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 Continuum1) 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).dT15. 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).dT15;
(b) the template-primer A(n).dT15, 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).dT15. In fact, in 1975, an International Conference on Eukaryotic DNA polymerases, which included Baltimore and Gallo1 defined DNA polymerase g, “a component of normal cells”, “found to be widespread in occurrence”, whose activity can be increased by many factors including PHA stimulation, as the enzyme which “copies A(n).dT15 with high efficiency but does not copy DNA well”; 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).dT15 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”, this is not the case, a fact accepted by some of the best known scientists. 4 “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 elements...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 viral RNA and DNA intermediates”.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 retrovirus-like particles, and retrovirus production by a factor of 10 to 50”). After 15 days RT activity of “HIV” strain, cells from a lymph node biopsy of a gay man with lymphadenopathy (lymphadenopathy syndrome [LAS]) were transfused vertically (in the germ cell line) and because they were from a patient at risk for acquired immune deficiency syndrome (AIDS). This virus is a typical reverse transcriptase-bearing entity other than retroviruses, including HIV, which may lead to the treatment of B infections as well as HIV.16 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.17 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)”,22 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 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)T15. The reverse transcription of A(n)T15 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 virus production by a factor of 10 to 50”). After 15 days RT activity of “HIV” strain, cells from a lymph node biopsy of a gay man with lymphadenopathy (lymphadenopathy syndrome [LAS]) were transfused vertically (in the germ cell line) and because they were from a patient at risk for acquired immune deficiency syndrome (AIDS). This virus is a typical reverse transcriptase-bearing entity other than retroviruses, including HIV, which may lead to the treatment of B infections as well as HIV.16 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.17 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?

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. Many scientists have found a virus in the treatment of B infections as well as HIV.16 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.17 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?

The “infected” umbilical cord lymphocytes as well as “HTLV-pro-
transcriptase activity and retroviral antigens.24-24 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 virologists, H. K. Kurth from the 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 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 antibody to donors or of laboratory workers. The protein was absent in supernatants of uninfected T lymphocytes, T- or B- cell lines” . They 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 blood cells” from CEM and HTLV-II. On the cultured (labelled) them with radioactive cysteine, 35S 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 line they formed “A cell line containing both normal T lymphocytes and the MOLT-4 cell line”, which was “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 protein is 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 patient 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.25 This conclusion can be extended to several grounds. Sufficient 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. Curiously, this was interpreted as 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 antigens, and the proteins with which these antibodies reacted 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 colleagues29 claimed that although p45/41 reacted with patient sera, this protein was absent in supernatants of uninfected T lymphocytes, T- or B- cell lines, but the virus when grown in vitro was found to contain the envelope protein gp41. Moreover, in a study published in 1987 by 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 env gene ... the envelope protein gp120”, and “gp41 is 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”, they reported that, “Although the overall sizes and structures of the seven surface proteins are rather similar, the deduced amino acid sequences differ in length and in some of the amino acids. gp41, which is composed of the two most conserved 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 with the protein gp41 present in HLTV-II (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 leukocytes;

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

Following the discovery of HL23V, some researchers attempted to determine its prevalence utilising antibody tests56 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 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 SS-AV, 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, SSSAV 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 in the population”. In a report published in 1989, 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 not 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, 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”),(ii) the title of the paper was, “Antibodies in human sera reactive against an internal structural protein of human T-cell lymphoma virus”.41 In this paper Gallo and his colleagues described the finding of a “novel glycoprotein of a mammalian structural protein (p24) of HTLV-I”, and claimed that such antibodies were “specifically directed at HTLV-I 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 difficulty in identifying 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 blotting is a sensitive and 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 the isolation of the 120-160 kDa “murine type-C virus proteins”,43 “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 the carbohydrate residues of the glycoprotein”.40

(i) All HIV researchers agree with Montagnier and Gallo that gp160 is the “HIV pol protein”, p31/34. "The "HIV gag protein", p24" 5.6 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 infected and apparently uninfected individuals, and there is no evidence 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.44 Since, according to the AIDS clinical laboratories, an individual who shows a positive reaction...
existing 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 between retroviruses pol and gag which are generally conserved among retroviruses.”23

