Parameters for a research mobilization against AIDS

New diseases like AIDS mean that mankind's survival now depends on the launching of a "Biological Strategic Defense Initiative." First of a two-part series by Jonathan Tennenbaum.

In mankind's continuing battle against dangerous infectious diseases, victory has always depended upon two essential elements: 1) Public health measures, to slow or stop the spread of infection, by identifying and isolating infectious persons, eliminating vectors such as insects and contaminated water, and increasing levels of nutrition, sanitation, hygiene, and medical care of the population. 2) Scientific research, to create effective treatments, vaccines, disinfectants, and sanitary procedures against the microbial agents of the disease.

Acquired Immune Deficiency Syndrome (AIDS) is no exception to this historical rule. Even under the most optimistic assumption, that a "miracle" cure were to be found within the next five years, failure to enact effective public health measures *now* would mean that AIDS would spread out of control, infecting and killing millions *before* the (hypothetical) cure could be tested, marketed, and administered. No new epidemic in history has been stopped by a cure or vaccine. On the other hand, millions of persons already infected must be saved from a horrible death, and the AIDS virus itself must finally be eliminated from the face of the Earth, by effective treatments and vaccines.

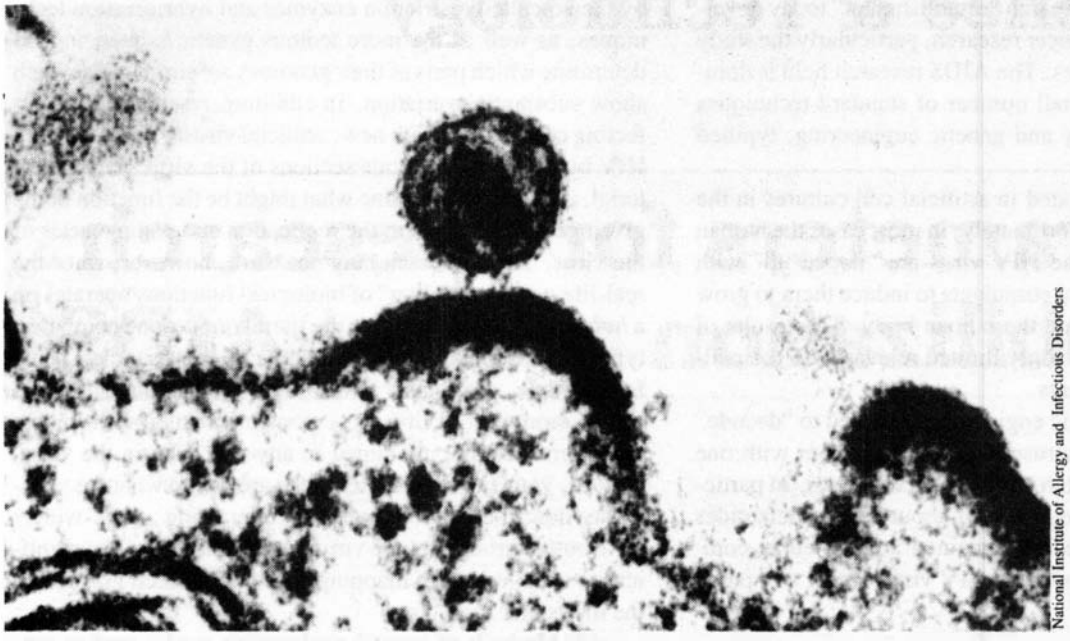
The following article is devoted to laying out fundamental parameters for a scientific research mobilization against AIDS. First, we briefly review the present state of AIDS research and pinpoint some key areas where new, more powerful methods must be introduced. Next, we present an overview of the decisive area of optical biophysics (otherwise known as "nonlinear spectroscopy"), which promises to revolutionize not only AIDS research, but much of biology and medicine as well. Finally, we discuss how the scientific crash program is to be organized.

The current status of AIDS research

In the report *Confronting AIDS*, released last October by the U.S. National Academy of Sciences, the committee of authors, representing the top level of medical research in the United States, came to the following conclusions:

"In the brief period since the first description of HIV and its unambiguous identification as the cause of AIDS, a tremendous amount has been learned about the genetic structure and transmission of the virus. Much less is known, however, about how it initiates infection, how it maintains infection, and what determines the progression and diversity of the resulting illness. . . . [Our] insights, however impressive, are only the beginning of what promises to be a long and difficult path toward effective therapeutic interventions to minimize or eliminate the debilitating effects of HIV infection and toward limiting the spread of the virus by means of safe and effective vaccines. . . .

