Chapter 33

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A Retrovirus Aetiology for CFS?

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Dr. Holmes is a clinical immuno-virologist who qualified in Medicine at Liverpool, then spent seven years with the British Antarctic survey. After spells at the UK Common Cold Unit and the Clinical Research Centre, Harrow, he submitted his MD thesis and went to work for NASA on the Viking project. He is now Senior Lecturer at Otago Medical School, New Zealand and has been working on the peripheral blood cytology of CFS for the past four years. In 1986-87, work by Dr Holmes was the first to suggest the possibility of reverse transcriptase activity in CFS T cell cultures. (Several local graduate study papers were written describing this discovery.)

This discussion paper is offered more as a vehicle for airing limited findings and inferential material rather than for making any hard claims that we have established a retrovirus aetiology for CFS. The data are provocative and, in the light of other contemporary observations, would seem to hold up very well. I am, however, still 'flying a kite'. The findings we have rest on the results of a limited pilot study run in July and August 1986. It was shelved when funds ran out and, though we have been trying to obtain more ever since, they have not yet been forthcoming.

Briefly, we took 6 of the CFS patients referred to my laboratory by professor Murdoch of the Department of General Practice, Otago Medical School, in Dunedin, New Zealand, age and sex-matched them with 6 controls, and set up a series of matched patient and control lymphocyte cultures from specimens of peripheral blood taken by venepuncture from the antecubital vein.

Lymphocytes were separated on a Ficoll-Conray interface and cultured for extended periods in an at-

tempt to demonstrate evidence of chronic infection by a lymphotropic virus. Cultures were examined fro cytopathic effects in the lymphocytes, evidence of the presence of structures resembling virus particles and by assay of the culture supernatants for the presence of reverse transcriptase using an assay modified in this laboratory to enhance its sensitivity.

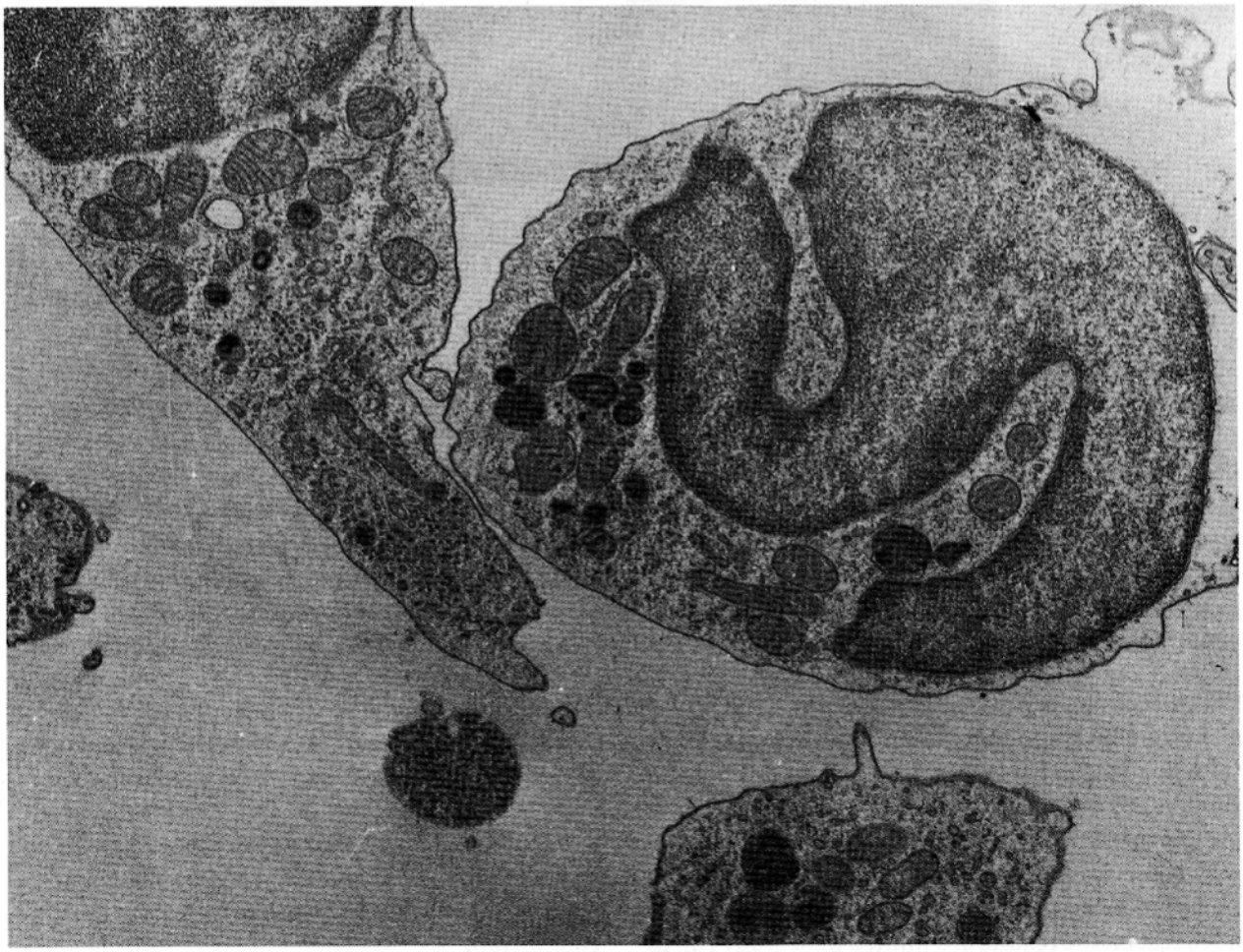
Before setting up the cultures, cytospin preparations were made and phenotyped by immuno-peroxidase labelling, according to the method of Moir and colleagues[1].

