THE VIROME PROJECT, A 'MANHATTAN PROJECT' FOR BIOTERRORISM. I INTRODUCTION AND BACKGROUND. Version 1.0

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Introduction

The greatest credible threat to mankind is a new or modified, lethal and highly contagious human viral pathogen, produced by either man or nature, for which we have no specific tests, no antiviral drugs, no effective vaccines, no native immunity, and therefore no effective defense.

Such viruses have been produced by nature, but can now be more efficiently made in the laboratory.

No systems, processes, collection of methods, or either government or private organizations exist, or are now planned, that can abort a pandemic caused by a new lethal virus while it is occurring. There is, therefore, little hope, under present conditions and arrangements, of reducing the mass mortality that would ensue.

The objective of the Virome Project proposed here is to provide protection against a new viral pathogen within as little a three weeks of its original detection. In this paper we begin to examine both the technologies and organization that will be required to achieve this goal.

Background

Given time, new infectious pathogens are isolated, sequenced, and either grown to produce a killed vaccine, modified to make a live vaccine, and the genes for one or more viral proteins expressed to make a subunit vaccine.

I propose that each event involved in this detection and response (D/R) process can be systematically and experimentally analyzed, and that an organized attempt be made to determine the minimum time required for each step now, and, with concentrated research and development, accelerate each step and develop new versions of it. These can then be organized into one continuous process, to be kept in constant operation and continuously improved and optimized. For reasons to be discussed, viruses are considered to constitute the major threat.

In all past outbreaks, the time required to decide that a virus is indeed new, to sequence the virus, and to then develop and distribute specific analytical reagents to diagnose new patients has been longer than the epidemic itself. A much longer time, which may be as long as seven years, has been required to make, test, approve and distribute a new vaccine.

As with the Maginot line of WWII, our present massive uncoordinated biodefense efforts may provide temporary comfort, but provide no effective defense in the end.

Given the enormity of the threat of viral pandemics of natural, deliberate, capricious or accidental origin, it is of importance to ask:

1. Is a viral pandemic indeed the prime credible natural or terrorist threat to the United States?
2. Will current plans and operations provide credible means to abort a viral pandemic should it occur?
3. Is an alternate project structure required, possibly modeled after the Manhattan Project, as has been repeatedly proposed?
4. Does the technology exist, or can its creation be reasonably expected, to provide an active in-time response to a new viral pandemic, i.e., is defense possible?

In this, the first paper in an online series, I propose to review each of the above topics. Subsequent papers will describe in more detail each of the technologies required to create a Virome Project that could provide an active detection and response system to answer the most important question of this century, which is: ***Is it technically possible to abort a lethal pandemic caused by a new virus before it has run its full course***?

This series and format is an experimental one, seeking to both inform, to obtain scientific input, and to affect policy. Articles, especially those dealing with experimental results, will be updated as new experiments are done and new ideas emerge. These will be web published as numbered versions, and this is 1.0 in the series. The second, 2.0, will be concerned with rapid virus detection and counting. Following articles will deal in greater detail with subprograms and their technologies.

The conclusion at the end is that rapid detection and rapid defense (RD/RR or "Rapid Cures") are indeed possible and that much of the technology, though almost forgotten, exists. A new organizational structure may be required to put an effective RD/RR system into operation in time to provide effective protection.

Risk Based Prioritization of Homeland Defense

Secretary of Homeland Defense Michael Chertoff[1] has proposed that prioritization of homeland defense activities be based on the evaluation of risk, realizing that risks may be differently evaluated over time. Even if human activity were countered and controlled, nature would persist in creating new and novel biothreats.

The number and complexity of the threats identified in the original organization of homeland defense was very great, and more than one response could be made to each, suggesting that an almost endless number of threat/response combinations are possible. Not all threats are equally dangerous, and not all responses equally cost effective, however. While initial responses may be based on the most publicly perceived threats, long term planning must be based on objective estimations of relative risk, with the realization that a deliberate attack may be specifically designed ***not*** to exploit the greatest calculated risk.

Identifying the Overriding Threat

While economic, social and political disruptions must figure in threat triage, death is, in the end, the deciding metric. Gingrich[2] has recently proposed that biological threats are approximately four times as serious as nuclear and chemical combined, suggesting that biological threats should be our first consideration.

This view is supported by the facts that infectious agents are produced by both man and nature, may start with only a single index patient, and may then spread to escape human intervention and kill millions. No other threat can span such a wide dynamic range or kill as many people. Nature does not produce nuclear weapons or, except in volcanic eruptions, produce large amounts of deadly chemicals, but does consistently produce new infectious agents. (In these assessments toxins are usually grouped with chemical threats, which are considered minor by comparison.)

If we accept the overriding importance of biothreats, the question arises as to which one or ones are the most serious. The CDC has listed human pathogens in order of the threats they pose to the public, and to staff working with them[3]. These agents can be further subclassified in terms of the rapidity with which they spread, their case mortality rates, and the availability and effectiveness of counter measures. A more useful classification, however, may be in terms of their basic biological structure, and whether or not they are living organisms.

Note that in the initial Homeland Defense anti-bioterrorism program, classifications were derived almost linearly from earlier biological warfare doctrine with an emphasis on a small number of weaponizable agents originally chosen for use under battlefield conditions, and with a limited number of detectors assigned to detect agents released in comparatively high concentrations over defined areas.

Homeland Security efforts have therefore not started with clinical laboratories to ask how clinical virology might be improved nationally, how viral infections may be rapidly distinguished from bacterial infections in the physician's office, how totally new infectious agent may be detected, or even with the fundamental question - is an infectious agent present in a presenting patient at all?

Evaluation of Program Effectiveness

While program initiation may be based on risk, program evaluation must be based on some real-world results. For the ABM program, performance is based on shooting down real targets in space. For the Virome Project I propose evaluation based on the continuous detection of all outbreaks of infectious diseases in the United States, on rapid isolation of the agents involved whether known or unknown, on continuous sequencing of their genomes, and on the continuous production and testing of experimental vaccines. The project should include both man and farm animals, since vaccines produced can be directly tested in animal subjects. A key aim of the program should be the isolation and characterization of all viruses to produce a complete sequence database of all human viral pathogens in circulation.

Real world evaluations help rule out the adoption of systems and methods that work only under laboratory conditions, and only provide a theoretical defense against a theoretical threat.Thus the existence of the Virome Project should be publicly visible, and its effectiveness be seen in the clinic, producing measurable public benefit. If biodefense does not revolutionize the field of infectious diseases, and especially virology, as argued here, it must be deemed a failure.

Living vs. Non-Living Agents

Bacteria, yeast, molds, and mycoplasmas have complex genomes with hundreds to thousands of genes, are generally not rapidly spread from person to person, usually grow readily in the

laboratory, can be identified by a variety of immunological and biochemical methods, and can be seen in the light microscope. All agents of this class are alive, can be genetically modified and manipulated, but have never been completely synthesized in the laboratory.

They are also susceptible to one or more of the dozens of antibiotics that are available. While bacterial agents have caused deadly plagues in the past, nearly all are susceptible to antibiotics or to passively given antibodies, can be controlled by vaccination, and can be limited by quarantine and public health measures. They are unlikely to cause serious pandemics now of the scope seen in the middle ages, although their terror value remains and they are an increasing threat as the number of antibiotic-resistant organisms increases.

Viruses are an almost totally different matter, and must be considered separately. Viruses are non-living molecular machines that subvert the molecular machinery of living cells, causing cells to support viral replication inside them. Viral infections are usually very specific, and most viruses will infect only a limited range of species, often attacking specific organs, although some range more widely and can even cross phylum barriers.

All viruses have a nucleic acid core surrounded by one or more protein coats, and the outer coat may contain lipoproteins. Viruses contain only a few genes, and these code for a limited number of proteins. They are not susceptible to the antibiotics that can kill bacterial cells. A few antiviral drugs are available, but none have the broad effectiveness of antibiotics. For an epidemic involving a highly infectious virus, there is a point of no return past which nothing can be done to stop it. Many statistical studies have been done on the viral epidemiology to model viral pandemics, however each model depending on assumptions as to the number of cases initially infected, the basic reproduction number, and case mortality rates, and none reliably predict what might actually happen.

In the design of strategic defenses, one must assume a worst credible case, and it must be concluded that viruses constitute the most serious threat to mankind on earth.

Urgency

The urgency of the Virome Project proposed here derives from the fact that new lethal human viruses both continually appear naturally[4] , and can now be easily made in the laboratory[5]. And the basic tools and the basic design information required to make them are becoming widely available, often on the web or by overnight delivery. As more and more viral pathogens are sequenced and the functions of their genes determined, an annotated database of the genes required to make more lethal viruses is being gradually developed. Genes for increasing infectivity, for making a virus target specific organs, for doing more serious molecular injury to cells and tissues, for more efficient transport through the aerosols, for longer persistence in the environment, and for increasing lethality by down regulating the host immune system[6] (as well, fortunately, as the genes for epitopes that could produce protective immunity) can all now be identified and catalogued. This database will only increase in size with time.

Given the vast resources of nature, and astronomical scale of marine virology[7] , which is able to produce the equivalent of thousands of human genomes-worth of new sequence information per day, and to create large numbers of mutant viruses, it might be thought that human efforts to produce new agents would be eclipsed by nature. Unfortunately that is not now the case, and man-made viruses may be more dangerous than any produced naturally.

Since it is now feasible to construct a human viral pathogen from scratch, genes of ones own choosing from many different sources can be included. This ability is new, and does not exist

in nature on the scale and range possible in the laboratory. The atomic bomb test was the defining moment in the history of mass destruction for the last century. The synthesis of a new lethal virus from simple chemicals is the defining experiment of the 21st century, and could end it for mankind.

This basic technology is widely understood and used. Almost the entirety of genetic engineering and much of molecular biology is based on the ability to put foreign genes into viral vectors. Modified human adenoviruses, for example, are widely prepared to include human or other genes for transfection into human subjects. And the Human Genome Project and all other sequencing projects depend on the ease with which human genes may be parked in viral vectors for amplification, storage, and transport. In fact, all human genes are now available in viral vectors. While gene transfer may occur between viruses in nature to provide new combinations, an event anxiously awaited for bird flu, it is highly unlikely to occur between a large number of different highly virulent viral types simultaneously. In the laboratory, however, the most dangerous genes from all known pathogens can now be all assembled in one virus - an ideal project for a Ph.D. in a "Center of Excellence for Biodefense and Emerging Infectious Diseases[8] ".

Defenses against known viruses can be put in place now, to be ready if an attack or outbreak occurs. For a new virus, all conventional responses would be too late. The threat of a new massively lethal virus is with us now. Sir Martin Reeves, UK astronomer royal, has bet that at least a million individuals will be killed by a natural or man-made infectious agent within the decade, and he gives mankind only a 50:50 chance of surviving this century[9] . Since evolutionists estimate that over 99.99% of all species that have ever lived on earth are extinct, one may reasonably surmise that some of them may have been exterminated by viruses. Hence viral extinctions may occur in the normal course of events, but are more likely now to result from human activity.

