the misstatements in the paper as resulting from editorial changes he did not control (see story on this page).

While Popovic has issued the sharpest denunciations of the draft report, nearly everyone outside of OSI who has read it seems to have harsh words for it. Some members of the panel convened by the NAS—known as the "Richards Panel"—initially complained that the draft report was too harsh with Popovic and not tough enough on Gallo, although the panel has since accepted the substance of the report. But the panel remains critical of the report's format, joining Healy in complaining that it is poorly written and that it undercuts its own points by failing to state clearly the issues, the findings, and the conclusions (p. 732). OSI is now drafting an Executive Summary for the report that will provide just such a concise statement.

In addition, Healy and the Richards Panel have accused OSI of committing technical violations of its policies and procedures while conducting the investigation. Mary Jane Osborn, a Richards Panel member, told *Science* that trying to figure out OSI's guidelines for conducting investigations was "a continuing problem." For instance, she said, OSI's preinvestigation inquiry seemed to take far too long, but the panel was never able to obtain written guidelines for the conduct of intramural inquiries. "The business of seeming to operate in a vacuum has always been a major concern," she said.

Whatever its faults, completion of the draft report signals that a new phase of the Gallo-Popovic affair is beginning. The investigation of the *Science* paper, which forms the substance of the OSI draft report, is only one of several ongoing probes into Gallo's isolation of HIV. Other issues yet to be addressed include the validity of the U.S. patent on the HIV blood test and the question of whether or not Gallo actually misappropriated the virus he called HTLV-IIIB from French researchers at the Pasteur Institute.

An intriguing window into these two questions opened early this week when *Chicago Tribune* reporter John Crewdson published an article describing the contents of Hadley's memo, which could affect the validity of the government's patent on the AIDS test. (*Science* later obtained a copy of the confidential memo.) Written in early June, the memo lays out numerous contradictions between official statements in patent filings and legal declarations and the findings of the OSI investigation. While one NIH source sympathetic to Gallo insists that "the patent is solid" despite the OSI findings, other sources point out that willful false statements in a patent application can be grounds for invalidating the patent.

Perhaps the biggest surprise in Hadley's memo is the news that the first virus Gallo's laboratory managed to grow in a continuous cell culture was none other than LAV—an isolate supplied by the Pasteur Institute. Gallo's declaration states that while Popovic did attempt to infect two cell lines with LAV in mid-October 1983—at least a month before establishing the cell line that produced HTLV-IIIB—"both transmissions were only temporary in nature." Hadley, however, notes in her memo that LAV continued to grow in Gallo's laboratory until Gallo ordered Popovic to freeze the cultures away in January 1984. These transmissions of LAV "were no more 'temporary' or transient than HTLV-IIIB, which was nurtured with fresh cells as well as virus to keep it alive," Hadley wrote.

The future of OSI's investigative work in this case remains uncertain, given the rough ride the misconduct office has taken in recent weeks. The controversy over the draft report and an ongoing administrative and management review at OSI seem likely to add considerably to the delay in releasing the final report. And even then, legal and procedural challenges will be waiting. OSI's long trek is far from over. ■ **DAVID P. HAMILTON**

Popovic Blasts Accusers, Demands Report Be Withdrawn

THE DRAFT REPORT FROM THE OFFICE OF Scientific Integrity (OSI) is particularly hard on Mikulas Popovic. Indeed, *Science* has learned that the report concludes Popovic is guilty of scientific misconduct as the result of false statements and data in the May 1984 *Science* paper in which he, Robert Gallo, and their colleagues at the National Cancer Institute reported that they had succeeded in growing the retrovirus that causes AIDS in permanently established cell cultures.

But Popovic isn't accepting that verdict. Far from it. In a written statement and exclusive interview provided to *Science*, Popovic, along with his lawyer, Barbara Mishkin of the Washington, D.C. firm of Hogan and Hartson, argues that "there is absolutely no basis for any conclusions of falsification, fabrication or misrepresentation" in the *Science* paper. Furthermore, they add that "because of the numerous procedural and factual errors" in the OSI draft, "we have asked that the report be withdrawn."

More is at stake than credit for isolating the virus. The 1984 paper was the first description of a crucial step in the development of a blood test for the AIDS virus: a technique for growing sufficient quantities of the virus to mass-produce the test. OSI investigators have questioned several claims made in the paper.

