A Scientific Case for the Elimination of Chimpanzees in Research

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Executive Summary

Chimpanzees are *Homo sapiens'* closest evolutionary relative. If any animal is an appropriate model of humans for research into diseases such as AIDS, Alzheimer's, Parkinson's and drug testing; it would have to be the chimpanzee. Therefore, an examination of the scientific preconditions surrounding such use is justified.

Historically, chimpanzees and other animals were successfully used to learn things about humans. In the past centuries, we have learned basic anatomy, physiology, and biochemistry from such research. Even today, chimpanzees can be used as bioreactors, for example to grow hepatitis B or other viruses that are difficult to grow in culture medium. These uses have their scientific as well as ethical downsides. The use of chimpanzees is based on their genetic similarity to humans. Because chimpanzees are our closest relatives, one would expect their response to drugs and disease to mirror ours. Obviously, this similarity also has ethical implications.

Today, science is seeking answers to very different questions than when chimpanzees were dissected in the 2nd century AD by Galen. As our examination of living systems has become increasingly fine-grained, we have found that subtle differences between organisms tend to outweigh gross similarities, as explanations for biologic activity. Science successfully used chimpanzees and other animals to shed light on shared functions, however, today we are studying drug response and disease at the level that defines not only a species, but in many cases the individual.

Differences in gene regulation and gene networks, as predicted by evolutionary and molecular biology, explain why even two nearly identical complex systems, such as a chimpanzee and a human, or even identical twins, may respond differently to the same stimuli (e.g., medication), and hence why one complex system, or species, cannot reliably predict response for a different complex system, or species. Current biomedical research is studying disease and drug response at the level where the differences between complex systems, be they two different species or even two different humans, manifest. Hence using animals as causal analogical models for human disease and drug testing is a scientifically invalid paradigm.

We are living at the beginning of the age of *personalized medicine*. Soon your genetic profile will be known to you and your physician. This will allow tailor-made treatment. You can take measures to avoid diseases for which you are at risk and the most appropriate medications will be selected for you. You will be prescribed dugs which complement your genetic makeup, rather than fight it. If we are to expand and refine our current gene-based treatments, our medical research must be more narrowly focused, not broadly focused for example on an entirely different species such as *Pan troglodytes*.

Introduction

Research involving chimpanzees can arguably be dated back to the 2nd century A.D. when Galen dissected them to gain understandings of a fundamental nature. Chimpanzees were undoubtedly studied from time to time after the 2nd century A.D. but their routine use in research can be dated to the middle half of the 20th century.

The growth of biomedical research using chimpanzees resulted from promises rather than results. Robert Mearns Yerkes wrote in 1943:

> …I am wholly convinced by my own experience and as well as by that of others that the various medical sciences and medical practice have vastly more to gain than has yet been achieved, or than any considerable number of medical experts imagine, from the persistent and ingenious use of the monkeys and anthropoid apes in experimental inquiry.[1]

While Yerkes' dream of persistent use have been realized to a degree he may not have imagined, his promise of vast achievements in human healthcare as a result of using chimpanzees and other primate species have been unrealized.

In *Comparative Pathology Bulletin,* Richard Dukelow and Leo A. Whitehair wrote:[2]

> The first major primate facility in the United States was established in 1928 by Professor Robert M. Yerkes at Yale University. This unit was devoted almost exclusively to behavioral observations and was later moved to the warmer climes of Orange Park, Florida.
>
> In the spring of 1940, Dr. James Watt visited Puerto Rico to investigate an outbreak of Shigella in a large colony of rhesus monkeys held on an island named Cayo Santiago. In 1956, then director of the National Heart Institute, Watt visited the world-renowned U.S.S.R. Institute of Experimental Pathology and Therapy at Sukhumi. From these experiences Watt recognized the importance of large, government-supported primate research facilities to aid in cardiovascular studies. Under his guidance, efforts began to develop a primate research facility in the United States.
>
> Within the National Institutes of Health (NIH), a planning committee was formed under the chairmanship of Dr. George Burch of Tulane University and met on September 25, 1957, in Washington, D.C. Immediately, heated discussions arose on whether other biomedical disciplines (besides cardiovascular) should be included and whether the development should be of a single "primate center" or of several "regional" primate centers.
>
> The committee was large, but among its members were Drs. Leon H. Schmidt (Cincinnati), Harry F. Harlow (Madison), and Theodore C. Ruch (Seattle). It is