The very small size of viruses should not mislead us into thinking that there are only a small number of different types of viruses to choose from, or that they are rare entities. The viral population of the world, the ***virome***, is extremely large. Nearly all species of animals and plants are infected with as least one and usually many different viruses, and the oceans of the world and all natural waters contain around a million or more virions per ml, yielding a world virion mass measured in millions of metric tons. Marine viruses constitute by far the majority of viruses on earth and are the major mutation engine of the planet, most turning over about once a day. Hundreds or thousands of viruses, potentially pathogenic in man, may be somewhere on earth awaiting accidental transfer to humans.

Capricious Virology

Computer viruses are of a complexity quite similar to biological viruses, and no one, at the beginning of the computer age, thought or proposed that they might either be created or become a problem. However, without over a billion dollars spent per year, and vigilance on the part of thousands of individual users, computer viruses would by now have destroyed the whole internet system and rendered most computers useless. It is estimated that over 60,000 such viruses have been written, and the rate at which they are produced is rapidly increasing. Whatever it is that motivates malicious or capricious computer virus authors to anonymously do as much damage as possible can be expected to infect biomedical virologists in the future. It requires an astonishing act of faith to believe this will not happen.

Time: The Key Dimension

The question therefore is, can existing and new technologies be organized and integratedinto a fast detection and response system capable of giving us a real time defense, analogous to that

expected from an anti-ballistic missile system?

The time between identification of the first index cases to the production of a vaccine is now estimated, as mentioned, to be about seven years. The overall objective must therefore be to compress this process by about two orders of magnitude.

No detailed analysis of the average time required to complete each and all steps in the course of detection and response in previous epidemics or pandemics is available, and there does not appear to be any laboratory that has undertaken to model the entire D/R process.

As a general rule, defense systems remain credible by conducing real time exercises to demonstrate readiness, by training personnel, and by insuring that all components are operational. Something better than a pick up band is required to respond do a threat that may become real at any moment.

Are Current Efforts Sufficient?

As noted by Schuler, Fitzgerald, Inglesby and O'Toole[10] in a recent review entitled, Executive Government Positions of Influence in Biodefense: The Biodefense Plum Book;

"Biodefense activities are widely spread throughout the federal government, with more than 26 biodefense positions that are appointed by the president and confirmed by the Senate (so called Plum positions) located in more than a dozen government agencies and government organizations administering more than $5.5 billion in funding in FY2004. This dispersion of responsibility across multiple agencies differs from other large government national security initiatives, such as the Missile Defense Agency (MDA), for example. MDA's head, who is appointed by the Secretary of the Department of Defense, commanded a budget of approximately $7.7 billion in FY2004. The budget for the Missile Defense Agency is approved by the Undersecretary for Acquisitions, Technology, and Logistics, a presidentially appointed, Senate-confirmed position."

"The comparative diffusion of biodefense programs reflects the multifaceted nature of biodefense activities, but this organizational structure also presents particular challenges to efforts to design, implement, and oversee a coherent, coordinated, and efficient biodefense strategy."

As pointed out to me in detail by Dr. John LaMontaigne, the now deceased former head of bioterrorism R&D for the NIAID, funds spent by the NIH can only be spent on peer reviewed projects, mostly in response to requests for proposals. The major RFPs have taken approximately one year to formulate, and an additional year or more before grants are reviewed and activated. There is no individual charged with the initiation and execution of an active biodefense program with the requisite authority required to carry it out. Given the Bayh Dole Act regulations regarding intellectual property, there is no one who **can** have current access to the range of technologies that would be necessary to effect rapid detection and response. The assignment of biodetection activities in DOD to the Air Force, and the development of responding vaccines to the US Army, and the long evident divisions of interest and activities between the NIAID and the CDC do not facilitate either actually setting up or even knowing how to set up an integrated D/R system. The Bioshield Act is specifically designed to insure that technological choices in the end are made by the marketplace, and that R&D be fully reimbursed.

The evident leisurely pace mandated by legislation spawned a Rapid Cures Act[11] aimed to provide the basis for system integration and for a real-time response to a new pandemic. However this proposal has not as yet received serious attention and has not become law.

There is, therefore, no one individual specifically charged with assembling, digesting and understanding all of the available technologies, or who has the mandate, budgets and authorities to mount an effective rapid detection and response system, analogous to that being planned and executed for missile defense.

This paper is neither a full or detailed review of the deficiencies of current HS bioterrorism-related projects, but it does indicate that serious problems remain to be addressed. There is no convincing evidence that the future promises better.

It is understandable, therefore that there have been repeated calls for a Manhattan Project type effort[12] in biodefense, preferably before depopulation has occurred.

The Key Delays

Even without a detailed analysis of the past timelines for detection/response (D/R) scenarios it is evident that great delays exist between the infection of the first index case or cases, the appearance of overt illness, the conclusion that a novel virus may be present, initiation of a response, the appearance of special forces on the ground, culture of the infectious agent, and completion of sufficient sequencing to confirm that the agent is new. The delays incident to the development of a PCR or other specific diagnostic method, and the production of a vaccine of any type, or of an antiserum are highly variable, and depend on a variety of accidents and events that cannot now be predicted. Several years elapsed between the initial conclusion that AIDS might have a viral cause, and the production of the virus in the laboratory, and a vaccine is still not available.

One cannot escape the conclusions that if it were possible to isolate the trace amounts of an infectious agent from the blood or tissue of affected individuals ***by purely physical means***, and if that quantity were sufficient for direct sequencing and conceivably for direct vaccine production, the entire process could be vastly foreshortened. This suggests that physical or chemical separations processes, on both a micro and macro scale not previously available or applied, could accelerate the detection/response process.

A Manhattan Project in Viroterrorism

At first glance the only commonalities between biodefense (more specifically virodefense) and the Manhattan Project would appear to be a large budget and an urgent task. Closer examination, however, shows surprising parallels and overlaps.

The first objectives of the nascent Manhattan Project were to obtain uranium ore and to produce pure uranium compounds. The fissionable isotope of this element , U-235, constitutes only 0.7% of the natural mass, so enrichment of this isotope became an essential, very challenging, and large scale part of the program. All conceivable enrichment processes were considered, rapidly explored on a bench scale, and then the most promising rapidly scaled up in parallel. The Manhattan Project lasted a little over two NIH grant cycles starting from initial consideration of an RFP topic, and cost a fraction of the current bioterrorism budget.

If a viral pandemic started, the first task would be to find and concentrate the virus. As reviewed here, centrifuges were considered very early in the Manhattan Project, but the technology was considered insufficiently advanced for immediate application. However, of all the technologies associated with the Manhattan Project, those involving centrifugation are the most relevant to the present discussion.

In the larger scheme of things, ranging from astronomical to subnuclear dimensions, viruses and uranium atoms are in the same general range of sizes. For many viruses, the position of every atom in the hollow protein shell holding the nucleic acids (the capsid) is known, and the complete sequences of many viral genomes are also known.

The Manhattan Project was concerned first with separations, and second, with the construction and delivery of a product (a bomb) derived from those separations. A viroterrorism project would be concerned with the separation of viruses, and the delivery of vaccines (a cure) derived from them.

This glosses over enormous complexities, but there is a point. If one had the task of quickly finding and concentrating new viruses, and of making a vaccine against these viruses very rapidly on a large scale, why not try a Manhattan Project style organization in an actual Manhattan Project (AEC) laboratory, instantly putting the whole idea to a test in the real world. In the early 1960s I thought this was a good idea so I tried it.

Before launching further into the history of the Joint NIH-AEC Zonal Centrifuge Program at Oak Ridge, which aimed to use the staff, facilities, and technologies of a three billion dollar AEC gas centrifuges program to isolate viruses, it is essential to understand the state of centrifugal techniques when virus isolation was started in Oak Ridge. Centrifuges for isotope separation and centrifuges for virus isolation have some factors in common, and other factors unique to each. It is essential to understand what these are.

Differential vs. Rate Zonal Centrifugation

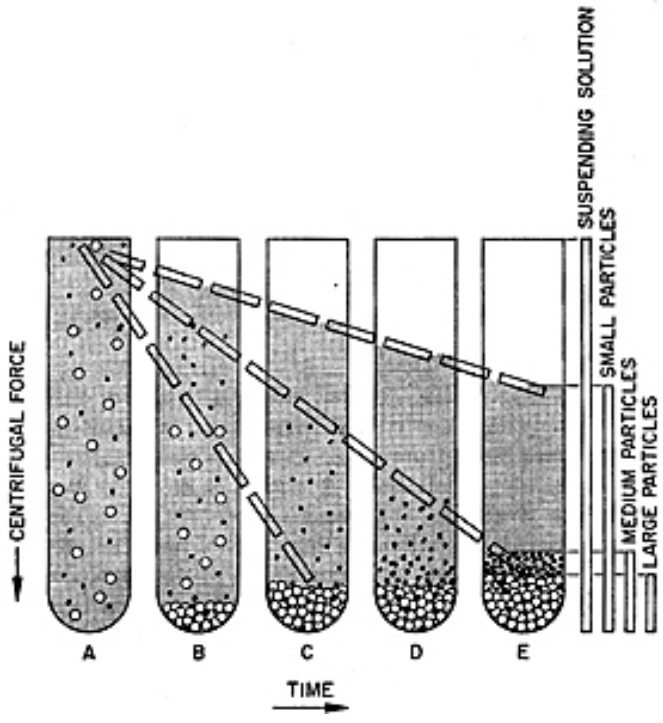

Figure 1. Differential Centrifugation in Swinging Bucket Centrifuge Tubes.

I review first centrifugation as applied to biological separations. Differential centrifugation, illustrated in Figure 1, is the classical technique for centrifugal separations, is widely known and used, and can play a part in virus purification, especially when used for precentrifugation. In differential centrifugation, which includes the vast majority of all centrifuge procedures, centrifuge tubes are usually completely filled with a particle suspension, and a series of spins are done at successively higher g forces or longer periods of time, with removal of supernatants at each step, usually with pellets sequentially resuspended for the repeat spins. All pellets are cross-contaminated, and only the last fraction sedimented, and only part of it, can be obtained in a relatively pure state. Schemes involving recentrifugation of pellets improve resolution, but no differential centrifugation procedure approaches the resolution required here,

which aims to recover trace amounts of virus from large and heterogenous particle collections such as blood or tissue homogenates. These require purification factors may approach a million or more. However the dynamic range of differential centrifugation and the particle S range in tissue homogenate are so large that differential centrifugation can be applied effectively to initial fractionation steps.

