriched library of 10^6 recombinant phage, and two of these were plaque-purified and characterized. Figure 1 illustrates the restriction maps of these two clones, designated lHXB-2 and lHXB-3. The overall length of the HTLV-III provirus is approximately 10 kilobases...To determine whether the HTLV-III genome contains sequences homologous to normal human DNA, the viral insert of lXB-2...was isolated, nick translated and used to probe HTLV-III-infected and uninfected cellular DNA. Under standard conditions of hybridization...this probe hybridized to DNA from H9/HTLV-III cells as well as other HTLV-III-infected cells, but not to DNA from uninfected H9 cells, uninfected HT cells (the parent line from which H9 was cloned), or normal human tissues (data not shown). This finding is in agreement with the results of other experiments in which the unintegrated (replicative intermediate) form of HTLV-III was used as a probe and demonstrates that HTLV-III, is an exogenous retrovirus lacking nucleic acid sequences derived from human DNA".[96]

6.2.3 In 1984, Levy and his colleagues cultured PBMC from patients suffering from Kaposi's sarcoma with IL-2, polybrene and PHA. The supernatant was tested for RT, the cells for reaction with serum from the Pasteur Institute patient BRU and "some cultures were examined for virus by electron microscopy". The finding of a positive result with "any of these tests" was considered proof of virus isolation. The supernatant from one of these cultures was "inoculated into fresh human PMC stimulated 3 days before with phytohemagglutinin". Within 6 days the supernatant of this culture had high RT activity and this was said to represent "the virus isolate ARV-2".[97] The HUT78 cell line was cultured with "ARV-2". In the HUT78 "Virus production was monitored by measuring reverse transcriptase activity". When there was maximum RT activity, the supernatant was centrifuged and the resuspended pellet, after treatment with DNAase, was centrifuged in sucrose gradients. The nucleic acid from each fraction was electrophoresed on agarose gel. The region in the gel containing an "9kb RNA species was cut out" and used to obtain "a radioactive cDNA probe". The DNA from the HUT78 cell line cultured with "ARV-2" was digested with restriction enzymes, electrophoresed in agarose gel and Southern blotted using the "radioactive cDNA probe". "No specific bands were detected in several digests of DNA from uninfected cells...whereas bands were seen in infected cells...undigested DNA from infected cells contained a species at 5.5 kb, a faint species at 6kb and a broad band at the exclusion limit of the gel (>15kb). We suggest that the DNA species 5.5kb and 6kb represent unintegrated viral DNA in a circular configuration containing respectively one and two long terminal repeats (LTRs); the upper broad band (>15kb) represents provirus integrated into the host cell DNA". In an additional experiment "whole-cell DNA from cells infected with ARV-2 was partially digested with E_{CO}RI; 9-15 kb cell DNA was cloned into an EMBL-4 bacteriophage l vector and recombinant phage were identified with the virus-specific cDNA probe". Among the recombinant phage obtained were l-9B and l-7A, each of which was 9.5 kb.[98]

6.2.4 SUMMARY AND DISCUSSION

It is obvious that although Montagnier, Gallo and Levy and their respective colleagues refer to virion or virus particles purification or isolation, none of these groups have presented evidence for the isolation of retrovirus particles or even the isolation of virus-like particles, the first and absolutely necessary step in proving the existence of a retroviral genome. (At the time of writing, neither has any other group of HIV/AIDS researchers). Finding some RNA which bands at 1.16 gm/ml, selecting from it a poly(A) rich fraction, or a fragment of a given length, even if always found to be the same length and sequence, and referring to it as HTLV-III, LAV, ARV does not constitute such proof. It must be stressed that even if the RNA is incorporated in a particle which in sucrose density gradients bands at 1.16 gm/ml, this is still not proof that it is retroviral RNA. According to John Coffin, one of the best known experts on the retroviral genome, there are particles "with a full complement of viral proteins, but the particles contain a collection of cellular RNAs and only about 1% genomic RNA...assembly of particles does not require the genome...in its absence other RNA molecules may be substituted".[83] It is important to note that although all groups, Montagnier's, Gallo's and Levy's refer to the material from the culture supernatants which in sucrose density gradients bands at 1.16 gm/ml as viral particles, virions, and to the RNA and proteins at that density as "particle-associated" RNA or proteins, not one of the groups presented evidence for the existence at this density of any particles, retroviral-like or otherwise, pure (isolated) or otherwise. Instead these researchers cultured lymphocytes from AIDS patients and stimulated (activated) them with a wide variety of agents. Reverse transcription of A(n).dT_{15} in the culture supernatant was considered proof for infection with a retrovirus or even proof of isolation. Supernatants from these cultures were introduced into cultures of leukaemic or transformed cell lines. With the supernatants from these cultures they performed two types of experiments:

(a) The supernatants were banded in sucrose density gradients. At the 1.16 gm/ml band (and sometimes at other band(s) – at least in Montagnier's group's experiments, this is not made clear), they found fragments of RNA of certain lengths (although no two had the same length) or were rich in adenine, (poly(A) rich fragments), and called these "HIV RNA", the "HIV genome". Using a (dT) primer the "HIV RNA" was transcribed into a complementary DNA (cDNA);

(b) The supernatants were introduced into another set of the transformed and leukaemic cell lines as well as into stimulated cultures of normal T-cells. The DNA from these cells, as well as the DNA from the cultures to which no supernatant was added, were hybridised using probes from the cDNA. Positive results were obtained only with the DNA from the cells to which the supernatants were added. This evidence was interpreted as proving that the "HIV DNA", the retrovirus, originated from the AIDS patients and in fact that these patient acquired it from the outside, that is, the retrovirus was exogenous.

There are many problems associated with these experiments and their interpretation. Among the many questions their conclusion raises the most obvious are:

1. HIV is said to be a retrovirus and retroviruses are particles

which contain among other things, RNA. How then is it possible to claim that the RNA which banded at 1.16 gm/ml, "HIV RNA", is the genome of a retrovirus without proof that it is a constituent of a particle, viral or non-viral which bands at this density?

2. RT is not specific to retrovirus and in fact A(n).dT_{15} can be reverse transcribed by all cellular DNA polymerases a, b and g. Is it possible then to consider reverse transcription of A(n).dT_{15} as proof for HIV isolation or even detection of a retrovirus? Even if the process of reverse transcription were specific to retroviruses, can the detection of a process ever be considered proof for the isolation of an object, in this case, retroviral particles?

3. Cell culture supernatants will contain both DNA and RNA including some enclosed in cellular debris (fragments) especially if cellular viability is not one hundred percent as is the case in cultures used by the three groups. The RNAs may include messenger RNA (which is adenine rich), as well as high molecular weight heterogenous nucleic RNA. These RNAs, in addition to having high molecular weight and heterogeneity in size, also have poly(A), with the poly(A) attached at the 3' end of the molecule, and may be RNAase resistant. Actinomycin inhibits its synthesis and also interferes with its proper processing and breakdown.[99] From animal virology it is also known that non-retroviral RNA and DNA also bands at 1.16 gm/ml.[100] How is it then possible to claim that just because an RNA bands at 1.16 gm/ml and is adenine rich or has a certain length, it is "HIV RNA"? If this RNA is "HIV RNA", then what is the other RNA and the DNA which also bands at this particular density? If the latter are cellular why not the poly(A)RNA as well?

4. By definition, retroviruses are infectious particles which contain only RNA. When they enter a cell the RNA is reverse transcribed into DNA, which is then integrated into cellular DNA as a provirus, which means that "HIV DNA" will be present only in the cell and nowhere else. Yet many HIV experts including Gallo have shown that both the supernatants of "infected" cell cultures and the "HIV particles", that is, the material which bands at 1.16 gm/ml, contains "HIV DNA" which "may integrate directly into the host chromosomal DNA".[101-103] The question then arises, is the "HIV DNA" the result of "HIV RNA" reverse transcription or is it vice versa?

5. It is accepted that the HIV RNA is localised in a condensed core surrounded by a "lipid-bilayered envelope derived from the cellular membrane of the host cell, studded with virally encoded gp120 and myristylated protein, p17. The so-called core-envelope link (CEL) attaches the core to the envelope".[103] One of the best know facts in biology is that condensed cores (chromatin) is transcriptionally inactive. This is one of the reasons why viruses, including retroviruses, to multiply, must first enter cells where their chromatin is decondensed. However, in a paper published in 1993, Hui Zhang and colleagues including Poiesz, from Suny Health Science Center at Syracuse, New York, wrote: "We have shown that in the absence of detergent, large amounts of DNAase-resistant viral DNA can be synthesized within intact HIV-1 virions, indicating that this phenomenon is not dependent on perturbation of the viral envelope. [Not to mention decondensation of chromatin]. Nascent viral DNA synthesis also occurred in purified virions incubated at 37° in cell-free human physiological fluids including seminal plasma, breast milk, and fecal fluids".[103]

This means that either:

(i) the "intact HIV-1 virions" perform a function that no other biological system with very condensed and protected chromatin can perform; or

(ii) the "HIV RNA" found in the supernatants or in the "purified virions" is present in an unembodied form; or

(iii) the "HIV RNAs" are de novo synthesised in the cell cultures (see 6.3.5);

6. At present there is ample evidence that any RNA or DNA present in the supernatant, irrespective of its origin, especially when cells are stimulated by polycations and oxidising agents, will be taken up by the cells (see 7.1). How is it then possible to claim that a positive hybridisation signal in cells cultured with the same "HIV DNA" containing supernatant as the supernatant from which the "HIV DNA" probe originated but not in other cells is proof that the "HIV DNA" is the genome of an exogenous retrovirus?

7. The first, absolutely necessary step in proving that the "HIV DNA" originated from the lymphocyte cells of AIDS patients and those at risk, is to perform hybridisation experiments using the DNA of their fresh, uncultured lymphocytes and the "HIV DNA" as a probe. It is hard to understand why neither Montagnier's nor Levy's group reported such experiments. Gallo's group did and the results were negative (see 6.4.4). How is it then possible to claim that "HIV DNA" is the genome of an exogenous retrovirus which originated from AIDS patients and those at risk?

8. Reading the seminal paper on HIV isolation entitled "Detection, Isolation and Continuous Production of Cytopathic Retroviruses (HTLV-III) from patients with AIDS and Pre-AIDS", one gets the impression that the leukaemic HT cell line which Gallo, Popovic, and their colleagues used was a new cell line and one which they established. The Gallo inquiry revealed that the HT (H9) cell line is the same as that used by Levy's group, HUT78, a leukaemic cell line established in another laboratory. However, the abundant evidence for the existence of endogenous human retroviruses has largely been obtained from experiments on leukaemic and transformed cells. Evidence exists that both H9 and EBV-transformed B lymphocytes release retrovirus-like particles even when not "infected with HIV".[104] Furthermore, the HUT78 (H9) cell line was established from a patient with "malignancies of mature T4 cells", a disease which, according to Gallo, is caused by the exogenous retrovirus, HTLV-I. Indeed, as far back as 1983, he claimed to have shown that the HT (H9) cell line contained HTLV proviral sequences.[105] According to some American researchers, EBV-transformed normal human peripheral blood B lymphocytes contain HTLV-I related transcripts.[106] Since all retroviral particles by definition band at 1.16 gm/ml, assuming that all the groups had a retrovirus at this density, how is it possible to claim that the retrovirus originating from the HUT78 and EBV-transformed B-lymphocytes is a new retrovirus HIV, and not one which was already present? Can one claim that the "HIV RNA" and thus the probes and primers originating from it are the RNA and probes and primers of a unique exogenous retroviral genome?

9. The biological dogma states that DNA is synthesised on a DNA template, RNA on a DNA template, and proteins on an RNA template. In other words, the only way for a cell to acquire new nucleic acid entities is for them to be introduced from the outside, exogenously either from another cell type, an infectious agent or a synthetic nucleic acid. If the biological dogma is correct then the "HIV RNA", be it a cellular or viral molecular entity, should have originated either from the patients' lymphocytes or the transformed and leukaemic cell lines. However, when "HIV cDNA" was used a probe, not one of the groups reported positive hybridization results from any of the cells, not even from the lymphocytes of AIDS patients. The question then arises, does a unique molecular entity, "HIV DNA" exist? What does it mean and from where did it originate?

6.3. SPECULATIONS ON "HIV DNA"

If one wishes to speculate on the nature and origin of RNA (cDNA) derived from the cultures containing tissues of AIDS patients and those at risk, and which bands at 1.16 gm/ml, there are many possibilities including:

6.3.1 Although to date no such evidence exists, it is possible that the stretch of RNA, presently called "HIV RNA", is the genome of an exogenous retrovirus, HIV. However, for this to be considered proven in addition to satisfying all the requirements in 6.1 one must also show that:

(i) the unique stretch of RNA can be obtained only from cultures of particular individuals;

(ii) when the RNA (or cDNA) is used as a probe to test fresh, uncultured lymphocytes, a positive test is obtained only from the fresh cells of individuals who also have a positive culture;

(iii) that in animals or humans, the retrovirus is horizontally (animal to animal, person to person) transmitted.

6.3.2 The genome of an endogenous retrovirus, that is, a stretch of RNA with a corresponding DNA template 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.

