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continuum

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Where are these three or four papers? Where is even one paper where there is electron micrographic evidence revealing particles of any shape or form at the density of 1.16 gm/ml, the density that defines retroviral particles, let alone retrovirus-like particles with "No apparent differences in physical appearances' as Sinoussi and Chermann wrote in 1973 or, as Beard much earlier wrote, "homogeneous with respect to particle kind"?

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As far as "HIV particles look different" is concerned, in cultures of tissues from AIDS patients one can see a "zoo" of particles with varying morphologies. For example:

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In view of the above, the question then arises if the particles with the "unique" morphology considered to be HIV represent an exogenous retrovirus originating from tissues of AIDS patients or those at risk, then what is the origin and role of the many non-HIV particles and which, if any, of these particles or the "HIV particle" band at 1.16 gm/ml?

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One cannot talk about "HIV antibodies" as being synonymous with "HIV infection" unless one has proof that the antibodies present in sera are specific to HIV. The only way to obtain such scientific proof is to use HIV isolation as a gold standard. To date, since HIV has not been iso-lated, no such proof exists<sup>21,22</sup>. However, as far back as 1934, Andrews, addressing the Royal College of Physicians in London on the subject of the Rous sarcoma retrovirus presented data that anti-retroviral antibodies are non-specific: "Most viruses evoke the production of antibodies which are demonstrated by their power of neutralising the virus in question when mixed with it in vitro...Normal fowls, particularly as they grow older, may develop in their sera varying amounts of similar neutralising properties...It is likely, therefore, that the antibodies in the birds with chronic tumours represent only an enhancement of a property occurring to a varying degree in normal birds"

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The main immunogenic (antibody generating) retroviral proteins are said to be coded by two genes, gag and env. From the beginning it was known that the gag gene of retroviruses is present in all cells, including those that do not have retroviral particles and in fact this observation forms the basis of the oncogenic theory of cancer. In 1970, Huebner, one of the originators of

this theory wrote: "Natural history studies of the prevalence of the gs [gag] antigen [protein] in virus-free laboratory mice revealed gs antigens in high titers in the hematopoietic tissues of individuals of most mice strains"<sup>24</sup>. One year later Robin Weiss wrote: "The idea that normal cells of chickens might contain avian tumor virus genomes first arose from the observation that normal embryonic tissues of some "leukosisfree" chicken strains possessed an antigen which was indistinguishable from the groupspecific (gs) antigen of avian tumor viruses' The p17/18 and p24 proteins of "HIV" are said to be coded by its gag gene. The evidence that the p18 and p24 proteins (and antibodies) are non-specific is overwhelming and can be illustrated by a few examples:

(a) Genesca et al conducted WB assays in 100 ELISA negative samples of healthy blood donors; 20 were found to have HIV bands (antibodies) which did not fulfill the then (1989) criteria used by the blood banks for a positive WB. These were considered as indeterminate WB, (WBI), with p24 being the predominant band, (70% of cases). Among the recipients of WBI blood, 36% were WBI 6 months after transfusion, but so were 42% of individuals who received WB-negative samples. Both donors and recipients of blood remained healthy. They concluded that WBI patterns "are exceedingly common in randomly selected donors and recipients and such patterns do not correlate with the presence of HIV-1 or the transmission of HIV-1", "most such reactions represent false-positive results" (b) According to researchers from Germany and the United Kingdom (Wellcome Research Laboratories), "Western blotting should not be used as a screening assay because rates of up to 20% indeterminate results are found in blood donors"<sup>2</sup>

(c) In most cases, by "HIV isolation" is meant detection of p24 in cultures. However, in cultures with whole unfractionated blood, positive results have been reported in 49/60 (82%) of "presumably uninfected, but serologically indeterminate" individuals and in 5/5 "seronegative blood donors"<sup>28</sup>;

