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Of Mice And Men; Viral Etiology Of Human Cancer:
A historical perspective

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About the author:

Etienne deHarven worked in electron microscopy (EM) primarily on the ultra-structure of retroviruses throughout his professional career of 25 years at the ***Sloan Kettering Institute*** in New York and 13 years at the ***University of Toronto***. In 1959 he was the first to report on the EM of the Friend virus in murine (mouse) leukaemia, and in 1960 to coin the word "budding" to describe steps of virus assembly on cell surfaces. He is a member of the Scientific Advisory Board of ***IFAS*** and the ***Presidential AIDS Advisory Panel of South Africa***.

The hypothesis according to which some human cancers might be caused by viruses is placed in an historical perspective. The contrast between the period 1945-1970, dominated by electron microscopy, and the post-1970 era, dominated by molecular biology is emphasised. Demonstrating association with viruses is far from enough to establish an etiological relationship. The overall impression is that possible etiological relationships belong more to DNA than to RNA viruses. Pitfalls in the molecular approach to the study of RNA viruses, and in particular of retroviruses, are underlined in reference to to-day's HIV research.

The hypothesis according to which some human cancers might be caused by filterable micro-organisms such as viruses is almost one hundred years old. It was indeed in 1903 that Borrel, in France, suggested such a possible relationship. To put this hypothesis in an historical perspective one should refer to the book "The Riddle of Cancer" *(1)* which Charles Oberling published in 1952 and in which the possible role of viruses in human cancer was presented with extensive references to contributions of initial pioneers such as Rous, Shope, and Bittner. Since our purpose is, to some extent, focussed on the evolution of methodologies which cancer researchers utilised in attempts to verify the hypothesis, one should emphasise that the approach of Rous and his followers was essentially based on establishing the difference between transmission of tumours and leukaemias by cell transplants, i.e. grafts, or by cell-free filtrates. Transmission of tumours in experimental animals by cell-free filtrates was always interpreted as demonstration of viral etiology.

During the past fifty years, viral oncology has been studied in almost all cancer research centers, world wide. Practical results in terms of effective therapy of human malignancies have been nil. But still, recent issues of all the main oncology publications contain numbers of studies related to viral oncology, clearly indicating that the hypothesis still has considerable momentum!

The past fifty years can be analysed in two distinct periods. The first, between 1945-1970 was dominated by electron microscopy; the second, from 1970 until now, being dominated by molecular biology.

Electron microscopy (EM) contributed a considerable amount of data, which can briefly be summarised as follows.

- EM can readily demonstrate associations between viruses and cancers of several laboratory animal species, such as chickens and mice *(2, 3)*.
- However, EM data by themselves do not prove any role of these viruses in the etiology of the tumours *(4)*. The EM data did, however, trigger microbiological experiments, based on ultrafiltration, which frequently brought scientific evidence for etiological relationships.
- As pointed out by André Lwoff et al. *(5)* in 1962, electron microscopy is probably the most efficient approach to viral classification.
- Viruses shown to be associated with several cancers of laboratory animals belong to various families of viruses (herpes, vaccinia, papova, retroviruses, DNA, RNA,...) and are not restricted to any one family.
- Viruses associated with some cancers and those responsible for infectious diseases look identical. There is no such thing as a family of oncogenic viruses, a terminology, which never appears in general classification of viruses and should actually be regarded as a misnomer.
- Practically, EM is essential to monitor the level of success in the sequential steps leading to virus isolation and purification. Therefore, the success of biochemical characterisation of viral markers depends on electron microscopy to ascertain the purity of viral isolates and the

absence (or minimal amounts) of non-viral contaminants.

- Finding particles with typical viral morphology does not mean that these viruses are pathogenic. Actually, there are probably many more non-pathogenic than pathogenic viruses. This point was well illustrated in a special conference sponsored around 1960 by the New York Academy of Sciences under the title "Viruses in Search of Disease".

- Viruses, infectious or cancer-associated, rarely satisfy all the Koch postulates which, incidentally, were presented before viruses were discovered.

- While the association between viruses and numerous malignancies of laboratory animals has been readily demonstrated by electron microscopy, and in spite of considerable efforts, similar associations have never been observed in human cancers *(4)* (with very rare exceptions such as common wart and molluscum contagiosum...)

"Of Mice and Men"...

Research in viral oncology changed drastically around 1970, when methods of molecular biology took the lead, while electron microscopy was relegated to a distant background.

Dedicated "virus hunters", as Peter Duesberg *(6)* would call them, were obviously not discouraged by the negative results of twenty years of active search for viruses by electron microscopy in many types of human cancers. To the contrary, large research programs were initiated, based primarily on the identification of viral, molecular "markers" such as enzymes, nucleic acids or proteins identified most frequently in cell cultures derived from human malignancies, rarely directly from the tumour tissues or blood plasma. The fact that viruses had never been directly observed in human tumours by electron microscopy was conveniently explained in terms of virus latency, and/or by integration of a provirus in the genome of tumour cells.