For many decades it has been known that animal DNA contains sequences "closely related or identical with those of infectious viruses". However, the human genome was considered to be an exception and as late as 1994, both Gallo and Fauci were of the opinion that "...there are no known human endogenous retroviruses".[107] In fact, in the 1970s and in the 1980s after Gallo's claim of the discovery of HL23V, HTLV-I and later HTLV-II, and especially after Montagnier's claim of the discovery of HIV, considerably greater interest was engendered in retroviruses with the result that it became "increasingly clear that the DNA of man, like that of other vertebrates, contains many integrated retroviral genomes",[25, 108] and that in many cases the genes are expressed, "including mRNA transcripts related to full-length endogenous retroviral DNA" [109, 110] with open reading frames for the gag, pol and env proteins.[111] By 1987, many researchers reported the expression of the genome of the human endogenous retrovirus, HERV-K, homologous to the mouse mammary tumor virus (MMTV). "In several cell lines, HERV-K genome was expressed as an 8.8 kilobase poly(A)+ RNA which appears to be the full-length tran-

script of this genome". When the human breast cancer cell line T47D was "grown in RPMI 1640 supplemented with 10% fetal calf serum, HERV-K genome expression was slight". However, when the cells were treated with estradiol and then progesterone, they produced "retrovirus-like particles and soluble protein sharing antigenic determinants with MMTV env gene product".[112] In support of their thesis "that a human endogenous RT might mediate gene movements leading to leukemia and cancer", researchers from Hahnemann University, Philadelphia, including David Gillespie, a long time collaborator of Gallo "demonstrated the presence of a reverse transcriptase-like enzyme in retroviral particles from patients with essential thrombocythemia, polycythemia vera, and chronic myelogenous leukaemia. It was subsequently shown that the human genome contains 50 copies of HERV-K. HERV-K is a human endogenous class I retroviral element that contains gag, pol and env open reading frames...as well as intact LTR regions...Expression of a 9 kb genomic HERV-K RNA transcript was detected in human cell lines...We were able to show for the first time the expression of HERV-K pol gene in human blood leukocytes. The HERV-K pol gene was expressed in peripheral blood cells from two sets of non-leukemic individuals. The first set consisted of seven normal donors, while the second set consisted of 3 patients with PV, all of which expressed HERV-K pol gene. Five different nucleotide sequences were obtained from the seven normal donors. Four of the five normal sequences contained heterogenous open reading frames for pol as detected by both RT-PCR and RNAase protection. Unlike normal donors which randomly express HERV-K proviruses, analysis of HERV-K pol from PV patient showed selective expression of a restricted family of related proviruses".[113] By 1995, Gallo admitted that the human cell does contain retroviral genomes but he still insisted they are defective, "Retroviruses are transmitted either genetically (endogenous forms) or as infectious agents (exogenous forms). As do many other animal species, humans have both forms...The DNA of many species, including humans, harbor multiple copies of different retroviral proviruses. The human endogenous proviral sequences are virtually all defective, and comprise about one percent of the human genome".[114] The view regarding defectiveness is not shared even by Reinhard Kurth who, with his colleagues, has extensively studied the human endogenous retroviruses[115] and have shown that HERV-K sequences are transcribed and that a human teratocarcinoma cell line, GH, which contains these sequences, when examined by EM was found to produce "human teratocarcinoma-derived retrovirus (HTDV) particles". By 1993 Kurth and colleagues reported that in the GH cell line, "Four viral mRNA species could be identified, including a full-length mRNA. The other three subgenomic RNAs are generated by single or double splicing events...Sequence analysis of expressed HERV-K genomes revealed non-defective gag genes, a prerequisite for particle formation. Open reading frames were also observed in pol and env. Antisera raised against recombinant gag proteins of HERV-K stained HTDV particles in immunoelectron microscopy, linking them to the HERV-K family". Discussing their findings they wrote: "In Northern blots, expression of HERV-K could only be demonstrated in teratocarcinoma cell lines but not in other human lines. Preliminary RT PCR studies suggest, however, that HERV-K may be expressed in many if not all human cells at levels too low to be detectable in Northern blots. The basis of the significant quantitative differences in expression between teratocarcinoma cells and other cell lines is not clear. It is intriguing to speculate that a cellular factor(s) may regulate the synthesis of HERV-K mRNA depending on the cell type or the state of differentiation. In this context, it should be remembered that other retroid elements [ERV-9, RTLVL-H, LINE-1] are also preferentially expressed in human teratocarcinoma cells".[116] It is of interest to note that Montagnier and his colleagues reported their "HIV genome" from a transformed cell line, that Levy and colleagues' HUT78 cell line is a human leukaemic cell line and that Gallo and colleagues' H9 cell line is none other than HUT78, and thus must have HTLV-I as well as endogenous retrovirus. It is equally important to note that although Kurth et al found no sequence homology between HERV-K and "human T-lymphotropic virus" or HIV, many researchers reported HTLV-I sequences in the human genome including in cell lines derived from teratocarcinoma.

In a paper published in 1985 researchers from a number of institutions in the USA including the Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, reported that "Human DNA contains multiple copies of a novel class of endogenous retroviral genomes. Analysis of a human recombinant DNA clone (HLM-2) containing one such proviral genome revealed that it is a mosaic of retroviral-related sequences with the organization and length of known endogenous retroviral genomes. The HLM-2 long terminal repeat hybridized with the long terminal repeat of the squirrel monkey virus, a type D virus. The HLM-2 gag and pol genes share extensive homology with those of the M432 retrovirus (a type A-related retrovirus), mouse mammary tumor virus (a type B retrovirus), and the avian Rous sarcoma virus (a type C retrovirus). Nucleotide sequence analysis revealed regions in the HLM-2 pol gene that were as much as 70% identical to the mouse mammary tumor virus pol gene. A portion of the putative HLM-2 env gene hybridised with the corresponding region of the M432 viral genome". The pol region of HLM-2 showed homology with HTLV-I which, according to the authors "is not endogenous to human cells but is transmitted horizontally as an infectious tumor-inducing virus of humans".[117]

In 1987 researchers from Canada reported the finding of a "Human Endogenous Retrovirus-like Genome with Type C pol sequences and gag sequences related to the Human T-cell Lymphotropic Viruses", HTLV-I and HTLV-II.[118] In 1989 researchers from the Department of Biochemistry, New York University showed that "human DNA contains a wide spectrum of retrovirus-related reverse transcriptase coding sequences, including some that are clearly related to human T-cell leukaemia virus type I and II, some that are related to the L-I family of long interspersed nucleotide sequences, and others that are related to previously described human endogenous proviral DNAs. In addition, human T-cell leukaemia virus type I-related sequences appear to be transcribed in both normal human T cells and in a cell line derived from a human teratocarcinoma".[119] In a paper published in 1989, researchers from the USA summarised their experimental findings as follows: "Human T-cell leukemia virus (HTLV) type I-related endogenous sequences (HRES) have been cloned from a human genomic library. HRES-1/1 is present in DNA of all normal donors examined. By nucleotide sequence analysis, HRES-1/1 contains two potential open reading frames capable of encoding a p25 and a p15. A 684 flanking region 5' from the first ATG codon of p25 contains a TATA-box, a poly-adenylation signal, a putative tRNA primer binding site, and inverted repeats at locations which are typical of a retroviral long terminal repeat...The HRES-1/1 genomic locus is transcriptionally active in lymphoid cells", including EBV-transformed normal human peripheral blood lymphocytes, leukemic cell lines, melanoma cells and embryonic tissues.[106] In a paper published in 1992 by researchers from Hungary and Britain entitled "Human T-cell lymphotropic virus (HTLV)-related endogenous sequences, HRES-1, encodes a 28-kDa protein: A possible autoantigen for HTLV-I gag-reactive autoantibodies", the "presence of a human T-cell lymphotropic virus (HTLV)-related endogenous sequence, HRES-1, in the human genome was documented. The HRES-1 genomic locus is transcriptionally active and contains open reading frames...Antibodies to HRES-1-specific synthetic peptides were noted in patients with MS, progressive systemic sclerosis (PSS), SLW, Sjogren syndrome (SJS), and essential cryoglobulinemia (ECG). The data suggest that HRES-1 may serve as an autoantigen and correspond to a natural target of HTLV-I core protein-reactive autoantibodies".[120]

6.3.3 The genome of a retrovirus de novo assembled by genetic recombination and deletion of:
(a) endogenous retroviral sequences;
(b) retroviral and cellular sequences;
(c) non-retroviral cellular genes.

In the virological literature there is ample evidence which shows that when a cell contains two proviruses, progeny may be found that possess the genome of one but the structural proteins of either or both viruses present. Conversely, the RNA may be viral but at least some of the proteins may be cellular. In other instances, the particles do not have a genome at all, or one or more genes are missing (genetically defective viruses). The genetic mixing can be between viral genomes or between viral and cellular genes.[83, 121] According to distinguished retrovirologists such as Weiss and Temin, new retroviral genomes may arise by rearrangement of cellular DNA caused by many factors including pathogenic processes, a view that proposes retroviruses as an effect and not the cause of disease.[122, 123] According to Varmus, "Retroviral genomes recombine at high frequency (estimates range as high as 10 to 30% for each cycle of multiplication), and heterodimeric RNAs are thought to be intermediates, with recombination taking place during reverse transcription. Recombination appears to be strongly favoured by homology, but joining also occurs occasionally between unrelated sequences, e.g., during the latter phase of genetic transduction by retroviruses. When viruses are grown in cells that contain related endogenous proviruses, packageable transcripts from those proviruses may participate in recombination reactions with the exogenous virus. This is most dramatically revealed by the repair of deletion mutations in the genome of an exogenous virus in a fashion that superficially resembles gene conversion". In some animals proviruses have been acquired "during recent breeding of the strains in the laboratory" and "in a few

instances, endogenous proviruses have been established or increased in number during experimental observations"[121] (italics ours).

As far back as 1974, based on the then available evidence, Howard Temin proposed that the retroviral (ribodeoxyviruses) genomes originate from "normal cellular components. The relationships between the different ribodeoxyvirus groups reflect the relationships among the cellular components from which the viruses evolved and the convergent evolution of the viruses. In other words, there are relationships among ribodeoxyviruses because the ribodeoxyviruses evolved from cells which themselves had relationships deriving from common ancestors. A possible mechanism of this evolution is described in Fig. 5". In the legend to Fig. 5 Temin wrote. "A section of a cell genome becomes modified in successive DNA (W) to RNA (-) to DNA transfers until it becomes a ribodeoxyvirus genome. First, these sequences evolve as part of a cellular genome. After they have escaped as a virus, they evolve independently as a virus genome. The time scale may be millions of years in germ-line cells and days in somatic cells".[122] Temin reinforced his view in a more recent publication.[124]

In 1975, Gallo, Gillespie and their colleagues wrote: "Even though RNA of class II [exogenous] retroviruses shows minimal homology to uninfected host cell DNA, hybridization of nucleic acids among class II leukemia viruses from different species gives a pattern which is the same as the phylogenetic relatedness among their natural hosts...We have proposed that these and other results favor the interpretation that all RNA tumor viruses are derived from cell genes, a proposal in agreement with the virogene theory...By analysis of the RNA of viruses infecting and replicating in a new host, evidence has also been obtained which indicates that the genome of type C viruses can be substantially changed by the host, probably by recombination with host DNA".[125] A few years later, Coffin wrote: "The close relationship of virion proteins as well as overall nucleic acid homology must mean that both exogenous and endogenous avian tumor viruses [retroviruses] derive from a common ancestor".[126]

In 1991 researchers from the New York University published a paper entitled, "Evolutionary Implications of Primate Endogenous Retrovirus". Discussing the presently available data they wrote, "A recent detailed phylogenetic analysis of exogenous and endogenous retroviruses (including retrotransposons) strongly suggests that a pool of endogenous retroviral sequences periodically contributes to the generation of exogenous viruses, and that the presence of endogenous primate retroviruses is probably more directly related to exogenous viruses that might have been thought";[127]

6.3.4 The "novel" RNA found in the cell culture supernatant and the material from it banding at 1.16 gm/ml, the "HIV RNA", may have nothing to do with a retroviral genome. It may be 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.[128, 129] Not only can retroposition "shape and reshape the eukaryocytic genome in many different ways"[128] but the nonviral retroelements may be similar to the retroviral elements. According to Doolittle et al from the University of California, San Diego,"...the entire group of reverse transcriptase-bearing agents, including retrotransposons and genuine retroviruses, has recently been dubbed, "retroids". Sequence comparisons by many other workers leave little doubt that the reverse transcriptases of all the "retroids" considered here are homologous, which is to say, the sequence resemblances are not the result of chance or convergences. Our own comparisons confirm that general notion, not only for reverse transcriptases, but also for the ribonucleases, endonucleases and proteases, although it should be understood that not all "retroids" contain all four enzymes...All of these elements have additional features in common with retroviruses including characteristic LTRs (long terminal repeats) and primer sites that are complementary to various tRNAs. Like retroviruses, most contain distinctive nucleic acid-binding and core particle proteins; in electron micrographs there is a remarkable likeness to retroviral capsids...About the only feature that regularly distinguishes many of these retrotransposons from genuine retroviruses is the absence of an envelope protein".[17]

6.3.5 Although 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, at present it is still generally accepted that any time one finds a particular stretch of RNA in a cell, for example, in a T-lymphocyte, unless RNA or DNA has been introduced from outside, all T-cells, regardless of their physiological state or stresses to which they are subject, will contain a corresponding stretch of DNA. In other words, the DNA (genes) in a cell are invariant and all RNA molecules in the cell are subservient to a matching length of DNA. However, according to McClintock, the genome can be restructured and not only by transposition. In her Nobel lecture of 8th December 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"[130] [italics ours and see this reference for examples].

In the 1980s a number of phenomena have been discovered which brought startling changes in concepts including the following:
Up until the late 1970s, the prevailing concept was that a discrete, contiguous stretch of DNA is a structural gene encoding the genetic information to specify the manufacture of a single protein, and that the linear sequence of the nucleotides in this stretch of DNA corresponds directly to the linear sequences of the RNA nucleotides and to the amino acids in the protein. The first discovery which contradicted this belief was the discovery that the DNA base sequences which coded for a given protein were not in a continuous stretch of DNA but may be interspersed with other, non-coding base sequences, that is, the genes are split, "genes-in-pieces". A number of mechanisms have been postulated to account for this observation. In one such explanation it is hypothesised that the entire stretch of DNA is transcribed into a piece of RNA, then the non-coding regions (introns) are excised and the coding regions (exons) are spliced together to make the appropriate messenger RNA.[131] There are no rules setting an upper limit on the number of introns in a "gene", some genes may have up to sixteen or more introns. Nor are there any rules regarding the length of introns, although in general, introns are much longer than exons, the length of exons "peaking at about 40 or 50 amino acids...the shortest intron being 50 bases long, the longest extending out to some 50.000 bp".[132]

According to Gilbert introns represent "hot spots" for recombination and new genes can be created "through the coupling of exons by intron-mediated recombination", "introns are lost and more complicated exons are formed".[133] At present evidence exists showing that at least some introns are mobile genetic elements, transposable elements, they self-splice, they often contain reading frames capable of encoding a protein including "regions of homology to reverse transcriptase scattered over a roughly 250-amino acid stretch in the middle of each intron ORF".[134] The discovery of split genes "shows that the genetic apparatus of the cell is more complex, more dynamic than any of us had suspected".[132]

Another strongly held view was the belief that all cellular reactions and thus gene splicing were catalysed by a protein enzyme. In the early 1980s it was found that RNA can cut, splice and assemble itself, as well as assemble RNAs other than itself.[135-138]