Rate Zonal Centrifugation Using Density Gradients

A very large improvement in resolution was provided by Brakke[13], who showed that high resolution separations could be obtained by putting a thin layer of a viral suspension on top of a liquid density gradient and then centrifuging for a defined period of time. Different particles having different sedimentation coefficients sediment at different rates, and the centrifuge rotor is brought to rest when the optimal separations has been obtained. If the rotor is run longer, all particles that had not already reached their isopycnic densities (or floatation level) would have moved further. The separation is therefore a dynamic, rather than an equilibrium one. Note that in differential centrifugation the tubes are full of sample, while in rate zonal centrifugation most of the centrifuge tube volume is occupied by the gradient. The best resolution is obtained with a very thin sample zone having a low concentration of particles.

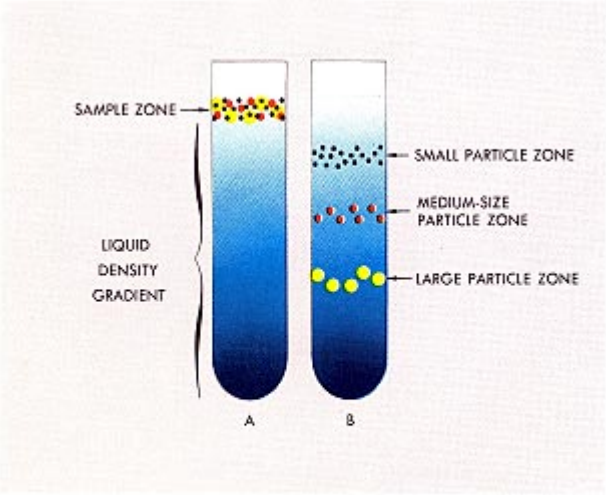

Fig. 2. Rate-zonal centrifugation in a conventional swinging bucket centrifuge tube. Initially a thin sample zone is layered over a liquid density gradient (A) with the tube at rest. After centrifugation (B) particles having different sedimentation rates are separated into zones at different density levels in the gradient.

Rate zonal centrifugation is illustrated diagrammatically in Figure 2.

Liquid density gradients are very sensitive to movement. A gradient set up at rest must be carefully moved into a horizontal position during acceleration and centrifugation, and then this process reversed at the end of a run. This is done in swinging bucket rotors, however at high speed, the strength of available materials severely limits rotor size and speed.

Scaling up low speed rate-zonal centrifugation in swinging buckets was done using glass tubes of modified sector-shape as shown in Figure 3, where a rat liver homogenate has been fractionated into the zones indicated[14].

An effort was initially made to scale up swinging bucket rotors, and a variety of gradient making devices, gradient recovery systems and tube designs were explored. A low speed rotor system was also developed in which gradients were formed and samples inserted during rotation. When gradients are made at rest, only the normal gravitational field stabilizes the gradients and they must be made very slowly to avoid mixing.

If the gradient could be introduced into a rotor during rotation, the gradient would be greatly stabilized by centrifugal force. Svedberg, in his early development of the analytical ultracentrifuge, had shown that a sample layer could be introduced over a slightly denser fluid in milliseconds using a synthetic boundary cell, without detectable mixing, in high centrifugal fields. The interfaces were razor sharp immediately after they were formed, but were soon widened by

diffusion. At 100,000 x g, two solutions differing in density by 0.1 g/ml would act as air and mercury do at 1 g. Hence there is much to be gained by loading gradients and samples into rotors during rotation, and by similarly recovering the gradients while the rotor is still spinning at the end of a run.

The first rotor that could be loading during rotation is shown at rest in Figure 4. The hub of the rotor included a distributor that connected to ports on top of the swinging buckets that channeled incoming fluid from the distributor to the bottom of each of the six swinging bucket tubes.

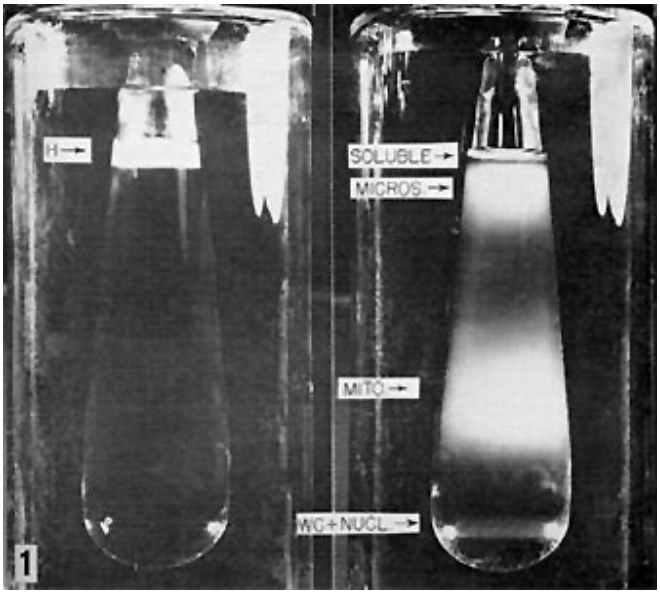

Figure 3. Fractionation of a Rat Liver Homogenate in Glass Modified Sector–Shaped Centrifuge Tubes

During rotation, with the centrifuge tubes horizontal, the sample was also arranged to flow into the distributor disc, where it was distributed evenly to the bottom of each tube. This was quickly followed by one large density gradient run in light end first, starting with liquid less dense than the sample layer. When all fluid was in, the rotor could be accelerated to maximum speed, and the exact speed was determined using the stroboscopic disc in the center of the distributor hub. This system demonstrated that sample and preparative gradients could be dynamically loaded. All the tubes had to be separately unloaded after the rotor came to rest.

A typical contemporary swinging bucket centrifuge rotor and a centrifuge tube for it is shown in Figure 5. The body of the rotor is massive to provide support for the small buckets at high speed, and to provide rotational stability. It illustrates directly that only very small samples can be centrifuged in tubes at high speed using tubes.

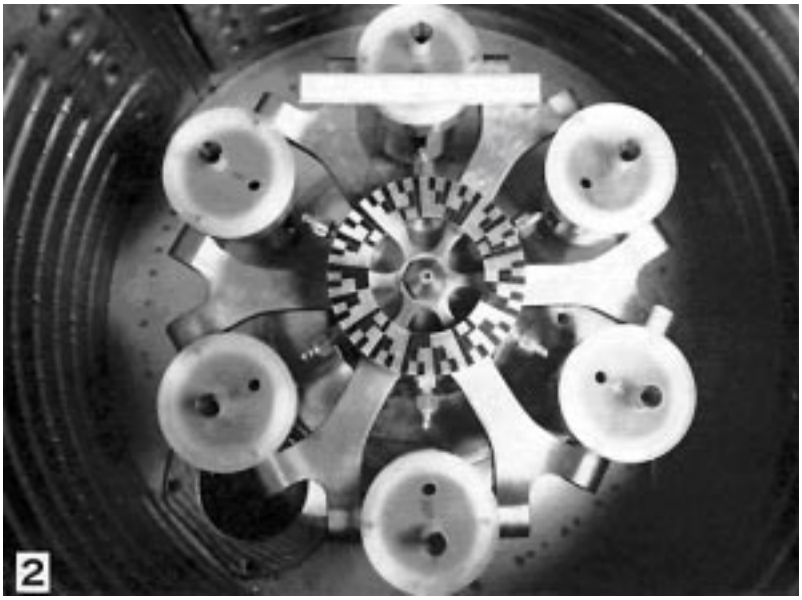

Figure 4. Large Preparative Swinging Bucket Rotor Loaded During Rotation

Making and handling gradients is an art in itself. In the rotor shown in Figure 5, the volume per tube is 13.2 ml, and the average sample size per tube is ~2 mls, making the sample size 12 ml.

To use such a rotor preparatively requires making a large number of gradients, and the gradient in each tube is recovered separately. Only recently have methods been evolved for making and loading precision gradients in small tubes[15].

Thus, in practice, rate-zonal centrifugation in swinging bucket rotors neatly defeated itself by severely limiting first, the sample volumes per tube since it must be present as a narrow zone; and secondly, by limiting the total volume per tube that can be spun at high speed. It initially appeared that basic laws of physics prevented transgression of volume and speed limitations set by swinging buckets. Going from the few milliliter sample volumes of swinging bucket tubes to 100 liter sample volumes did not initially appear possible.

Figure 5. Contemporary Swinging Bucket Rotor (Beckman SW41 Ti). Maximum Speed: 41,000 rpm, Maximum Centrifugal force:288,000 x g, Maximum Volume: 79.2 ml, Number of Tubes: 6

Isopycnic Banding in Gradients

For isopycnic banding the particle capacity problem was not as serious, since the gradients may be self forming, banding gradients are not easily overloaded, and the particles may be initially distributed throughout the entire liquid volume. There is therefore no need for a sharp starting zone, and such gradients have a relatively high capacity. Isopycnic banding is also relatively insensitive to tube orientation, and can be done in angle head or vertical tube rotors. Basic principle is illustrated diagrammatically in Figure 6.

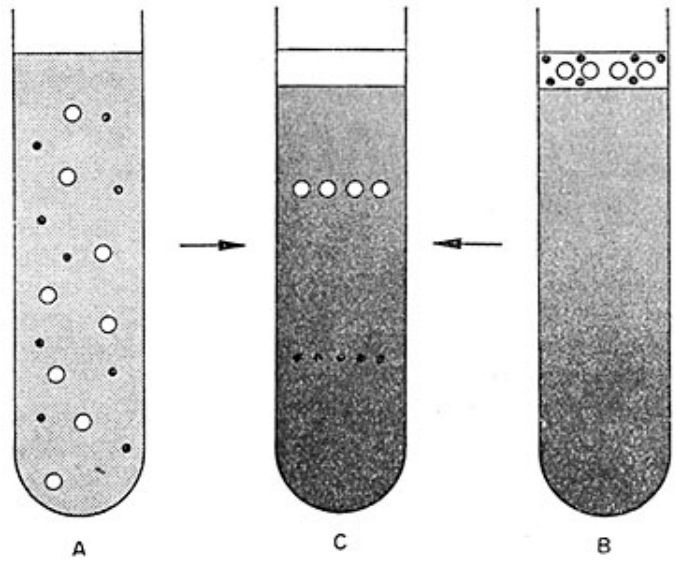

Figure 6. Isopycnic Banding in a Self-Forming Gradient A, or in a Preformed Gradient B. Equilibrium Banding is shown in C.

As shown in Figure 6A a centrifuge tube may be filled with a sample containing a solute that will form a density gradient after prolonged centrifugation. Initially in A the particles are distributed throughout the tube. After high speed centrifugation the particles are banded at their isopycnic points as shown in C. Alternatively, the gradient may be preformed and the sample containing particles introduced on top as in tube B, and centrifuged to move the particles directly into the configuration shown in C. Less time is required to reach equilibrium starting with a preformed gradient as in C.