"Infectious viral agents remain a major threat to human health both in the United States and around the world. Recent years have therefore seen considerable effort directed toward the development of new antiviral agents for the treatment of acute viral infections. However, even though there are now many examples of successful chemotherapeutic conquests of bacterial infections, there are only a few examples of drugs that are effective in the treatment of viral diseases. Much of the difficulty in treating viral infections is a consequence of their nature as intracellular pathogens—that is, they replicate within the cells of their chosen host. Because viruses use many of the host's synthetic pathways in their reproductive processes, specifically inhibiting their replication without also severely compromising the metabolic activities and health of their hosts is difficult.



National Institute of Allergy and Infectious Disorders

Here the AIDS virus (HTLV-III/LAV) is shown budding off from a T lymphocyte.

"As a virus that appears to cause persistent lifelong infection, HIV must be approached as a member of the class of viruses for which successful treatment may be most difficult to find. Furthermore, as a member of the family of retroviruses, HIV represents a type of viral pathogen whose therapy has never been attempted in humans. Because the contemplated development of drugs to treat HIV infection and AIDS represents such a novel and difficult challenge, predictions of ultimate success are presently impossible to offer. . . .

"Development of an effective vaccine to prevent HIV infection must be a prominent goal in any program designed to halt the continued spread of the AIDS epidemic. However, it is also the most difficult to realize. Active immunization has proven to be an extremely effective means to limit or eliminate the exceptional morbidity and mortality inflicted upon human populations by many types of viruses, but the derivation of an effective vaccine against a human retrovirus has never been seriously attempted, much less achieved. Similarly, experience in the production of vaccines for retroviruses of other animals has been rather limited and often disappointing. . . . Biologically, the characteristic genomic diversity and persistence of infection by HIV may present serious obstacles to the generation of broadly effective immunity. Vaccine development is also constrained by the presently limited basic understanding of the immune response to HIV infection, its apparent impotence in clearing the viral load, and the ways it might be bolstered through protective immunization. Should the biological and scientific obstacles be surmounted . . . the scarcity of available chimpanzees to test the safety and efficacy of candidate vaccines may compromise or delay adequate preclinical evaluation. Initiation of testing in human populations will also present serious ethical and practical considerations, which will undoubtedly affect the clinical evaluation of an HIV vaccine. . . .

"[In conclusion], an effective vaccine may be very difficult, if not impossible, to produce. Should an effective vaccine candidate become available, there are significant social concerns that may limit or prevent its testing and use. Therefore, a vaccine may not reasonably be expected to be available in less than 5 years. Even for the next 5 to 10 years, the committee believes that the probability of a licensed vaccine becoming available is low."

This sober assessment of the prospects of AIDS research should teach a lesson to those politicians and public health officials who have hoped that a "miracle cure" from some U.S. laboratory would permit them to avoid facing the unpleasant problem of instituting public health measures against AIDS. No, there isn't going to be an easy way out of the problem! The U.S. National Academy of Sciences report proposes a large expenditure on AIDS, reaching $1 billion a year for research alone, and $1 billion a year for public health measures, by the year 1990. This amount, however, is only a small fraction of the estimated $10 billion a year which the treatment of AIDS patients will cost the United States in that same year, 1990.

Methodological weaknesses of AIDS research today

As the Academy of Sciences admits in their report, *a scientific solution to AIDS may not be possible at all, using the presently known methods of virology and genetic engineering*. Fortunately, there are more powerful scientific weapons within reach. Before going into these new methods, let us briefly examine the limitations of present AIDS-related research. (See **Figure 1** for a schematic representation of prevailing views on retrovirus replication.)

Much of the AIDS research "establishment" today developed out of the area of cancer research, particularly the study of cancer-inducing viruses. The AIDS research field is dominated by a relatively small number of standard techniques and concepts of virology and genetic engineering, typified by the following:

(i) Viruses are cultivated in artificial cell cultures in the laboratory (*in vitro*). Unfortunately, in most cases the human cells used to cultivate the HIV virus are "doped up" with special nutrient media and stimulants to induce them to grow and replicate virus outside the human body. The results of such *in vitro* studies have only limited relevance to the real-life events in actual patients.

(ii) Methods of genetic engineering are used to "decode" the genetic material of viruses, to compare them with one another, and even to "patch together" new viruses. In particular, the entire genetic sequence of about 9,600 nucleotides of the HIV virus has been determined and stored in computers. Different variants of the HIV virus can be compared by the so-called restriction enzymes and hybridization techniques, as well as the more tedious genetic sequencing, to determine which parts of their genomes are similar and which show substantial variation. In addition, researchers are infecting cell cultures with new, artificial viruses obtained from HIV by "excising" various sections of the virus genetic material, in order to determine what might be the function of the given genetic sections in the replication and pathogenesis of the virus. This approach may not work, however, since the real-life genetic "coding" of biological functions operates on a *holographic* principle, not the literal one-to-one computer-type code assumed by most molecular biologists today. Thus, for example, virologists have found that the characteristics of the common cold viruses, responsible for their well-known symptoms, are not contained in any one gene in the virus. Instead, your sniffles and headache are due to what the virologists describe as a complex of interacting genes widely distributed throughout the virus genetic material. The significance of holographic mappings has not yet been grasped by the molecular biologists.