6.3.6 One of the strongest held views in biology is the belief that nucleic acids have an inherent ability of instructing their own synthesis and that nucleic acids cannot be synthesised in the absence of a nucleic acid template. Manfred Eigen and his colleagues in Germany conducted extensive theoretical and experimental work on molecular self-replication.[139] In their experimental work they used the bacterial virus (phage) Q_b. In addition to its genome, a simple strand RNA molecule of 4500 nucleotides, the virus has an RNA molecule of 220 nucleotides known as "Spiegelman's minivariant" which, like the genomic RNA, is reproduced in cell-free laboratory systems by an enzyme called Q_b replicase. By mixing Mg^{2+} ions, the nucleoside triphosphates ATP, GTP, UTP, CTP, Q_b replicase and template RNA, they could obtain RNA replication but a totally unsuspected finding was that even the absence of the template, RNA was still synthe-

sised. They performed many experiments to prove this phenomenon and to exclude the possibility of the presence of an initial RNA template and concluded, "Finally we were convinced we had before us RNA molecules that had been synthesised de novo by the Q_b replicase enzyme. What was most puzzling, the de novo product had a uniform composition which in each trial turned out to be similar to or even identical with Spiegelman's minivariant". When the template free mixture was then divided into several isolated compartments where optimal conditions for de novo synthesis were maintained they found that "each component had a uniform population of de novo product, the products differed from compartment to compartment. Further analysis revealed however that the different sequences were not completely unrelated...There was a definite, uniform final product for any set of experimental conditions, but here were as many different optimal products as there were different experimental conditions. One of the optimal products appeared to be Spiegelman's minivariant...Other products of optimization were adapted to conditions that would destroy RNAs, such as high concentrations of ribonuclease, an enzyme that cleaves RNA into pieces...Some variants were so well adapted to odd environments that they had a replication efficiency as much as 1000 times that of variants adapted to a normal environment...Any RNA formed by noninstructed chemistry would be reproduced by template-instructed chemistry at a rate proportional to the current RNA concentration. The result would be exponential growth. Furthermore, even if only a single template were formed initially by noninstructed synthesis, there would soon be a host of different sequences because errors (point mutations, insertions and deletions) would inevitably be made in the course of replication. Hence in each generation there would be not only a larger number of RNA strands but also a greater variety of RNA sequences. What would happen then? Some of the mutants would be copied more rapidly than others or would be less susceptible to errors in copying, and their concentration would increase more rapidly. Sooner or later these faster-growing mutants would take over...Hence the results of the self-replication competition had to be the master sequence together with a huge swarm of mutants derived from it and from which it had no way of escape...We call this entire mutant distribution a quasispecies. It is the quasispecies mutant distribution that survives the competition among self-replicating RNAs and not just one master sequence or several equivalent ones that are the fittest genes in the distribution. The essence of selection then is the stability of the quasi-species".[140] According to Eigen and his colleagues, the maximum length of an RNA master sequence is of the order of 10,000 nucleotides.[139, 141]

6.3.7 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!".[142-144] 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".[145]

6.3.8 CONCLUSION

The finding of a novel stretch of RNA or DNA and proteins in:

(a) lymphocytes of sick individuals or individuals who have been "shocked" with agents such as physical or chemical mitogens, carcinogens or oxidising agents in general as is the case with AIDS patients and those at risk;[77, 79, 90]

(b) lymphocytes in cultures or co-cultures (which could lead to the appearance of hybrids) which have been additionally "shocked" with sometimes multiple, similar agents;

is not proof that the given stretch of RNA comes from the outside, irrespective of its length, the presence of poly(A) and number of ORF ("genes").

From Montagnier's, Gallo's and Levy's and their colleagues' evidence it is not possible to conclude that the "HIV RNAs" they found are a "new species" of RNAs induced by "shocking" the cells or by one or more of the other phenomena which have come to light in the 1980s. Nor is it possible to conclude that their RNAs are the genome of an exogenous retrovirus as they did. However, a number of predictions can be made:

(a) If the "HIV DNA" is indeed 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 fragment of "HIV DNA" or "HIV cDNA" is used for hybridisation studies all infected people should give a positive result.

(b) If the selected RNA which was found to band at 1.16 gm/ml, the "HIV RNA", is the genome of a retrovirus which exists "in all of us", endogenous retrovirus, then again evidence must prove the existence of a unique molecular entity, "HIV RNA", ("HIV DNA"). When hybridisation studies are conducted using the full length of the unique molecular entity as a probe, positive results should be found "in all of us";

(c) If the RNA found by the three groups, "HIV RNA", is the genome of a retrovirus assembled de novo from DNA already existing in the cells, as the result of in vivo or in vitro conditions, evidence must also prove the existence of a unique molecular entity. When the whole length of the unique fragment of nucleic acids is used as a hybridisation probe, a positive result should only be found in cells which are subjected to exactly the same in vivo or in vitro conditions as those from which the "HIV RNA" at 1.16 gm/ml was obtained. When only fragments of "HIV RNA" are used for hybridisation, the probability of finding a positive result will increase;

(d) If the "HIV RNA" is a unique non-viral molecular species of RNA resulting from the transcription of a unique molecular species of DNA then when the whole fragment of "HIV RNA", ("HIV cDNA") is used a probe for hybridisation studies, a positive result should be found only in the cells of the same type as those from which the "HIV RNA" originated, in all individuals;

(e) If the "HIV RNA" is neither the genome of a retrovirus nor a faithful transcript of a fragment of DNA present in the cells from which it has been obtained, but is the result of the "shock" to which the cells have been exposed, either in vivo or in vitro or both, or as a result of the phenomena discovered in the 1980s then:

(i) since it is not possible to exactly reproduce the conditions in vivo or in vitro to which the cells are subjected, it would prove difficult if not impossible to always obtain a unique molecular entity "HIV RNA", that is, to always obtain a fragment of RNA or DNA of identical length and sequences;

(ii) when the full-length fragments of "HIV RNA" or "HIV cDNA" are used as hybridisation probes there will be only a low probability of finding a positive result. However, the probability will increase if only small fragments of the "HIV RNA" or "HIV cDNA" are employed.

6.4. EVIDENCE THAT THE "HIV RNA" BELONGS TO AN EXOGENOUS RETROVIRUS

The Montagnier, Gallo and Levy groups claimed that the special RNA which they selected from the total RNA which in sucrose density gradients banded at the density of 1.16 gm/ml was novel to the lymphocytes and that in fact belonged to an exogenous retrovirus. Although they did not present evidence to prove this assertion, the possibility cannot be excluded that indeed this may have been the case. Since at present their claim is generally accepted one would have thought that by now they or other researchers should have been able to provide ample confirmatory proof. This does not seem to be the case:

6.4.1 If the RNA originates from a retrovirus either endogenous or exogenous then evidence must exist which proves that such RNA is a constituent of particles which possess at least the most basic morphological and physical features of retroviruses, that is, "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)".[82] To date not only has nobody shown that the "HIV RNA" belongs to such particles, there is no evidence that particles of any kind are present in the material from cell cultures/cocultures which bands at the retroviral density of 1.16 gm/ml and from which the "HIV RNA" is selected. Furthermore, although particles have been demonstrated in cultures, cultures contain many different types of particles but none display BOTH principal morphological characteristics, that is, "a diameter of 100-120 nm" AND surfaces which "are studded with projections (spikes, knobs)".[146]

6.4.2 If the "HIV RNA" is the genome of an exogenous retrovirus then, like the "exogenous animal retroviruses", one should be able to find it in infected material without the necessity to revert to the use of co-cultivation or mitogenically stimulated cultures. However, none of the phenomena which are thought to prove the existence of HIV can be detected unless one employs mitogens or co-cultures or both (and sometimes additional "shock"), a fact accepted by both Montagnier and Gallo.[78, 147]

6.4.3 One cannot claim that "HIV RNA" is the genome of a unique retrovirus, HIV, unless evidence is presented to prove that 'HIV" is a unique molecular entity.

By 1985 it was known that "the env genes of ARV and HTLV-III differ by more than 20 percent" and that "the Gallo group has sequenced another HTLV-III isolate and finds that it differs from the first by about as much as ARC".[114, 148] By 1986, Gallo and his colleagues accepted that the "HIV genome" has a "far greater variability" as "compared to HTLV" and in fact "The rate of genetic change for the AIDS virus is more than a millionfold greater than for most DNA genomes and may even be tenfold greater than for some other RNA viruses including certain retroviruses and influenza A virus".[149] At present it is accepted that "no two isolates are identical. Each isolate contains many variants".[150] In one and the same patient the genomic data in monocytes differs from that in T-lymphocytes.[151] There are "striking differences" between the proviral DNA and cDNA in one and the same PBMC sample "which could not be explained by either an artefact of reverse transcriptase efficiency or template selection bias".[152] The genetic data obtained in vitro do not correlate with the data obtained in vivo, "to culture is to disturb".[153] According to the researchers from the Pasteur Institute "an asymptomatic patient can harbour at least 10^6 genetically distinct variants of HIV, and for an AIDS patient the figure is more than 10^8.[154, 155] The "HIV genome" varies with time; in one case where clones were obtained 16 months apart all the clones detected in the second sample were distinct from the clones in the first sample.[156] It is also accepted that up to 99.9% of the "HIV genomes" may be defective.[157]

According to Levy, "The mechanism responsible for generating these varying strains of virions is puzzling. One theoretical possibility is that the unintegrated proviral copies of HIV that accumulate during acute replicative infection can undergo efficient genomic recombination leading to the evolution of infectious variants."[158] In Robin Weiss' view, "the source of variation is the infidelity of reverse transcription, which has no editing mechanism for transcriptional errors", as well as "genetic recombination" especially when cell fusion takes place.[159]

By the late 1980s, researchers from the Pasteur Institute concluded, "it is increasingly clear that it will be very difficult to describe correctly the characteristics of HIV viruses using single molecular clones". "It is evident that HIV, either in vivo or in vitro, is extraordinarily complex and that a population-based approach", a quasi-species approach as defined by Eigen, must be used to describe HIV. They also added, "Even with a population-based approach, only small regions of the HIV genome can be studied... Given such complexity and the evident differences between quasispecies in vivo and in vitro, the task of defining HIV infection in molecular terms will be difficult".[153, 160] The data which have been published since confirm their conclusion. Prior to the 1990s, the HIV sequences were classified as African and USA/European with sequence differences of 20-30 percent between these two groups.[161] In the 1990s, HIV researchers started to divide the "HIV genome" into subtypes A, B, C, D, E, etc. The basis for this classification system is:

"(a) subtypes are approximately equidistant from one another in env (a 'star' phylogeny);

(b) the env phylogenetic tree is for the most part congruent with gag phylogenetic trees;

(c) two or more samples are required to define a sequence subtype". However, "Subtype naming problems have arisen for several reasons. A small but not insignificant number of viral sequences are hybrid, clustering with one sequence subtype in gag and another sequence subtype in env, for example; or, to take another example, clustering over different stretches with two or more subtypes in env...Naming becomes problematic when highly divergent forms of a given subtype arise: such forms are sometimes designated A', B', F', etc. It is increasingly necessary to have sequence data from both gag and env coding sequences when a new form or subtype is being claimed".[162]

By the middle of this year "at least ten" (A-J) prevalent major (M) and a low prevalence, O, HIV-1 genotypes were described and new genotypes are still reported.[8, 163]. According to researchers from the Henry M Jackson Foundation Research Laboratory and Division of Retrovirology, Walter Reed Army Institute, USA, "The great majority of genotypic consignments for HIV-1 are based on subgenomic sequence segments, typically encompassing 2% to 30% of the genome", and not by comparisons of the whole genome. This is because, "it remains impractical to obtain full length genomic sequences of HIV-1 isolates as a routine genotyping method, due to the low abundance of HIV-1 proviral DNA in clinical samples and virus cultures on PBMC substrate, and to the relative inefficiency of the polymerase chain reaction when amplicons become large". "The designation Human Immunodeficiency Virus Type-1 (HIV-1) encompassed an unanticipated complexity of viral forms".[163]. According to researchers from the Los Alamos National Laboratory, "while a subtype designation based on a gene or gene fragment may be correct, recombination may have occurred. Therefore, care should be taken to not over interpret the subtype designation. If one is to discuss the subtype designation of viral isolates based on the data presented here, they should refer to the designation as 'B-like over V3 loop region' rather than as 'subtype-B'".[164] One and the same person may be "infected" with more than one subtype.[165] This means that at present it is not possible to say what are the sequence differences, both qualitative and quantitative, between different HIV-1 subtypes. Nonetheless, some suggestive data does exist. In 1993 researchers from several institutions "reported that in the A-G HIV-1 genotypes the intra-genotypic gag distances averaged 7% whereas the inter-genotypic distances averaged 14%...The maximum level of variability in gag is still well below that observed for the envelope region of HIV-1".[166] "Two HIV-1 strains, designated ANT70 and MVP5180 were isolated in 1987 and 1991 respectively from patients in Cameroon". They were classified as HIV-1 subtype O. By 1994 evidence was presented which "indicated that subtype O was endemic in Cameroon and Gabon".[167] "DNA sequence analysis of MVP-5180 showed that its genetic organisation was that of HIV-1, with 65% similarity to HIV-1 and 56% similarity to HIV-2 consensus sequences. The env gene of MVP-5180 had similarities to HIV-1 and HIV-2 of 53 and of 49% respectively...Comparison of the MVP-5180 amino acid sequence with that of the Gabon chimpanzee virus showed similarities of 70, 78 and 53% in the gag, pol, and env genes, respectively; similarities of 70, 76 and 51% to the Uganda HIV-1 (U455) and of 54, 57 and 34% to the HIV-2 isolate D205 were found". The researchers from Germany and Cameroon who conducted this study expressed the view that "Even more divergent HIVs may exist. Such divergent HIVs are likely to be transmitted by the usual routes (sexual and blood contact and mother-to-infant transmission), leading to wider distribution. They will have to be taken into account in vaccine development and diagnostic test sensitivity and specificity".[168] Indeed, this seems to be the case. Last year, David Ho and his associates[169] studied an Australian patient with "primary infection". "Since seroconverters generally harbor a relatively homogenous population of viruses", they were surprised when they found that he was "co-infected", "by multiple subtype B HIV-1...The average genetic distances between group I and II, I and III, and II and III were 9.6, 16.5 and 18.4% respectively...One population of sequences was clearly distinguishable from the others on the basic of phylogenetic analysis. In addition, sequences suggesting recombination between two of the three distinct viral populations were also found".

That the "HIV DNA" may be "Even more divergent" than has been generally accepted is best illustrated in a study published this year by researchers from the United States. Because protease inhibitors are becoming the drugs of choice for the treatment of "HIV infected" individuals, and because "naturally occurring mutations in HIV-1 infected patients have important implications for therapy and the outcome of clinical studies", these researchers performed a "sequence analysis of the pr gene [protease gene] in 167 HIV-1 viral strains from 102 protease inhibitor naive patients collected from different geographic regions of the United States". "Given the enzyme's relative small size and the constraints on its structure imposed by function, it was reasonable to conclude that sequence variability in HIV-1 would be limited". To their surprise it was found that "A total of 41% of the nucleotides and 49.5% (49/99) of the amino acids were variable. The amino acid diversity seen in these USA viral isolates is much greater than that previously reported for HIV-1 clade B viruses" and is also greater than that seen in pr genes for all HIV-1 clades (40 out of 99, 40% of amino acids varying)"![170] At present, more so than in 1986 when Gallo and colleagues reached their conclusion that "The rate of genetic changes for the AIDS virus is more than a million fold greater than for most DNA genomes and may even be tenfold greater than for some other RNA viruses including certain retroviruses and influenza A virus", and in 1989, when the Pasteur researchers reached their conclusion that "the task of defining HIV infection in molecular terms will be difficult", there is no evidence which proves the existence of a unique molecular entity "HIV RNA" ("HIV DNA").

