Medical College of Virginia
Virginia Commonwealth University

7/30/86

Perry A. Chapdelaine, Sr.
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Rt. 4, Box 137
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Dear Mr. Chapdelaine:

I have just received a letter from you dated 7/23/86 which has me both puzzled and disturbed. You presented three issues which I must emend.

First, with regard to the presence of brass at the circumference of the screen in our thermotropic isolation device: Copper salts ("copper in ionic presence," as you phrased it) are poisonous to most forms of animal and plant life. Protozoa may even have a lower threshold for copper toxicity than higher organisms. But brass is not poisonous. Although an alloy made of copper and zinc, any good chemistry textbook will inform you that copper and zinc readily form a homogeneous alloy, and by means of chemical analysis, brass is not simply a mixture of the two metals. Brass is an *intermetallic* compound of copper and zinc. It is highly resistant to chemical action and corrosion, and there is no ionic copper (the form of copper obtained, for example, by dissolving copper sulfate in water) in brass.

The ordinary rules do not hold for *intermetallic* compounds. You don't need a textbook to verify this. Consider such toxic heavy metals as lead and mercury. Solder is an alloy of lead and tin, universally used in making sweat joints linking *copper* pipe that conveys drinking water. Pewter, another alloy of lead and tin, contains antimony, also toxic. Dental amalgams are composed of "deadly" mercury and silver alloy.

But putting the academic arguments aside, we have done the laboratory experiments which prove that nothing in our thermotropic isolation apparatus has a deleterious effect on amebae. These I discussed in my letter of 7/23/86 to Dr. Pybus. To reiterate, during the prospective phase of our studies, in determining that amebae could migrate through a 5 micron filter, we also tested to be certain our procedure was not injurious to amebae by culturing the emigrants. Since these amebae grew as well as an unmanipulated control inoculum of the same size, *we knew before the first clinical specimen was processed* that the overall procedure was without pernicious effects. Retrospectively, as also detailed in my letter to Dr. Pybus, the presence in cultures

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of the very same brass rings employed in the apparatus had no noxious effects on several species of amebae. I would be glad to send you a set so that you may have this fact verified. In the face of these simple and direct experiments, conjecture that our methods for isolating amebae were affected by the brass retaining rings (*never in direct contact with the tissue and suspended above the collecting cup, I might add*) is disproven by the facts.

Negative findings were also obtained with tissues never placed in the thermotropic isolation unit. Among the various culture media we employ are Balamuth's and Nelson's media. For each biopsy sample, tubes of these were inoculated with fresh minced tissue (as well as others with the thermophilic filtrate), plus and minus heat-killed bacteria as food organisms. In histologic sections, using chemical and immunofluorescent staining techniques on formalin-fixed tissue and frozen tissue, amebae could not be detected. These procedures are not susceptible to interference by copper. Thus, all things considered, the suggestion that because brass contains copper our findings are invalid is specious at best, not supported by the facts concerning brass as an alloy, and contradicted by the laboratory evidence.

Regarding the second issue which was raised in your letter: We have done complete work-ups on thirty-two biopsy tissue samples to date. Considering that the work could not be initiated until funding from the Foundation was received in mid-September, and the time required in the process of hiring a technician, tooling-up, and validating protocols with positive controls of pathogenic, free-living amebae, I think this is a laudable achievement. Synovial tissue was the only source of patient material used in attempting to verify the Wyburn-Mason hypothesis. How the misconception that "very few (perhaps one)" biopsy specimens were studied, or that sera or knee effusions were used, is an enigma to me. Perhaps you could explain.

You are correct in your third assertion - we have not yet studied malignant tissue. I have focused exclusively on synovium from rheumatoid arthritis patients for the following reasons: 1) Cancer is not a rheumatoid disease. 2) It is abundantly clear from his writings that the organism which Wyburn-Mason construed as a limax ameba was present in the vast majority of rheumatoid patients examined. 3) The Foundation's stated purpose is "For the eradication of rheumatoid disease." 4) The double-blind clinical trials are being conducted using rheumatoid arthritis patients. Hence, in my judgement it was most logical and consistent to focus in the first year of the investigation on rheumatoid arthritis.