(iii) Methods of genetic engineering can be used to synthesize specific proteins encoded by parts of the virus genome, such as the outer envelope protein. Such techniques might, it is hoped, eventually lead to a form of vaccination in which artificially produced antigenic proteins are used to stimulate the host's creation of effective antibodies against HIV. This approach might be rendered ineffectual, by the high variability of the HIV viruses.

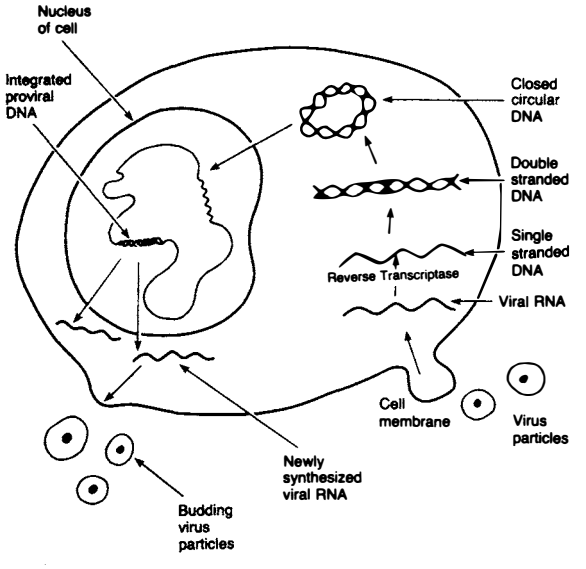
(iv) Electron microscope images are employed to determine the morphology of the virus and of tissue infected by the virus. Unfortunately, cells must first be killed, then sliced very thinly and coated with metals, before they can be viewed by the electron microscope. As a result, no one has actually seen "live" virus replicating in living cells.

(v) Special radioactive and fluorescent "tagged" substances are applied to "search out" and selectively bind with specific proteins or specific bits of DNA. Thus, for example, artificially produced fluorescent antibodies are used to identify the cells attacked by HIV. Using these kinds of techniques, the Pasteur Institute's Professor Chermann was able to show that insects, captured in Central Africa, contained the genetic sequence of HIV in their tissue. Related techniques provide the best means at present for precise testing for HIV infection in humans. Unfortunately, the high variability of the HIV virus may cancel many of the potential advantages of DNA-probe and related genetic-engineering tests for AIDS. The more precise the genetic test, the less able it will be to catch the myriad mutant forms of the virus. We might need a separate test for each variant.

(vi) "Classical serology" employs a basic technique known as "electrophoresis" to separate out the various protein components in the sera of a given individual. In this way, for example, the presence of a specific pattern of antibodies, characteristic of the immune response to a given virus, can be ascertained. Unfortunately, these methods often lack the

FIGURE 1

Reproductive cycle of a retrovirus



The retroviruses form a bridge between the RNA viruses and the DNA viruses. Outside the host cell, the retrovirus exists as a particle that contains RNA as its genetic material. Inside the cell, the virus exists as a proviral DNA sequence within the genetic material of the cell nucleus, or as free circular DNA within the cell nucleus. Normal uninfected animal cells carry genes called virogenes, which are capable of giving rise to RNA viruses such as retroviruses and which can then go on to infect other cells. When the virogene-produced retrovirus infects another cell, the inserted genetic material is called a provirus. Thus, normal uninfected cells can produce new viruses and infected cells can pass on their acquired virus genes to succeeding generations of cells, which will then express these inherited viruses under the appropriate circumstances.

precision necessary for basic research, providing only an indirect footprint of the viral infection. In AIDS testing, serological methods have the disadvantage, that they miss the cases of persons newly infected with the virus, who have not yet developed a significant immune reaction.

Taken as a whole, these methods permit researchers to accumulate large amounts of detailed data concerning the genetic makeup and variability of the HIV virus, the proteins involved in its construction and replication, and the so-called "receptor molecules" at which the virus binds and is absorbed into the target cell. The same methods may permit a large-scale production of therapeutic substances and vaccines, once these have been discovered.

However, present-day techniques of molecular biology all suffer from a systematic limitation: They focus upon the structures of individual biomolecules, but are powerless to measure or describe the *life process itself* within which these molecules play the role of singularities. The molecular biologist is at best like the paleontologist who tries to deduce the habits of a dinosaur from some fragments of bone. The paleontologist has little choice but to do so, since the animals in question are long dead. If he possessed a live dinosaur, he would hardly chose to kill it and separate out all the individual bones for study, as the molecular biologist does; instead, he would first want to study the live animal! Similarly, knowledge of the DNA genetic sequence tells us only something about the "skeleton" of a biological process, and very little about its living reality.