(d) Detection of p24 has been also reported in organ transplant recipients. In one kidney recipient (the donor was negative for p24 antigen) who, three days following transplantation developed fever, weakness, myalgias, cough and diarrhoea, all "Bacteriological, parasitological and virological samples remained negative [including HIV PCR]. The only positive result was antigenaemia p24, positive with Abbot antigen kits in very high titers of 1000pg/ml for polyclonal and 41pg/ml for monoclonal assays. This antigenaemia was totally neutralizable with Abbott antiserum anti-p24...2 months after transplantation, all assays for p24-antigen became negative, without appearance of antibodies against HIV. Five months after transplantation our patient remains asymptomatic, renal function is excellent, p24 antigenaemia still negative and HIV antibodies still negative"29 Using two kits, the Abbott and Diagnostic Pasteur, in one study, p24 was detected tran-siently in 12/14 kidney recipients. Peak titres ranged from 850 to 200,000 pg/ml 7-27 days post-transplantation. Two heart and 5/7 bone marrow recipients were also positive, although the titres were lower and ranged from 140-750 pg/ml. Disappearance of p24 took longer in kidney (approximately 6 months) than in bonemarrow (approximately 4-6 weeks) recipients. According to the authors: "This may be related to differences in immunosuppression therapy" Discussing their findings they wrote: "The observation of a 25-30kD protein [the French researchers report p24 as p25] binding to polyclonal anti-HIV human sera after immunoblots This with reactive sera raises several questions. protein could be related to a host immune

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from the Veterinary Medical Teaching Hospital, University of California, Davis" tested in commercial Western blot assays, "reacted with one or more HIV recombinant proteins [gp120— 21.5%, gp41—23%, p31—22%, p24— 43%]<sup>"34</sup>.

(g) According to Philip Mortimer and his colleagues from the UK Public Health Laboratory Service: "Experience has shown that neither HIV culture nor tests for p24 antigen are of much value in diagnostic testing. They may be insensitive and/or non-specific"<sup>35</sup>.

Regarding antibodies found in human sera which react with the envelope proteins (p41, p120, p160), in 1981 Gallo accepted the evidence that the antibodies which reacted with retroviral glycoproteins were directed not against the proteins "but against the carbohydrate moieties on the molecule that are introduced by the host cell as a post-transcriptional event, and which are therefore cell-specific and not virus-specific"<sup>36</sup>. This is amply confirmed today for the HIV envelope glycoproteins by many HIV researchers including the 1994 studies of Essex and his colleagues<sup>37</sup>.

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What has been published is pictures of viruslike particles present in cell cultures where several types of particles are present and some are arbitrarily said to be HIV. There are no published EMs of material banding in sucrose density gradients.

### 11. "...is next to impossible to remove all other debris from the culture..."

It may not be possible for "HIV" but animal retroviruses have been isolated by banding in density gradients (see EM in Pasteur/Spectra publications).

12. "...it's like saying that it is impossible to

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#### identify a German shepherd dog by its unique appearance, if it happens to be surrounded by a pack of poodles".

How does one look at a zoo and know one has a German shepherd or a poodle? The differentiation between a German shepherd and the remainder of the universe including poodles is possible only because German shepherds are obtained separate from all other objects in the universe and shown to possess unique morphology, constituents and behaviour such as walking, barking and biting. The analogy with HIV is more like someone who does not know what a German shepherd is but who looks at an aerial photograph of a zoo, expects to see dogs (retroviruses) but all he sees is many objects some of which look like animals (viruses) and decides that one of the objects is a dog, in fact a dog with unique composition and behaviour without first showing the object is:

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If the virus-like particles seen in cultures of tissues of AIDS patients and those at risk are HIV, what then are the particles seen by Weiss and his colleagues in cultures of patients with common variable hypogammaglobulinaemia "which on electron microscopy showed a retrovirus morphologically indistinguishable from HTLV-III/LAV [HIV] and animal lentiviruses? Supernatant from this co-culture was positive by reverse transcriptase, and the cells were positive by immunofluorescence with serum from a patient with AIDS and with the anti-HTLV-III monoclonal antibodies to p24 and to p19 (from Dr. R. C. Gallo) indicated that the viral genome showed homology to HTLV-III/LAV"38 According to Weiss: "It has long been known from electron microscope and immunofluorescent studies (24) that HIV is found in massive amounts in the lymph nodes, even in the asymptomatic phase of infection"<sup>2</sup> Firstly, the authors of reference 2439 did not claim to have proven the existence of HIV par-"retrovirus-like particles". If the virus-like particles seen in the lymph nodes of AIDS patients and those at risk are HIV, then what are the particles with identical morphology seen with the same frequency in the enlarged lymph nodes of patients who do not have AIDS and who are not at risk of developing AIDS? In a study conducted by O'Hara and colleagues from Harvard, "HIV particles" were found in 18/20 (90%) of patients with enlarged lymph nodes attributed to AIDS. However, the identical particle was also found in 13/15 (87%) of patients with enlarged lymph nodes not attributed to AIDS leading the authors to conclude, "The presence of such particles does not, by themselves indicate infection with HIV"40

### 13. "...the insistence that the experiment must start with pure particles makes this unattainable".

If the proof of the existence of pure particles is unattainable then:

(a) how can one claim virus purification or isolation? Isolation means obtaining an object separate from everything else that is not that object;

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(c) how can one claim that the effects, if any,

of "HIV" are caused by "HIV" and not by impurities?