Initial Work on Zonal Centrifuges at Oak Ridge

In the 1950s high speed centrifuges were all severely limited by both the strengths of materials available, which in turn limited the size of the tubes used. Sample volumes therefore decreased rapidly as speed was increased. As mentioned, use of gradients greatly increased resolution for rate-zonal work, but also severely limited the sample size, in most cases, to a few milliliters.

I conceived the idea of using a closed hollow bowl rotor, and of keeping the rotor spinning while introducing the gradient(s), making the separation, and while unloading the rotor[16]. This would ultimately require that there be liquid lines to both the center and the edge of the rotor, leading back to a coaxial fluid-line seal. Fluid could be pumped in to the edge initially to introduce the gradient, light end first. Flow was then reversed and the sample introduced to the center, and moved out clear of the core by a still lighter fluid. This left the sample layer as a liquid cylinder inboard of consecutively denser rings or zones of liquid. The rotor bowl cavity was divided by vertical radially-oriented septa into sector-shape compartments, which, as initially pointed out by Svedberg, are ideal for sedimentation studies. This projected configuration was approached in stages.

Note that a fraction of sedimenting particles hit the wall in a swinging bucket tube, and that such wall effects are absent in sector-shaped compartments. In Figure 7 only one sector is shown, but it is evident that the complete 360^0 of the bowl can be utilized to produce a new type of rotor called a zonal rotor.

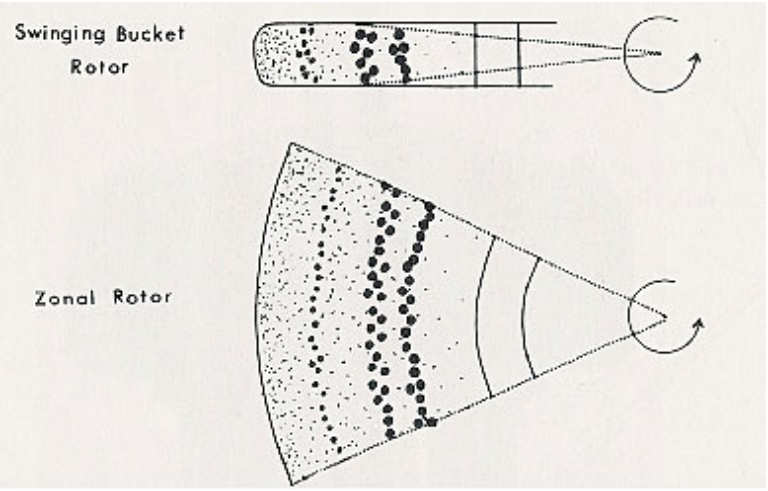

Figure 7. Comparison of Centrifugation in Tubes and in a Sector-Shaped Compartment of a Hollow Bowl Rotor.

The first zonal rotor is illustrated in diagrammatically Figure 8. It exploited the full capacity of a hollow bowl zonal rotor, and was loaded during rotation through a distributor disc in the rotor center which connected directly to the internal edge of the rotor, and accelerated fluid to the tangential velocity existing there. The sample layer was introduced manually as shown through a center opening in the rotor during continued rotation. The rotor was unloaded by introducing a dense fluid to the rotor edge, using the same distributor and lines used to run in the gradient. The displaced fluid concentrically approached the axis, and drained out of the rotor into a stationary collecting ring.

The A-I rotor, as it was subsequently named, was slow and difficult to clean and operate, but did demonstrate that a complete loading and unloading cycle could be carried out during rotation. The actual rotor is shown in Figure 9 in an International Equipment Company PR-2 refrigeratedcentrifuge. A much smaller rotor, the A-II shown in Figure 10, was used to demonstrate dynamic loading, and unloaded in a closed rotor at speeds up to 10,000 rpm. None of these rotors reached speeds were they could be really useful, especially for ribosome fractionation and virus isolation. I therefore turned to Dr. Pickels, of the Beckman Instruments to build a rotor to my

specifications that could spin in a vacuum chamber, be dynamically loaded and unloaded, which brought fluid lines back close to the axis, and would have a large enough capacity for preparative work.

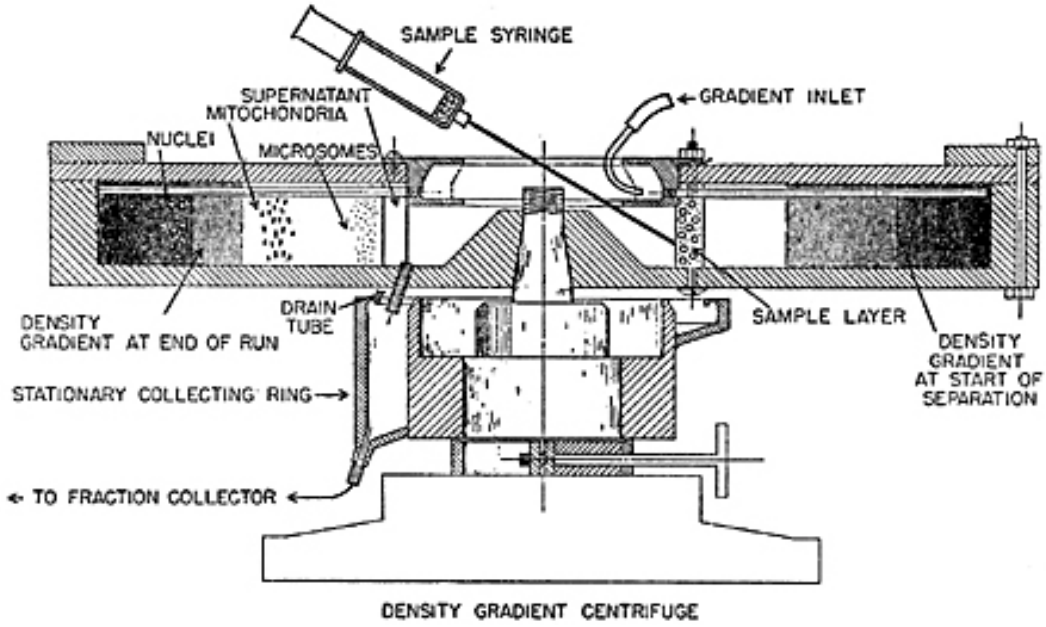

Figure 8 A-1: The First Zonal Centrifuge Rotor Design

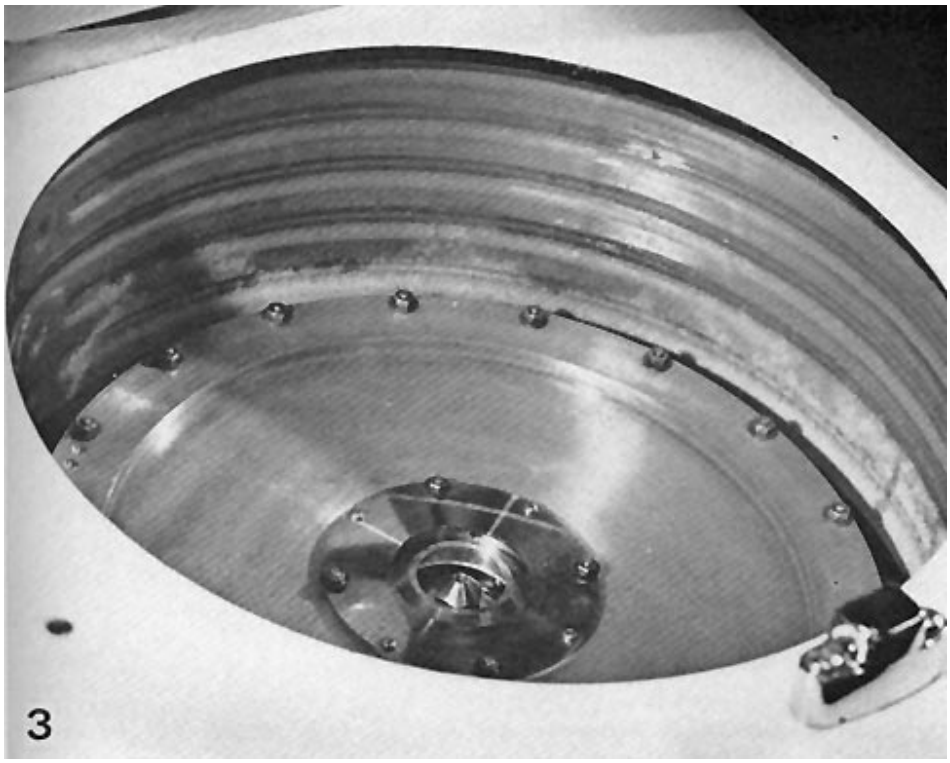

Figure 9. A-1 Rotor in PR-2 Refrigerated Centrifuge

This rotor was jointly developed and delivered to Oak Ridge where it was found to be quite unstable as it approached high speeds. The rotor[17] is shown diagrammatically in Figure 11. Dr. Pickels at Spinco had observed the instabilities, and had developed a theoretical analysis which suggested that stability would be approached if the internal cavity was divided into a number of sector-shaped cavities equal to the rpm divided by 10^3.

This meant that to reach the designed speed of 36,000 rpm routinely, there had to be 36 cavities.

The core configuration is shown in Figure 12, and Figure 13 shows how zones approaching the core body are funneled into exit rings at two levels. Having to clean and reassemble all of the core components was time consuming and tedious, and the necessity for many septa further limited the volume of liquid that could be spun in the rotor.

A concentrate of Type II polio virus suspended in culture medium was kindly provided by Dr. Jonas Salk, and the results of a run in the B-II rotor are shown in Figure 14. The small peak in the center of the plot is the polio virus.This demonstrated that viruses could be successfully purified in zonal rotors.

Figure 10. A-II Rotor.