This systematic limitation is reflected in the extremely narrow approach to the AIDS problem prevalent in most present research. The implications of the elementary fact, that viruses can only replicate in living cells, are often forgotten, as researchers focus their attention on the "mechanics" of the virus particle per se, instead of the life process without which viruses could not exist. This problem is reflected in the very *language* of virology and molecular biology, as typified by such terms as "reverse transcriptase," "transposons," "promoters," etc. These terms ascribe to dead objects—molecules or fragments of molecules—qualities of action that only exist in the context of a living cell, and in which the molecular object in question represents only an included feature. Thus, "reverse transcriptase" does not really exist, except as a linguistic illusion. It is the living process which organizes the molecules, not vice versa. We shall return to this point at the end of the article.

The limitations of a purely molecular-biological approach to fundamental medical research, are clearly revealed in the case of cancer. In spite of the explosive development of molecular biology and genetic engineering over the last 30 years, the prospects of survival for persons diagnosed with cancer have not dramatically improved, on the average, during the same period.

All of the questions concerning the underlying dynamic of HIV infection remain unanswered and largely inaccessible to present methods of virology. How does the virus get into the cell? How is the virus-associated genetic material transported into the nucleus of the cell, and exactly what happens there? What processes determine whether that material remains dormant in the nucleus during a given period, or is activated to participate in the production of new virus particles? What causes the cytopathic effects associated with the HIV virus? What is the relationship between viral infection and the various forms of HIV-related disease? How are the processes of synthesis of viral components in various distant regions of the cell coordinated with one another, and how is the final assembly of the virus particle organized? How does the virus replication process interact with the immune system of the host, from initial infection through to the final disastrous stages of the AIDS disease? All of these crucial questions force the astute scientist to turn his or her attention back to the original, central focus of biology: the organization of living cells and particularly of their characteristic activity, cell division or mitosis.

Optical biophysics: key to a revolution

Historically, breakthroughs in medicine and biology have always gone hand-in-hand with major progress in physics and technology. Seventeenth-century developments in optics led to the creation of the *light microscope* and the first investigations of microbial life by Leeuwenhoek and Hooke. The vast development of electricity and magnetism in the 18th and 19th centuries was launched, in large part, by the discoveries of Galvani and Volta on the electrical properties of living tissue. This led finally to such modern technologies as the *electroradiograph* and *electroencephalograph*. Louis Pasteur's revolutionary breakthroughs in microbiology and medicine began with his discovery of the asymmetric optical action of substances produced by living cells. Pasteur began his life's work as a physicist, in what was then the frontier area of physics—crystallography and the wave theory of light. Röntgen applied his discovery of *x-rays* immediately to medical diagnostics, astounding doctors with the first pictures of internal broken bones in living patients. The initial development of wave mechanics and particle beam technology in the 1920s and 1930s gave the biochemist the powerful tool of *mass spectroscopy,* and led to the creation of the *electron microscope* and the first photographs of virus particles. The rapid growth of radar technology and nuclear physics during World War II led to the techniques of *nuclear magnetic resonance* (NMR) spectroscopy and *radioactive isotope tracing*. Together, the techniques of x-ray diffraction, electron microscopy, mass spectroscopy, and radioactive tracing, aided by the *ultracentrifuge* technology developed during the wartime Manhattan Project, provided the basis for the vast expansion of molecular biology in the postwar period.

Today, with the evolution of *lasers* and other sources of coherent electromagnetic radiation over the entire spectrum from radio waves into the soft x-ray region (x-ray laser), *we stand at the threshold of a new era in biology and medicine.*

New scientific instruments, far more powerful than those hitherto applied in molecular biology and genetic engineering, are now becoming available. These instruments, combined with a new scientific assault on the understanding of basic living cell processes such as mitosis, constitute mankind's most powerful weapons in the battle against AIDS.

How spectroscopic methods can lead to a cure for AIDS

Before discussing the technology and methods of optical biophysics in detail, we shall briefly indicate, in highly simplified language, how effective treatments against AIDS could be discovered by these methods.

In the simplest terms, the principle is this: Living processes are organized around certain sets of *characteristic frequencies,* analogous to the spectra of atoms and molecules. The cell spectrum is manifested, broadly speaking, in nonlinear, "resonant" interactions with specific frequencies of electromagnetic radiation, as well as by emissions from living cells themselves. Certain of these frequencies remain relatively constant (for a given cell and tissue type), while others change during the life of the cell. Particular modes of the cell process, including pathological modes (disease), are associated with particular spectral features such as the presence of particular "spikes" in the frequency spectrum. The crucial "target" process for detailed spectroscopic investigation is *mitosis*. So, regarding virus replication as a process taking place essentially within or in association with mitotic processes, the question arises: "What are the particular spectral 'spikes' corresponding to the viral reproduction process in an infected cell?" Research to date leaves little doubt, but that such specific spectral features actually are present and can, in principle, be detected using suitable instrumentation.