The cultures were sampled with each media change, aliquots of the supernatants tested for reverse transcriptase (RT) activity by conversion of the mixed oligonucleotide to its deoxy form in the presence either of magnesium or manganese ions. Duplicate experiments using the deoxy form of the oligonucleotide were carried out to assess the possible non-specific effects of cellular nucleases. All specimens were tested in triplicate, blind and randomised with the controls. A positive control of caprine arthritis-encephalitis virus RT derived from infected goat synovial membrane primary cell cultures was included with each experiment.

From each specimen, aliquots of cells were removed from the MLC for thin-section low power electron microscopy (EM) of the cellular morphologies. The EM preparations were not examined at high power for virus particles since at this time the machine was faulty and losing definition at higher magnifications.

A Powerful RT Inhibitor

Our findings were puzzling. At 6 days, that is, just before we added mitogen, RT activity was detected in culture supernatants from 4 out of the 6 patients at

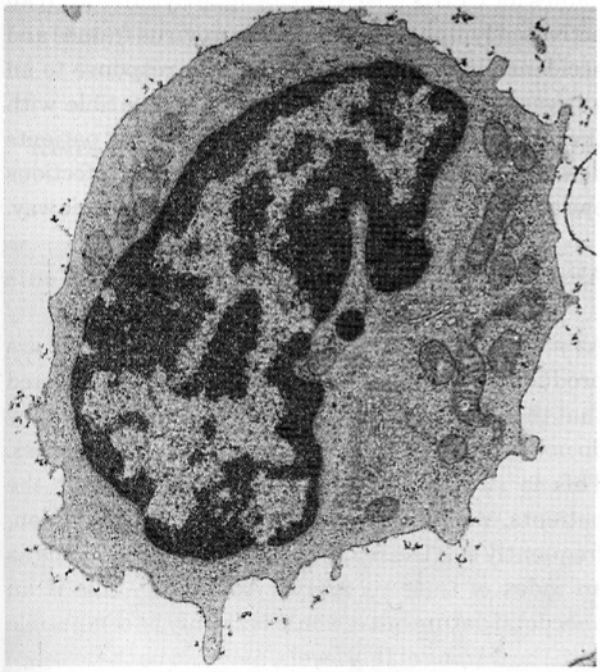


Abnormal Convoluted Nucleus of a Mononuclear Cell from a M.E. / CFS patient. This would be similar to what would be expected from an HIV/ARC syndrome patient.

levels from 1.5 to 4 times those of controls. Since each retrovirion has only two molecules of RT, this inferred that a significant quantity of virus was being produced, especially when the replication rate of this group is normally so slow. The DNA oligonucleotide controls were very low, so we were confident of our technique. However, at subsequent samplings the RT levels of the whole group were lower than those of the negative controls. It was not until some time later that we identified a powerful inhibitor in the mitogen preparation.