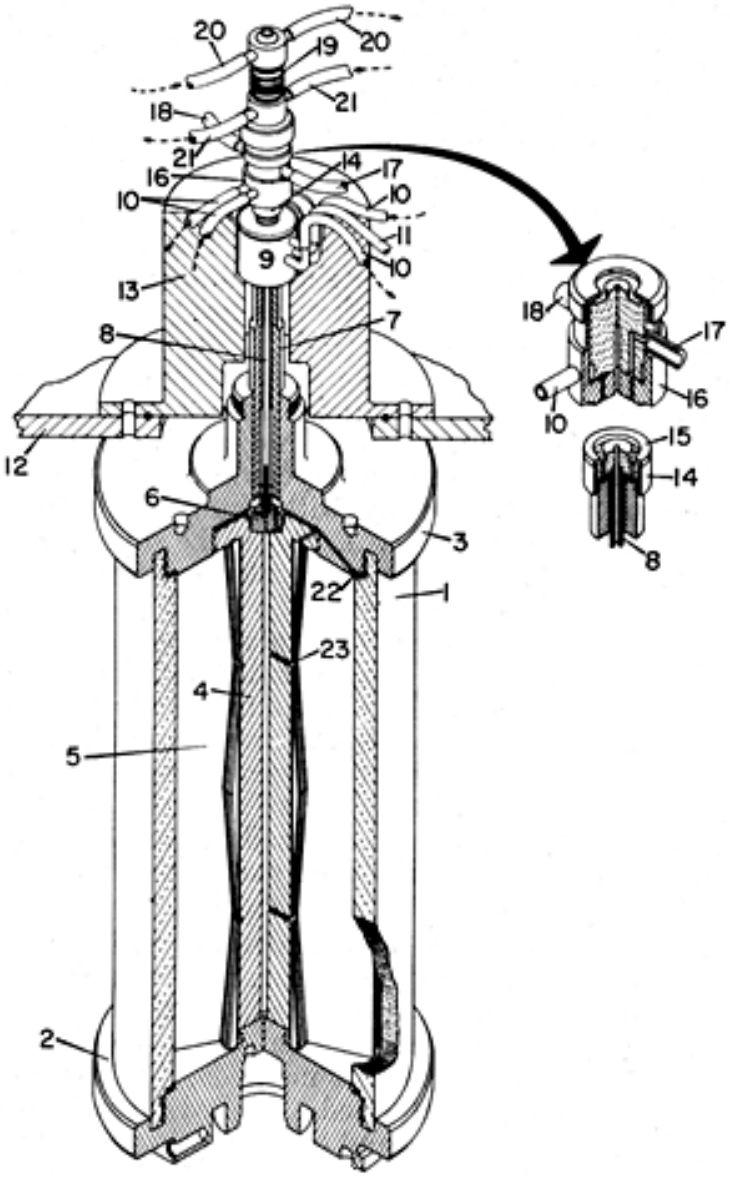

Figure 11. Schematic drawing of a B-1 rotor.
(From Anderson, N.G., J. Phys. Chem. 66:1984, 1962)

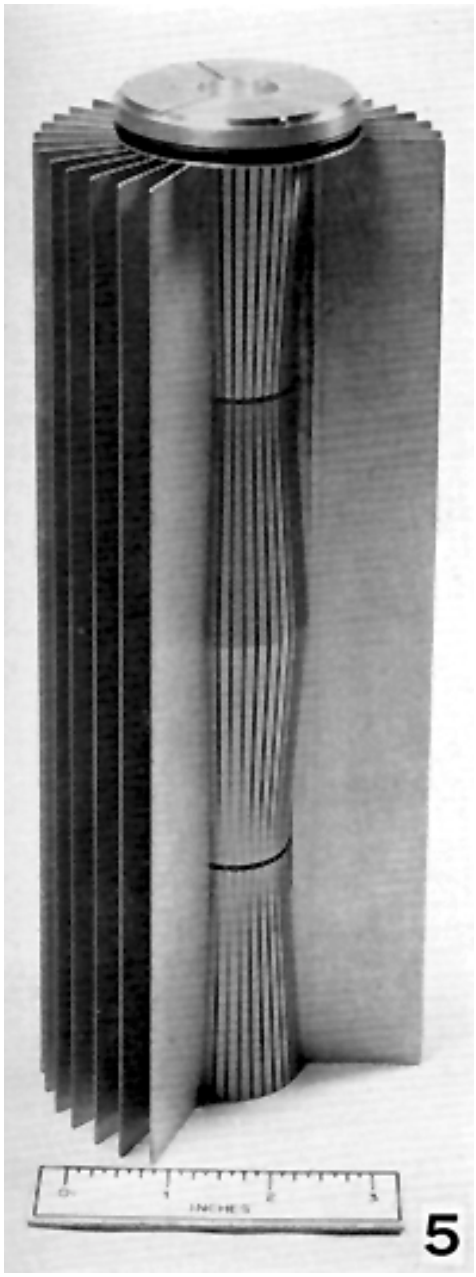

Figure 12. Core of The B-I Rotor

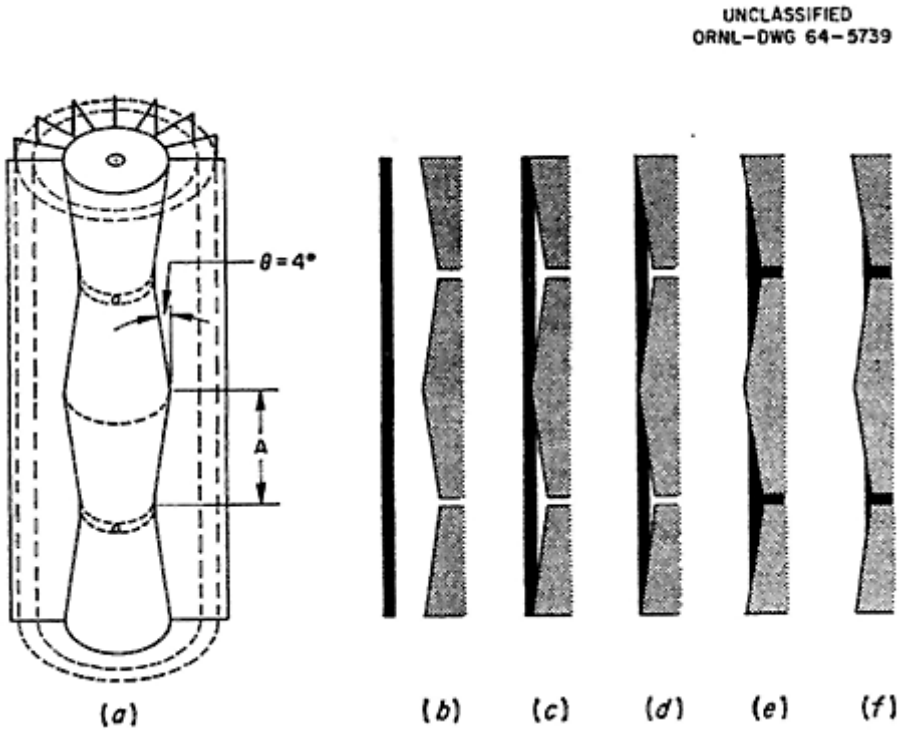

Figure 13. B-II Core Configuration. (a) shows general configuration, and (b)-(f) show how a zone is funneled into the two exit rings of the core.

Proposal to the NIH

In the early 1960s I proposed to Dr. Kenneth Endicott, then Director of the National Cancer Institute, that he fund a project at the Oak Ridge nuclear facility to develop physical methods for finding and isolation human cancer viruses, for isolating appreciable masses of virus from infected human tissues, and for concentrating and purifying human cancer virus grown in vitro to make a vaccine to vaccinate the US population against cancer, i.e., ***for doing exactly what a Virome Project for Homeland Defense would have to do.*** While this project was described in the scientific literature of the time, that literature is largely beyond the horizon of detailed electronic literature search, and is effectively forgotten. Hence the review included here.

The NCI had sponsored a Special Virus Task Force that grew to become a large program funding many investigators both in the US and abroad to find human cancer viruses, and, while many animal viruses were found in animals, providing model systems to work with, no virus causally associated with a major human cancer was discovered. This was not for want of trying.

I suggested, as had others, that such viruses might be present, and simply might not grow in any of the animals or tissue cultures tested. This idea had found support in descriptions of "virus like" particles in sections of human cancer tissue studied with the electron microscope. If the idea was correct, then biological discovery methods, based on virus growth in an animal model, or in available tissue culture systems, might fail, while attempts to find and isolate particles by physical means might work. The initial problem was simply that there were no good physical methods that were up to the problems posed.

I had spent the entirety of WWII working on special problems with the photographic branch of the US Navy, and had experienced the pleasures of interdisciplinary R&D with extensive

technical support, and I though this might translate into research on viruses in an AEC laboratory. I had obtained a Ph.D. at Duke working on centrifugal cell fractionation, a new and promising field at the time, and thought this might also contribute to virus purification. I had also gotten to know Dr. Gordon Sharp (who later invented electron microscopic virus counting) while a student at Duke after the war, and had admired the fact that he built his own ultracentrifuges and Tiselius apparatus. He held the arcane notion that real scientists built (or could build) their own instruments.

I felt comfortable about my proposal to the NCI because I had already invented the zonal centrifuge, but had found no interest in centrifuge development at Oak Ridge.

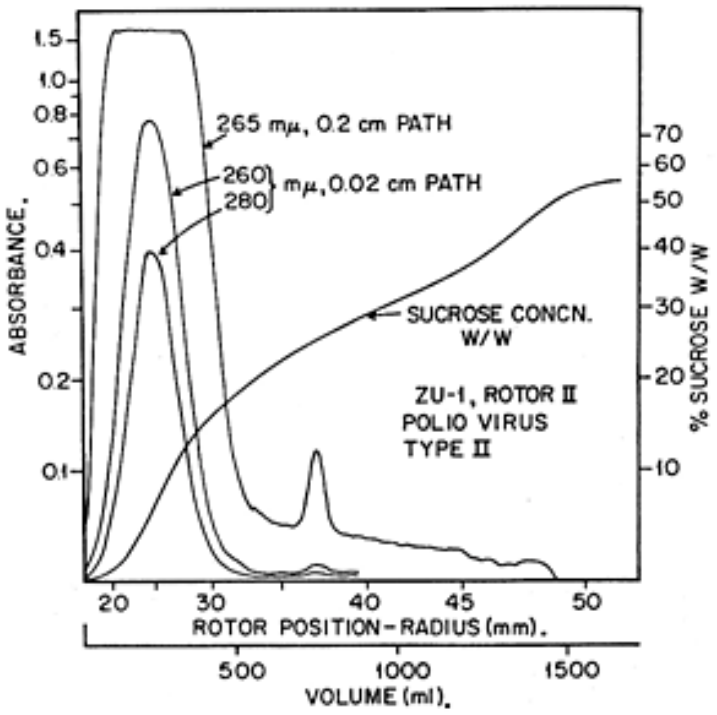

Figure 14. Sedimentation of Polio Virus in B-II Rotor

This missing piece was accidentally provided by Dr. Alvin Weinberg, Director of ORNL, who asked me to attend a meeting to discuss a new centrifuge the US Army wanted to build for a biological warfare laboratory to be built at Pine Bluffs in Arkansas. It soon transpired that the requirement was for a high speed system that would be remotely operated and repaired, that could safely work with highly infectious materials, and that no US company would bid on building it. As a last resort Vitro Corporation, in charge of the planning the project, approached the AEC - and hence the meeting. Present were a number of Oak Ridgers I had seen around town, but I had not known what they did. It was now obvious that they knew very much about ultracentrifuges, and their design and construction. These, it turned out, were gas centrifuges for uranium enrichment.

The Biology Division, to which I was assigned, was, by accident of space availability, in the Y-12 weapons plant, some 12 miles from the Gaseous Diffusion Plant that housed the Separations Division and the gas centrifuge project, but was intellectually estranged from large scale engineering. In the Vitro meeting I found a group to which I could relate, and who were willing to be part of my proposed project with the NCI.