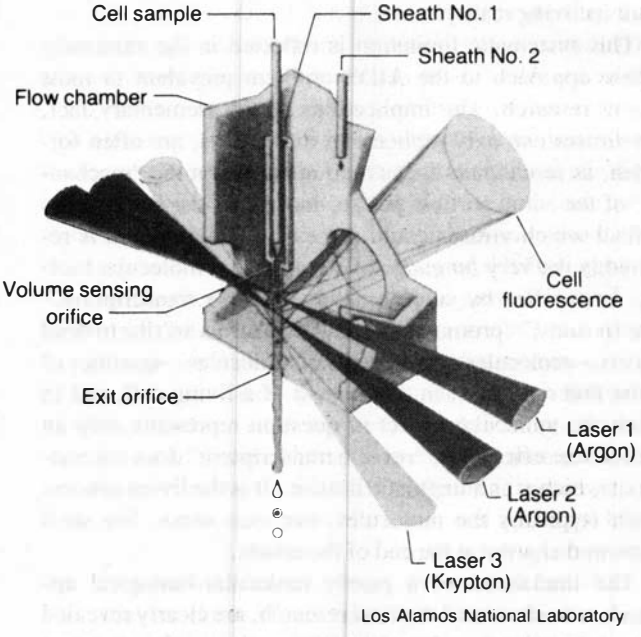
Once we have located a certain pattern of "spikes" characterizing the AIDS process within the spectrum of cell mitosis as a whole, we then seek the means to selectively block, or "jam," the pathological AIDS-associated processes—by means analogous to "electronic countermeasures" in modern warfare. The most straightforward method for doing this is indicated by recent biophysical researches into the mechanism of action of various drugs. The relevant work reveals that in many cases, drugs act via their *optical activity,* and not primarily via chemical properties such as affinity to form compounds. ("Optical activity" refers to the changes in intensity, direction, polarization, and frequencies of electromagnetic radiation absorbed and re-emitted by a physical system.) The second stage of the project consists, therefore, in "spectroscopic engineering": to design a molecule whose optical activity exactly fits the "spikes" of the AIDS process, so that the presence of that substance will selectively interfere with the disease, but not with healthy tissue function. In addition, the optical activity of substances can be used to restore healthy functions, by strengthening frequencies around which those functions are organized.

This simplified sketch is intended only to identify the bare concept of the project. The concept of "tuning" of biological processes provides the simplest access to a highly complex domain of investigation, to be reviewed in more detail in the course of this document.

In principle, the instrumentation required for both phases of the proposed approach either already exists or is in advanced phases of development today; we shall discuss some of the key technologies in the following section of our report. Although the basic principle is very simple, the actual development of effective drugs against AIDS by such spectroscopic methods will require a research mobilization of major dimensions. We are talking about an effort comparable to the Apollo program which put the first man on the Moon. We have to map the entire spectroscopy of normal and pathological forms of mitosis—the most complex process ever submitted to detailed physical investigation. The development of computer systems able to rapidly process the required masses of spectroscopic data, will itself be one of the major challenges of the project. While the total cost of the project may run into the tens of billions of dollars, a revolution in medicine will have been accomplished: Once the "proof of principle" of spectroscopic design of drugs against AIDS has

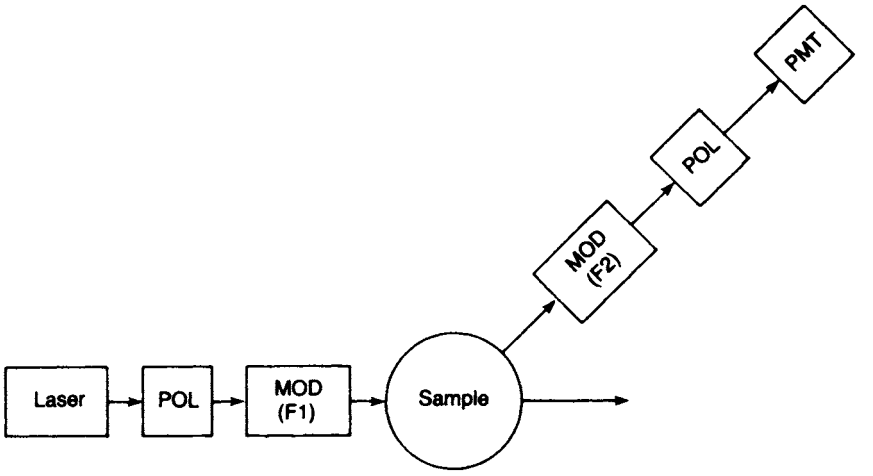
FIGURE 2

Multilaser flow cytometer



This is a cutaway view of a Los Alamos National Laboratory device that uses three different lasers to excite a biological sample and then read its signature. On entering the chamber, the cells stained with fluorescent dyes and suspended in normal saline solution intersect a krypton laser beam and two argon laser beams. The fluorescence emitted by the cells is measured with sensors capable of detecting light in one, two, or three wavelength regions as selected by various filter combinations.