Gallo always maintained that homology exists between the HTLV-I, II and HIV gag genes46 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 and monkey) antisera and antibodies. Indeed, p24 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-interspecies). The many 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.”48 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 proteins in the membranes of “HIV particles”, 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.56

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? The proteins in the 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 superants 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 those “HIV antibodies” and “HIV antigens” 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;69

(b) at present there is ample evidence that the plethora of cellular proteins [26 methods] and the a and chains of human lymphocyte antigen (HLA) DR, CD71, CD63, CD43, CD8, “the major leucocyte adhesion receptors LFA-1 (CD11A/CD18) and CD44] which are present in the “HIV particles”, include actin and myosin.46 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-1la” 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”67. 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, “the F-actin linking 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 where the F-actin is enriched. Further, as actin/myosin function, polarization of F-actin might change the cell membrane configuration or cell fragility, which may be essential for HIV release”67.

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.67-69

(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 with oxidising agents.77 Ten years ago Montagnier wrote, “Indeed, LAI 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” phenomenon can be observed.79 80 Indeed in at the 1997 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 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 harmless blood cells and not respectively but nonetheless predict infection with Epstein-Barr virus and Treponema pallidium. 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 ("With HIV-1 DNA and HIV-2 DNA... 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 this RNA is associated with the morphological, physical and replicative characteristics of a retroviral particle. Proof of these properties can only be 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 100-1200 nm 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 no matter how many times the experiment is repeated; 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” (supematant 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 immunocompromised.
6. (d) the appearance of endogenous retrovirus can be accelerated and the yield increased a million fold by stimulating the cultures with mitogens, 89 mutagens, chemical carcinogens and radiation. 88, 89 If test cultures are exposed to or employ such agents so should the controls.
7. Since AIDS patients and those at risk of developing the syndrome are exposed to strong oxidising agents, 79 the control cells should also originate from such patients;
8. 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 uninfected 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 regimes were tried. The one that gave rise to continuous productive invasion of LAV was the following. Whole lymphocytes of F. R. were first stimulated for 24 hours with concanavalin A and then cultured with both and 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 FR8. 20 Reference 1 to which Montagnier refers is the 1983 paper in which Montagnier et al. described the first isolation of the virus.”

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, dTTP, dCTP including 32dCTP and an oligo(dT) primer. From the CDNA thus obtained, three clones “pLAV13, 75 and 82,” carrying inserts of 2.5, 0.6 and 0.8 kilobases (kb), respectively, were characterized. All three inserts have a common restriction pattern at one end, indicative of a viral particle... "The PstI derived (bp) common HindIII-P1 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 slot-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 peripheral blood...”. Furthermore, they tested 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. "Third, the experimental 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 peripheral blood..."
In May 1984, Gallo and his colleagues published four papers. To “isolate” HIV they used a leukemic 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. In contrast, in the Gallo et al. paper, 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. The Gallo investigation found this procedure to be “of dubious scientific rigor.” One scientist described the procedure as “really crazy.” In 1985, Gallo and his colleagues wrote, “The HT cell line, initially derived from an HTLV-I-infected T-cell line HT, following co-culture with T lymphocytes obtained from several AIDS patients, and contains many different HTLV-III forms.”

The detection of reverse transcription of (Aln)4dT15 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 “selected for being a healthy donor as a feeder.” 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 g/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 oligo(dT) cellulose. The resulting matographed on an oligo(dT) cellulose column. The resulting extract was hybridised to poly(A)-containing RNA was used as template to synthesise 3P-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 from HTLV-I and HTLV-III infected cells, both of these 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-I and HTLV-III, as well as identical patterns of RNA in homologous RNA sequencing from the same supernatants as those from which the probe was obtained and were like those with HTLV-III.”

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 3P-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 that the unintegrated DNA exhibited a broad band (>15 kb) in the undigested DNA represents the linear form of unintegrated HTLV-III.” In yet another study Gallo and his colleagues reported that, “Since the HTLV-III provirus was found to lack Xba I restriction sites, the HTLV-III unintegrated DNA was hybridised to Xba I digested H9/HTLV-III DNA, and this was screened with an HTLV-III cDNA probe. Identical patterns of RNA in homologous RNA sequencing from the same supernatants as those from which the probe was obtained and were like those with HTLV-III.”