In fact, there are a number of reasons why the myriads of incommensurable "HIV DNAs" cannot be even described "in terms of popu-

lations of closely related genomes, referred to as a quasispecies".[153] These include:

(a) Eigen and his colleagues developed the quasispecies model to describe the distribution of self-replicating RNAs. However, the "HIV RNA", is said not to be a self replicating RNA, but replicates through a DNA intermediate;

(b) the self-replicating RNA of the RNA viruses appears to "demonstrate remarkable stability in some situations. The type 3 Sabin poliovirus vaccine differed from its neurovirulent progenitor at only 10 nucleotide positions after 53 in vitro and 21 in vivo passages in monkey tissues. In 1977, H1N1 influenza A virus reappeared in the human population after 27 years of dormancy with sequences mainly identical to those of the 1950s virus". Although Eigen's quasispecies model has been used to describe the genome of RNA viruses, even 1% sequence differences in these genomes are considered to represent "extreme variability". "Many selective forces may stabilize virus populations. These stabilizing factors may include the need for conservation of protein structure and function, RNA secondary structure, glycosylation sites, and phosphorylation sites. Even third-codon changes can be subject to selective pressures. Recently, remarkable conservation of certain protein domain sequences has been observed between completely unrelated RNA viruses.[171] Is it possible then to describe the "HIV DNA" even if it has variation of 10% , not to mention 20 or 30 or 40% as is the case, as a "population of closely related genomes, referred to as a quasispecies"?;

(c) Defining the concept of a quasispecies Eigen wrote: "In the steady state that is eventually reached the best competitor, designated the master sequence m, coexists with all mutant sequences derived from it by erroneous copying. We designate this distribution of sequences as quasispecies". However, to date, nobody has proven that:

(i) there is an "HIV" quasispecies which is ever in equilibrium;

(ii) the "closely related HIV genomes" are derived from a master sequence;

(iii) a master sequence has ever existed.

6.4.4 If the "HIV RNA" stretch is the genome of an exogenous virus which infects individuals with AIDS or those at risk, then this RNA (or cDNA) should be present in fresh uncultured tissue from all these individuals and in nobody else. Furthermore, if in these individuals there is massive HIV infection, as some of the best known HIV experts claim,[172, 173] Southern blot hybridisation should be more than sufficient to detect it.

The first such study was conducted by Gallo and his colleagues in 1984. Using a Southern blot hybridisation technique they tested many tissues from AIDS patients, including lymph nodes. 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 referred to by Gallo). "However, as shown herein, HTLV-III DNA is usually not detected by standard Southern blotting hybridization 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 in 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".[96] These studies 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".[96] This alternative explanation has been ignored by everybody, including Gallo. However, at a 1994 meeting held in Washington sponsored by the US National Institute of Drug Abuse, 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".[174] Data which has come to light since 1984 suggest that Gallo's and his colleagues' alternative explanation may be a fact:

(a) at present there is ample evidence showing that normal human DNA contains sequences related to HTLV-I and HTLV-II (see 6.3.2);

(b) apparently, up until 1993, Gallo was unaware of the existence of endogenous human retroviruses,[107] which means that by "virus distantly homologous to HTLV-III" they could have meant none other than the exogenous retroviruses Gallo claimed to have discovered earlier, that is, HTLV-I and HTLV-II. However, at present even Gallo admits that the human endogenous proviral sequences "comprise about one percent of the human genome";

(c) some of the best known HIV experts including Montagnier, Blattner and Gelderblom agree that the pol and gag genes "may be highly conserved between subtypes of virus" (see 5.6). In a paper published in 1996 by Reinhart Kurth and his colleagues one reads, "Retrotransposons evolved in a variety of organisms ranging from protozoa to human beings. In these elements, RT genes are linked to genes that code for polyproteins with the potential to self aggregate and to form core particles. These proteins are the equivalents of the retroviral capsid proteins usually designated group-specific antigens (gag)...They [retrotransposons] may be either the derivative or predecessors of retroviruses. Retroviruses differ from retrotransposons by the presence of at least one additional coding region, the envelope (env) gene".[175] In 1984, Gallo's group reported that the "HIV genome" hybridised with the "structural genes (gag, pol, and env) of both HTLV-I and HTLV-II.[56] Obviously, the finding of a positive hybridisation "signal" at least with an "HIV" gag or pol probe is no proof for the existence of the "HIV genome";

In fact, at present evidence also exists which shows the presence of "HIV" sequences in non-infected tissues:

(i) although it is no longer accepted that HIV is transmitted by or is present in insects, in 1986 researchers from the Pasteur Institute found HIV DNA sequences in tsetse flies, black beetles and ant lions from Zaire and the Central African Republic;[176]

(ii) in 1985 Weiss and his colleagues reported the isolation, from the mitogenically stimulated T-cell cultures of two patients with common variable hypogammaglobulinaemia, a retrovirus which "was clearly related to HTLV-III/LAV" Evidence included positive WB with AIDS sera and hybridisation with HIV probes;[177]

(iii) DNA extracted from thyroid glands from patients with Grave's disease hybridises with "the entire gag p24 coding region" of HIV;[178]

(iv) In a study designed to address the question whether the neuronal cells of patients with AIDS dementia complex are infected with HIV, "the brains from 10 patients with AIDS and neurological evidence of viral encephalitis and the brains from five patients without HIV-1 infection" were examined using an HIV gag probe. "The antisense riboprobe hybridized to cells known to be infected with HIV-1. It hybridised to HIV-1-infected A3.O1 cells as well as splenic and renal lymphocytes obtained at autopsies from patients known to have AIDS. The probe did not, however, hybridize to neurones in the brain sections from 10 patients with AIDS...Surprisingly, when we applied the control sense HIV-1 gag probe to the brain sections from patients with AIDS, we observed specific hybridization to neuronal cells. Similarly, when brain sections from five individuals not infected with HIV-1 were examined, the HIV-1 sense probe detected transcripts in neuronal cells. Our Northern blot analysis confirmed these results and demonstrated the presence of a 9.0-kb polyadenylated transcript in brain tissues".[179] Thus, either the positive hybridisation signals obtained with the antisense probe are non-HIV-specific or, as the authors concluded, there is a neurone-specific 9.0-kb transcript that shows extensive homology with antisense gag HIV-1 sequences and this transcript is expressed in neuronal cells of both HIV-1-infected and noninfected individuals;

(v) Horowitz et al, "describe the first report of the presence of nucleotide sequences related to HIV-1 in human, chimpanzee and Rhesus monkey DNAs from normal uninfected individuals". They have "demonstrated the presence of a complex family of HIV-1-related sequences" in the above species, and concluded that "Further analysis of members of this family will help determine whether such endogenous sequences contributed to the evolution of HIV-1 via recombination events or whether these elements either directly or through protein products, influence HIV pathogenesis".[180]

The inescapable conclusion therefore is that the hybridisation studies do not prove that T-cells or any other cells of AIDS patients and those at risk contain a unique molecular entity "HIV DNA".

6.4.5 In the second half of the 1980s, in order to rescue the concept of an "HIV genome", the HIV experts made extensive use of a newly discovered process known as the polymerase chain reaction (PCR). Although the PCR is a very useful tool in molecular biology there are many problems associated with its use in studying the "HIV genome": (a) The PCR is an extremely sensitive technique. Writing of his Nobel prize winning discovery, Kary Mullis, himself rather ironically sceptical of the HIV/AIDS hypothesis wrote, "Beginning with a single molecule PCR can generate 100 billion similar molecules in an afternoon".[181] With such amplification it is not difficult to detect even very low levels of the "HIV genome". However, "a striking feature of the results obtained" by 1990 with PCR as with the standard Southern/Northern hybridisation, was "the scarcity or apparent absence of viral DNA in a

proportion of patients".[182] In a further effort to rescue the "HIV genome", in the 1990s researchers from the Department of Genetics, University of Edinburgh, introduced a modified version of PCR, the double PCR method or nested PCR. "The double PCR overcomes the problem of limited amplification of rare template sequences". They reported that, "Using a double polymerase chain reaction which allows the detection of a single molecule of provirus and a method of quantifying the provirus molecules, we have measured provirus frequencies in infected individuals down to a level of one molecule per 10^5 PBMCs...As a general rule, only a small proportion of PBMC contain provirus (median value of samples from 12 patients one per 8,000 cells)"...samples from 7 of our 12 patients (60%) contained one or more provirus per 104 cells...while samples from all (100%) of our patients contained one or more provirus per 80,000 cells". They concluded, "The most striking feature of the results is the extremely low level of HIV provirus present in the circulating PBMC in most cases".[182]

There is no doubt that PCR can "amplify a DNA-needle into a DNA-haystack" but even PCR cannot perform miracles.

In a review of Neville Hodgkinson's book, AIDS, The Failure of Contemporary Science: How a Virus That Never Was Deceived the World,[183], Sir John Maddox wrote, "the virus that never was has been made more tangible" early in 1995 when "it became apparent that even in the earliest stages of infection by HIV, the virus is far from dormant".[184] Maddox is referring to two papers published in Nature in 1995. One by Ho et al where the authors claim to have shown that in patients who have not received antiviral treatment the "plasma viral levels ranged from...15 X 10^3 to 554 X 10^3 virions per ml";[172] the other by Wei et al where it is claimed that the "plasma viral RNA levels in the 22 subjects at baseline ranged from $10^{4.6}$ to $10^{7.2}$ molecules per ml" and concluded that their study "suggests that virus expression per se is directly involved in $CD4^+$ cell destruction. The data do not suggest an "innocent" bystander mechanism of cell killing whereby uninfected or latently infected cells are indirectly targeted for destruction by absorption of viral proteins or by autoimmune reactivities".[173] These claims raise two obvious questions:

(i) "The majority of exogenous pyrogens are microorganisms, their products or toxins", and "endogenous pyrogens are polypeptides produced by a large variety of nucleated host cells including monocyte/macrophages" and "lymphocytes, endothelial cells, hepatocytes, epithelial cells, keratinocytes, and fibroblasts, as well as other cells...generally in response to initiating stimuli triggered by infection or inflammation". In addition, "many endogenous products result in the release of endogenous pyrogens, thereby causing fever. Such endogenous substances include antigen-antibody complexes, complexes with complement, complement cleavage products, steroid hormone metabolites, bile acids and some cytokines".[185] Since "the virus ["HIV"] is replicating 24 hours a day and from day one",[155] and "2x10^9 $CD4^+$ cells [are] produced and destroyed each day", and fever and "many of the associated features of fever can be reproduced by infusions of purified cytokines, including back pain, generalised myalgias, arthalgias, anorexia and somnolence",[185] it is indeed surprising that such "massive" infection and cellular destruction may remain largely, if not totally, asymptomatic for prolonged periods of time in HIV seropositive individuals;

(ii) If there is such a "massive" HIV infection, why is it not detected by standard hybridisation procedures and why, in order to detect such "massive" infection, did not the authors use PCR which can "amplify a DNA-needle into a DNA-haystack" or even nested PCR but were obliged to determine "Viral RNA" with novel assays, "modified branched DNA (bDNA) or RT-PCR assay and confirmed by QC-PCR" for which no details are given?

One of the many problems[186, 187] associated with the Ho and Wei studies and the methods they employ is illustrated in a presentation at the XIth International Conference on AIDS. Researchers from the Medical School, Camden, New Jersey took a single plasma sample from a patient "with a CD4 cell count of 123 cells/cmm" and divided it into ten aliquots. The RNA from each sample was reverse transcribed and the cDNA "was then amplified with an internal control DNA (mimic) using gag primers...cDNA was also pooled from the initial 10 individual RT reactions and QC-PCR was performed 10 times on pooled cDNA". They reported that "The mean HIV-1 copy number for the 10 individual plasma aliquots was 136,000 RNA copies/ml with a standard deviation of 76,9000 copies/ml (range 74,2000 copies/ml to 334,600 copies/ml). The mean HIV-1 copy number for the pooled cDNA assayed 10 times was 145,900 copies/ml with a standard deviation of 61,900 copies/ml (range 84,500 copies/ml to 259,300 copies/ml)...the RT is not the source of variability in HIV-1 QC-PCR. Rather, variability is likely due to differences in amplification of the target template and internal control used in the QC-PCR assay".[188]

According to Maddox and Wain-Hobson both Ho and Wei and their colleagues were able to reach their startling conclusions only after a decade of HIV research because they teamed up with mathematicians and because they were able to use "New techniques for assaying the low levels of virus involved"! (italics ours). It is ironic then that the strongest criticisms of these studies have emanated from mathematicians such as Frank Buianouckas from the Department of Mathematics and Computer Science, City University, Bronx, New York USA and Mark Craddock, School of Mathematics and Statistics, The University of Sydney, Australia. "What is this viraemia of billions of RNA particles that can only be seen with an undocumented branch-PCR or PCR but not with a functional infectivity test?".[189] "My question is this: Just what exactly will it take to get people doing HIV research to turn away from high tech, unproven methods, arcane speculations about molecular interactions etcetera etcetera and ask themselves 'do any of us have the faintest idea what we are doing?'."[190] One can argue that criticism of the Ho and Wei papers by individuals from the HIV/AIDS dissident movement is not to be unexpected but it is unheard of for one group of HIV experts to criticise another as it happened with the Ho and Wei studies.[191] In July 1995, as a result of "misgivings" about the claims of Ho and Wei and their colleagues, "two dozen AIDS researchers congregated in Berkeley, California...to challenge the establishment, swap copies of their own manifestos, and enjoy the bonhomie of hanging out for two days with fellow 'alternative' thinkers", who concluded that Ho et al and Wei et al "were short on compelling evidence that their ideas were correct".[192]

(b) According to researchers from the Walter Reed Army Institute of Research, "the extensive use of the polymerase chain reaction (PCR) to recover HIV-1 proviral DNA has favoured analysis of the short amplicons that are most efficiently recovered by this technique".[193] In fact, in the vast majority of cases the presence of the "HIV genome" is proven by amplifying short "invariant regions" of a "viral gene", usually of the gag gene. However, since it is accepted that a significant proportion of the "HIV genomes" are defective, finding a fragment of a gene is not proof of the existence of the whole gene and even less so for the existence of the whole genome "HIV DNA" or "HIV RNA", a point accepted by many HIV/AIDS researchers.