Relevant Background of the Gas Centrifuge

I digress briefly to mention aspects of the history of gas centrifugation, because it bears on the question of whether or not that technology could contribute to progress in virology. The concept of using centrifuges for isotopic separations was suggested by F.A. Lindemann and F.W. Aston as early as 1919, but was not reduced to practice until 1927 when Dr. Jesse Beams at the University of Virginia separated ^{35}Cl from ^{37}Cl, and, in 1941 succeeded in separating uranium isotopes. Estimates at that time for the energy requirements were such as to preclude ever building a large centrifuge

plant. and in 1944 the US Army discontinued support of the gas centrifuge project. It was reappraised in the late 1940s when news of gas centrifuge work in Russia reached the United States.

Dr. Gernot Zippe, a Lufwaffe engineer, was captured by the Russians at the end of WWII, sent to a special prisoner camp for sorting, and ended up assigned to a gas centrifuge laboratory at Sukhumi where he learned of the power requirement problems. He evolved a unique design that was simple, and which used, in the terms of one of the Oak Ridge engineers, gnat power. Zippe had come to the west after the war, and the AEC wanted to learn about the design, and, if it showed promise, extend it. My contact with the Oak Ridge Gas Centrifuge program occurred after Zippe had spent two years at the University of Virginia, and before the AEC fully appreciated Zippe's work. On my part, I had invented the zonal ultracentrifuge to attempt to solve the problem of scaling up density gradient centrifugation but had not previously found the engineering support needed to develop sophisticated liquid ultracentrifuges.

Gas centrifuges spin, as the name indicates, a volume of gas, in this case uranium hexafluoride, in a hollow tubular rotor rotating around its long axis. The essential components of the system are the rotor, the electric drive, the outer casing, a vacuum system, suspension systems and a column that contains the feed, tails and product lines. These are shown diagrammatically in Figure 15.

The outer casing encloses the centrifuge and provides leak tightness to maintain a vacuum and to provide physical protection in case of a rotor explosion. At the speeds employed, air friction would rapidly heat up the rotors, hence it is necessary to spin them in a vacuum. Since the actual mass of gas in the rotor is very small, the cylindrical rotor walls were very thin, and gas centrifuges are now made of carbon fiber. An axial air gap motor is generally used to drive the rotor, while the partially magnetic suspension system holds the rotor upright, and helps absorbs runout due to slight imbalances. The entire aim is to generate sufficient centrifugal force to drive the slightly heavier U-238 isotope closer to the outside wall than the lighter U-235 (desired) isotope. The separation factor in gas centrifugation is proportional to the absolute mass difference between the two isotopes, while in the gaseous diffusion process, the relative, rather than the absolute molecular mass difference provides the separative effect.

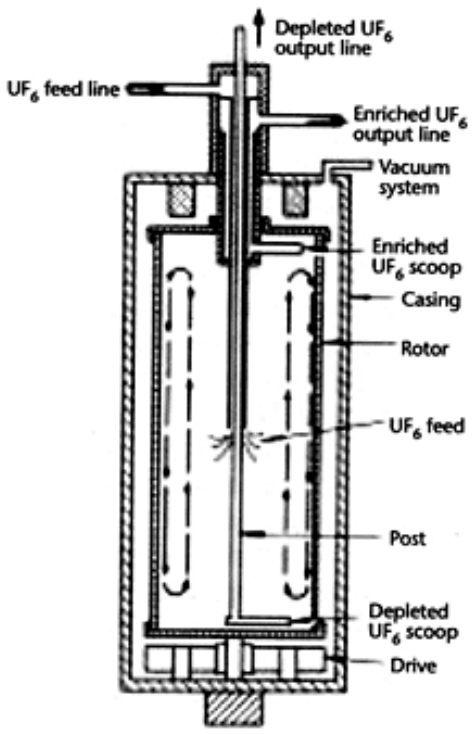

Figure 15. Schematic Diagram of a Zippe-Type Gas Centrifuge[18]

In operation, the UF_6 gas is introduced near the rotor center where it is subject to two separate force fields, which are the centrifugal field produced by high speed rotation, and a countercurrent circulation of gas produced by a thermal gradient along the length of the rotor. The

gas at the hot (lower) end becomes lighter and moves toward the rotor center, while the opposite process occurs at the upper cold end of the rotor. The combination of these two fields results in a rather large difference in isotopic ratio between the top and the bottom. A thermal gradient was provided by keeping the lower end of the rotor hotter than the top. This provides a constant gas circulation during use. Gas is extracted to produce an enriched fraction coming out the top scoop and a depleted fraction at the bottom scoop. The enrichment per centrifuge stage is about one hundred times as great as per stage in a gaseous diffusion process.

Gas centrifuges are arrayed in cascades to achieve the degree of enrichment required. Since the actual mass processed by one cascade per unit time, for example per year, is rather small, thousands of centrifuges are required.

All rotor systems are limited by mass of sample to be centrifuged, by the strengths of materials available, by the critical frequency of the rotor. In addition, rotors are least stable if the length and diameters are equal, which means that one must choose between a pancake or a tubular configuration.

The enormous differences between the weight of a gas and an aqueous gradient means that gas centrifuges can be made very much larger and lighter than liquid centrifuges. Gas centrifuges must be designed for years of continuous operation, while liquid centrifuges have been almost always used intermittently (an exception will be described in a subsequent paper).

Fluid line seals are unique to liquid zonal centrifuges, although some of the basic considerations evolve from seal design in gaseous diffusion plants.

Evolution of the Liquid Centrifuge Project at Oak Ridge

Several planets now conjoined in favor of a virus centrifuge project at Oak Ridge. Before describing these, I mention briefly the problem of blithely setting large sails with incomplete knowledge as to where to go. The large Oak Ridge facilities had originally been set up with underlying theories on which many experts agreed. There were few if any who thought that anything serious would emerge from a few biologists working with an engineering group, especially one as specialized as the gas centrifuge team. But, given the blind alleys of cancer virus research, and the evident mystique of the whole nuclear enterprise, it seemed worth a try. The conceptual base of all uranium enrichment Oak Ridge was simply that there was a differences between U-235 and U-238 that, although very tiny, it could be exploited for large scale separations. And the differences came down to differences in atomic size and mass.

The entire proposed liquid centrifuge program depended on finding exploitable differences between the biological units to be separated. For uranium these differences were physical, and were obvious from an inspection of uranium isotopes in the atomic table. For plutonium, the problem was to find and exploit the chemical differences between the new synthetic element plutonium and both the uranium reactor fuel and the non-plutonium fission products present. The latter problem led directly to the development of ion exchange technologies which, as described in subsequent papers, were of great importance in the develoment of molecular biology.

Unfortunately there was initially no useful data set or theoretical basis for designing separations systems for viruses and other biological particles from scratch.

The Virion Window

All viruses (with a few exceptions such as the recently described 400nm Mimivirus) range in size

from ~ 20 to ~250 nm, with buoyant densities ranging from ~1.14 to ~1.54 S depending on composition of the suspending medium, sedimentation coefficients ranging from ~80 to 300 S, and have nucleic acid cores enclosed in a protein or lipoprotein coat. Not all virions of one species are biophysically identical, however the range of these properties for one virion species is not large.

Following the lead separations technology had provided, I decided to plot the sedimentation coefficients (S) of many different viruses against their banding densities (ρ), including the same data for all the biological particles that might be present, and see if anything interesting fell out. When I first did this in the early 1960s[19] I was surprised to find that viruses generally fall in a region of these so called S-ρ plots that I termed the virus window. A more recent version of this plot is shown in Figure 16.

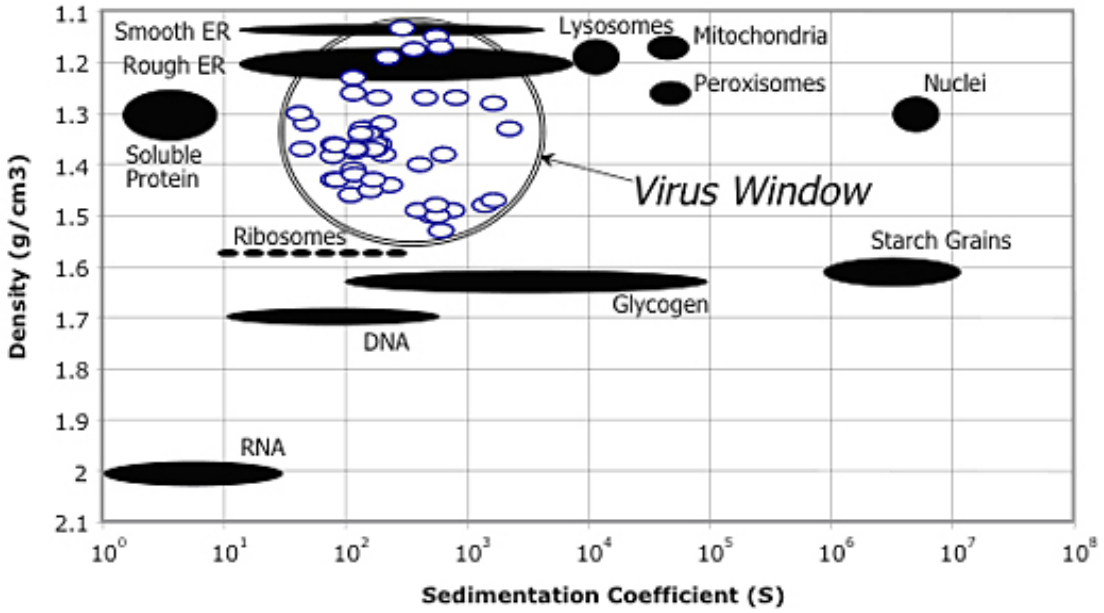

Figure 16. S vs Rho Plot of 53 Different Viruses. (Data provided by Dr. Peter Spragg)

This plot says two things. First, if high resolution methods for two-dimensional centrifugal separations could be devised, then it should be technically feasible to isolate most viruses from almost any source in a high state of purity by purely physical means. And those low density viruses that overlapped the endoplasmic reticulum could, in theory be isolated by consecutive banding in media of different composition, one in cesium chloride, and the other in a nonionic iodinated medium. Only particles that had nucleic acids should be differentially separated. Further, DNA and RNA band at very different density levels, and hence it might also be possible to separate RNA from DNA viruses on this basis.

It is evident that if this plot correctly reflects nature, and if it were possible to make sharp separations based on these parameters, then the entire virus area, originally termed the "virus window" could be isolated from almost any source. Further, if the cuts could be made sharply enough, then an individual virion species could be similarly isolated, even from infected tissues. The major contaminants in S rate separations would be expected to be smooth and rough endoplasmic reticulum. Many viruses survive passage through the digestive tract, and are hence

exposed to many lytic enzymes. The nucleic acids in most subcellular fractions are readily digested with nucleases, and subcellular membranes can be similarly digested with proteases. If the viral mixture that might be obtained from isolation of the "virus window" space were exposed for controlled intervals to such enzymes, host nucleic and proteins would be digested to smaller, more slowly sedimenting particles.