> significant that these three individuals would eventually become the first directors of three of the seven regional primate research centers.
>
> In consultation with several influential politicians, primarily Senator Lister Hill and Congressman Ralph Fogarty, it was believed that the establishment of the centers program would have a better chance of funding if a regional approach was adopted. In 1960, the House and Senate Appropriations Committees agreed to appropriate $2 million to the NIH to establish two primate centers. Over the next five years, appropriations were added to allow the construction of six regional centers and a "National Conditioning Center." The latter center's mission was to carry out research on husbandry, transportation, and management of various species of nonhuman primates.

Looking back at the history of ape-based studies we find claims made by researchers using chimpanzees that promise much relief from human suffering. An example of such claims can be found in the twenty papers published by cancer researcher Dr. Hilliard F. Seigler, M.D. of Duke University Medical Center between 1970 and 1989. Speaking in 1976, at a tribute to Robert Mearns Yerkes, the father of American chimpanzee research, on the occasion of the 100th anniversary of his birth, Dr. Seigler summed up his talk with strong assertions that chimpanzee-based studies held great promise for curing cancer.

> The importance of biomedical research in human cancer is more evident today than ever before, the obvious important role that the subhuman primate plays in this continued research is evident. Research data accumulated using this experimental animal, so close to man, has in the past and will continue in the future to be directly applicable to the human situation and thus, permits vital investigation that for moral and ethical reasons could have never been considered using human volunteers.[3]

Today, Dr. Seigler continues to conduct research on melanoma but, like the research community at large, has given up on chimpanzees as models of human cancer. Indeed chimpanzee models of cancer are no longer used today. Nor are chimpanzee models of cardiac and vascular disease, two other areas in which chimpanzees were touted as being excellent models for humans.

Again, Dukelow and Whitehair:

> Probably the most impressive role of the [primate] centers was demonstrated in the early 1980s when the AIDS epidemic became the scourge of the world. The regional primate research centers rose to the occasion quickly by their altering research objectives to study and develop the simian immunodeficiency virus (SIV) macaque model for AIDS, the standard and best animal model available for basic research on this disease.

Although the above is disusing monkeys not chimpanzees the concept holds true. Chimpanzees in regional primate centers were used extensively to study HIV/AIDS in the

1980s and 1990s. Considering the lack of substantive progress made in treating AIDS, based on research in chimpanzees and monkeys, the most impressive role of such use makes the whole appear scientifically weak.

This is an inauspicious beginning for our study of chimpanzees in research.

If researchers' claims regarding the utility of using animals as models of human disease and drug response are true and accurate, then the chimpanzee's response to drugs and disease should model human reactions more closely than other nonhuman species due to our close evolutionary relationship. The chimpanzee should be the most productive animal model used, and further, we should expect this use to have escalated as a result of this high utility. It is unreasonable to expect that productive animal models of human disease and drug response have been abandoned.

As the closest living relative of *Homo sapiens*, chimpanzees should be near-perfect models for studying human disease and drug reactions. If a nonhuman animal is going to predict response in a human, it would have to be the chimpanzee. If chimpanzees are not reliable indicators for humans then a *prima facie* case can be made that no animal will be. There is evidence on both sides of the scientific validity question. Chimpanzees are remarkably resistant to AIDS, Alzheimer's, hepatitis B virus (HBV), and malaria. That there are no shared HLA class I alleles between humans and chimpanzees, could account for some of the differences in response to infectious disease.[4] However, scientists have successfully incubated hepatitis A (HAV), HBV and hepatitis C (HCV) in chimpanzees. Chimpanzees were used to determine HBV vaccine potency, sterility, pyrogenicity, and purity. (Cell cultures were also used to make some of these determinations.)