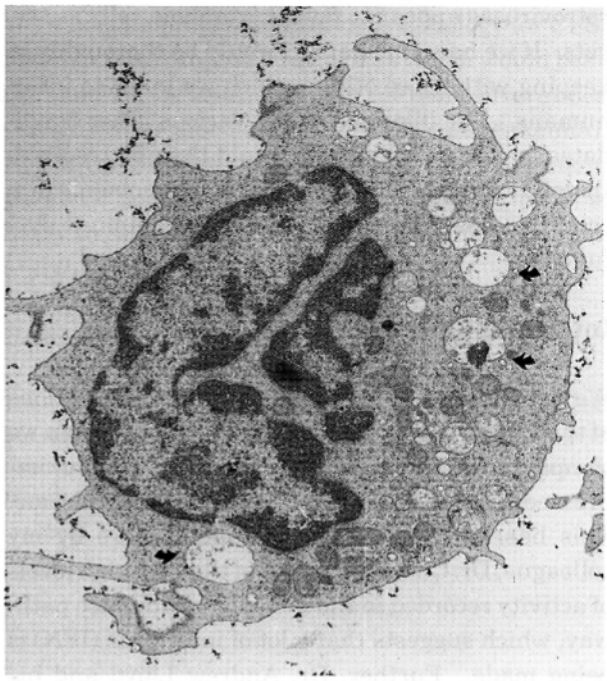
Abnormal Mononuclear Cells

The EM morphology studies also proved suggestive. In the earliest samples we found a proportion of mononuclear cells with convoluted nuclei comparable to those described in the ARC syndrome. These were not present in controls.



Mononuclear Cell, Normal Control (1986)

At 6 days some of the mononuclear cells had begun to develop vacuoles which had a reticular appearance and which also we were unable to observe in the controls. Later specimens began to show degenerative changes which we did not observe in controls, although cell losses began to increase by day 14 in both patient and control groups.



Vaculated Mononuclear Cell, CFS patient (1986)

The only other finding from this pilot study was a T helper cell cytopenia in the CFS patients which we later confirmed in more extensive investigations.

I would like to draw your attention to these observations in a broader context. The RT activity we recorded is not decisive but certainly merits further consideration and the apparent abnormalities we found in the white cells themselves would also benefit from a closer scrutiny. Within the limits of the trial we could scarcely have found anything more suggestive of retrovirus activity that was still consistent with the symptomatology of CFS. However, we would like to propose a retrovirus aetiology for CFS based not only on this pilot but on the train of deductive observation which led us to consider it in the first place.

Coxsackie Virus and Candida Albicans as Opportunists

First, I would ask you to consider the various pathogens from *Candida albicans* to coxsackie-viruses which have been proposed as causative agents. Practically all of them are highly persistent or latent organisms which tend to be found in immunologically embarrassed patients. They are not classical opportunists but they are not far off. Many human

retroviruses appear to favour lymphoid cells as targets. If we have one here, it would be thoroughly in keeping with those others which we know to infect humans. The dilemma of the various other candidates for the agent of CFS would then be resolved. Instead of rivals, they become epiphenomena in a condition now recognised as including immune dysfunction.

Interferon Poisoning

We were impressed by our patients' own descriptions of their condition as 'Poor man's AIDS' and, when we compared symptomatology, were even more impressed by the similarities to the ARC syndrome. This has been given a new impetus here by my colleague, Dr. Cheney's, report of the very high levels of activity recorded in the 2'-5' oligosynthetase pathway, which suggests that a lot of interferon (IFN) is being made. Further, Dr. Andrew Lloyd and his colleagues have pointed out the striking similarities of CFS symptoms to IFN poisoning. Let us consider, therefore, the possibility that our patients are making a lot of interferon.

A Chronic Persistent RNA Virus

If the CFS symptoms are due to IFN poisoning, they must have a prolonged stimulus to sustain IFN induction. They must, in fact, have a chronic infection. It must also be an intracellular infection, because IFN is induced only by the presence of foreign nucleic acids *inside* a cell. Furthermore, the most powerful IFN inducers are RNAs. In this scenario we therefore have a chronic, persistent, lymphotropic, RNA virus. We also have a plethora of low-grade, persistent, opportunist, epiphenomena which suggests a degree of immune dysfunction and we now have independent serological evidence of the latter.