(c) If a unique molecular entity "HIV DNA" exists, then the same primers would be able to amplify it, irrespective of where such unique DNA is found. According to the same researchers, "Due to the extensive genetic diversity of HIV-1, opportunities to identify a single primer pair capable of amplification of diverse subtypes are limited".[193, 194] In fact, amplification results obtained with primers for different genes from one subtype are not in complete agreement. For example, in the first "HIV" PCR, two primer pairs to amplify the gag gene were used and it was found that "some samples scored positive with only one of the two primer pairs".[195] It is said that in the USA and Europe individuals are almost exclusively infected with subtype B. Yet researchers from the University of Edinburgh found that "The results obtained with the gag and env primers were not in complete agreement. In 5 of the 28 replicates, either the gag or an env sequence was amplified but not both".[182] A PCR study of 40 individuals using primers from the LTR, gag and env regions was performed by French researchers including researchers from the Pasteur Institute. Out of 38 positive samples, "34 were gag positive (90%) whereas env and LTR were detected in fewer cases 24 samples (63%) and 18 samples (47%) respectively...11 of 40 samples were positive with three primer pairs, 16 with two primer pairs and 11 with only one primer pair".[196] Such discrepancies may be due to:

(i) "a false-positive reaction", which the authors themselves suggest but which they say is unlikely;

(ii) "the known genomic variability of HIV". If this is the case then one cannot talk of the "HIV genome" as being a unique molecular entity. Indeed, if such variability is entertained then it may be only the lack of an immense variety of primer pairs that prevents all of Homo sapiens from being "infected with HIV";

(iii) the genome is defective.

(d) No meaningful information can be obtained from a test unless the test is standardised and it is shown to be reproducible. No such data is currently available for the PCR. In fact, since there are so many "HIV" subtypes and one has to use different primers for different subtypes or even for the same subtype, it makes it extremely unlikely that such data can ever be obtained.

(e) By far the most important parameter of a test is its specificity, that is, how often a test is negative when the condition sought is absent. For PCR one must have proof that the primers:

(i) belong to a unique retrovirus as defined in the procedures described in 6.1;

(ii) the primer sequences are found only in the unique retrovirus and nowhere else;

No such evidence exists for the "HIV" primers. In fact, since it is not

possible to say what the "HIV DNA" sequences are, it follows that it is also not possible to be specific about what the primers represent. Even if one assumes that the "HIV DNA" and thus the primers are specific to a retrovirus since:

(a) most of the "HIV" primers originate from the leukaemic cell lines HUT78 (H9), CEM, and EBV-transformed cells;

(b) there is evidence that leukaemic cells and EBV-transformed cells contain endogenous retroviruses, including the CEM cell line;[88]

(c) "release of endogenous retroviruses can be induced by the methods used to "isolate HIV";

(d) Gallo himself reported that the HUT78 (H9) cell line "contained HTLV[-I] proviral sequences";[105]

(e) no method exists to separate one retrovirus from another;

it is impossible to say that the "HIV DNA" probes are HIV, or DNA probes of an endogenous retrovirus or even an exogenous retrovirus HTLV-I;

(iii) in a DNA (RNA) sample the primers bind only to HIV sequences and not to any other non-HIV homologous or non-homologous sequences. Again, no such data exists.

Furthermore, given the facts that:

(a) "about one percent of the human genome" consists of endogenous retroviral sequences;

(b) homologies exist between the genes of endogenous and exogenous retroviruses, especially in the gag and pol genes, and between these genes and cellular retroelements;

specific binding of the "HIV" primers is most unlikely.

Even if (i)-(iii) are proven one must still determine the specificity of the PCR reaction, that is, show that no positive results are obtained in individuals who are not infected with HIV. This can only be determined by using HIV isolation as an independent gold standard, that is, by comparing PCR with the procedures listed above (see 6.1). This has not been done, a fact accepted by one of the best known HIV/AIDS researchers, William Blattner "One difficulty in assaying the specificity and sensitivity of human retroviruses [including HIV] is the absence of a final 'gold standard'".[59]

(f) At present some evidence obtained without the use of a gold standard illustrates that the PCR procedure is non-specific:

(i) There has been only one study in which the reproducibility, sensitivity and specificity of PCR were examined. In this study, the gold standard used was not HIV isolation but serological (HIV Western blot) status. In this investigation, Christine Defer from the Laboratorie d'Ingenierie Moleculaire, Centre Regional de Transfusion Sanguine including colleagues from the Pasteur Institute, studied PCR testing proficiency in "Seven French laboratories with extensive experience in PCR detection of HIV DNA". Four groups of individuals were tested: those with "unequivocal HIV-positive test results" (ELISA confirmed with Western blot); "individuals at low risk of HIV infection who presented with a persistent and isolated anti-p24 antibody on Western blot"; "HIV-1 seronegative (on ELISA) individuals at low risk of HIV infection (blood donors)", and "seronegative (on ELISA) individuals at high risk of HIV infection (homosexual contacts of an HIV-seropositive partner". From "two different peripheral blood mononuclear cell panels...each consisting of 20 samples", the authors compared PCR results in both seropositive and seronegative subjects. The PCR was found to be non-reproducible, "False-positive and false-negative results were observed in all laboratories (concordance with serology ranged from 40 to 100%)", and "the number of positive PCR results did not differ significantly between high-and low-risk seronegatives";[197]

(ii) The finding of positive PCR in eosinophils has been interpreted to "suggest that eosinophils may act as host cells for HIV-1". [198] However, "Formaldehyde-fixed eosinophils nonspecifically bind RNA probes despite digestion with proteolytic enzymes and acetylation...When preparations are treated with amounts of ribonuclease adequate to destroy viral RNA, the eosinophilic binding remains";[199]

(iii) One group of researchers reported that "While evaluating a nested PCR procedure for the detection of HIV, we found that primers for the env gene of HIV-1 amplify human satellite DNA sequences in a small proportion of blood donors to produce a fragment that is close in size to the genuine HIV PCR fragment in ethidium-bromide-stained gels";[200]

(iv) Controls and even buffers and reagents may give positive HIV PCR signals;[201]

(v) Monocytes from HIV+ patients in which no HIV DNA can be detected, even by PCR, become positive for HIV RNA after cocultivation with normal ConA-activated T-cells";[202]

(vi) it is generally accepted that once infected with HIV, always infected. However, a positive PCR reverts to negative when exposure to risk factors is discontinued.[203]

In a study of 327 health care workers exposed by needlestick injuries to the "human immunodeficiency virus", four had "one or more positive" PCR tests. An additional seven had "an indeterminate PCR test result on the initial specimen". Later samples for all 11 were negative "none seroconverted or developed p24 antigenemia" and "all of the subjects remained healthy".[204, 205] While the evidence for such occurrence in adults is sporadic, it is much more often reported in children. However, PCR is not used for routine diagnosis of HIV infection in adults and rarely, if ever, is repeated. Unlike in adults, PCR is very often used in children, this being the case because "HIV diagnosis" is "complicated by persistence of passively acquired maternal antibody".

By 1995 numerous studies in children[206-209] revealed the conversion of a positive PCR to negative. One of the most recent reports was published in 1995 by French researchers. In a six year cohort of 188 "infected" children which was 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 seven) 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 neutralizing 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".[210]

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 shown in Fig. 1 function well in the PCR amplification of HeLa DNA even at annealing temperatures around 60°...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".[119] In an article published this year, 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 test and should be rejected unless they fulfil a need that antibody testing fails to meet".[211] 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".[200] 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,[89] one has no choice but to agree with Shoebridge et al that "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".[212] In analysing the "HIV" molecular biology one cannot help reflecting on the words of Sir 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".[213]

CONCLUSION—The present data do not prove the existence of a unique molecular entity "HIV DNA" which constitutes the genome of a unique, externally acquired retrovirus, HIV. Neither is there any proof for the existence of an "HIV quasispecies". Nor is it possible to say what exactly the different "HIV DNAs", the probes and primers derived from these DNAs and the sequences in the cellular DNA with which they hybridise represent.

7. "Isolation of HIV: The existence of a retrovirus HIV predicts that HIV can be isolated from the chromosomal DNA of infected cells. This prediction has been confirmed as follows: Full-length HIV-1 and HIV-2 DNAs have been prepared from virus-infected cells and cloned in bacterial plasmids (Fisher et al., 1985; Levy et al., 1986; Barnett et al., 1993). Such clones are totally free of all viral and cellular proteins, and cellular contaminants that copurify with virus purified by conventional density gradients. Indeed, these clones are even free of genomic HIV RNA. Infectious HIV-1 and HIV-2 DNA clones productively infect human cells to initiate HIV replication (Fisher et al., 1985; Levy et al., 1986; Barnett et al., 1993). Such infected ("transfected") cells contain HIV-specific DNA, and produce particles that contain reverse transcriptase; HIV specific antigens (Fisher et al., 1985; Levy et al., 1986), have diameters of 100 nm under the electron microscope (Fisher et al., 1985), as expected for retroviruses".

7.1 Before the cited evidence is discussed in detail, to avoid misunderstanding, it will be helpful to define some terms including cloning of DNA, transfection and virus cloning, as well as the evidence that must be presented to claim proof of these phenomena:

Plasmid– freely replicating, circular chromosomal elements present in bacteria. They duplicate independently of the main chromosomal element and are frequently used to "carry" a DNA fragment into a cell.

DNA cloning– the production of identical copies of a DNA fragment, any DNA fragment, from an ancestral DNA fragment by splicing it into a suitable cloning vehicle, for example, a bacteriophage or plasmid.

Transfection– the introduction of exogenous DNA into cells and its ability to replicate and express itself in these cells, that is, transcription of DNA into RNA, translation of RNA into proteins. The genetic material does not have to be of viral origin and transfection can be achieved by various methods. As far back as 1969 it was known that these methods may include "infection of cells with bacteria and viruses, formation of hybrids of two cell types by fusion, transplantation of isolated single nuclei in eggs and embryos, microinjection of nuclei and mitochondria fractions, and pinocytic uptake of purified DNA". In that year Margit Nass from the University of Pennsylvania, taking advantage "of the phagocytic properties of mouse fibroblasts (L cells) grown in suspension culture" demonstrated that, "Mouse fibroblasts (L cells) in suspension culture incorporated isolated chloroplasts of spinach and African violets and isolated mitochondria of chicken liver...Green cells divided like normal cells. Green chloroplasts were followed for five cell generations or five days, at which time hybrid cells were greatly outnumbered by nongreen progeny cells".[214] By 1989 it was realised that the delivery of DNA into cells could be facilitated by polycationic reagents such as poly-DEAE dextron and poly-ornithine. "An aliquot of the aqueous reagent is simply added to the tissue culture experiment together with the DNA or RNA of interest".[215] (It is of interest that cultures/cocultures derived from tissues of "HIV positive" and AIDS patients are treated with the polycation polybrene and/or oxidising agents which may lead to the formation of cations). In 1990, researchers from the University of Wisconsin showed "that injection of pure RNA or DNA directly into mouse skeletal muscle results in significant expression of reporter genes within muscle cells...RNA and DNA expression vectors containing genes for chloramphenicol acetyltransferase, luciferase, and b-galactosidase were separately injected into mouse skeletal muscle in vivo. Protein expression was readily detected in all cases, and no special delivery system was required for these effects. The extent of expression from both the RNA and DNA constructs was comparable to that obtained from fibroblasts transfected in vitro under optimal conditions".[216] One year later another group of researchers from the USA showed that after direct injection into animal hearts "of the firefly luciferose gene coupled to the myosin heavy chain...the heart can be transfected in vivo with greater efficiency than the skeletal muscle".[217]

Virus cloning–the introduction into cells of genetic material, DNA or RNA, which has been proven beforehand to be the genome of a virus followed by the appearance in the same cells of viruses identical in every aspect to the viruses from which the genomic material originated. Before one can claim proof of cloning of a retrovirus one must:

(a) Obtain a particle(s) separated from everything else (isolated) and show that the particle contains, among other molecules, proteins and nucleic acids (RNA), and that the particle(s) is indeed an infectious particle (see 6.1);

(b) Show that there is a direct relationship between the particles' nucleic acids and proteins, that is, the proteins are coded by the nucleic acids (the viral genome);

(c) Introduce the viral genome (RNA or DNA) into cells and show that the DNA (cDNA) is integrated into the cellular DNA and is transcribed into RNA and the RNA is translated into proteins (transfect the cells);

(d) Show that the cells produce particles and that the particles' proteins are coded by the particles' nucleic acids;

(e) Show that the particles' nucleic acids and proteins are identical with those of the ancestral particle and that they too are viral particles;

(f) Because all cells contain retroviral genomes, which under appropriate circumstances may be expressed in culture, that is, both the cells in the culture from which the original particles were obtained as well as the transfected cells may release identical retroviral particles even if there is no cloning, when one attempts to clone a retrovirus a control culture is of quintessential significance. The only difference between the control and the cells transfected with the viral genome should be that in the control cultures one should use some other genes for transfection. This is because, under suitable culture conditions, the very act of transfection may result in retroviral expression including the production of retroviral particles. It is obvious that retrovirus cloning is not synonymous with retrovirus isolation, in fact, for cloning one must isolate the virus twice, the first time to obtain the viral genome and the second time to prove that the particles, if any, released by the cell after introduction of the viral genome, are identical with those from which the genome was originally obtained.

7.2 In 1985 Fisher, Gallo and their colleagues published an article entitled, "A molecular clone of HTLV-III with biological activity".[94] "The phage clone lHXB-2 [see 6.2.2] which contains full-length provirus (10 kilobases, kb) with cellular flanking sequences (12.7 kb total length)" was inserted into the plasmid pSP62. "Similarly, a 13.7 kb Eco RI fragment of lCH-1 (a molecular clone containing 9.0 kb of HTLV-I proviral sequences) was inserted into" another plasmid, pSV2gpt. "These plasmid constructs [pHXB-2D, pCH-agpt] were then transfected into DH-1 bacteria and used in protoplast fusion experiments". pCH-1gpt and yet another plasmid containing "no HTLV sequences (pSVneo)" were used as controls. (No reasons are given why they used three different plasmids). PHA stimulated cord blood mononuclear cells "were then fused with bacterial protoplasts carrying the plasmids". "Three parallel fusions using cells from different individuals were established for each plasmid". (It is not clear if they used cells from three or nine individuals, if the latter, this is an additional reason why the cloning conditions could not have been identical).

(a) Spent medium "was concentrated 10-fold and assayed for the presence of reverse transcriptase" using A(n).dT_{15}, at days 5, 11, 14 and 18 after fusion. If the conditions used for transfection were identical and if transcription indicated the presence of a retrovirus, then one would expect RT to be present in the cultures with pHXB-2D and the three cultures with pCH-1gpt. However, DNA synthesising activity was reported only in two cultures with pHXB-2D, (the activity in one of them was less than half the other at each sampling point), and no mention is made regarding the activity in the third culture. Furthermore, for some unknown reason, the DNA synthesising activity was reported only for 18 days after transfection when it was said to be maximum. Unlike RT activity, the viability of the cells in the cultures was determined repeatedly starting before transfection and up to 32 days afterwards. The results were reported as the mean of the three cultures for each plasmid. If the viability of the cells was determined by the expression of retrovirus present in the cultures and if HIV and HTLV-I possesses the biological properties attributed to them, then one would expect the number of cells in the cultures containing $pSV2_{neo}$ to remain constant, in the cultures containing pHXB-2D to decrease, and in the cultures with pCH-1gpt to increase. They reported that between day 18 and 32 the number of viable cells decreased in all cultures. The decrease was most pronounced in the culture with the "HIV clone", and appeared earlier: "By day 18, however, the number of viable cells in cultures transfected with pHXB-2D has fallen dramatically". In other words, the highest cell death occurred before maximum HIV (RT) production and even before the full "HIV DNA" was integrated into the cellular DNA (see below). Furthermore, since apparently no RT activity was detected in one of the three cultures with pHXB-2D, in this culture the cell number should have remained constant.