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 existence of retrovirus particles or even the isolation of virus-like particles, the first and absolutely necessary step in proving the existence of a retrovirus. (At the time of writing, neither has any other group of HIV/AIDS researchers. Finding some RNA which bands at 1.16 g/ml, selecting from it a poly(A) rich fraction, or a fragment of a genomic fragment, even if it is a so-called ‘provirus’ is not enough to be considered a ‘proviral 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 g/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.” 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 g/mg/ml or viral particles, virologists refer to the particles obtained after sucrose density gradient purification or to the RNA purified from the culture supernatants which is “homologous RNA...assembly of particles does not require the genome...in its absence other RNA molecules may be substituted.”

Within 6 days the supernatant of this culture had high RT activity and was inoculated into fresh human PMC stimulated 3 days before with phytohaemagglutinin. Within 6 days the supernatant of this culture had high RT activity and this was said to represent “the virus isolate ARV-2.” 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 resulting cell-free supernatant containing reverse transcriptase activity was purified twice in sucrose gradients. The nucleic acid from each fraction was electrophoresed on agarose gel. The region in the gel containing a “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 “radioactively labelled” and used to hybridise to the 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 P CLI RI; 15-15 kb cell DNA was cloned into an EMBL-4 bacteriophage I vector and recombinant phage were identified with the virus-specific cDNA probe”. Among the recombinant phage obtained were I-8B and I-7A, each of which was 9.5 kb.

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which contain among other things, RNA. How then is it possible to claim that the RNA which banded at 1.16 g/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).dT₁₅ can be reverse transcribed by all cellular DNA polymerases α, β and γ. Is it possible to claim reverse transcription in the absence of endogenous DNA polymerase? How then can one be sure 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 the genomic material (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 heterogeneous nuclear RNA. These RNAs, in addition to having high molecular weight and heterogeneity in size, also have poly(A), with the poly(A) attached to the minus strand of the molecule, and may be RNase resistant. Actinomycin inhibits its synthesis and also interferes with its proper processing and breakdown.¹⁹ From animal virology it is also known that non-retroviral RNA and DNA also bands at 1.16 g/ml.¹⁰⁰ How is it then possible to claim that just because a RNA bands at 1.16 g/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 may then be transcribed as RNA. This RNA may be present in the cell and elsewhere. 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 g/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”.¹²¹ 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, showing 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 (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 the particular patient’s lymphocytes or of the patient. 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 Retrovirus (HTLV-III) from patients with AIDS and Pre-AIDS”, one gets the impression that the HT (H9) 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 g/ml, assuming that all the groups who tested HIV 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 nuclear 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 g/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 cells 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 DNA 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 “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 HIV-2, 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 DNA sequences.”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).¹¹² “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-
expression of a restricted family of related proviruses. It has been shown that Montagnier and his colleagues reported their "HIV genome" from the first ATG codon of p25 contains a TATA-box, a polyadenylation 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, melanoma cells and embryonic tissues. In 1989 researchers from the USA summarised their experimental findings as follows: "Human T-cell lymphotropic 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 contains a TATA-box, a polyadenylation 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, melanoma cells and embryonic tissues. In 1989 researchers from the USA summarised their experimental findings as follows: "Human T-cell lymphotropic 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 contains a TATA-box, a polyadenylation 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, melanoma cells and embryonic tissues.