Figure 16 laid the basis for a two-dimensional or S-rho process, in which each of the fractions from the first separation are then subjected to the second. Note that the order of these separations is not critical. However an S separation requires the construction of many small S gradients, which is inconvenient.

The basic equations for gas centrifugation determine gas centrifuge design, and S-rho plots similarly suggest the requirements for high resolution liquid centrifuge design. It is evident that we require centrifuge systems that can achieve high resolution separations over an extended sedimentation coefficient range, and resolve particles over a similarly extended range of banding densities, and which can also process large masses of materials.

It has also become evident in the intervening years that a series of systems and designs are also required to go from hundreds of liters of starting sample to concentrates whose volumes may be measured in milliliters or microliters.

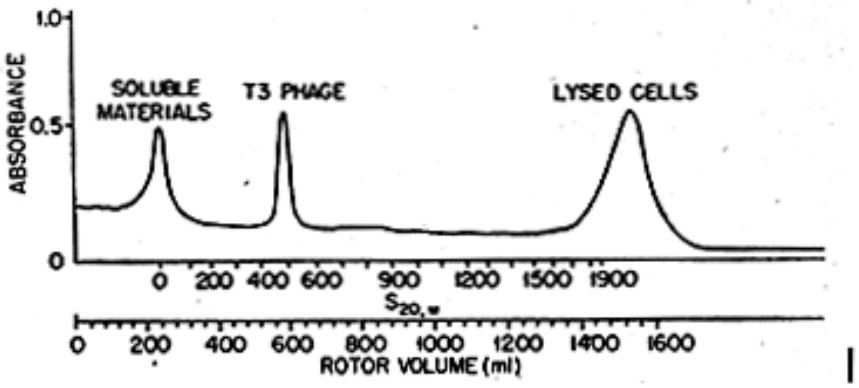

Figure 17. Rate zonal isolation of T3 bacteriophage in the B-1 rotor.

One additional card can be played, which is to change the density of nucleic acids in intact virions by changing the cation species present, conventionally substituting cesium or rubidium for sodium or potassium, thus vastly changing the isopycnic banding density of viruses without changing the densities of contaminating subcellular debris. This trick of changing the density of the component sought, while others remain constant, can be exploited best if a single virus is to be isolated.

Examination of Figure 16 suggests that if a rate-zonal first dimension separation could be made that could isolate fractions having a peak width at half height of ~50-100 S, then almost any virus could be isolated by S-rho technology from almost any source. A preconcentrated lysate of E. coli infected with T-3 bacteriophage was therefore centrifuged in the B-I rotor, and the recovered peak, which, from a virologist's point of view, contains a massive amount of virus, had a width at half height of ~50S (Figure 17). Starting with that observation, it was evident that there is no technical reason why any patient should ever succumb to a viral disease and not have it identified.

Early in the program it was also realized that density gradients could be formed at rest in tubes or hollow bowl rotors, and reoriented during careful acceleration, reoriented during equally careful deceleration, and the separated fractions recovered at rest, as illustrated diagrammatically in Figure 18. This laid the basis for the development of vertical tube centrifugation[20]. In practice septa are essential in hollow bowl configurations to avoid mixing due to Coriolis forces.

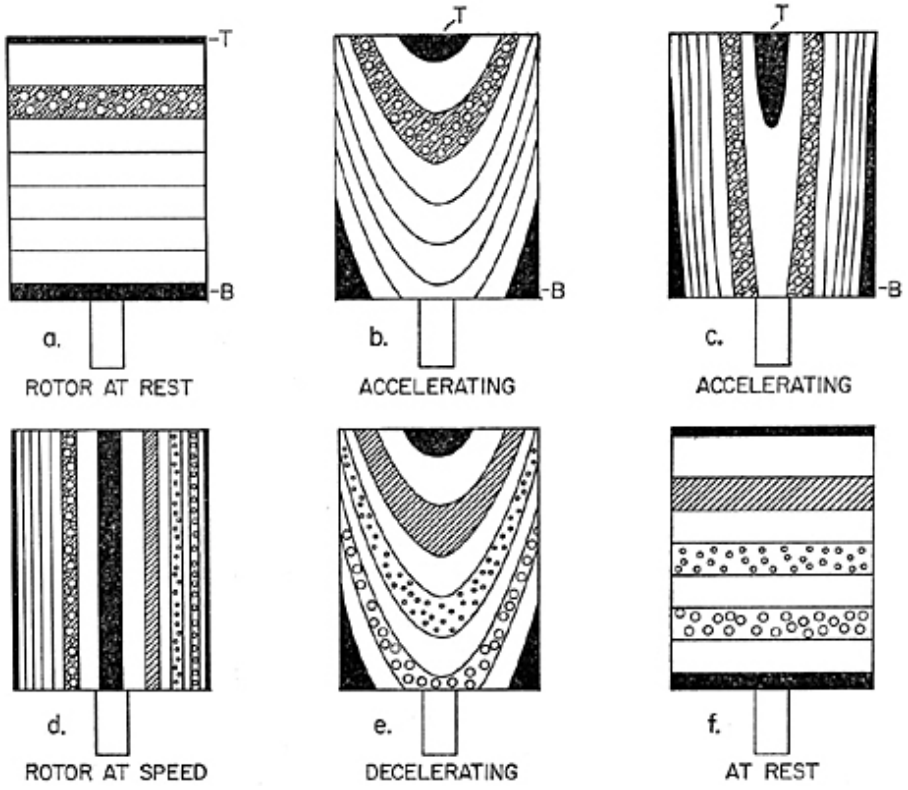

Figure 18.

Isolation of Viruses from Infected Tissues

In an outbreak involving a new virus, the highest viral load would be expected to be in the tissue most affected. It was evident that if S-ρ centrifugation could be developed to a precision preparative system, as is suggested diagrammatically by Figure 19, then enough virus to make a provisional killed vaccine could be isolated. Pasteur had shown that by drying the spinal cords of rabies-infected rabbits, a series of extracts could be made that, if given in the order of length of drying, i.e., as an inactivation series, protection could be provided. If this can be done without any purification whatsoever, then purified virus should also work, and should work better.

First responders require faith that protection is possible and that it will come. A Pasteur-type vaccine provides the only possible protection against a new lethal viral pandemic, and, as will be discussed, commercial vaccines prepared from patient-derived materials have already been made. In practice an inactivation series would be injected, ranging from overkilled to possibly live. The choice between no vaccination, and one of uncertain effectiveness would have to be made at the time.

This approach assumes the presence of operational systems and proven isolation procedures. These cannot be worked out during a pandemic, but, like an ABM must be present and tested.

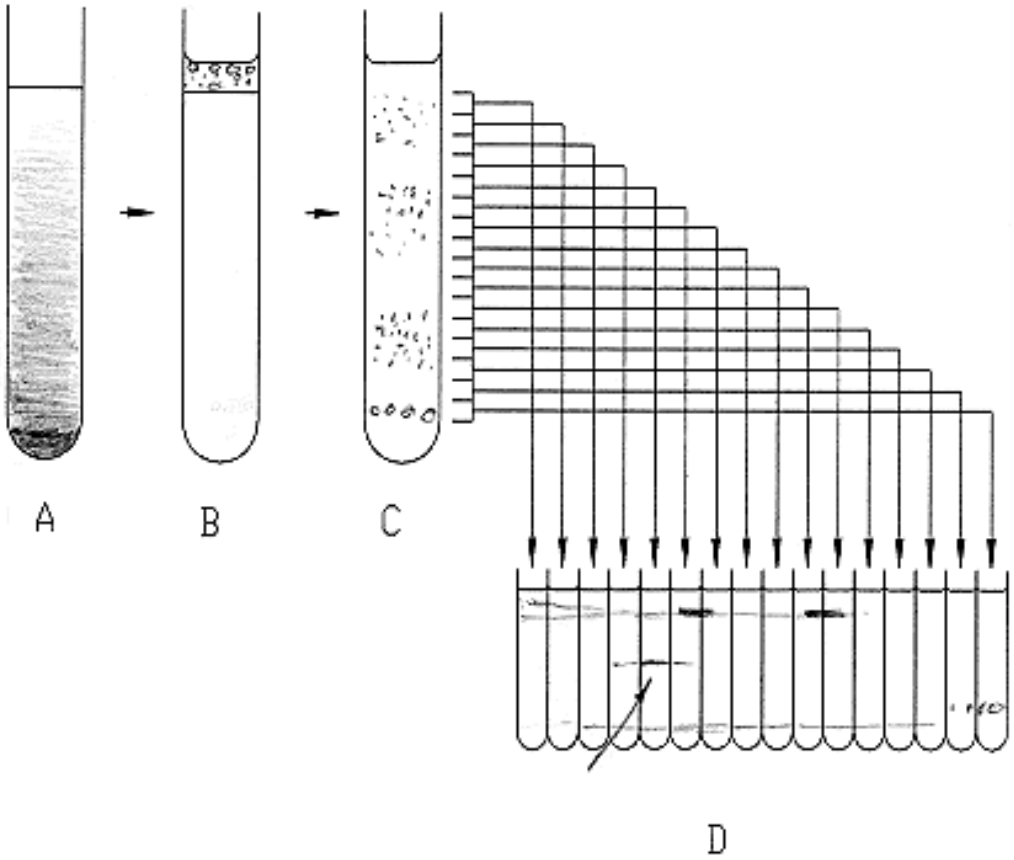

Figure 19. Diagrammatic Representation of S-Rho Separation. Isolated virus shown by arrow.
A. Swinging bucket centrifuge tube with liquid density gradient. B. Narrow sample zone placed on top of the gradient. C. Separation achieved after high speed centrifugation. D. Individual zones recovered from tube C are centrifuged to equilibrium banding positions in a series of smaller tubes D. The banding tubes D are lined up and photographed to produce a pattern congruent with that shown in Figure 15. It is evident that only a very small volume of starting sample, as shown in B, can be processed at one time.

The Manhattan Project (MP) Approach

So many different individuals have suggested that a new approach modeled after the Manhattan Project is now required.

Unfortunately, few of the key organizers of the Manhattan Project appear to have discussed in detail either why or how the organization was set up as it was, and few are available now with direct experience with it. This debate may benefit from numerous discussions I have had with individuals with the project from the beginning, from my thirty post-WWII years in national laboratories derived from the project, and from my experience in setting up and directing scientific programs in those laboratories. I therefore include here a brief review of some of the unique advantages the project offered, and ask whether the same general approach could be reasonably adopted now, or could even be fitted into current legal frameworks.

The Manhattan Project involved a dedicated effort by a group of scientists and engineers, many the best available in their fields, to solve an exceedingly sharply defined problem as rapidly as money, power, and human intellects allowed. Everything imaginably necessary was provided, including rights to all intellectual property, and arrangements for acquisition of staff, land,

equipment, and for design and construction of new facilities. No one had a little company on the side, had any other serious known distracting objectives, or expected, with a very few exceptions, to be subsequently reimbursed for their inventions and discoveries.