(b) Results of the hybridisation studies are given only for pHXB-2D, and even there for only one of the three cultures with this plas-

mid. "The presence of HTLV-III sequences was demonstrated by Southern blot analysis" using "insert" from the molecular clone lBH-10, "an incomplete viral clone of HTLV-III". "A 10-kb band, corresponding to unintegrated linear virus, was detected in undigested DNA samples prepared 14 days after transfection. Digestion with XbaI revealed three distinct band at 11, 10 and 5.2 kb...these bands probably represent the nicked circular, linear and closed circular forms of unintegrated HTLV-III respectively...Digestion with HindIII, an enzyme which cuts the HTLV-III genome of pHXB-2D six times, yielded bands at 4.5, 2.0 (doublet), 1.7 and 0.6 (a doublet)...This restriction pattern is clearly different from that of H9/HTLV-IIIB...High relative molecular mass 'smears' were not observed when DNA was digested with BamHI. Therefore, we have no direct evidence that transfected HTLV-III DNA is integrated in the host cell genome...In time-course experiments (Fig. 36), DNA isolated from a single culture 6, 11, 14, 18 and 31 days after transfection with pHXB-2D, was digested with BamHI and analysed for HTLV-III sequences. Six days after transfection an 8.6 kb DNA fragment was detected as a faint band; 18 days after transfection it was possible to detect a 1.5 kb DNA fragment in addition to the 8.6 kb fragment...No HTLV-III sequences were detected 31 days after transfection". Despite these findings, the time-course experiments were interpreted "as evidence that cells originally transfected with pHXB-2D are able to produce fully infectious virus which is then transmitted within the culture"!

(c) The pHXB-2D transfected umbilical cord lymphocytes were reacted with "monoclonal antibodies against the HTLV-III-gag-related proteins p24 and p15...maximum expression was observed 15 days after transfection, when 4-11% and 5-9% of cells were reactive with antibody to p15 and p24, respectively (data not shown)...In comparison, among H9/HTLV-III cultures, a much larger proportion of cells (70-90%) was positive for p24 and p15". In addition to the many problems associated with the interpretation of a positive antibody/antigen reaction, especially with umbilical cord cells and the gag antigens (antibodies), as proving HIV infection, it is also interesting to note that:

(i) maximum antibody/antigen reactions preceded maximum reported RT activity and hybridisation bands;

(ii) no mention is made regarding the antibody reactivity with the pSV2-neo transfected cells but "cord blood cells removed 18 days after transfection with pCH-Igpt (HTLV-I clone) were not labelled by these antibodies". However, if as Gallo claims:

(a) the gag genes of HIV and HTLV-I are homologous;

(b) there is cross-reactivity between the p24 proteins of the HTLV-I and HIV-1;

the reported finding that the "monoclonal antibodies against the HTLV-III gag-related proteins" did not react with the pCH-Igpt transfected cells is inexplicable.

Their immunological findings led them to write: "The finding that, at any stage, only a minor population of the transfected cells are apparently infected by the virus (<15% express viral proteins) suggests that the cytopathic effects may not result solely from direct viral infection". However, if the dramatic fall of viable cells in the pHXB-2D transfected cultures where only a minority of cells are "infected" is caused either directly or indirectly by "the clone of HTLV-III with biological activity" (cytopathic effects), why are such effects not also observed in the H9/HTLV-III cell line where a much higher percent of cells is "infected" but such cells divide indefinitely? Especially when one considers the fact that the H9 (HUT78) cell line originates from a patient who "had malignancies of mature T4 cells"[6] and HIV is said to specifically destroy the T4 cells.

(d) Fisher and colleagues published an electron micrograph showing extracellular but not budding, virus-like particles some of which had a diameter of 100nM. However, they did not prove that the particles were viral particles or even that they had any of the other morphological and physical characteristics of retroviral particles.

7.3 In 1986 Levy and his colleagues published a paper entitled "AIDS retrovirus (ARV-2) clone replicates in transfected human and animal fibroblasts".[218] The molecular clone l9-B of ARV-2 (see 6.2.3) was inserted into the plasmid pSp65. The p9B-7 thus obtained and l9B-7 were used to transfect the human monocytic cell line U937 as were the Jurkat and HUT-78 cell lines. ARV was detected by the presence of "RT activity in the culture supernatant...ARV production was detected in the Jurkat and U937 cells at 36 to 44 days after transfection by the presence of reverse transcriptase (RT) activity...Virus replication was detected at 5 days in the HUT-78 line, with RT activity reaching over 200,000 cpm/ml...Virus from each culture was subsequently passed to mitogen stimulated normal human peripheral mononuclear cells (PMC)...Reverse transcriptase activity increased to over 10^6 cpm/ml within 14 days after the virus from the HUT-78 cells was passed to fresh human PMC". The NIH 3T3 (mouse), MIL (mink lung), COS-7 (African Green monkey), and RD-4 rhabdomyosarcoma (human) cells were also transfected. In all cells RT activity was detected within 5 to 14 days after transfection. "The detection of virus was enhanced by cocultivation of the fibroblast cells with mitogen-stimulated normal human PMC...added every 3 to 6 days".

Protein extracts of "PMC infected with virus recovered from transfected MIL cells", COS-7 cells and HUT-78 were electrophoresed and reacted with "serum positive for antibodies to ARV...Extracts of the infected HUT-78 cells and PMC contained all the antigens of ARV as demonstrated by immunoblotting (Fig. 2). These included the envelope proteins gp160, gp120, gp41, and the gag proteins of molecular weight 55K, 25K, and 16K". No such reactions were reported with the "non-infected" PMC.

However, even Montagnier reported that at least one protein, gp41 from non-infected cells reacted with patient sera. The difference may be due to the fact that apparently Montagnier stimulated the non-infected cells but Levy did not. Again, while in normal non-stimulated cells patient sera do not react with a p16-18 protein, the same proteins are detected in normal, non-infected but stimulated cells.[219-222] Levy and his colleagues also found that "The virus recovered from all the cells was cytopathic for HUT-78 cells...The virus produced in HUT-78 cells showed cytopathic effects (fusion, balloon degeneration) typical of AIDS retroviruses".

If the cytopathic effects are caused by an HI virus which appeared as a result of cloning then Levy et al managed to prove an effect of HIV on HUT-78 (H9) which to date nobody else has managed to demonstrate. (It is true that in 1986 nobody apart from Gallo and his colleagues knew that HUT78 is actually HT (H9)).

7.4 In 1993 Barnett, Levy and their colleagues published a paper entitled "Distinguishing features of an infectious molecular clone of the highly divergent and noncytopathic human immunodeficiency virus type 2 UC1 strain". This study by Barnett, Levy et al refers to HIV-2. Since HIV-2 is said to be totally different from HIV-1, its isolation or cloning, even if true, in not proof for the isolation or cloning of HIV-1. Nevertheless, since it has been cited a few comments may be worthwhile. The "molecularly cloned virus (HIV-2_{UC1mc} or $UC1_{mc}$)" was obtained as follows: The cellular DNA of "UC1-infected SupT1 cells", was "subjected to partial digestion with EcoRI. The digestion products were size fractionated on NaCl gradients and then ligated to EcoRI-digested EMBL4. Plaques were screened by hybridization to a mixture of DNA probes including simian immunodeficiency virus from macaque, HIV-2_{ROD} env cDNA clone E2, and an HIV-1_{SF2} preparation enriched for gag-pol sequences... Approximately 2 million plaques were screened, and 12 positive plaques were obtained following successive rounds of plaque purification and hybridization. Of these 12 positive clones, only one was found to contain full-length HIV-2 proviral DNA following restriction enzyme analyses. Lambda-cloned UC1mc was transfected into RD cells by calcium phosphate precipitation, and infectious virus was recovered following cocultivation of these cells with phytohemagglutinin-stimulated normal PBMC" and this "virus" was used to transfer to other cell lines. Proof for virus cloning and the existence of "infectious virus" was obtained as follows: "Culture supernatants were assayed every 3 or 4 days for reverse transcriptase activity. Cell samples were also tested for viral protein expression by an indirect immunofluorescence assay. Cultures were examined at 2- or 3-day intervals by light microscopy for cytopathic effects such as the appearance of syncytia, large cells, ballooning cells, and cell debris. Cell viability counts were determined by trypan blue dye exclusion. Immunoblot analyses were performed as described previously by using virus lysates prepared from cell culture supernatants of virus-infected Molt4/8 cells. The sera came from HIV-infected individuals or from a rabbit immunized with recombinant HIV-2_{ST} gp120". They reported, "UC1mc grew well in the Supt1, Molt4/8, and HUT78 T-cell lines but did not exhibit productive infection of Jurkat or CEM cells...UC1mc demonstrated relative inability to induce syncytium formation, kill cells, and down-modulate surface CD4 expression in infected cells [do Levy and his colleagues now agree with us[80] that the apparent loss of CD4 cells is not due to their destruction by "HIV", but to the ability of the cultures to "down-modulate surface CD4 expression"?]...The molecular sizes of the UC1mc viral proteins and their reactivities with various sera were determined by immunoblot analysis. While most of the UC1 and UC1mc viral proteins were reactive with sera from HIV-2 infected individuals, the cell surface Env glycoprotein (gp140: SU) was usually poorly reactive with these sera compared with the gp140s of other HIV-2 strains (e.g., HIV-2_{UC3}) shown). In contrast, the UC1mc and UC1 gp140 molecules appeared to react well with Env-specific rabbit antiserum raised against recombinant HIV-2_{ST}SU protein". For the molecular characterisation of UC1mc, "The entire UC1mc genome was subjected to DNA sequence analysis to determine its genetic structure and the relatedness of its deduced protein structure to those of other known

HIV strains. The proviral DNA sequence of UC1mc was found to be 10,271 bp long, and its overall genetic structure appeared to be similar to that of other sequenced HIV-2 strains...By sequence analysis, UC1mc appeared to diverge substantially from most other HIV-2 strains. The differences were most noticeable in the very low percentages of identity of the amino acids sequences of Env; viral regulatory proteins Tat, Rev, and Nef; and viral accessory proteins Vif, Vpx and Vpr. The divergence of UC1mc was more subtle but nevertheless significant in the generally more conserved Gag and Pol proteins"[223] (italics ours).

7.5 COMMENTS

Neither Fisher et al, Levy et al nor Barnett et al satisfied the conditions absolutely necessary to claim cloning of a retrovirus, HIV. Nor was it possible for them to so do. To molecularly clone a retrovirus first one must obtain the retroviral RNA and this can only be obtained by isolating the retrovirus. NO ISOLATION NO CLONING. However, to date not only has no researcher isolated a unique retrovirus from fresh tissues of AIDS patients or even from cultures/cocultures containing material from these patients but neither has any researcher proven the existence of particles, viral or non-viral, which satisfy the principal morphological and physical properties of retroviruses.[146] Fisher et al, Levy et al and colleagues, by various means, but with no proof that it belonged to a particle, any particle, selected fragments of DNA, no two of which were the same either in composition or length and called it "HIV DNA" (see 6.2). Subsequently, they attempted to introduce the "HIV DNA" into cells using well known techniques by which it is possible to introduce any DNA, viral or non-viral, into cells. Irrespective of what is meant by "HIV DNA", given the techniques they used, it is highly probable that they succeeded. However, proof can only be claimed by sequencing "HIV DNA" both before and after cloning into the cells and none of these groups did so. The only evidence presented by the above workers to this effect and indeed to virus cloning was:

(a) The detection in cell cultures of RT activity (transcription of $A(n).dT_{15}$);

(b) The finding in cells of proteins ("the envelope proteins gp160, gp120, and gp41, and gag proteins of molecular weight 55K, 25K and 16K") which react with antibodies to p24 and/or with sera from AIDS patients.

However, thus far, nobody has proven that any of the above proteins which are present in cell extracts and which may react with AIDS patient sera are actually coded by the "HIV" env and gag open reading frames (see 5). Neither are the presence of viral-like particles in the culture supernatants nor transcription of $A(n).dT_{15}$ proof for the existence of HIV or of any retrovirus endogenous or exogenous (see 3.0). Even if there was proof that the particles were actually retroviral and that reverse transcription of $A(n).dT_{15}$ was induced by a retroviral enzyme, the proteins were retroviral proteins and the antibodies were specifically directed against such proteins, their finding in cell cultures is not proof of transfection of "HIV DNA" and even less of "HIV" cloning. All of these phenomena may be caused by an endogenous retrovirus, especially if one considers the type of cells used, leukaemic and umbilical cord lymphocytes, and the conditions, chemical stimulation and co-culture techniques. According to Kurth and his colleagues, "indirect evidence has accumulated over the past years that some endogenous proviral loci must also be expressed in humans... Expression of retroviral information was also suggested by the demonstration of reverse transcriptase activity and by the detection of antigens cross-reactive with animal retroviral antigens in a variety of human cells and tissues".[116] AIDS patients' sera contain antibodies directed against many self and non-self antigens including lymphocytes[89, 224, 225] and sera from 70% of AIDS patients react with antigens of "The viruses in all of us", that is, endogenous retroviruses.[175] In a 1989 publication by researchers from Sweden, Japan and the USA one reads: "In the 1960s and 1970s new techniques (morphological, immunological, and molecular biological) became available...not only to find exogenous or endogenous retroviruses, but also to correlate retrovirus expression with certain human diseases...Electron microscopic studies revealed particles with a retroviral morphology in several normal and neoplastic human tissues and also in milk, urine and several other effusions. Sensitive radioimmunoassays were developed which led to the detection of antigens [including gag proteins in umbilical cord blood sera] related to the proteins of known exogenous murine and primate retroviruses and reverse transcriptase (RT) was found in different normal and neoplastic tissues".[108] "Three HERV-R [human endogenous retrovirus-R] polyadenylated mRNAs (9, 7.3 and 3.5 kilobases) are expressed in first trimester and term placentae villi. A comprehensive survey of HERV-R expression in human tissues revealed that most other tissues also express the 9- and 3.5-kilobase mRNAs at a level of about 10% of that in the placenta...The greatest expression besides the placental villi was in the monocytic leukemia cell line U937", one of the cell lines employed by Levy et al. Another of the cell lines used by Levy et al in the 1986 study, COS-7, was from an African Green monkey. Since then it has been shown that African Green monkeys are "infected with SIV" and even earlier, 1983 they were said to be infected with "adult T-cell leukemia virus".[226]

The RD cell line used by Levy is a human rhabdomyosarcoma cell line and for many years these cells have been known to express viral information and to release retroviral-like particles.[227] For cloning, Fisher et al and Levy et al obtained their "HIV DNA" from the HUT78 (H9) cell line. This is also the cell line from which Fisher and her colleagues obtained most for their evidence for "HIV-1 cloning". Even if one assumes that the "HIV DNA" is indeed retroviral, for which there is no proof, it cannot be assumed to be the "genome of HIV". According to Gallo the HUT78 (H9) cell line is infected with HTLV-I.[6] If so, then all HUT78 cell cultures, and the clones derived from it, "infected with HTLV-III" or non-infected, and the material from these cultures which bands at 1.16 gm/ml, should contain HTLV-I, and thus RT and retroviral particles. Furthermore, because about 25% of AIDS patients have antibodies to HTVL-I, and the immunogenic proteins of HTLV-I and HIV have the same molecular weights, then approximately 25% of the non-infected HUT78 (H9) cultures in addition to RT and particles, should have, in the Western blot, the same bands as those of the "HTLV-III infected" cultures. Thus, the cell extracts from the HUT78 cells and the Western blots will erroneously appear positive for HTLV-III. Both Gallo's and Montagnier's groups showed that the gag and pol genes of HTLV-I and HIV-1 are homologous. This means that the HUT78 cell line should have "HIV DNA" sequences even when not transfected with "HIV DNA".