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instances, endogenous proviruses have been established or increased in number during experimental observations".12

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 among these components 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 (Y) 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 uninfect ed 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 relationships among their natural hosts...We have proposed this to account for the interpretation that all RNA tumor viruses are derived from cell genes, a proposal in agreement with the viogene 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 "Implications of the Endogenous Retrovirus". Discussing the presently available data they wrote, "A 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 into the genome. These sequences have a number of characteristics that are reminiscent of retrotransposons, 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.129, 130

According to Gilbert introns represent "hot spots" for recombination and new genes can be created "through the coupling of exons by intron-mediated recombination", "intron is lost and more complicated exons are formed".131 At present evidence exists showing that certain introns can act as genetic elements, "introns and other genetic elements, they self-splice, they often contain reading frames capable of encoding a protein including a region or domains that again will bring startling changes in concepts"132 [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, it was generally believed that the cellular RNA could be identified and that the RNA was simply a copy of the 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.133 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 of 450 amino acids...". Intron between 50 bases long, the longest extending out to some 50,000 bp.134

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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.135 136 137

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.138 In their experimental work they used the bacterial viral RNA (Qb). In one of the cases where the triphosphate ATP, GTP, UTP, CTP, Qb 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 synthet-
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 O₃ 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 to the model of 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 endogenous cutting enzyme. 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, the normal results would 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 is that 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 escaping... We call this entire mutant distribution as 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 quasispecies.”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 these RNA molecules, non-templated changes occur, 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 stable transcript that lacks a parent template composed of 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 the tests. The discovery of reverse transcriptase amended but did not challenge the central dogma of how genes make proteins; introns qualified the conclusion that genes are the isomorphically 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;70, 60 (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”.

(c) 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”;

(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 “in all of us”;

(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 for hybridisation studies, 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)”15 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 now known to prove the existence of HIV can be described in this manner. One employs mitogens or co-cultures of bone marrow cells sometimes additional “shock”, a fact accepted by both antignier and Gallo.8, 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-II 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 HTLV genome is 0.1 differences per million years whereas the HIV-1 genome 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”.141 In one and the same patient the genomic data in monocytes differs from that in T-lymphocytes.152 There are “striking differences” between the proviral DNA and cDNA in one and the same PMBC sample “which could not be explained by either an artefact of reverse transcriptase error 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”.151 According to the researchers from the Pasteur Institute “an asymptomatic patient can harbor at least 104 genetically distinct variants of HIV in their blood”.152 The AIDS patient figure is more than 106.149 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.152, 156

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.”152 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.153

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 opposed to a single clone analysis, is required. In this case, the HIV sequences were sequenced. 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 quasi-species in vivo and in vitro, the task of defining infection in molecular terms will be difficult”.153 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 and gag genes.
(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, 0, HIV-1 genotypes were described and new genotypes are still reported.8, 151 According to researchers from the Henry M. Jackson Foundation Research Laboratory and Division of Retrovirology, Walter Reed Army Institute, USA, “The major genetic divergence 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 population based approach” from amplicons (genes).8, 164 “The designation Human Immunodeficiency Virus Type-1 (HIV-1) encompassed an unanticipated complexity of viral forms”.164 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 interpret the results of these subtypes as a true indication of 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.164 This means that at present it is not possible to say what are the sequence differences, both qualitative and quantitative, between 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”.161 “Two HIV-1 strains, designated ANTO70 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 35% similarity to HIV-2. In contrast, ANTO70 was more closely related to HIV-2”.167 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 HIV-2 isolate 10125 were found.”161 “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”.165 Indeed, this seems to be the case. Last year, David Ho and his associates168 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. The population of the primary infection was clearly different from the others on the basis 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 Pasteur Institute and the University of California. Because protease inhibitors are becoming the drugs of choice for the treatment of “HIV infected” individualls, 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 treatment-naïve patients”.169 The results showed that the nucleotide and amino acid sequences of the protease region of HIV-1 had “the greatest 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 than so 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 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 myths of incomensurable “HIV DNAs” cannot be even described “in terms of popu-
lutions 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 virus5. 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 repre-
sent “extreme variability”. “Many selective forces may stabilize virus
populations. These stabilizing factors may include the need for con-
servation of protein structure and function, RNA secondary structure,
global conservation of codon usage, and ribosomal adaptation to
changes can be subject to selective pressures. Recently, remarkable
conservation of certain protein domain sequences has been observed
between completely unrelated RNA viruses;”171 it is possible then to
describe the “HIV DNA” even if it has variation of 10%, not to men-
tion 20 or 30 or 40% as is the case, as a “population of closely relat-
ed 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, designat-
ed 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 uncuturled 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, as normally is usually not detected by standard
Southern blot 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 sar-
coma 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”.5 These studies were confirmed by
many other researchers. The finding that when the results were posi-
tive 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
generated acceptance, although Gallo and his colleagues had an
alternative explanation: “Theoretically, this low signal intensity
could also be explained by the presence of virus distantly hetero-
gous to HTLV-III in these cells”.
94 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”.
124 DNA obtained with the antisense probe are non-HIV-specific or, as the
authors concluded, there is a non-HIV-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”.154 Korower 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-relat-
ed sequences” in the above species, and concluded that “Further
analysis of members of this family will help determine whether such
sequences are derived from an endogenous retrovirus”.
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) PCR is an expensive and time-consuming technique with a high
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”.
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". 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 use of multiple molecular primers for quantifying the provirus molecules, we have measured provirus frequencies in infected individuals down to a level of one molecule per 10^6 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 cell whilst uninfected controls (12 samples from patients contained one or more proviruses 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.”