If, in spite of the unanimity of negative findings from the laboratories of myself and the other investigators, the

Board of the Foundation still feels that malignant tissues should be examined, we will fulfill that portion of the original proposal. All of the implementations are in place; there are no foreseeable problems in going from synovial tissue to tumor tissue. (Indeed, it is easier to mince and to cut for histologic sections.) My appointment at MCV is in the Division of Surgical Oncology, and my chairman assures me of his full cooperation.

Frankly, however, it is not obvious to me why the Foundation would be inclined to divert it's focus from rheumatoid disease to cancer. Finding pathogenic, free-living amebae in tumorous tissue would be intensely provocative, but except for partial vindication of the Wyburn-Mason hypothesis, in my view such an eventuality has little bearing on premises made by Wyburn-Mason and the Foundation with regard to rheumatoid disease. I have understood you to say that Dr. Wyburn-Mason suggested that malignant disease in a tissue may merely serve as a niche for the establishment of an opportunistic amebal infection, and that he could not be certain whether infection was a cause or a consequence of the disease. An analogy might be *Pneumocystis carinii*, an opportunistic parasite found in many AIDS victims, which is a complication of the disease but not the cause. If pathogenic, free-living amebae are prevalent in tumors, I would hypothesize a strong statistical correlation between cancer and rheumatoid arthritis. The data do not support such a link. But, to be consistent I must say that these are essentially "negative data," and therefore do not rule out the possibility.

Perhaps the most important finding from Wyburn-Mason's work, and certainly the most applicable, is the effect of clotrimazole and metronidazole on rheumatoid arthritis. Whether one holds that rheumatoid arthritis is caused by a pathogen (amebal or otherwise), an autoimmune syndrome, or indicative of some other physiologic imbalance, what is clear is that elements of the immune system (B cells, T cells, and macrophages) are involved in the pathologic progression of the disease. Clotrimazole is chemically related to levamisole, which has been shown to have immunoregulatory activity.

From the onset of my proposal I realized that the screening of rheumatoid or tumor tissues for a potential amebae pathogen would be like a deep-sea fishing expedition - the only truly meaningful results are if we "catch" something. If we do, then the reward is great. If we don't, then we have little to show for it. Because we had no positive findings, during Dr. Pybus's visit little time was spent re-examining the hundreds of histologic sections that I had already looked-through and found devoid of amebae. I did give Dr. Pybus a demonstration of the power of the immunofluorescent antibody technique in detecting amebae in a positive control. Most of our time together (nearly 5

hours) was constructively spent discussing and contemplating avenues of investigation that might be more promising. Judging from his reception of my ideas, I thought we were in accord that 1) *in vitro* studies to examine the immuno-modulating effects of clotrimazole and metronidazole, and 2) studies using animal models of arthritis to complement and extend the clinical trials, would be most likely to yield meaningful results.

We are poised to immediately begin the *in vitro* studies on the effects of clotrimazole and metronidazole on the cells involved in the immunologically-mediated pathogenesis of rheumatoid arthritis. Our initial experiments would assess the drugs' effects on the production of three principle mediators of the rheumatoid inflammatory response - interleukin 1, interleukin 2, and *gamma*-interferon (i.e., afferent effects of the drugs). Subsequent experiments would determine the influence of the drugs on the biologic effects caused by these mediators in their respective target cells (i.e., efferent effects of the drugs). The *in vivo* studies using animal models of arthritis would be conducted in consultation with members of the MCV School of Pharmacology, and could be initiated within six weeks. At this point in the investigation, however, I would like to have a mandate from the Board of the Foundation - should the search for amebae be extended to malignancy, or should we change course to gather scientific evidence relevant to the immunomodulatory properties of the Foundation's affirmed therapeutics? Either set of studies will be carried out promptly and diligently. I look forward to the Foundation's anticipated support for the coming year.

Sincerely yours,

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cc: Dr. Paul Pybus