FIGURE 3
Multiparameter light scattering spectrometer



In this schematic, light from a laser passes through a polarizer (POL) *and then through a photoelastic modulator* (MOD) *operating at frequency* F1, *which converts linearly polarized light to alternately right- and left-circularly-polarized light. Scattered light from the sample then passes through a second photoelastic modulator operated at another frequency,* F2. *The light leaving the second modulator represents a series of terms at frequencies corresponding to various sums and differences of* F1 *and* F2. *After passing through a second polarizer, the light then falls on a photomultiplier tube* (PMT). *The signal from the photomultiplier then goes to a bank of lock-in amplifiers, each tuned to one of the frequency components of interest.*

been obtained, the same approach may permit science to conquer any other virus afflicting the human race.

The instruments of optical biophysics

The following examples illustrate some of the leading features of optical biophysics technologies, which promise to revolutionize much of biology and medicine over the coming 25 years. All of these instruments measure *characteristics of emission, absorption, and transformation of electromagnetic radiation by living organisms*. After examining some of the leading work internationally on these technologies, we shall indicate how they provide the key to answering exactly those questions about AIDS, which today's molecular biology is unable to resolve.

1. Laser Raman spectroscopy and the "tuning" of living tissue: Careful experiments carried out in the 1960s and 1970s demonstrated that low-level microwaves of specific frequencies can have dramatic effects on growth rates and rates of synthesis for various metabolic products in living tissue. These effects were obtained at intensities far below those at which significant increases in temperature could be detected (typically less than 10 MW per square centimeter), and showed sharp resonance peaks at various wavelengths. This confirmed the view, that the processes of living cells are "tuned" in such a way that exceedingly small amounts of energy, injected at the right frequencies, can interfere strongly with cell function. Unfortunately, the tremendous variability of living processes and their sensitivity to countless external influences rendered these microwave effects notoriously difficult to replicate. An intrinsic difficulty lies in the fact, that living processes evolve in irreversible fashion: The cell culture of today "remembers" what the experimenter did to it yesterday! However, with the advent of *laser Raman spectroscopy* (see discussion below), we now have a tool with which high-frequency internal oscillations in cells can be conveniently studied in their full time dependence. Just recently, a group of Chinese researchers was able to replicate both the *positive* and the *negative* results of previous laser Raman studies of cell tuning, by showing that biological effects of the weak electromagnetic fields emitted by *the measuring equipment itself* were responsible for the discrepancy in reported results.

The most spectacular microwave effects reported were obtained by the Italian radar engineer A. Priore, working in Bordeaux, France from the 1950s until his death in 1983. As far as can be determined, the Priore apparatus produced microwaves in the 10 gigahertz (GHz) range, modulated at radio frequencies in the megahertz (MHz) range. This radiation was applied in combination with a powerful magnetic field slowly pulsed at approximately 1 Hz. Through the choice of specific modulation frequencies, Priore reportedly obtained dramatic biological effects, such as increases in growth rates of plants and rapid reduction and elimination of tumors in laboratory animals. Later, it was shown that the "Priore radiation" did not kill cancer cells directly, but worked by *stimulating the immune system* of the host animal. Exposure to suitable modulation frequencies permitted laboratory animals to recover from otherwise deadly inoculations of trypanosomes, the parasites responsible for sleeping sickness. Trypanosomes characteristically defeat the immune system by constantly changing their antigenic characteristics. Hence, the reported results indicate a spectacular effect on immune response of the irradiated animals.

At the time of these experiments, there was no adequate theoretical framework for understanding such effects, nor the technical means to precisely determine the characteristics (in

particular, the polarization and strength of magnetic components) of the radiation involved. The Priore results became the subject of a destructive controversy. Remarkably enough, the controversy concerned not so much the *existence* of the effects (which were repeated hundreds of times by many groups of researchers over more than a decade), as the circumstance, that Priore maintained close secrecy over the operating parameters of his machines. The proximity of Priore's work to areas of research into weapons applications of microwaves, may be responsible for much of the mystery and controversy surrounding an otherwise most promising direction of biophysical investigation.