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, 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.” Maddox is referring to two papers published in Nature in 1995. One by Ho et al, where the authors claim that in even patients who have not received antiviral treatment the “virus reveals viral levels ranging from...15 X 10^3 to 554 X 10^3 virions per ml”; the other by Wei et al where it is claimed that the “viral DNA levels in the 22 subjects at baseline ranged from 10^6 to 10^7 molecules per ml” concluded that “the vast majority of infected patients are infected by the same provirus 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 immuno-reactives.”

These claims raise two obvious questions:

(i) “The prototype virus’ are microorganisms, their products or toxins”, and “endogenous pyrogens are polypeptides produced by a large variety of nucleated host cells including macrophages and ‘lymphocytes, endothelial cells, hepatocytes, epithelial cells, keratinocytes, and fibroblasts, as well as other cells...in response to initiating stimuli triggered by infection or inflammation”.

(ii) “The results suggest that virus expression by standard hybridisation procedures and why, in order to detect such “massive” infection and cellular destruction may remain largely, if not totally, asymptomatic for prolonged periods of time in HIV seropositive individuals”.

Thus, “the ‘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 define ‘Viral RNA’ with novel assays, ‘modified branched DNA (BDNA) or RT-PCR assay and confirmed by QC-PCR’, for which no detailed genes were given.

One of the many problems associated with the Ho and Wei studies and the methods they employ is illustrated in a presentation at the Xth 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 ranging from 45,000 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.” 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 Buianoukcas from the Department of Mathematics, University of Sydney, Australia. “What is this viremia of billions of RNA particles that can only be seen with an undocumented branch-PCR or PCR but not with a functional infectivity test?”

One question is this: Just what exactly will it take to get people doing HIV research to turn away from the Ho and Wei studies? 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.”

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.” In fact, in 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”. 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 suggest that the UK and Ireland contain a globally unique combination of HIV subtypes”. Researchers from 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?”

(ii) the primer sequences are found only in the unique retrovirus species (“HIV” RNA), a point accepted by many HIV/AIDS researchers.

(iii) 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.” 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”. 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 suggest that the UK and Ireland contain a globally unique combination of HIV subtypes”. Researchers from 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?”

(iv) “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. (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”. In fact, in 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”. 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 suggest that the UK and Ireland contain a globally unique combination of HIV subtypes”. Researchers from 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?”

(iii) The genome is defective.

(iv) No meaningful information can be obtained from a test unless the test is standardised and 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.

(v) For the most part the performance 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 non-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 exogenous retroviruses, including the CEM cell line;

(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 "contains HIV sequences";

(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 other 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 the gag and pol genes and cellular retroelements;

(c) 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 an "independent gold standard".

(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 Laboratoire 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 (heterosexual 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".

(ii) The finding of positive PCR in eosinophils has been interpreted to "suggest that eosinophils may act as host cells for HIV-1". 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".

(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 hybridizes to the genuine HIV PCR fragment in ethidium-bromide-stained gels".

(iv) Controls and even buffers and reagents may give positive HIV PCR signals.

(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.

(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.