■ The draft report says that although the paper claims that a continuous culture was achieved in November 1983, in fact that did not happen until sometime in January 1984. In between, the report says, Popovic and his colleagues added fresh virus and cells to the culture to keep it going. In the interview, Popovic claimed that the dispute is purely semantic. He concedes he added fresh virus to cell cultures when the percentage of infected cells in the culture dropped below 10% to 30%. He did this to hasten the development of cell lines continuously producing virus—and not because he worried that the virus would stop growing in the infected cultures. Popovic contends that he had used the technique of "refeeding" cultures in the past and that no one had ever challenged his subsequent use of the term "continuous production." (See letter on next page for details.)

■ The OSI report points to an error in the paper when it says that the infected cell cultures continuously produced virus "for over 5 months." When the paper was written the virus had been cultured for only 4 months (even granting the November starting date for continuous culture). Popovic says others in the lab are responsible for that error. He says his original draft read "over 4 months."

Gallo has acknowledged that the draft of the paper submitted to *Science* on 30 March 1984 did read "over 5 months"—which, at the time of submission, was an overstatement. Gallo says he approved the change from Popovic's language because he knew that by the time the paper came back to his lab in galley form, the virus would have been in culture for more than 5 months.

■ The OSI draft report also raises questions about whether the virus isolates used to create the continuous culture had been tested for the presence of reverse transcriptase (RT), an enzyme produced by retroviruses. The virus described in the paper was grown from a pool made from viral samples from ten different AIDS and pre-AIDS patients. OSI investigators say that by using the words "first shown" to have RT activity, the paper implies that each of the ten samples had been tested individually for the presence of RT. Popovic says that, once again, imprecise language is to blame: All the samples were tested serologically for the

presence of virus, but only the pooled sample was tested for the presence of RT, not the individual samples. He acknowledges that, subsequently, remaining portions of the original ten samples were tested for the presence of RT, and two failed to show any.

■ A key table in the 1984 paper—Table 1—presents data on the virus samples grown in culture. Notes to the table explain the notation "ND" as meaning "not done." But in fact, OSI investigators say, some of the tests marked ND were done—but the results were not usable. Popovic argues that his lack of fluency in English (he is a Czech who came to the United States in 1980) partly explains the discrepancy. In his past scientific work, he says in his statement to *Science*, he had used ND to mean "not determinable," and in the 1984 paper he intended ND to mean "either incomplete or inconclusive." He adds: "My lack of fluency in the English language prevented my recognizing that 'not done' would be interpreted differently from my understanding."

■ OSI examined the question of why there were no comparisons between LAV and HTLV-III in the 1984 paper. French

Mikulas Popovic's Letter on The HIV-1/HTLV-IIIB/83

Science (B. J. Culliton, News Report, 22 June 1990, p. 1494; News & Comment, 19 Oct. 1990, p. 368) and recently Nature (*1*) have published comments criticizing the methodology I used to establish the H9 cell line chronically infected with the human immunodeficiency virus 1 (HIV-1)$_{IIIB}$ human T cell lymphomal leukemia virus (HTLV)-III$_{B/83}$ isolate (*2*). I wish to respond by explaining the rationale of that methodology, in particular, the pooling technique.

Luc Montagnier and his colleagues stated that lymphadenopathy-associated virus (LAV) could not be grown in permanent T cell lines (*3*). Nonetheless, on the basis of the experience that I and others had gained from studies of the avian sarcoma virus (ASV)–mammalian host cell system, pioneered by Jan Svoboda, I pursued experiments to determine whether neoplastic cells were permissive for the putative acquired immunodeficiency syndrome (AIDS) virus. It was my thought that the AIDS virus could be propagated indefinitely in neoplastic counterparts of the cell type that appeared to be its natural host in vivo, the CD4$^+$ lymphocyte. Work I performed from 1967 through 1969 that demonstrated the continuous long-term production in vitro of infectious ASV/B77 by rat tumor cell lines (*4*) provided both the theoretical and technical impetus for my experimentation.

My work with Ersell Richardson at the Laboratory of Tumor Cell Biology, National Cancer Institute (LTCB, NCI) with HTLV-I(human T cell lymphomal/leukemia virus)–harboring peripheral blood cells from an AIDS patient also suggested the feasibility of adapting the AIDS virus to neoplastic CD4$^+$ cells. [Only later was this HIV-1 isolate also grown in H9 cells and characterized; it has been designated HIV-1$_{cc/83}$ (*5*).] From February through August 1983, we maintained cell cultures from this patient which clearly contained immortalized cells and in which we consistently observed cytopathic effects, an apparent contradiction. At that time it was known that HTLV-I could immortalize CD4$^+$ cells, and it had been suggested that CD4$^+$ lymphocytes were permissive for the putative AIDS virus and that the virus had a cytopathic effect. I recognized that the significant cell death observed in these and other cultures we initiated from the blood of AIDS patients was not typical for HTLV-I (*6*). I therefore concluded that whether these cultures contained an unusual cytopathic variant of HTLV-I or a second unique retrovirus (the AIDS virus), it should be possible to maintain cultures containing this cytopathic virus when at least some immortalized cells were present.