What claims are made for the chimpanzee model? According to *Chimpanzees in Research: Strategies for Their Ethical Care, Management and Use*:[5]

> Chimpanzees have been used in biomedical research to gain an understanding of various diseases that result in substantial morbidity and mortality. The value of chimpanzees in studies designed to make it possible to prevent or treat diseases is due in large part to their genetic similarity to humans. In the case of some infectious diseases, such as hepatitis B, chimpanzees are the only nonhuman species that can be infected with the causative microorganism. Furthermore, some important therapies for diseases not caused by microorganisms have been developed only because they were evaluated in chimpanzees when other species proved to be unsuitable or provided suboptimal results. Because situations like these are likely to arise in the future, chimpanzees should continue to be available for research protocols that benefit human health and well-being. Furthermore, the possibility of a national emergency due to a new infectious agent that presents a major hazard to human health and for which no obvious prophylaxis or therapy is available is a compelling reason to maintain a population of chimpanzees for biomedical research.

With this perception in mind, we will evaluate the scientific sequelae of using chimpanzees in research over the last one hundred or so years.

Models, Genetics and the Scientific Underpinnings of Animal Models

Before we analyze individual cases where chimpanzees are used in research, an explanation of what is meant by *animal models* and the scientific arguments for using animals, such as chimpanzees, as models is needed. This examination is really the crux of the case against using any nonhuman animal species in biomedical research. It is more tedious than merely pointing out differences between species in disease and drug reaction, but it is also more useful in explaining, scientifically, why chimpanzees are not needed or even useful, in biomedical research. A case-by-case analysis is illustrative, but it is more informative and enlightening to investigate the paradigm.

This is a scientific examination of using chimpanzees in biomedical research as models for humans. In order to understand the reader will need to be familiar with four somewhat unrelated areas of scientific study: 1) The origins of scientific study and how it was applied to medical research; 2) What models are and how they are used in medical research; 3) A general knowledge of evolutionary biology and how it pertains to our topic; and 4) An appreciation of what complex systems are and how their relate to this discussion. In what follows, we attempt to briefly discuss each of the four but refer the reader to general texts on the various subjects for more in-depth understanding. The relationship between the four sections should become evident as the reader continues.

Newton and Causal Determinism

Animal use in modern medical science arose during the time of Newtonian physics vis-à-vis reductionism and determinism. Newton said: "Therefore to the same natural effects we must, as far as possible, assign the same causes." Newton went on to explain that this rule applies, ". . . to respiration in a man and in a beast, the descent of stones in Europe and America, the light of our culinary fire and of the sun, the reflection of light in the earth and in the planets."[6]

Both Newton and Claude Bernard (the father of animal-based research) subscribed to the theory that similar causes yield similar effects. Indeed, this theory was one of the breakthroughs that led to the systematic method of inquiry known as the *resolutio-compositive* method or method of analysis and synthesis.

This is the concept of *causal determinism*; it rests on two claims. First, all events have causes, and second, for qualitatively identical systems, the same cause is followed by the same effect. Causal determinism is a presupposition of much scientific activity, notwithstanding indeterministic quantum phenomena. The idea that results in the laboratory can be extended to form expectations about qualitatively similar systems outside the laboratory is embodied in this idea, as is the demand that experiments should be replicable.

This was how science viewed the universe, including animate bodies, when the animal model was embraced by science in the 17th century.

Reductionism was part of that environment. Reductionism is the belief that any complex set of phenomena can be defined or explained in terms of a relatively few simple or primitive parts.

Reductionism has long been the method of analysis and synthesis for biomedical research. Reductionism basically takes complex systems, breaks them into parts, analyzes the properties of parts, analyzes structural relations among parts, analyzes dynamical relations among parts, and synthesizes the knowledge of the parts into integrated knowledge of the complex system. For example, if one understands the cells that compose the organs, anatomy, and how the organs interact, physiology, one understands the human body.

Another example would be atomism. Atomism is a form of reductionism as it holds that everything in the universe can be broken down into a few simple entities or elementary particles and is governed by laws and interactions among them. Modern chemistry reduces chemical properties to ninety or so basic elements and their rules of combination.

Reductionism was widely accepted due to its power in prediction and formulation. It is at least a good approximation of the macroscopic world, although it is completely wrong for the sub-microscopic world as demonstrated by quantum physics, for example, and even in the macroscopic world, it can be taken to extremes.