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. This initial sequence of PCR results for the "nearly non-converted or developed p24 antigenemia" and "all of the subjects remained healthy". 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, cases being the cause because of "diagnosis" is "complicated by persistence of a partially required material antibody".

By 1995 numerous studies in children 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 189 "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 rounds of PCR with separate amplification circuits were used for DNA extraction, PCR-buffer preparation, amplification and blotting. Amplifications were never transferred in the area reserved for unamplified sequences. Thus, positive PCR results are unlikely to be due to contamination...Nevertheless, as our assays are performed on unamplified cells, which are considered negative by PCR, positive PCR results is impossible...We therefore consider that the probability of repeated contamination on successive samples from the same child is scatter". The authors “could not find any correlation between either neutralizing or antibody-dependent cellular cytotoxicity-mediating antibodies and HIV clearance". Of 139 born children to HIV-positive mothers, 49 (35%) had at least one PCR positive result. The authors reported that "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".

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 gene of all HIV strains can give a positive signal 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 exogenous sequences are considered here...The results 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. 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-1negative intravenous drug users". If PCR needs to be matched against the "HIV" antibody test because it is less reliable than serology given the implication that it is present there is no evidence which shows that a positive "HIV" antibody test is proof of HIV infection, 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". In analysing the "human molecular biology of HTLV-I" 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".
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 would anneal, 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., 1989). Such clones could be used to identify free DNA derived from viral genes and cellular contaminants that copurify with virus purified by conventional density gradients. Indeed, these clones are even free of genomiv 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 can be used to produce HIV-specific secretory products (HIV-specific transcripase; 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 to present 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 dextran and polyethyleneimine. An important result of a standardised protocol in 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 “the transfection of pure DNA or RNA 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 β-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 luciferase 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 and showing that the particle contains, among other molecules, proteins and nucleic acids (RNA), and that the particle(s) is/are an infectious particle (see 6.1).

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

(c) Prove the introduction of DNA (or RNA) into cells and show that the DNA (or RNA) 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) Prove that the particles’ nucleic acids and proteins are identical with those of the parental particles, 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 and those released by the 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. Hence 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”.24 "The phage clone HXB-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 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 (pSVneu)” 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.)

(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 and those released by the 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. Hence 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.
mid. “The presence of HTLV-III sequences was demonstrated by Southern blot analysis” using “the molecular clone IBH-10, “an incomplete viral clone of HTLV-III”. “A 10-kb band, correspond- ing 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 HTLV-III gag-related proteins”.

7.3 In 1986 Levy and his colleagues published a paper entitled “AIDS retroviruses: Induction of syncytium formation, killing cells, and down-modulating surface markers”. They reported, “UC1mc grew well in the Supt1, culture supernatants of virus-infected Molt4/8 cells. The sera came from AIDS patients...and infectious virus was recovered following cocultivation of these cells with phytohemagglutinin-stimulated normal PBMC”. In contrast, the UC1mc and UC1 gp140 molecules demonstrated by immunoblotting (Fig. 2). These included the env 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.

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 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 demonstration would mean that it is unlikely to be the virus type 2 UC1 strain”. However, if the reported finding that the “monoclonial 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 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 were transfected? The culture supernatants of virus-infected HUT-78 cells showed cytopathic effects (fusion, balloon degenera- tion, and infectious virus was recovered following cocultivation of these cells with phytohemagglutinin-stimulated normal PBMC” and “the virus” was used to transfer to other cell lines. Proof for virus cloning and the existence of “infectious virus” was obtained as fol- lows: “Culture supernatants were assayed every 3 to 4 days for reverse transcriptase activity. Cell samples were also tested for viral protein expression by an indirect immunofluorescence assay. Cells were examined for Env glycoprotein expression by immunofluorescence 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 Mo/4/8 cells. The sera came from HIV-infected individuals and were incubated with recombinant HIV-2, gp120”. They reported, “UC1mc grew well in the SupT1, Mo/4/8, and HUT78 T-cell lines but did not exhibit productive infec- tion of J urkat 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 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 of UC1mc was not reactive with sera from these individuals compared with the gp140s of other HIV-2 strains (e.g., HIV-2UC3) shown). In contrast, the UC1mc and UC1 gp140 molecules appeared to react well with Env-specific rabbit antiserum raised against recombinant HIV-2, gp120 protein”. For the molecular character- ization 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

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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 and the highly conserved Vpr gene (c. 90% of identity). The divergence of UC1mc was more subtle but nevertheless significant in the generally more conserved Gag and Pol proteins222 (italics ours).