Success or failure at the end would be objectively determined, in plain sight of the world. While there was tight lateral compartmentalization for security purposes, nearly everything was accessible and visible from the top. No attempt was made to build any lasting physical or organizational structures, and funds and even facilities migrated to where they were needed. There was remarkably little top level administrative structure, with General Groves operating out of a couple of offices in Washington, and with some of his most senior scientific staff resisting even having offices, preferring to operate directly in the field. The structure was biological, in a sense, unfolding over time to meet needs as they were seen. In general, a double organizational arrangement evolved, with a scientist and an Army officer working in tandem at upper levels. Much attention was given to support structures, including machine shops, instrument shops, optical facilities etc, often reduplicated in adjacent plants. There was no hesitation to shut down operations that were redundant or did not function as needed, and, where problems remained unsolved, it was not unusual to set up a competing facility. The real sin was to hide an unexpected problem, especially one that would cause delay. There was an academic overlay that provided, at least in theory, an intellectual home for scientific staff so that physicists belonged to a Physics Division of which there could be several, chemists to a Chemistry Division of some type, etc. For an operational project, a Director (usually the project initiator) could be assigned, who could choose a staff from members of many different divisions for a temporary assignment that could last from months to years. This provided great operational flexibility, and also provided a scientific home at the end of the project, unless the project became a "permanent" operating unit. Given the size of these laboratories, it was usually possible to find an expert in detergents, tribology, strain gauges, titanium machining, or some abstruse branch of mathematics on the spot, or to find some one who knew a world expert somewhere else.

There were, in short, few reasons short of confronting basic laws of physics, for failing to make progress. Underlying the whole operation was an army engineering structure that provided a basic operating system, organization and rules, and knew how to work closely with industry. Groves, after all had built the Pentagon. Managing scientists was what was new.

NASA may provide an alternative model for organizing against bioterrorism, and Webb has left a treatise[21] on organization and management in the space age which deals with the organization of a program that had some military overtones, had a defined objective, but did not have the full benefit of war as a driver.

It should be emphasized that the Manhattan Project could function as it did only with a central, overriding, agreed upon objective, such as atomic bomb. Once that was achieved, the unifying and motivating force largely faded away, and there are few remnants remaining of the original Manhattan Project "operating system".

To reasonably examine the proposition that we now require a Manhattan Project in bioterroriism we must ask what the specific scientific aims of such a project would be, and what aspects of the Manhattan Project apply.

K-703

How the joint centrifuge project actually became located at the Oak Ridge Gaseous Diffusion Plant is an interesting story. Both Sabin and Salk had been apprised of the proposed

project, and Salk had provided polio virus samples for initial studies. However the masses of material required for large scale experiments vastly exceeded what either Salk or Sabin could supply. American Home Products Corporation produced a killed polio vaccine at that time, and had

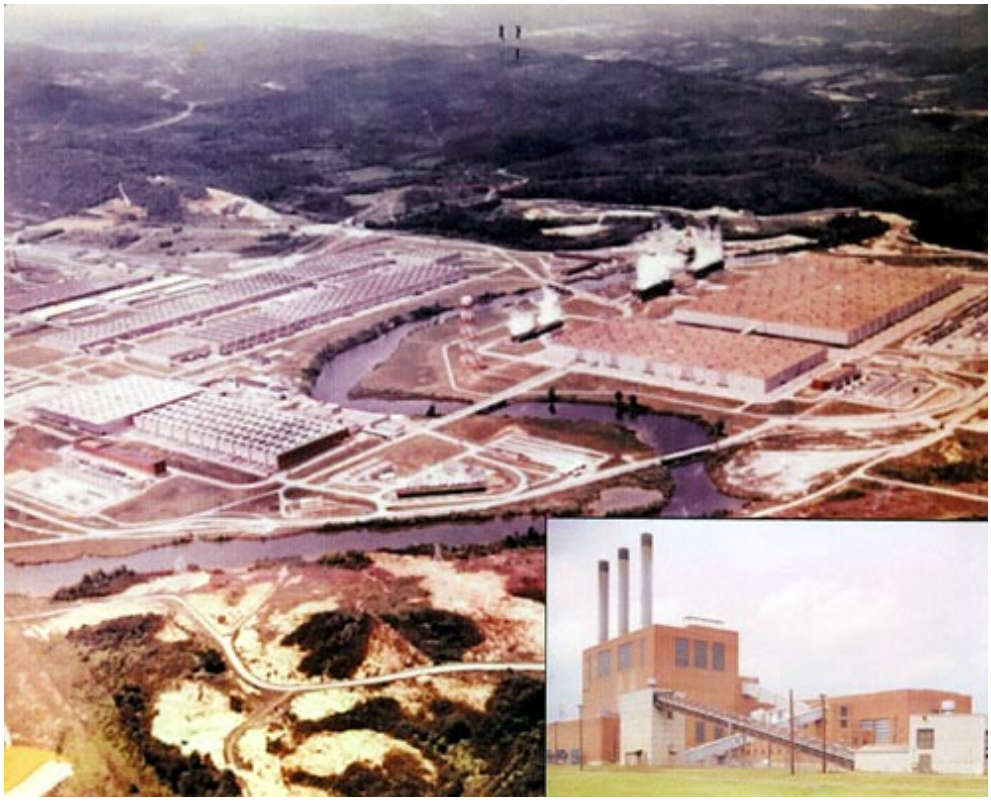

Figure 20. Oak Ridge Gaseous Diffusion Plant in the 1960s. Insert is Building K-703, the site of the Molecular Anatomy Program and the Joint NIH-AEC Zonal Centrifuge Program.

prepared a large batch in which the three different polio strains used had been inadvertently mixed together before all the prescribed tests had been done on the separate preparations. The FDA would not allow this batch to be sold, and I arranged for it to be sent frozen to Oak Ridge. When it arrived at Y-12, the size of the shipment was larger than had been expected, and it was difficult to find a place to store it frozen, thus bringing the situation to the attention of management at many levels. A new difficulty now arose.

A simian virus, SV40, had been discovered that would produce cancer if injected into newborn mice. It was present in some batches of polio vaccine. The Biology Division was home to two very large long-term mouse experiments that might be at risk if masses of polio virus that might contain SV-40 was being fractionated in the same buildings without biological containment. Hence Dr. Alexander Hollaender, Director of the Biology Division initiated an effort to find some other place in the Oak Ridge Complex where the virus centrifuge work could be done. The most distant site was on a spit of land partially surrounded by the Clinch River beyond ORGDP which contained the radioactive remains of a nuclear aircraft engine project, the thermal diffusion project under Dr. Phillip Abelson, later editor of Science, and Building K-703, a 238 megawatt coal or gas fired electrical generating plant built as an alternative standby source of electricity in case of failure of the TVA grid. John L. Lewis, a prominent union leader had threatened to shut down the coal industry, even in wartime, and, since it would cost more to restart the Oak Ridge

Gaseous Diffusion Plant if it were ever shut down than to build it, General Groves considered that a local alternative energy source was essential. The K-703 plant had never been used, and it contained office and laboratory space that was instantly available, just over the fence from the classified ORGDP.

In 1962 the virus laboratory in Y-12 was moved to K-703, and work under Joint NIH-AEC Zonal Centrifuge Program began. Additional support was provided by Vaccine Development Board of NIAID, and by NIGMS to develop systems subsequently described for possible use in clinical chemistry laboratories.

Figure 21. Gas Centrifuge Cascades at ORGDP. Photograph circa 1978.

I asked for the assignment of engineers with experience in the Navy CBs and found several who understood field engineering. The office and laboratory spaces in K-703 were cleaned up, and we drew out the laboratory designs with chalk on the floor and walls, and very shortly had three floors of laboratories ready for occupancy.

Figure 21 indicates the very large difference in the potential size of gas centrifuges as compared to liquid centrifuges, and further indicates the general scale of engineering operations at ORGDP.

At the point where operations started in Building K-703, initial zonal centrifuge development had progressed to the point where a general theory (the S-rho theory) of virus isolation by physical means had been developed, the zonal centrifuge had been invented and carried through to proof of principle using the B-1 rotor system, high resolution preparative rate-zonal isolation of viruses had been demonstrated, support for an enlarged program with NIH and AEC support had been arranged, and administrative approval of a collaborative arrangement with the Separations Systems Division of the Oak Ridge Gaseous Diffusion Plant was in place. However, the B-1 rotor had serious problems, especially with core design, and there were no containment facilities for handling infectious materials.

A basic approach for finding human cancer viruses by physical means was in place, but it was not clear how centrifugation and especially S-rho technology could be applied to the purification of viruses from large batches of cancer viruses (if and when they were found), and if they could be grown on a large scale for vaccine purposes. On the nuclear side of the Oak Ridge enterprise a key technology was mass spectrometry, which allowed elemental isotopes to be discovered, their masses accurately measured, and the ratio of isotopes of one element to be determined. The degree of enrichment for one diffusion stage in the gaseous

diffusion plant, or one centrifuge in a gas centrifuge cascade is extremely small, and, without an extraordinarily sensitive analytical method for measuring enrichment, it would not have been possible to know that the method indeed worked at all. It was therefore logical to go from the minuscule beam throughput of analytical mass spectrometers to the giant Calutrons of Y-12, which ultimately allowed tons of uranium to be beam processed, because the basic idea was so well proven.

For viruses there were no general detection and counting methods analogous in their generality to mass spectrometry. Gordon Sharp, whom I had known as a graduate student at Duke after WWII had developed a method for virus counting that involved sedimenting viruses onto electron micoscope grids[22]. This required that the viruses be in a relatively pure form, have known defining morphologies, and be present in a concentration higher than 10^5 virions/mL.

For further work it was important then, and more important now, to develop facile direct methods for virus counting that can be applied to any virus discovery or isolation process.

Therefore the historical continuity of this series is interrupted and the following paper describes current work on virion detection and counting. Later virus isolation from tissues and large scale virus purification for vaccines will be described, together with a variety of other projects that were part of the general project.

Naphy[23] has described the reasons why governments, especially those in Italy during the middle ages, could function during the plague years, and he concluded the reason was that the citizenry believed that everything that could be done was being done.

Once a rational approach to viroterrorism, such as is described here, is proposed and is the subject of public discussion, it can contribute to panic and chaos if known and not taken seriously, and to public survival if it is and succeeds.

Conclusions

To this point, it had become evident that both the basic theory and the basic technology for rapid virus discovery and isolation were in hand, but that extensive further innovation and development was now required. Further reviews will concern virus counting, rapid high resolution mass isolation of viruses from infected tissues, global screening for human viral pathogens, the development large-scale vaccine purification centrifuges, the engineering of containment systems, and ancillary innovations required to make very rapid detection and response to viral pandemics a reality.

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