Unlike Fisher et al, Levy et al did not perform hybridisation studies. However, Fisher, Gallo and their colleagues could not find evidence that the "HTLV-III DNA is integrated into the host cell genome", a step absolutely necessary in cloning and production of retroviruses. Nor has anyone of these researchers shown that the DNA is transcribed into RNA. For transfection, in addition to proving integration of the "HIV DNA" into the host cell genome and its transcription into RNA, one must also prove that the RNA is translated into proteins.

CONCLUSION—To claim that "The existence of the retrovirus HIV predicts that HIV DNA can be isolated from the chromosomal DNA of infected cells", one must first have proof of the existence of a unique molecule of DNA which is the genome of a unique retrovirus particle, HIV-1, which can only be obtained by isolating the retroviral particle. At present there is no such proof. Fisher et al and Levy et al selected a portion of the RNA which from the supernatant of "infected" HUT78 cells banded at 1.16gm/ml or had a certain length, reverse transcribed it and called it "HIV-1 DNA" (see 6.2.2; 6.2.3). However, since neither they nor anybody else before or after them has shown that this RNA (cDNA) was even the constituent part of a particle, any particle retroviral or otherwise, the claim that the DNA is "Full length HIV-1" or "HIV-specific" cannot be substantiated. In the cell extracts of "transfected" cells Fisher et al and Levy et al found some proteins with molecular weights similar to the "HIV proteins" which reacted with AIDS patient sera. They also found reverse transcription of $A(n).dT_{15}$ in the cell supernatant but presented no evidence that the proteins or the RT were constituents of a particle, viral or otherwise, and thus cannot claim that they have proven that the "transfected" cells "produce particles that contain reverse transcriptase, HIV specific antigens". Although Fisher and colleagues had an electron micrograph showing virus-like particles in the culture supernatant, they did not prove that the particles were indeed retroviral particles, or even that they had some of the most basic morphological and physical features of retroviral particles and thus they "could reflect non-viral material altogether".

Fisher et al, Levy et al and Barnett et al did not start with RNA (cDNA) proven to be the RNA of a retrovirus and did not obtain retroviral particles proven to contain the same RNA, a most basic requirement for cloning. In fact, given their evidence they cannot even claim transfection of cells with a DNA, viral or non-viral.

8. "IDENTIFICATION OF HIV"

8.1 "The existence of HIV predicts that infected cells contain a unique, virus specific DNA of 9150 nucleotides that cannot be detected in DNA of uninfected cells".

The genome of a retrovirus cannot be identified on the basis of the length of a RNA (cDNA) fragment and its presence in some but not other cells.

8.1.1 Using fragments of "HIV DNA" as hybridisation probes or primers, positive results with both standard hybridisation and PCR have been obtained from DNA of "uninfected" human cells and

insects (see 6.4.4). It is a fact that:
(a) hybridisation of nucleic acids of "exogenous retroviruses" "from different species gives a pattern which is the same as the phylogenic relatedness among their natural hosts",[228] a relationship which led retrovirologists including Gallo to conclude that exogenous retroviruses "are derived from cell genes";
(b) The existence of human retroviruses has been "shown" using hybridisation probes derived from endogenous and exogenous animal retroviruses.
If this is the case and if "HIV DNA" is the genome of an exogenous human retrovirus, the non-infected human genome should contain sequences which will hybridise with "HIV DNA" probes. There can be two reasons why such findings have not been reported more often:

(a) Most HIV researchers ignore one of the most fundamental requirement of basic experimental research, that is, controls. In the rare instances where controls are used, they are not suitable (see 6.1). In the 1970s, Gallo, Gillespie and their colleagues were saying that the success of the "hybridization assay appears to depend on the biological history of the virus", and on the physiological state of the cells.[125, 228] In a large study published in 1975 entitled "Relationship between Components in Primate RNA Tumor Viruses and in the Cytoplasm of Human Leukemia Cells: Implications to Leukemogenesis", the aim was to show that human leukemia cells but not normal cells have properties associated with retroviruses including retroviral genomic sequences. It was reported that "The human leukemic blood cell cytoplasmic particle that contains reverse transcriptase activity is capable of synthesizing DNA in vitro, using endogenous RNA as both template and primer. This endogenous activity has been used to learn about the nature of the particle itself. Many intracellular cytoplasmic particles or organelles (described generally in Table 8) can carry out endogenous DNA synthesis in vitro. These include mitochondria, small cytoplasmic particles of low density, 1.10-1.16 g/cc in sucrose density gradients, and small cytoplasmic particles of higher density, 1.17-1.19 g/cc in sucrose density gradients...Small particles have been detected in the cytoplasmic fraction of phytohemagglutinin-stimulated lymphocytes from normal donors...These particles carried out endogenous DNA synthesis, and the resulting DNA population contained sequences related to genomes of RNA tumor viruses...Viral-related sequences were found in patients with several types of leukemia, including AML, CML, CML-A and CLL...Attempts to detect viral sequences in RNA of leukemic cells by hybridizing DNA synthesized by animal viruses to RNA isolated from cytoplasmic small particles (the reciprocal hybridization experiment) in our hands fails to find differences in sequences in RNA of leukemic and dividing normal [PHA stimulated] human peripheral white blood cells. It has been reported by others that radioactive DNA probes synthesized by $MuLV_R$ hybridize to cytoplasmic RNA from leukemic, but not normal white blood cells. A difference between our experiments and those previously reported is that the normal human cells used as a source of RNA are actively dividing while most of those used in previous studies were not" [125] (italics ours);

(b) The "HIV RNA" is not the genome of either an exogenous or an endogenous retrovirus or even the transcribed DNA fragment present in un-"shocked" cells.

8.1.2 Most of the positive results in "uninfected cells" have been found by using probes and primers for one or at most two genes or even gene fragments. The "great majority" of HIV studies, encompass "2% to 30% of the genome".[163] However, finding fragment of a gene or even a gene is not proof for the existence of the HIV genome.

8.1.3 Montagnier and his colleagues reported the "HIV DNA" to be 9 ± 1.5 Kb[91] whereas Gallo and his colleagues reported that "The overall length of the HTLV-III provirus is approximately 10 kilobases".[96] In Levy and colleagues' first study of the "HIV genome", the "broad band (>15 Kb) represents provirus integrated into host cell DNA".[98] In 1995, Pasteur researchers reported that "The complete 9193-nucleotide sequence of the probable causative agent of AIDS, lymphadenopathy-associated virus (LAV), has been determined. The deduced genetic structure is unique; it shows, in addition to the retroviral gag, pol, and env genes, two novel open reading frames we call Q and F".[229] In the same year, Gallo and his colleagues reported their results on the "HIV" nucleotide sequences using clone BH10 but also added, "The sequence of the remaining 182 bp of the HTLV-III provirus not present in clone BH10 (including a portion of R, V5, tRNA primer binding site and a portion of the header sequence) was derived from clone HXB2...Of note is the presence of a fifth open reading frame (nucleotides 8, 344-8991) designated 3' orf, present in clone BH8 but truncated in BH10". They concluded, "The complete nucleotide sequence of two human T-cell leukaemia type III (HTLV-III) proviral DNAs each have four long open reading frames, the first two corresponding to the gag and pol genes. The fourth open reading frame encodes two functional polypeptides, a large precursor of the major envelope glycoprotein and a smaller protein derived from the 3' terminus long open reading frame analogous to the long open reading frame (lor) product of HTLV-I and -II...The HTLV-III provirus is 9,749 base pairs (bp) long".[32] In 1990 the HIV genome was said to consist of ten genes.[230] This year Montagnier reported that HIV possesses eight genes[7] and Barré-Sinoussi,[8] HIV has nine genes.

To date, no two "HIV DNA" of the same length have been reported and moreover, it is accepted that most "HIV genomes" are defective. Even if all the genes can be amplified by PCR, it still does not mean that the "full-length HIV genome" is present. For example, in 1995 the nef gene of three of the blood recipient members of the Sydney "Bloodbank" cohort and of the donor were amplified by PCR. "The resulting amplified fragments for the three recipients ranged from 410 bp to 680 bp. One recipient yielded fragments of two sizes...The amplified fragment from the donor (D36) was 550 bp in length, indicating a deletion of 290 bp...compared with 840-bp fragment from the molecular clone pNL4-3".[231] In 1995 David Ho and his colleagues "analyzed by polymerase chain reaction and direct sequencing 57 viral sequences from 47 individuals of North American, Australian and Haitian origin infected with human immunodeficiency virus type 1 (HIV-1), focussing on the V1 and V2 regions of gp120. There was extensive length polymorphism in the V1 region, which rendered sequence alignment difficult. The V2 hypervariable locus also displayed considerable length variations, whereas flanking regions were relatively conserved".[232] As far as Gallo is concerned, it is not even a requirement that the "HIV" genome possess any genes whatsoever to be pathogenic, "This suggests that defective virions such as RNA-free particles and/or viral proteins expressed in the absence of particle formation contribute to AIDS pathogenesis".[114]

8.1.4 In searching the HIV literature it is striking that to date, not one single 9150 bp or any length of "full length HIV genome" from fresh uncultured cells has been sequenced. "The low abundance of HIV-1 proviral DNA in clinical samples is a barrier to full-genome analysis of HIV-1 provirus as it occurs in vivo". All the "full-length HIV genomes" sequenced so far have been from cultured cells; in fact "Completely sequenced full-length HIV-1 genomes in the current Los Alamos data base have been derived, almost without exception, from HIV-1 isolates adapted to growth in continuous [leukaemic or transformed] T-cell lines". As of late 1995 "only 19 sequences encompassing the full-length, 10-Kb HIV-1 genome have been reported, and most derive from HIV-1 isolates of genotype B expressed in continuous cell lines. Five of the eight most prevalent genetic subtypes of HIV are without a single, full-length, sequenced prototype".[193]
At present it is also known that:

(a) patients belonging to the AIDS risk groups are exposed to high doses of oxidising agents and that these agents have profound effects on DNA and RNA;[74, 79]

(b) in cultures "HIV" cannot be detected unless cultures are treated with chemical or physical oxidants including PHA;

(c) there are structural and functional abnormalities in the lymphocyte genome of AIDS patients. "AIDS patients have shown increased levels of spontaneous DNA repair synthesis (three times higher), increased quantity of single-stranded DNA breaks (11-18%), decreased ability to restore DNA damage (2-2.5 times lower) compared to healthy persons";[233]

(d) according to Chermann and his colleagues, "Different populations of distinct HIV-1 DNA fragments of highly variable size ranging from 600 bp to full length provirus were present in PBMC from HIV-infected persons... Defective genomes tended to gradually disappear after activation of PBMC with phytohemagglutinin";[234]

(e) According to the HIV experts, the defective genomes are "rescued" by recombination and this recombination is one of the main causes of "HIV DNA" complexity. If this is the case one may ask:

(i) can one exclude the possibility that the 19 "full-length HIV genomes" described so far, even if they all had the same length of 9150 bp and identical sequences are nothing more than a chance finding among the many molecular species present in the cultures, or even the uncultured lymphocytes, which have nothing to do with a retroviral genome and which appeared as a result of either in vivo or in vitro conditions or both and of natural selection?;

(ii) if there is such a high rate of recombination between the HIV genomes, is it not possible that the same process takes place between the endogenous retroviral genomes? If this is also the case, how does one know that the 19 "full-length HIV genomes" are nothing more than recombinations between endogenous retroviral sequences and cellular sequences, for example, non-retroviral retroelements?

As has been pointed out, HIV researchers seldom use controls and to date those that have, failed to use appropriate controls, that is, tis-

sues or cultures derived from similarly sick, non-AIDS individuals in which experimental techniques and conditions employed are identical apart from the presence of putative retroviral material. However, if HIV researchers or others capable of mounting such experiments were encouraged to put as much effort as they put into studying "HIV" from lymphocytes of at risk patients into studying lymphocytes from patients not at risk but:

(a) who are exposed to agents (other than "HIV") and doses similar to those in the high risk groups;
(b) which have similar structural and functional abnormalities as lymphocytes from AIDS patients or those at risk;
(c) using exactly the same methods and culture conditions as those used by "HIV" researchers;

can one exclude the possibility that in another ten years time these researchers will not be able to report "19 full-length HIV genomes" in these individuals?

8.2 "For example, Jackson et al. have tested blood cells of 409 antibody-positives including 144 AIDS patients and 265 healthy people. In addition 131 antibody-negatives were tested. HIV-specific DNA subsets – defined in size and sequence by HIV-specific primers (start signals for the selection amplification) – were found in 403 of the 409 antibody-positive, but in none of the 131 antibody-negative people (Jackson et al., 1990)".