7.5 COMMENTS

Neither Fisher et al, Levy et al nor Barnett et al satisfied the conditions absolutely necessary in the generally more conserved Gag and Pol proteins222 (italics ours).

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8. IDENTIFICATION OF HIV

8.1 The existence of HIV predicts that infected cells contain a unique, virus specific DNA. If such DNA is not present in infected cells, then it may be possible to detect it by hybridisation studies.

8.1.1 Using fragments of "HIV DNA" as hybridisation probes or explants, it became possible to detect HIV DNA in human cells and tissues.116 AIDS patients' sera contain antibodies specific to HIV antigens (e.g., anti-gag, anti-env, anti-p24) 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 antibodies to p24 and/or with sera from AIDS patients. No proof can be obtained 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)dt15); (b) The finding in cells of proteins (the envelope proteins gp160, gp120, gp41, and gag proteins of molecular weight 55K, 25K and 16K) which react with antibodies to p24.

There is no proof that the particles were actually retroviral and that reverse transcription of A(n)dt15 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 no proof of retrovirus cloning. Of course, it is also possible that the "HIV DNA" is integrated into the host cell genome and that the "HIV DNA" into the host cell genome and its transcription into RNA, for transfection, in addition to proving integration of the "HIV DNA" into the host cell genome, must be followed up by RT and retroviral particles. Furthermore, because about 25% of AIDS patients have antibodies to HTLV-I, and the immunogenic proteins of HTLV-I and HIV have the same molecular weights, then approximately 25% of the non-infected HTUT78 (H9) cells in culture and the clones derived from it, "infected with HTLV-III" or not-infected, and the material from these cultures which bands at 1.16 gm/ml, 116 should contain HTLV-I, and thus RT and retroviral particles. Furthermore, because about 25% of AIDS patients have antibodies to HTLV-I, and the immunogenic proteins of HTLV-I and HIV have the same molecular weights, then approximately 25% of the non-infected HTUT78 (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 HTUT78 cells and the Western blot 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 are homologous. Thus, it means that the HTUT78 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 and thus transfection of "HIV DNA" into the host cell genome and its transcription into RNA, must also prove that the RNA is transcribed into proteins.
insects (see 6.4.4). It is a fact that:

(a) hybridization of nucleic acids of "exogenous retroviruses" “from different species gives a pattern which is the same as the phylogenic relatedness among their natural hosts”;225 a relationship which led retrovirologists including Gallo to conclude that exogenous retroviruses "are derived from cell genes”.

(b) Inter-experimental human retroviruses has been "shown" using hybridization probes derived from endogenous and exogenous ani- mal 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 hybridize with "HIV DNA" probes. There can be two reasons why this has not been demonstrated: (a) Most of the positive results in "uninfected cells" have been associated with reverse transcription of RNA to DNA; 125 (italics ours); the normal human cells used as a source of RNA are actively dividing plasmic RNA from leukemic, but not normal white blood cells. A difference in the 1970s, Gallo, Gillespie and their colleagues were saying that the success of the "hybridization assay appears to depend on the biologi- cal history of the virus", and on the physiological state of the cells.125, 127 The 8.1.2 Most of the positive results in "uninfected cells" have been reported by themselves; (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.

8.1.3 Montagnier and his colleagues reported the "HIV DNA" to be 9 ± 1.5 Kb whereas Gallo and his colleagues reported that "the overall length of the HTLV-III provirus is approximately 10 kilobases".14 In Levy and colleagues’ first study of the "HIV genome”, the "broad band (>15 kb) represents provirus integrated into host cell DNA". 20 In 1995, Pasteur researchers reported that "the complete 9153- nucleotide sequence of the probable causative agent of AIDS, lymphadenopathy-associated virus (LAV), has been determined. The nucleic acid sequence from LAV is identical to that of HTLV-III in the 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 B10 but also added, "the sequence of the remaining 182 bp of the HTLV-III provirus is not present in the previously reported 9153 nucleotides. 20, 21 The previous HTLV-III proviral DNA was sequenced to almost full length".