(d) since no EM has been published showing virus-like particles in the material which bands at 1.16 gm/ml, how can one know that such particles, pure or impure, are present at the retroviral density?

### 14. "...grow HIV isolates..."

How can one grow HIV isolates when the virus has not been isolated?

#### 15. "HIV's genetic material, on the other hand, can be purified"

A critical analysis of the HIV literature shows that by "HIV genome" is meant nothing more than the selection of part of the RNA from cultures which bands at a density of 1.16 gm/ml. Since no evidence exists for the presence of retroviral particles at this density, it is impossible to say that such RNA belongs to HIV or even to a virus-like particle.

### 16. "Gene cloning techniques allow

### researchers to extract the viral genes found in HIV-infected cells

This cannot be the case unless one first has nucleic acids which have been proven to belong to a unique retroviral particle, which can be done only by isolating the particle

#### 17. "When the complete set of genes is reintroduced into healthy human cells in culture, the cells produce HIV particles

In the vast HIV literature there is not one paper with such evidence.

#### 18. "It would clearly be unethical to inject these particles into humans to see if they caused AIDS

If it is impossible to obtain such evidence, or to

### References

I. Beard JW. Physical methods for the analysis of cells. Annals of the New York Academy of Sciences 1957;69:530-544.

1957;69:530-544. 2. Toplin I. Tumor Virus Purification using Zonal Rotors. Spectra, 1973;No. 4:225-235. 3. Sinoussi F, Mendiola L, Chermann JC. Purification and partial differentiation of the particles of murine sarco-ma virus (M. MSV) according to their sedimentation rates in sucrose density gradients. Spectra, 1973;4:237-243. 4. Bader JP. Reproduction of RNA Tumor Viruses. In: Fraenkel-Conrat II, Wagner RR, ed. Comprehensive Virology. New York: Plenum Press, 1975; 253-331. vol 4 5. Ternin HM, Baltimore D. RNA-Directed DNA Synthesis and RNA Tumor Viruses. Advances in Virus Research

and RNA Tumor Viruses. Advances in Virus Research, 1972.17.129-186

6. Weiss R, Teich N, Varmus H, Coffin J. In: RNA Tumor

Weiss R, Teich N, Varmus H, Coffin J. In: RNA Tumo Viruses. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1982:
 Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Causer D. Factor VIII, HIV and AIDS in haemophili-acs: an analysis of their relationship. Genetica, 1995;95:25-50.
 Gelderblom HR, Uzel M, Hausmann EHS Winkel T, Pauli G, Koch MA. Fine Structure of Human Immunodeficiency Virus (HIV), Immunolocalization of Structural Proteins and Virus-Cell Relation. Micron Microscopica, 1988;19:41-60

Microscopica, 1988;19:41-60. 9. Frank H .Retroviridae In: Nermut MV, Steven AC, ed.

Perspectives in Medical Virology. New York: Elsevier, 1987: 253-256. vol 3). 10. Hockley DJ, Wood RD, Jacobs IP. Electron

Microscopy of Human Immunodeficiency Virus. Journal of General Virology, 1988;69:245s-2469. 11. Lecatsas G, Taylor MB. Pleomorphism in HTLV-III, the AIDS virus. South African Medical Journal, 1986;69:793-794.

1986;69:793-794. 12. Palmer E, Sporborg C, Harrison A, Martin ML, Feorino P. Morphology and immunoelectron microscopy of AIDS virus. Archives of Virology, 1985;85:189-196. 13. Dourmashkin RR, O'Toole CM, Bucher D, Oxford JS. The presence of budding virus-like particles in human lymphoid cells used for HIV cultivation. VII International Conference on AIDS. Florence: , 1991:122. 14. Barré-Sinoussi F, Chermann JC, Rey F. Isolation of a T-Lymphotrophic Retrovirus from a patient at Risk for Acquired Immune Deficiency Syndrome (AIDS). Science, 1983;220:868-871.