8.2.1. Apparently, up until 1987 Jackson et al considered the detection of RT (reverse transcription determined by transcription of $A(n).dT_{15}$) in cultures, synonymous with HIV isolation! However, they had an "isolation rate of 57% in patients with acquired immunodeficiency syndrome". By 1988 the "reverse transcriptase assay was replaced with the Abbot Laboratories HIV-1 antigen detection assay", which "primarily detects the p24 core antigen of HIV-1...A culture was considered positive for HIV-1 antigen if two serial supernatant samplings were positive, with the later sampling showing greater activity"! "HIV-1 was isolated from the PBMC of 141 (99.3%) of 142 HIV-1 antibody-positive patients".[235] In their 1990 paper Jackson et al reported that "Between February 1987 and October 1988, peripheral blood mononuclear cells (PBMC) from 409 individuals who were antibody-positive for HIV-1 by Western (immuno) blot (56 AIDS patients, 88 patients with ARC, and 265 asymptomatic individuals) were cultured". Using a sensitive technique previously described, the p24 assay noted above, they reported that "HIV-1 can be isolated from 100% (56 of 56) of AIDS patients, 99% (87 of 88) of ARC patients, and 98% (259 of 265) HIV-1 antibody-positive asymptomatic individuals". Not one of "131 HIV-1 antibody-negative individuals has a positive culture". Using the same p24 assay (Abbot) they tested the serum from 403 out of the 409 individuals. The test was positive in 23/56 (42%) AIDS patients, 31/88 (57%) ARC patients and 44/259 (17%) asymptomatic antibody-positive individuals. For unstated reason(s) a positive serum test is considered proof for the detection of "HIV-1 antigen in serum" while the same positive culture test is considered proof for "HIV-1 isolation" from the culture. There are many reasons to question the interpretation of the p24 assay:

(a) The p24 assay is an antibody/antigen reaction and is subject to background reactivity. In this context, even if there are "two serial supernatant samplings with the later sampling showing greater reactivity", even if double or triple, for example, 30 and 60 or 30 and 90, both readings may be nothing else but background readings. Jackson and colleagues' criteria are not even in agreement with those used by Ho et al which are equally as arbitrary; "A culture was considered positive if the concentration of p24 antigen in the supernatant exceeded 1000pg per milliliter (typical cutoff value approximately 30pg per milliliter) on a single determination or $\geq$ 200pg per milliliter on two or more determinations".[51] In this regard it is important to note that no amount of experimental variations and technological improvements in the p24 test can change the underlying nature of the test. The test solely detects antibody/antigen reactivity and the reason underlying such reactivity cannot be determined on the basis of an arbitrary cut off. A priori, there is no reason why conditions leading to non-specific reactivity should not be present at a sufficient level to drive the reaction above cut off, nor any reason to prevent the reverse, that is, specific reactivity below cut off. The only way to resolve this issue is to compare reactivity with the presence or absence of HIV as determined by virus isolation. To date, this has not been reported. Even without a gold standard, the non-specificity of the p24 antigen test is so obvious that it 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".[236] The fact that in experiments with "serial dilution studies of culture supernatants" the p24 test is more likely to be positive than RT is not proof that the p24 test is "at least 100-fold more sensitive that reverse transcriptase assays". Sensitivity for HIV can only be measured by the use of HIV isolation as a gold standard;[237]

(b) There are no scientific reasons and indeed no commonsense reasons why reactions such as reverse transcription or antibody/antigen reactions, even if specific for retroviruses, can be considered proof for viral isolation. If these phenomena are considered proof for virus isolation then both the pregnancy test, (measurement of the protein bHCG in blood or urine using antibodies), or estimation of cardiac enzymes in suspected myocardial infarction, must also be considered proof for "isolation" of placenta or heart respectively.

8.2.2 To improve on the p24 assay, the DNA extracted from frozen uncultured PBMC of their seven "antibody-positive culture negative subjects" and "23 healthy heterosexual HIV-1 antibody-negative, culture negative individuals" was assayed by PCR. In addition, "In order to compare the sensitivity and specificity" of the two tests, PCR and culture, the PBMC of 59 seropositive and 20 seronegative individuals were analysed by both tests. "Amplifications of HIV-1 were performed by using a primer pair, SK38-39, which amplifies a 115-base-pair conserved region of the gag gene (nucleotides 1551 to 1665 of HIV SF23: GenBank accession no. K02007). The amplified product was detected by oligomer hybridization, a technique in which a ^{32}p-end-labeled probe (SK19) to the nucleotide 1595 to 1635 gag region hybridizes in solution to one strand of the amplified sequence. The probe-target duplex was then resolved by electrophoresis on a 10% polyacrylamide gel and autoradiographed". None of the seronegative individuals was reported to have a positive PCR test. "All initial DNA samples from the seven HIV-1 antibody-positive, culture-negative patients" were reported positive. When the PCR and culture tests were compared, 57 of the 59 patients had a positive PCR and 57 of the 59 patients had a positive culture. The two PCR negative individuals had positive cultures and the two culture negative individuals had a positive PCR. The authors concluded, "We isolated HIV-1 or detected HIV-1 DNA sequences from the PBMC of all 409 HIV-1 antibody-positive individuals. None of 131 HIV-1 antibody-negative individuals were HIV-1 culture positive, nor were HIV-1 DNA sequences detected by PCR in the blood specimens of 43 seronegative individuals. In addition, HIV-1 PCR and HIV-1 culture were compared in testing the PBMC of 59 HIV-1 antibody-positive and 20 HIV-1 antibody-negative hemophiliacs. Both methods were found to have sensitivities and specificities of at least 97 and 100% respectively...Our ability to directly demonstrate HIV-1 infection in all HIV-1 antibody-positive individuals provides definite support that HIV-1 antibody positivity is associated with present HIV-1 infection".[52] In other words, Jackson et al used the antibody tests as a gold standard for both the culture and PCR tests and the PCR and culture tests as a gold standard for the antibody test.

Jackson et al's claims are not even confirmed by other laboratories. According to Jackson et al, up until 1990 only three small studies reported "100% isolation rates of HIV-1 from AIDS patients". In all the other studies, "HIV-1 was not isolated from 6 to 50% of HIV-1 seropositive AIDS cases reported. The culture recovery rate of HIV-1 from HIV-1 antibody-positive asymptomatic patients has generally been even lower, only 20 to 42% in some studies". The most recent situation is best illustrated by a large WHO study published in 1994. Between 1992-93 224 specimens were collected in Brazil, Rwanda, Thailand and Uganda from asymptomatic "HIV positive" individuals. Serostatus was first confirmed in the country of origin and then at the "centralized laboratories responsible for confirming serology, virus isolation, virus expression, and distribution of reagents (George-Speyer-Hans Chemotherapentisches Forschunginstitut (GSH) in Frankfurt, Germany; National Institute for Biological Standards and Control (NIBSC) in London, United Kingdom; and DAIDS/NIAID in Bethesda, Maryland, United States)". Using the method of Jackson et al, "of a total of 224 virus cultures, 83 were positive (Isolation rate=37%)".[238]

Jackson et al's PCR results, like their culture results, are not reproducible in other laboratories. For example, in the study conducted by Defer and her colleagues, where the same samples were tested in "Seven French laboratories with extensive experience in PCR detection of HIV DNA", the data revealed that of 138 samples shown to contain "HIV DNA", 34 (25%) did not contain "HIV antibodies" while of 262 specimens that did not contain "HIV DNA", 17 (6%) did contain "HIV antibodies".[197] In a paper published in 1994 by researchers from The Laboratory of Molecular Retrovirology Georgetown University, Chiron Corporation California, Retrovirology Section, US National Institutes of Health, Maryland, the authors noted that the PCR techniques are "exceedingly labor intensive and suffer from laboratory-to-laboratory variation due to differences in technique and operations" and that "in some reported studies there is no correlation between p24 antigen levels and measurements of infec-

tious virions. Similarly, a decrease in p24 antigen level is not necessarily associated with a positive clinical outcome". Because of this, to "Monitor Human Immunodeficiency Virus Type 1 Burden in Human Plasma", the authors used "the branched DNA signal amplification assay" which, "offers improved sensitivity" and compared it with the "two other standard assays for viral burden; end-point dilution plasma culture and immune complex-dissociated (ICD) serum p24 antigen". They reported that "HIV-1 DNA and ICD serum p24 antigen assays were done on serum samples from 102 seropositive (Western blot-confirmed) patients who were being screened for enrollment in clinical trials...of the 102 patients, 75 (74%) were positive for HIV RNA by the bDNA assay and 61 (60%) were positive by the ICD p24 assay. Only a subset of patients (n=56: CD4 cell range, 29-394; median 160) was tested for plasma viremia by viral culture; 34 (61%) were culture-positive, while 50 (89%) were positive by bDNA assay and 39 (70%) were positive by the ICD p24 assay".[239] How is it then possible to claim that "virtually all people who contain HIV DNA also contain antibodies against Montagnier's HIV strain" and "most, but certainly not all people who lack HIV DNA contain no such antibodies"?

CONCLUSION AND COMMENTS—Since Jackson et al did not test all 409 patients and all 131 antibody-negative individuals for the presence of "HIV DNA" using PCR, but tested only 66 patients and a maximum of 43 "antibody-negative" individuals; did not sequence the amplified segments and did not determine the specificity of the PCR by using the only valid gold standard, HIV isolation, it was not possible for them to report "HIV specific DNA subsets...in 403 of the 409 antibody-positive, but none of the 131 antibody-negative people". Furthermore, Jackson et al acknowledged that their PCR method did not prove the existence of the full-length HIV genome but only "that AIDS patients as well as HIV-1 antibody-positive asymptomatic individuals harbor HIV-1 genetic material". In addition, for their PCR determinations, Jackson et al used a small fragment of the gag gene as a primer. But:

(a) since the best known HIV experts agree that the gag genes of retroviruses are homologous, Jackson et al's negative PCR results in all 43 "antibody-negative" individuals who must at least have had the retrovirus present "in all of us", remain unexplained;

(b) finding a positive PCR result using a small fragment of the gag gene as a primer is not proof for the existence of the "full length HIV genome" or even for the existence of the "full length HIV gag gene".

As has been already mentioned, by 1989 researchers at the Pasteur Institute concluded that "the task of defining HIV infection in molecular terms will be difficult". In fact, as far back as 1973, retrovirologists were aware that the unusual nature of retroviruses "will prove a stumbling block to any genetic analysis of RNA tumour viruses".[240] Yet, at least some HIV experts, including Jackson et al insist on defining HIV infection in genetic terms. On the other hand, an analysis of the presently available data on retroviruses shows that all retrovirologists seem to agree that the single most decisive factor in proving the existence of a unique retrovirus is the existence of specific antibodies, its importance well illustrated by the history of the discovery and subsequent demise of HL23V (see 5.4). As far as HIV is concerned, it is well known that the only evidence considered to prove the HIV theory of AIDS is a correlation between the clinical syndrome and a positive antibody test. Less well known is the fact that in the four papers published in Science in May 1984, Gallo and his colleagues claimed that in contradistinction to Montagnier and his colleagues, he and his colleagues achieved "true isolation". However, it is of pivotal significance that the only difference between the experiments performed by the two groups is that Gallo's group employed a leukaemic cell line from which they were able to obtain abundant "HIV antigens" and thus could perform significantly more antibody tests.

Given the crucial status retrovirologists accord to specific antibodies proving the existence of a unique retrovirus and its pathogenicity, proof of antibody specificity would appear to be mandatory. The specificity of the HIV antibody tests can be determined only by the use of HIV isolation as a gold standard. To date this has not been done and at present would seem impossible because nobody has fulfilled even the first step in the only scientifically valid method for retroviral isolation, that is, electron microscopic demonstration of particles with the morphological characteristics of retroviruses banding in sucrose density gradients at the density of 1.16 gm/ml. In addition, "HIV" can only be "isolated" from a minority of individuals who have a positive antibody test. Furthermore, as in the case of HL23V, there is evidence that the antibodies present in human sera which react with "HIV proteins" are also non-specific:

(a) "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":[241]

(b) "The immunochemical determinants of the antigenic factors of Candida albicans display a high identity with the glycoprotein (gp) 120 of HIV-1: they contain (a1->2) and (a1->3) linked mannose terminal residues";[242]

(c) antibodies to the mannans of candida albicans "block infection of H9 cells by HIV-1" as well as the binding of lectins to gp 120;[242]

(d) 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 lymphotrophic 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;[242]

(e) "normal human serum contains antibodies capable of recognising the carbohydrate moiety of HIV envelope glycoproteins...from 100ml of human serum approximately 200mg of MBIgG was recovered [MBIgG=mannan-binding IgG]...MBIgG bound to HIV envelope glycoproteins gp 160, gp 120 and gp41";[243]

(f) researchers from the University of Rome infected healthy mice with an E. coli lipopolysaccharide (LPS) and reacted their sera with two synthetic peptides, one encompassing gp 120 V3 loop of "HIV-1 MN" and the other "representing a gp41 immunodominant epitope". (V Colizzi et al., personal communication).

(g) 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 crossreactivities with HIV-1 pol and gag 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".[244]

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),[245-247] contain carbohydrate (mannans). One hundred percent of AIDS patients (even those with "No candida clinically") have Candida 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".[248] It may also be of interest to note that in gay men the only sexual act which is a risk factor for seroconversion is passive anal intercourse (exposure to semen)[249] and that mannose is present in both sperm and seminal plasma.[250] Since antibodies to mannans react with the "HIV proteins" then, as Essex and his colleagues have 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". Given the fact that individuals with fungal and mycobacterial infections have antibodies which may produce a positive "HIV' antibody test even in the absence of "HIV", how can one assert that:

(a) PCP, candidiasis, cryptococcosis, coccidiodomycosis, histoplasmosis, tuberculosis or Mycobacterium avium-intracellulare 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[251]) which signify AIDS are caused by HIV on the basis of a positive antibody test?

(b) that a positive antibody test in individuals with fungal and mycobacterial infections proves HIV infection?

Indeed, as in the case of HL23V, is it only a matter of time before HIV researchers accept that there may be no such entities as specific HIV antibodies? As a consequence, will the compilation of phenomena inferred as proof of the existence of the human immunodeficiency virus, pass into history as "non-viral material altogether"?

ACKNOWLEDGMENTS

We would like to thank all our colleagues and especially Bruce Hedland-Thomas, Richard Fox, Livio Mena, Alun Dufty, Barry Page, Andrew Campbell, Jennie Brooks, Gordana Pelemis, Daphne Peters, Gladys Powell, Ron Hirsch, David Dawson, June Rider-Jones, Christine Sibley, the staff of the Royal Perth Hospital Library and the clerical staff of the Department of Medical Physics. We also thank Todd Miller, Christine Johnson, Philip Johnson, Harvey Bialy, Charles Thomas, John Lauritsen, Neville Hodgkinson, Gordon Stewart, Huw Christie, Tony Tompsett, James Whitehead, Volker Gildemeister, Michael Baumgartner, Michael Verney Elliot, Joan Shenton, Stefan Lanka, Michael Ristow, Fabio Franchi, Djamel Tahi, Richard and Rosalind Chirimuuta, Udo Schuklenk, Brian Peachey, Philip Adams and Hiram Caton, as well as any others we may have unfortunately omitted. We especially thank Peter Duesberg for all his help and encouragement and for his inspiring example of scientific courage and integrity.

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