It appeared to me that a reasonable method for sustained propagation of this seemingly cytopathic retrovirus would be to introduce it into a suitable neoplastic host cell. This approach would clearly take advantage of the fact that retroviruses integrate into the genomes of their host cells and thereby persist indefinitely.

My experience with the ASV–heterologous host cell system and other retroviruses suggested that success in productively infecting "unnatural" (neoplastic or heterologous) host cells depended on the use of a viral inoculum with a high multiplicity of infection (m.o.i.). Our early work with cultures from AIDS patients indicated that the level of virus expression was low. Therefore, to obtain a viral inoculum with a high m.o.i., I concentrated culture fluids using ultracentrifugation.

Generally, in retrovirus systems, a 100-fold (100x) concentration will increase infectivity by only approximately 10- to 30-fold, because centrifugation causes some loss of biological activity. Therefore, to obtain 0.5 to 1.0 ml of an appropriate (100x) viral inoculum, the starting volume of harvested culture fluids needed to be at least 50 to 100 ml. Obtaining a sufficient volume of culture fluids from a single AIDS patient's cell cultures was problematic. Not only was it difficult to expand these T cell populations sufficiently, given the cytopathic effects of the virus, but also many of the patients from whom we received blood were lymphopenic. Consequently, in many cases not enough cells were available to initiate cultures of a reasonable size. Most commonly we could recover 10 to 20 ml of culture fluid from a single specimen before the cells died. It seemed to me, therefore, that the most feasible and efficient way to obtain a sufficient volume for virus concentration was to pool culture fluids from different specimens (different individuals). In addition to the H9/HIV-1$_{IIIB}$ line, which arose from this pooling of culture fluids from different patients' samples, Elizabeth Read-Connole (LTCB, NCI) and I also established several cloned cell lines infected with HIV-1 isolates that were handled and maintained as individual isolates from a single patient. Five of these HIV-1 isolates were reported in the same paper in which my colleagues and I reported the H9/HIV-1$_{IIIB}$ (*2*). One of these, the HIV-1$_{RF/83}$ isolate, was propagated in H4/HUT-78 cells for a number of months almost in parallel with HIV-1$_{IIIB}$ and represented an alternative source of HIV-1 for large-scale production.

Contrary to some allegations, the data I reported on single isolates (*2*) indicate that I did, at that time, recognize the importance of the "pedigree" of a virus isolate. These reported data were overshadowed by Robert Gallo's decision to go ahead with HIV-1$_{IIIB}$ instead of the HIV-1$_{RF/83}$ isolate for large-scale production. His decision, as I understood, was dictated by the urgent need to protect donated blood supplies (E. Rubinstein, News Report, 22 June 1990, p. 1499).

There was another equally valid reason for pooling fluids from cultures from different individuals. It was known that most retroviruses exhibit some heterogeneity with respect to biological behavior (for example, the Schmidt-Ruppin strain of Rous sarcoma virus readily infects rodent cells, while the Bryan strain does so poorly). I therefore reasoned that an efficient way of finding a variant or variants of the AIDS virus which could infect and replicate in neoplastic CD4$^+$ T cells would be to pool virus from different sources (different patients), concentrate them to obtain a high m.o.i., and then use the resulting viral inoculum to infect CD4$^+$ cell lines. My reasoning proved to be correct. It is now known that only a limited number (10 to 20%) of HIV-1 isolates from patients are capable of productively infecting neoplastic CD4$^+$ cell lines to a significant degree (*7*).

Of course, the remarkable genetic heterogeneity among HIV-

AIDS researchers, led by Luc Montagnier, had isolated a virus in the spring of 1983 that they thought caused AIDS and had provided Gallo's lab with several samples of it. The first samples did not grow, Gallo has asserted, but a later sample did. "Originally, as I understood it, data would be included about the French virus in the manuscript," Popovic told *Science*. "Later Dr. Gallo said, 'No, we will publish later in a collaborative paper.'"

Gallo says OSI investigated this question and determined that there was no wrongdoing with regard to the discussion of LAV in the 1984 paper. He says he took responsibility for taking out the comparisons with LAV because he felt they would be more appropriate for later papers to be coauthored with the French scientists. In fact, Gallo says, these were written but never submitted for publication after a disagreement with the French team. In hindsight, Popovic says publishing the collaborative papers would have been a good idea. "Perhaps we wouldn't be under such very tough scrutiny," says Popovic.