1705.220.808-8/1. 15 . Klatzmann D, Barré-Sinoussi F, Nugeyre MT. Selective Tropism of Lymphadenopathy Associated Virus (LAV) for Helper-Inducer T Lymphocytes. Science, 1984;225:59-63. 16 Montanzia

16. Montagnier L. Lymphadenopathy-Associated Virus:

have an animal model, how can the claim that the cause of AIDS is HIV be justified?

### 19. "However, experiments with purified SIV, the monkey equivalent of HIV, have proved that the pure retrovirus causes the selective loss of CD4 cells resulting in an AIDS-like disease

(a) The evidence for SIV isolation and "purified" SIV is no better than that for HIV; (b) In most cases SIV, like HIV, has been "isolated" from cultures with the human leukaemic

cell line H9 (HUT78) a cell line which Gallo claims to have shown contains the HTLV-1 genome, a "human retrovirus"<sup>41</sup>.

(c) The effects obtained when animals are injected with "SIV" have nothing to do with the AIDS diseases. In fact, in many cases, they may represent nothing more than graft vs host effects.

(d) Even if the diseases were similar or identical to AIDS they may be the result of impurities in the "SIV preparations" and not to SIV.

#### 20. "Moreover, three American laboratory workers have been infected with purified HIV

How is it possible to prove this when the "insistence that the experiment start with pure particles" is "unobtainable"?

#### 21. "By 1993, all three had developed low CD4 counts and one had been diagnosed with PCP, proving the link between HIV, immune suppression and AIDS

Even if these individuals were proven to have repeatedly low CD4 counts and to have PCP diagnosed by lung biopsy and not by the nonspecific methods presently used, it does not mean that these abnormalities are caused by HIV. The existence of low CD4 counts and the

From Molecular Biology to Pathogenicity. Annals of Internal Medicine, 1985;103:689-693. 17. Gallo RC, Shaw GM, Markham PD. The etiology of AIDS. In: de Vita V, Hellman S, Rosenberg SA, ed. AIDS etiology, diagnosis, treatment, and prevention. New Vorty, J. P. Uppingert Composity, 1005: 21-51 York: J. B. Lippincott Company, 1985: 31-51. 18. Levy JA, Hoffman AD, Kramer SM, Landis JA, Shimabukuro JM, Oshiro LS. Isolation of lymphocyto-pathic retroviruses from San Francisco patients with

pathic retroviruses from San Francisco patients with AIDS. Science, 1984:225:840-842. 19. Munn RJ, Preston MA, Yamamoto JK, Gardner MB. Ultrastructural comparison of the retroviruses associated with human and simian acquired immunodeficiency syn-dromes. Laboratory Investigation, 1985;53:194-199. 20. Orenstein JM, Meltzer MS, Phipps T, Gendelman HE. Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony-stimulating factor-I-treated human mono-cytes: an ultrastructural study. Journal of Virology, 1988;62:2578-2586.

21. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM. Is a Positive Western Blot Proof of HIV Infection?

JM. Is a Positive Western Blot Proof of HIV Infection?
Bio/technology, 1993;11:696-707.
Papadopulos-Eleopulos E, Turner VF, Papadimitriou
JM. Has Gallo proven the role of HIV in AIDS?
Emergency Medicine [Australia], 1993;5:113-123.
Andrews CH. Viruses in relation to the aetiology of tumours. Lancet, 1934;ii: 117-124.
Huebner RJ, Kelloff GJ, Sarma S, Lane WT, et al. Group-specific antigen expression during embryogenesis of the genome of C-type RNA tumor virus: implications of ontogenesis and oncogenesis. Proceedings of the National Academy of Sciences of the United States of America, 1970;67:366-376.
Weiss RA, Friis RR, Katz E, Vogt PK. Induction of avian tumor viruses in normal cells by physical and

 Weiss RA, Fills RR, Katz E, Vogi PK. Induction of avian tumor viruses in normal cells by physical and chemical carcinogens. Virology, 1971;46:920-938.
 Genesca J, Jett BW, Epstein JS, Shih JWK, Hewlett IK, Alter HJ. What do Western Blot indeterminate pat-terior. IR, Alter HJ, What do Western Blot Indeterminate patterns for Human Immunodeficiency Virus mean in EIA-negative blood donors? Lancet, 1989;II: 1023-1025. 27. Weber B, Hess G, Enzensberger R, Harms F, Evans CJ, Hamann A, Doerr HW. Multicenter evaluation of the novel ABN Western blot (Immunoblot) system in compar-ison with an enzyme-linked immunosorbent assay and different uncertare blot. Inversel of Ciliaiden. different western blot. Journal of Clinical Microbiology 1992;30:691-697.