A Possible Retrovirus

Now, a virus which induces IFN production normally kills the host cell after a varying period of virus production. Before this happens, however, IFN can diffuse to neighbouring cells rendering them less amenable to infection. Also, during virus production, the cell becomes immunologically 'visible' and thus subject to immune surveillance. Unless a cell is latently infected with a non-productive virus infection it is destroyed, and this is the basis of the cell-mediated immune response. The production of IFN can therefore be seen, in one aspect, as an interim measure to contain an infection until host defenses can be mobilised to control it. This does not happen in CFS for years, although it seems to eventually. And there is one group of viruses which can make an immunologically invisible DNA provirus copy of themselves which is inserted into a host cell chromosome, namely the Retrovirus.

This provirus copy can lie latent for a long time and is activated only when the host cell reads the frame under the impression it is making a bit of RNA to do some housekeeping job like making a protein. What it actually does is make a new infectious virus which can then induce IFN production. A significant percentage of CFS sufferers relate the onset of their condition to a "dose of 'flu" and indeed, one of the names for CFS is Post-viral Fatigue Syndrome. If an activated lymphocyte is reading provirus frames and accidentally making retroviruses in response to an antigenic challenge, this would be compatible with the CFS picture. Furthermore, many of our patients describe relapses as triggered by an acute infectious episode, which would also be explicable in the same way.

Absence of Common Colds in CFS patients

As a coda to the suggestion that CFS patients are producing sustained levels of IFN, we have noted that the majority of our patients maintain that they do not suffer from common colds during their illness. This is such a feature of the condition that the patients, without any reference to clinical opinion, frequently cite the reappearance of common colds as an index of their recovery. Admittedly this is an anecdotal datum but it would certainly be compatible with the waning of IFN levels, as would be the absence of common colds while IFN levels were sustained.

Finally, there is the strange biphasic nature of the condition: limited outbreaks with a persistent intercurrent trickle of cases. This would also be consistent with a retrovirus aetiology. The RT is an enzyme which forces a reaction the 'wrong way'. It is much easier to make RNA from DNA than vice versa and the DNA copies are not good. They are *low-fidelity* copies (which accounts for the rapid antigenic evolution of HIV). Every so often, therefore, a nastier

strain is going to appear, but, because of the essential genetic instability of the virus, it is unlikely to persist for long. This would cause the limited outbreaks. Apart from HIV, the majority of retrovirus infections seem to be occult and this would also be compatible with the sporadic cases which are seen.

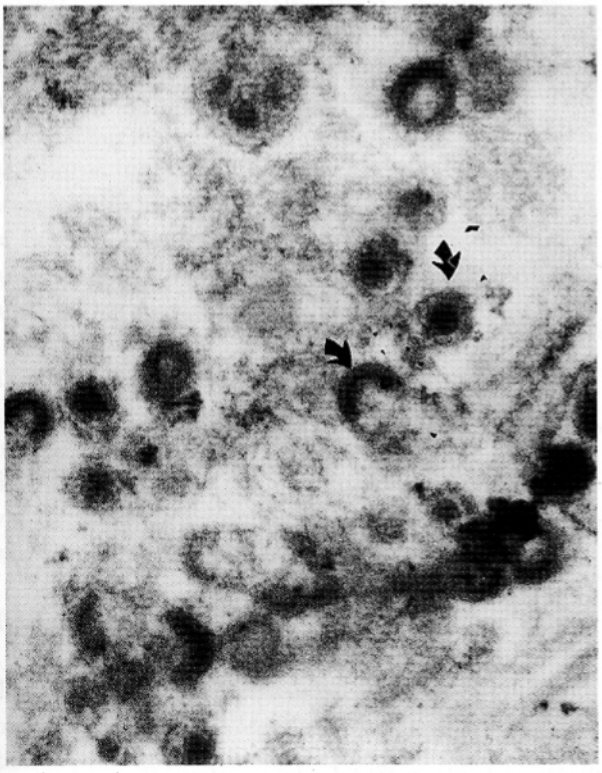
The sporadic cases would therefore represent the tip of the iceberg in a largely silent endemic or epidemic situation. The majority of infections would be occult and the unlucky patients would be those who triggered a whole series of latently infected cells with an ill-timed acute infection or an unhappy mutation with a bad provirus copy.