In the late 1960s, Sidney Webb and co-workers applied the new technique of laser Raman spectroscopy to study the tuning of living tissue. In this type of spectroscopy, an impinging laser beam exchanges energy with molecular vibrations and coherent excitation waves in the test material, causing upshifts and downshifts in the frequency of the scattered light (Raman effect). Webb et al. found that the Raman spectrum of living cells is entirely different from that observed from crystals or mere suspensions of organic molecules. First, they discovered an anomalous *lack* of Raman activity in "resting" cells, i.e., cells not undergoing metabolic activity. As soon as nutrients (oxidizable carbon sources) were added to the medium, strong Raman lines appeared, at frequencies characteristic of the cell type and nutrient used. However, the intensities of the lines changed with time, in such a way, that specific lines appeared at specific times during the cell division cycle. It was later found that the Raman shift frequencies are sums and differences of a small number of fundamental frequencies, in much the same manner as the spectral lines of atoms and molecules. The precise, time-dependent shifts of energy between the various lines, are peculiar to the life process.

Webb found further remarkable results when he studied the differences in Raman spectra between normal and tumor cells, and the relationship between spectra of "mother" and "daughter" cells in mitosis.

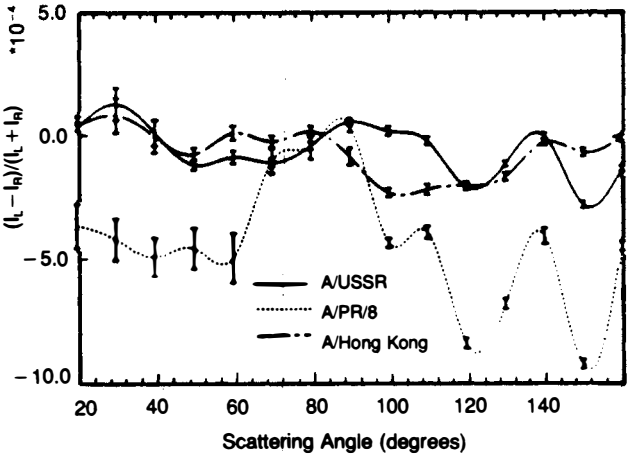
The potentials of *in vivo* Raman spectroscopy for fundamental research in biology and medicine, have hardly begun to be realized. Most important is the fact that this method, like the multiparameter light scattering (MLS), ultraweak photon emission (UPE), and nuclear magnetic resonance (NMR) techniques described below, allow us to directly observe the space-time characteristics of the relativistic (Riemannian) geometry of the living organism. Ordinary micrographs, in contrast, tend to reveal only distributions of object-like "structures," leaving unseen the underlying *life process* in the cell. The results of Webb and others indicate, in fact, that the observed Raman lines do not represent oscillations of isolated molecules, but coherent waves "binding up" the cell components into a unified process.

2. Multiparameter light scattering: When we look at a specimen in a microscope, the image we see consists of light and dark areas corresponding to varying absorption of ordinary light by regions of the sample. But, the microscope image represents only one small aspect of the complex process by which light is absorbed, re-emitted and scattered by the specimen. *Multiparameter light scattering* (MLS) instruments observe the entirety of the light scattered *in all directions* from a given sample, as a function of the wavelength of the light-source (a laser), the angle of scattering and the degree of left-handed or right-handed polarization of the light going into and emerging from the sample (**Figure 2**). Rather than providing a photographic image, the MLS technique produces a characteristic "signature" which can be used to identify microorganisms and even viruses in biological fluids in the remarkably short time of 5 minutes. Even closely related varieties of virus, which otherwise can only be distinguished by laborious cell culturing or biological engineering techniques, are easily distinguished by MLS methods.

Figure 3 shows a prototype version of MLS, called *Circular intensity differential scattering* (CIDS). In this instrument, light from a laser is first passed through a polarizing filter, then through a modulator, and finally directed onto the biological sample fluid in a cuvette. The scattered light is then measured by a photomultiplier tube preceded by a second polarizer-modulator combination, and mounted on a rotating arm which moves in steps through the angles from 0° to 180° to the axis of the original beam.