■ JOSEPH PALCA

Isolate and the Pooling Experiment

1 isolates from different patients was not known at the time I did this work, and the recent demonstration of the contamination of both HIV-1_{BRU} and HIV-1_{IIIB} with the HIV-1_{LAI} isolate (*8*) confirms that there are significant differences in the capability of different isolates to infect neoplastic CD4$^+$ T cells.

It is worth noting that in the past, microbiologists and virologists working at the forefront of newly developing fields performed "dubious" experiments in an attempt to isolate new infectious agents or demonstrate their biological effect(s), or both. One hundred years ago, David Bruce, a British microbiologist, in an attempt to transmit trypanosomes, inoculated large volumes of blood from 10 different wild animals into dogs. The earliest experiments performed by Emile Roux, a French microbiologist, demonstrated an effect of the diphtheria toxin on guinea pigs, but did not meet the scientific "standard" of the time. More recently, Montagnier *et al.* (*9*) reported the induction of multinucleated giant cells (polycaryons) by superinfection of one HIV-1–positive T cell culture with an HIV-1 isolate originating from another virus-positive individual. The basic procedures I used to productively infect neoplastic CD4$^+$ T cell lines with HIV-1 have since been reproduced successfully in a number of laboratories and are described in greater detail elsewhere (*10*).

There is another reason why large amounts of the virus were needed. Although in the case of some HIV-1 isolates, a single exposure of CD4$^+$ neoplastic cells to the viral inoculum can be sufficient for initiating and maintaining long-term continuous virus production, reexposure of the cells to the concentrated (high m.o.i.) viral inoculum is frequently necessary. This is because after the first or even the second exposure of CD4$^+$ neoplastic cells to the virus, the particular fraction of virus-producing cells that also has a capacity to proliferate can be small. Reexposure of the already infected cell population to the virus serves to maximize the number of infected virus-positive cells and, at the same time, to facilitate selection of those viral progeny that can readily infect CD4$^+$ neoplastic cells. It also minimizes the probability that the uninfected neoplastic cells will overgrow the culture (*10*). This phase of the establishment of HIV-1–infected cell lines is characterized by fluctuation in virus production. This has been observed not only in the case of neoplastic CD4$^+$ T cell lines but also in HIV-1–infected B cell lines (*11*). Because the viral genome integrates into the genome of its host cell, and because neoplastic cells can proliferate indefinitely, virus production can become permanent or "continuous." I used the term "continuous" in this context in 1969 (*4*) to describe production of ASV/B77 by rat tumor cell lines. In 1984, I applied it to the H9/HIV-1 system. In each case, I used the term to describe virus production that was consistently detected in culture fluids and which never became discontinuous. To my knowledge, that was an accepted use of the term in both 1969 and 1984.

The procedures I have described for establishing cell lines chronically infected with HIV-1 were applied not only in the case of H9/HIV-1_{IIIB} but also to several individual HIV-1 isolates. The stably infected cell lines H9/HIV-$1_{RF/83}$, H9/HIV-$1_{CC/83}$, and H9/HIV-$1_{MN/84}$ (developed by Read-Connole and myself) represent, in addition to H9/HIV-1_{IIIB}, the only isolates from Gallo's laboratory which are in large-scale production, have been deposited in the National Institutes of Health—National Institute of Allergy and Infectious Disease and World Health Organization repositories, and are extensively used worldwide. We established stably infected lines using not only the HUT-78–derived H9 cells, but also Molt-3 and KE-37. The practical significance of my approach to HIV-1 propagation is that, of the 48 detections and isolations of HIV-1 by more conventional methods reported by Gallo *et al.* (*12*), only one isolate, HIV-$1_{MN/84}$ adapted to H9 cells, is extensively studied in addition to those mentioned above.

Finally, I wish to point out that later apparent discoveries by others of several novel human retroviruses associated with specific diseases remain in question for the very reason that these viruses have yet to be convincingly transmitted. Consider, for example, the reports of novel retroviruses associated with multiple sclerosis (*13*), Kawasaki disease (*14*), human leukemia (designated as HTLV-V, not HTLV-I) (*15*), and human breast cancer (*16*). Clearly, the establishment of appropriate host cell systems for propagation of these viruses would permit the development of specific reagents essential to the unequivocal identification of a causal link between the virus and the disease. The information contained in our much-debated publication was essential for establishing just such a causal relationship; perhaps the publications claiming discovery of new human retroviruses collectively represent a more appropriate yardstick by which to measure the true value of our paper.

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