We therefore propose a retrovirus aetiology for CFS. Moreover, we propose a retrovirus which would bear the same sort of relationship to the more severe ones (such as those associated with AIDS and leukemia), that a common cold virus bears to its first cousin, paralytic poliomyelitis. We also suggest that it is likely to cause a high proportion of occult infections and that it is likely to be lymphotropic.

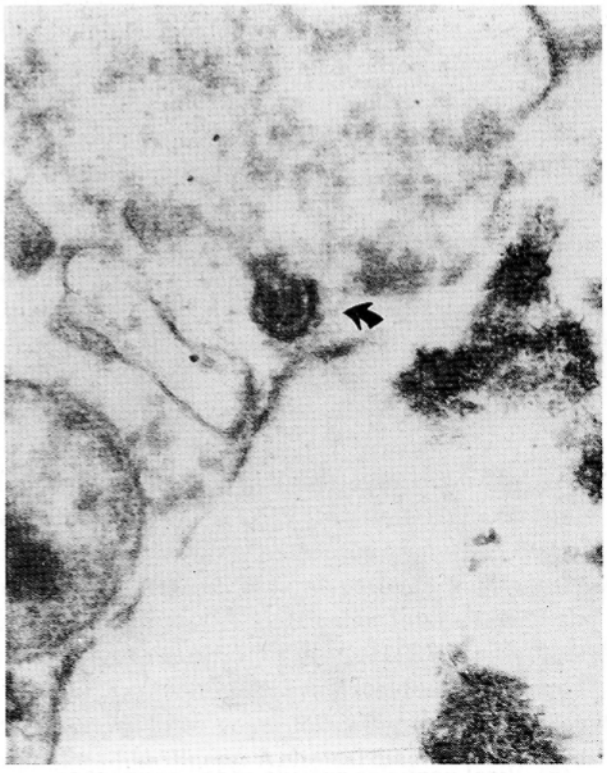
References

Moir, D.J., Ghosh, A.K., Abdulaziz, A., Knight, P.M. and Mason, D.Y. (1983). "Immunoenzymatic staining of haematological samples with monoclonal antibodies." **Brit. J. Haem. 55**: 395-410

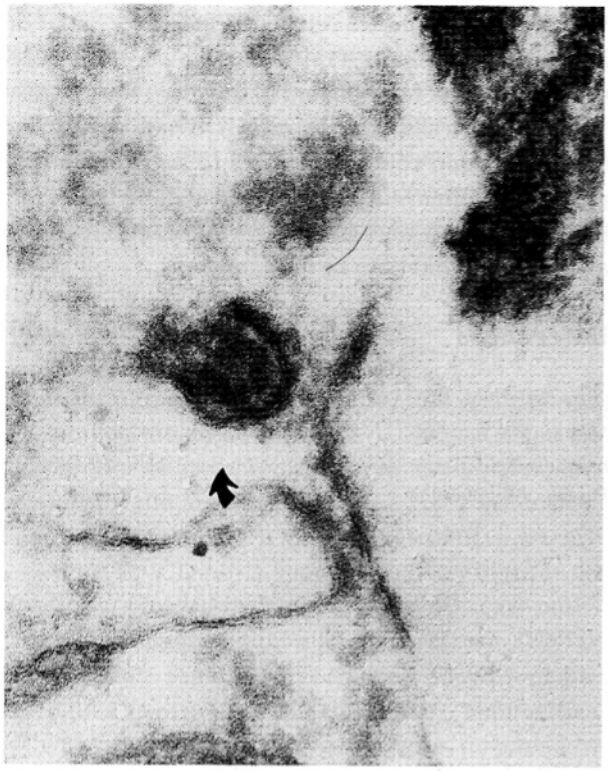
Retrovirus Particles (Presumed), Isolated and Grown by Dr. M. Holmes, from CFS patients