28 Schupbach J, Jendis JB, Bron C, Boni J, Tomasik Z. False-positive HIV-1 virus cultures using whole blood. AIDS, 1992;6:1545-1546.

29 Vincent F, Belec L, Glotz D, Menoyo-Calonge V, Dubost A, Bariety J. False-positive neutralizable HIV antigens detected in organ transplant recipients. AIDS,

AIDS-like diseases are nothing new and are not specific to HIV. Furthermore, a superficial glance at the AIDS literature shows that no relationship exists between CD4 cell counts and the syndrome<sup>42</sup>. Indeed, in those at risk, low T4 cell counts frequently antedate "infection" with HIV which can be interpreted as low T4 cell counts being the "cause of HIV" and not vice versa.

### CONCLUSION

Retrovirus-like particles including particles with morphologies attributed to HIV are ubiquitous. The first absolutely necessary but not sufficient step in proving that the particles represent a retrovirus is to show that in sucrose gradients the particles band at the retroviral density of 1.16 gm/ml. The first absolutely necessary but not sufficient step in claiming the existence of a retroviral protein and genome is to prove that each belongs to one and the same type of retrovirus-like particle such as type C, type D or Lentiviruses.

No such evidence exists for the "HIV" particles, proteins are nucleic acids.

1993:7:741-742

1993; /: /41-742
 Agbalika F, Ferchal F, Garnier JP, Eugene M, Bedrossian J, Lagrange PH. False-positive HIV antigens related to emergence of a 25-30kD proteins detected in organ recipients. AIDS, 1992;6:959-962.
 Courouce A, Muller J, Richard B. False-positive Western blot reactions to human immunodeficiency virus in blood deprose. Japacet, 1094:ii:021 022

Western blot reactions to human immunodeficiency virus in blood donors. Lancet, 1986;ii:92I-922 32. Stricker RB, McHugh TM, Moody D J. An AIDS-relat-ed cytotoxic autoantibody reacts with a specific antigen on stimulated CD4 + T cells. Nature, 1987;327:710-713. 33. Parravicini CL, Klatzmann D, Jaffray P, Costanzi G, Gluckman JC. Monoclonal antibodies to the human immunodeficiency virus pl8 protein cross-react with nor-mal human tissues. AIDS, 1988;2:171-177. 34. Strandstrom HV, Higgins JR, Mossie K, Theilen GH. Studies with canine sera that contain antibodies which recognize human immunodeficiency virus structural pro-

recognize human immunodeficiency virus structural pro-teins. Cancer Research, 1990;50:5628s-5630s. 35. Mortimer P, Codd A, Connolly J, Craske J, et al. Towards error free HIV diagnosis: notes on laboratory practice. Public Health Laboratory Service Microbiology Digest, 1992;9:61-64. 36. Kalyanaraman VS, Sarngadharan MG, Bunn PA,

 Kalyanaraman VS, Sarngadharan MG, Bunn PA, Gallo RC. Antibodies in human sera reactive against an internal structural protein of human T-cell lymphoma virus. Nature, 1981;294:271-273.
 Kashala O, Marlink R, Ilunga M, Diese M, et al. Infection with human immunodeficiency virus type 1 (HIV-1) and human T cell lymphotropic viruses among leprosy patients and contacts: correlation between HIV-1 cross-reactivity and antibodies to lipoarabinomannan. Journal of Infectious Diseases, 1994;169:296-304.
 Webster ADB, Malkovsky M, Patterson S, North M, et al. Isolation of retroviruses from two patients with "common variable" hypogammadlobulinaemia. Lancet, "common variable" hypogammaglobulinaemia. Lancet, 1986;i:581-582

39. Armstrong JA, Horne R. Follicular dendtritic cells and virus-like particles in AIDS-related lymphadenopathy.

virus-like particles in AIDS-related lymphadenopathy. Lancet, 1984;ii:370-372. 40. O'Hara CJ, Groopmen JE, Federman M. The Ultrastructural and Immunohistochemical Demonstration of Viral Particles in Lymph Nodes from Human Immunodeficiency Virus-Related Lymphadenopathy Syndromes. Human Pathology, 1988;19:545-549. 41. Wong-Staal F, Gallo RC. Human T-lymphotropic retroviruses. Nature, 1985;317:395-403. 42. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Hedland-Thomas B, Causer D, Page B. A critical analysis of the HIV-T4-cell-AIDS hypothesis. Genetica, 1995;95:5-24.