8.1.3 Montagnier and his colleagues reported the "HIV DNA" to be 9 ± 1.5 Kb whereas Gallo and his colleagues reported that "the overall length of the HTLV-III provirus is approximately 10 kilobases".14 In Levy and colleagues’ first study of the "HIV genome”, the "broad band (>15 kb) represents provirus integrated into host cell DNA”. 20 In 1995, Pasteur researchers reported that “the complete 9153- nucleotide sequence of the probable causative agent of AIDS, lymphadenopathy-associated virus (LAV), has been determined. The nucleic acid sequence from LAV is identical to that of HTLV-III in the 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 B10 but also added, "the sequence of the remaining 182 bp of the HTLV-III provirus is not present in the previously reported 9153 nucleotides. 20 The previous HTLV-III proviral DNA was sequenced to almost full length”.210

(c) there are structural and functional abnormalities in the lympho- cyte 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 repair DNA damage (2-2.5 times lower) compared to healthy persons”231.

(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 from 600 bp to full length provirus were present in PBMC from HIV- infected persons... Defective genomes tended to gradually disappear from 600 bp to full length provirus were present in PBMC from HIV- infected persons... Defective genomes tended to gradually disappear from 600 bp to full length provirus were present in PBMC from HIV- infected persons..."5 Defective genomes tended to gradually disappear from 600 bp to full length provirus were present in PBMC from HIV-infected persons... Defective genomes tended to gradually disappear from 600 bp to full length provirus were present in PBMC from HIV-infected persons... Defective genomes tended to gradually disappear from 600 bp to full length provirus were present in PBMC from HIV-infected persons... From 600 bp to full length provirus were present in PBMC from HIV-infected persons... .232 As far as Gallo is concerned, it is not even a requirement that the "HIV" genome possesses any genes whatsoever to be pathogenic, “This suggests that defective viros such as RNA- free particles and/or viral proteins expressed in the absence of parti- cle formation contribute to AIDS pathogenesis”114.

(e) According to the HIV experts, the defective genomes are “res- cued” 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 retro- viral sequences and cellular sequences, for example, non-retrovi- nal retroelements? As has been pointed out, HIV researchers seldom use controls and to date those that have, failed to use appropriate controls, that is, mis-
ues 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 others with similar but untested clinical evaluations, (a) who are exposed to agents (other than “HIV”) and does not exhibit 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 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?

8.2.2 To improve on the p24 assay, the DNA extracted from frozen uncultured PBMC of seven of their “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 culture, and the culture, the PBMC of 59 seropositive and 20 seronegative individuals were tested by both PCR and culture respectively. Culture was determined 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 7p-ended 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 acrylamide 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, the results were complementary. “Of the 59 positive PCR results, 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”. HIV-1 DNA was detected by PCR in the blood specimens of 63 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 hemophilics. 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 antibodies positivity is associated with present HIV-1 infection”. 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 study, the best illustrated in the WHA document, was Roland Kesper and colleagues. Between 1992-93 223244 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-Haus Chemotherapeutika Forschungsinstitut [GH] 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%)”. 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”...

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 positive. “Serial isolation of the same virus in mononuclear cells...supernatants” the p24 test is more likely to be positive than RT is not.
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 plaque count and immunofluorescence-dispersion assay” for relative comparison.

They reported that “HIV-1 DNA and ICD sera 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 small number of patients (5 < 10%) were positive by both the bDNA and ICD p24 assay. As expected, patients 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.”

How is it then possible to claim that “virtually all people who contain HIV DNA also contain antibodies against Montagier’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 segment from PCR products; did not test the sera of the patients 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 individuals with 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 retroviral antigen present, they are meaningless;
(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”. 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 the human immunodeficiency virus, pass into history as “non-viral material altogether”?

Indeed, as in the case of HL23V, it is 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”?

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