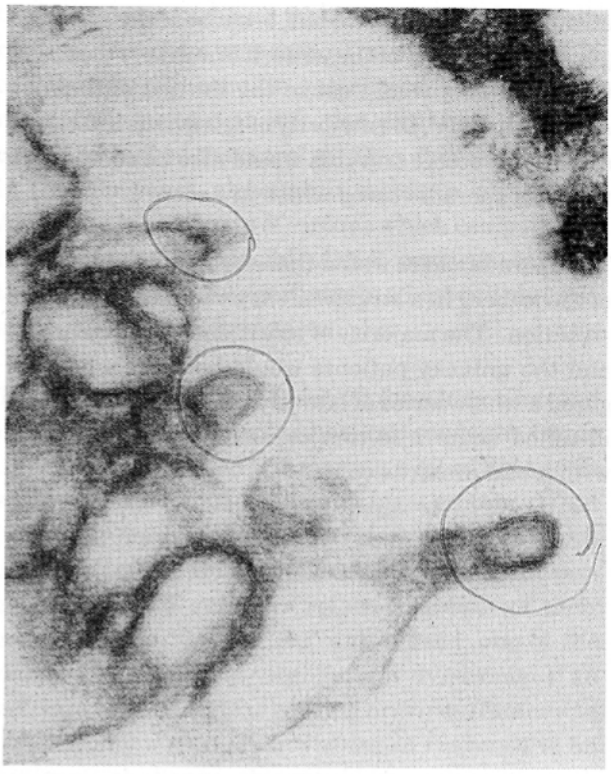
(1) Immature and maturing virions



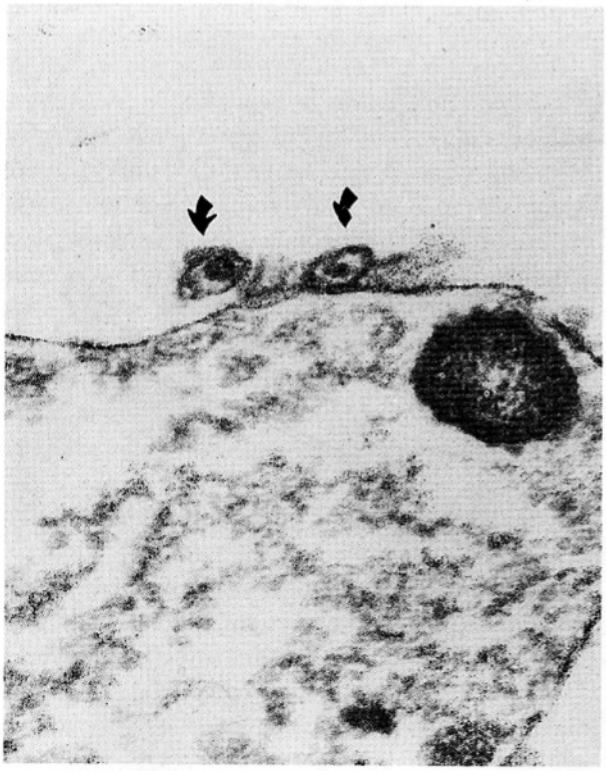
(2) Particle approaching cell membrane (low power)



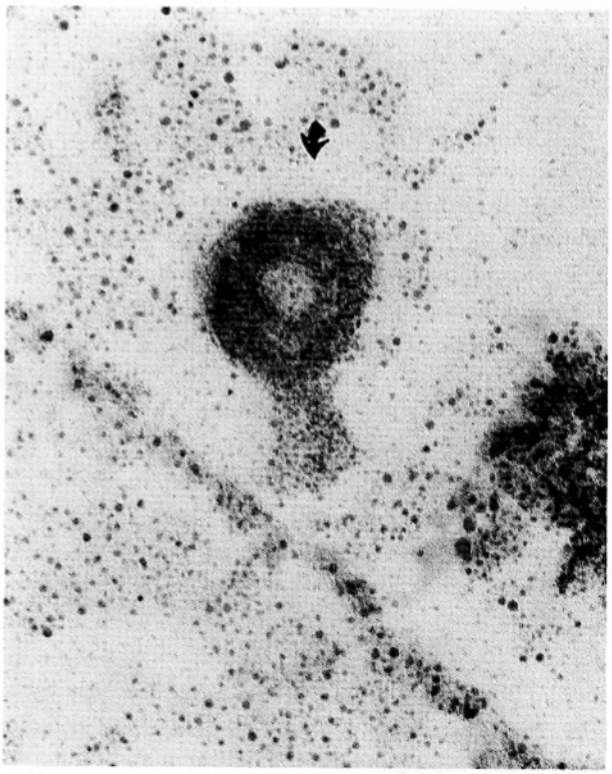
(3) Particle approaching cell membrane (high power)



(4) Particle budding across cell membrane.Note similarity of to budding retroviral particles of Dr. DeFreitas.



(5) Viral particles outside of cell membrane



(6) Particles just detached from the cell membrane (high power)