FIGURE 4

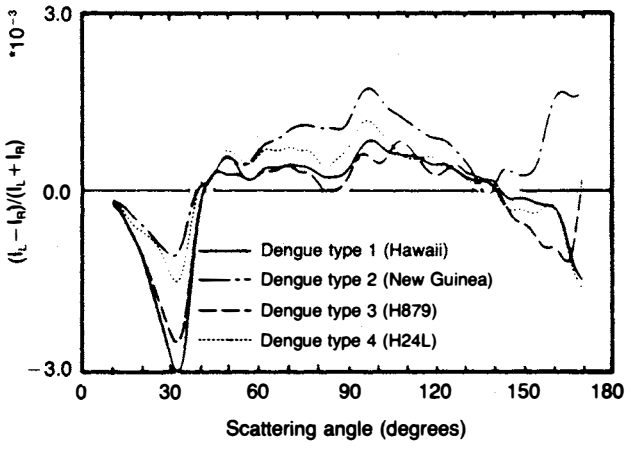
Typical CIDS spectra for three influenza viruses



The circular intensity differential scattering results are compared here for three types of influenza A virus at 488 nanometers. The vertical axis graphs the ratio of the difference between the measured intensities of left-circularly-polarized light and right-circularly-polarized light in the scattered light to the total scattered light in the given direction. The horizonal axis is the scattering angle. There is good discrimination among the three virus preparations around 60°, 110°, and 150°.

FIGURE 5

CIDS spectra for dengue fever viruses



Four types of dengue fever virus vaccines are plotted here at 360 nanometers, with the best resolution among them obtained at about 30°.

The CIDS signature is obtained by this instrument by measuring the ratio

$$(I_L - I_R)/(I_L + I_R)$$

of the difference between the measured intensities of left-circularly-polarized and right-circularly-polarized light in the scattered light, to the total scattered light in the given direction, as a function of direction. This parameter is highly sensitive to what are called *chiral structures*, processes in the sample which display left-right *asymmetry*. The basic principle behind this method was originally discovered by Louis Pasteur, who was the first to show that living organisms systematically "choose" between left- and right-handed forms of most molecules, in their metabolic processes.

Figure 4 shows typical CIDS spectra, obtained from virus preparations of three different varieties of influenza virus. **Figure 5** shows CIDS spectra of the dangerous tropical viruses Dengue 1, 2, 3, and 4. In both cases, the different varieties are easily distinguished by the different intensities of the CIDS parameter at certain angles of scattering. The sensitivity of the technique for viruses is presently about 10^7 virus particles per milliliter (ml), but can be improved.

The same technique produces clear signatures of various types of bacteria at concentrations of 10^5 per ml.

The CIDS technique is based on measuring a single parameter of the scattered light, out of a total number of 16 parameters which characterize scattered light (the so-called Mueller matrix). In the MLS technology, several or all of these parameters are measured, thereby obtaining much more information. Also, different wavelengths of input light produce very different signatures. In principle, the total scattering information for a sufficient number of input wavelengths should provide enough information to discriminate thousands of different microbes, even when several are present at once in a given sample. In addition, the MLS could be combined with a flow cytometer, permitting it to examine single cells of various types. This would open the way to a revolution in clinical medicine: Ideally, an automatic MLS instrument would feed a large computer storing the signatures of all the bacteria and viruses of clinical interest. Within minutes of obtaining a blood sample from a patient, the physician would obtain a "print-out" inventory of all microbes present in the sample, with approximate titers! This compares with hours or days of laboratory work often required to diagnose a sick patient today. Often, a physician must begin treatment for a suspected viral infection, days before the virus has been positively identified.

Much work will be required to develop the MLS-technique from its present "proof-of-principle" stage into a standard hospital instrument. In the estimation of one of its developers, Dr. Charles Gregg of the Life Sciences Division of Los Alamos National Laboratory, a commercial version of MLS for bacterial identification could be ready within one year. Identification of viruses is more difficult, but could be achieved within a few years given a crash program. The case of AIDS, where titers of virus are characteristically very low, will require technological innovations to increase the optical sensitivity and discrimination of the MLS instrument.

The interest of MLS goes far beyond its potential applications in clinical medicine. The scattering signature of a virus, bacteria or tissue cell gives critical information on the *geometrical structure*, or conformation, of genetic and related material—information which is missed by the standard techniques of molecular biology. Inside the nucleus of a human cell, the DNA is tightly wrapped into a complex hierarchy of helical structures. When fully "unwrapped," the DNA of a single cell would be about 1 meter long. Inside the nucleus, it occupies a space of less than 1/1,000th of a centimeter in diameter! The actual form of the DNA superstructure, which changes as the cell goes through various metabolic stages, is crucial to understanding how cell functions are regulated. Observation of changes in the DNA supercoiling may be directly important in determining what factors cause HIV retrovirus material "sleeping" within an infected cell nucleus, to become active.

Actually, the characteristic CIDS-MLS signature cannot be completely understood simply on the basis of the interaction of light with a single macromolecule such as the DNA. The signature represents a characteristic of phase-space geometry: the action of the tissue process as a whole upon incident light. Hence, MLS readings have a more fundamental significance than the mere determination of molecular conformation. A crucial direction of research, hardly touched upon so far, is to examine the changes in MLS signature as a function of the phase of a cell in its mitotic cycle.

To be continued.