The most significant example illustrating this drastic change in the approach is given by the reverse transcriptase enzyme, discovered in 1970 by Temin in purified Rous sarcoma virus *(7)* and by Baltimore in Rauscher mouse leukaemia virus *(8)*. This discovery was regarded as historical. It resulted in two Nobel prizes and in the renaming of all RNA tumour viruses as "retroviruses". DNA synthesis from an RNA template was indeed a very surprising observation in 1970. The enzyme was initially thought to represent a unique feature of RNA tumour viruses and was, therefore, regarded as a reliable "marker" for the presence of "retroviruses", even when retroviruses particles were never convincingly observed with the EM. We learned, later on, that reverse transcription is a common phenomenon, that the enzyme (RT) is present in many different cells *(9)*, and that demonstration of RT activity is far from enough to substantiate any claim for the isolation of any "retrovirus".

GENERAL CONSIDERATIONS ON STUDIES RELATED TO THE HYPOTHETICAL VIRAL ETIOLOGY OF SOME HUMAN CANCERS.

Contemporary viral oncology research is primarily based on the identification of viral markers such as proteins or nucleic acids.

However, the specificity of viral markers depends on the success of virus isolation and purification. Without fully demonstrated success in virus isolation and purification, identification of "viral markers" is extremely hazardous and can lead to severe misinterpretation of clinical data. A dramatic illustration of this is to be found in current HIV research. In this case, the virus (HIV) has never been properly isolated, since sedimentation in sucrose gradient at the density of 1.16 gm/ml was erroneously considered to yield "pure virus", systematically ignoring that material sedimenting at that density contains large amounts of cell debris and microvesicles *(10, 11)*. Therefore, proteins and nucleic acids found in such "1.16 bands" are very likely to be of cellular origin and cannot be used as viral markers. Such a faulty methodology has had extremely serious consequence, i.e. the world-wide use of HIV-antibody tests, Elisa and Western Blot, which dangerously lack specificity, as demonstrated in 1993 by Papadopulos et al. *(12)*, in Australia.

Admitting, however, that some viral markers can be specific, their presence within tumour cells will probably never show more than an association. Etiological relationships are unlikely to be demonstrated by the presence of markers, even if these markers are related to the viral genome. One has difficulties in following Levin and Levine *(13)* when they state that the identification of the viral genome in the tumour cells is "the strongest evidence for its activity as an oncogenic agent". This is reminiscent of an old problem when electron microscopy was only showing association with viruses, but never their etiological significance.

In microbiology, most viral diseases are highly contagious. If some forms of cancer had viral etiology, how is it that we don't see more cancer "clusters"? Clusters have been occasionally observed, but their number is very small and is certainly not compatible with the concept of primary infections. We know that EBV is a ubiquitous virus. And, as T. Osato *(14)* points out, "ubiquity and oncogenicity are seemingly incompatible". But we are not aware of the ubiquity of HHV-8, and we don't see any evidence for clusters of HHV-8 associated malignancies?

An area in which progresses have been highly significant is unquestionably that of apoptosis. Thirty years ago, viruses were regarded as either cytolytic or non-cytolytic. This property was considered as an intrinsic characteristic of the virus itself. Today, factors controlling cell cycle are much better understood, and the cell cycle appears as a fragile balance between apoptotic cell death and cell immortalization. Suppression of apoptosis may contribute to cancer. As studied at the Ludwig Institute in London *(15)*, it appears, for example, that over-expression of the anti-apoptotic BCL-2 protein is a key event in follicular lymphoma. Factors interfering with the progression through the cell cycle are

many. Some are endogenous, some are exogenous. Some are chemical in nature, others are physical. Some could probably be added by the activation of latent viruses, like EBV. However, all experiments supporting this view are in vitro experiments. And it will take considerable clinical skill to demonstrate that these in vitro observations are of any significance in the sudden development of tumours in latently EBV infected individuals.

If viral markers show only "association", without implying etiology, this does not mean that the presence of such markers within cancer cells is not of possible therapeutic usefulness. "Targeting" is an interesting approach to chemotherapy, or to CTLs lymphocytes. A significant example for this can be found in the paper by Roskrow et al. *(16)* on EBV-specific cytotoxic T lymphocytes for the possible treatment of patients with EBV-positive relapsed Hodgkin's disease.

But what about antiviral therapy? Could it possibly be that its eventual success would produce the evidence for oncogenicity of some viruses, which we are so eagerly trying to establish?
For DNA viruses associated malignancies, we have at hand effective antiviral agents of manageable toxicity. This is not the case for RNA virus associated diseases, and in particular for syndromes such as AIDS, hypothetically associated *(6, 12)* with infection with the HI-retrovirus. In these cases, the currently used combined antivirals are unacceptably toxic, making the so-called "therapy" worse than the disease itself! Moreover, the effects of anti-retroviral therapy are currently measured by "quantitative" PCR technology. Unfortunately, Karry Mullis PCR technology is not reliable to measure what has been erroneously labelled "viral load" in AIDS patients *(17, 18)*.

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