Determinism concerns the way systems change relative to time. Determinism has been closely associated with reductionism and is the philosophy that everything has a cause, and that a particular cause leads to a unique effect.

Another way of stating this is: that for everything that happens there are conditions such that, given them, nothing else could happen. Determinism implies that everything is predictable, given enough information. Since Newtonian or classical physics is rigidly determinist, both in the predictions of its equations and its foundations, there is no room for chance, surprise and creativity. Everything is as it has to be, which gave rise to the concept of a “clockwork universe”.

Newton's three laws were so successful that for several centuries after his discovery, the science of physics consisted largely of demonstrating how his laws could account for the observed motion of nearly any imaginable physical process.

Although Newton's laws were superseded around the year 1900 by a larger set of physical laws, determinism remains today as the core philosophy and goal of physical science. The animal model arose during a time when determinism, along with a very strict view of reductionism, was the view of the universe. Chaos and complexity, not to

mention relativity and quantum theory have destroyed this view of the universe. Not surprisingly, this has implications for the animal model as well.

(For a more in depth review of how animals are used as models, please see LaFollette and Shanks, *Brute Science,* Routledge, 1996 and Shanks and Greek, *Animal Research in Light of Evolution,* Rodopi 2006.)

We will next examine the concept of models in biomedical research.

Models

Animals are used in science in at least nine distinct ways: (1) as models for human disease; (2) as models to evaluate human exposure safety in the context of pharmacology and toxicology (e.g., ADMET in drug testing); (3) as sources of "spare parts" (e.g., aortic valve replacements for humans; collagen for humans); (4) as bioreactors (e.g., as factories for the production of insulin, or monoclonal antibodies, as a reservoir for viruses such as HBV, or the fruits of genetic engineering); (5) as sources of tissue in order to study basic physiological principles; (6) for dissection and study in education and medical training; (7) as heuristic devices to prompt new biological / biomedical hypotheses; (8) for the benefit of other nonhuman animals; and (9) for the pursuit of scientific knowledge in and of itself.

This essay is focused primarily on the practice of using chimpanzees as models of human biological and biomedical phenomena (items (1) and (2) above) as that is how their use is sold to the nonscientific public. Insofar as we are interested in the scientific issues raised by the use of one group of evolved, complex, hierarchically organized systems (a sample of an animal population) to draw conclusions about another group of such complex systems (typically a much larger and varied population of humans), our investigation might be described as an investigation into the logic of inter-species extrapolation. This problem is related to the problem of intra-species extrapolation (e.g., between varieties or strains of a given species or between distinct individuals of the same species).

This essay is not intended to be a criticism of the use of chimpanzees in the context of basic biological research. There can be no doubt that careful studies of chimpanzees have prompted important hypotheses about basic biological principles, and there can be no doubt that studies of chimpanzees have contributed greatly to our scientific understanding of life, and there is little doubt that studies on animals in general will continue to illuminate these matters in the future (items (7) and (9) above). But evolutionary processes which occur in accord with basic biological principles have modulated the manifestation of life in different lineages, and in this way, have contributed to biological diversity. One consequence of this is that superficial similarities between different species can be highly misleading.

A theory, or in this case a model, is reliable or *scientific* only if it has predictive value. The two types of animal-model use we are addressing (items (1) and (2) above) focus on *predicting* human response, hence our criteria of defining as *scientific* only models that

are predictive is justified. Researchers maintain that chimpanzees are a type of *causal analogical models* (CAMs) and can be used to study human disease. Causal analogies are a subset of analogy arguments in which causal assumptions arise based on the model. In using chimpanzees as CAMs we assume that chimpanzees are similar to humans in certain respects {*a…e*}. For example, chimpanzees and humans have a) immune systems, b) have 99% of their DNA in common, c) contract viruses, etc. A CAM has an additional property "*f*" e.g., HIV reproduces very slowly in chimpanzees. Researchers then conclude that if both humans and chimpanzees share properties *a thru e* then both should also share property *f*, that is, HIV also reproduces slowly in humans.

When used as CAMs, chimpanzees have misled researchers many times.

The *causal/functional asymmetry* theory implies that causal mechanisms may differ between species, and thus call into question the probability of success from using chimpanzees as CAMs. *Causal disanalogies* compel caution in extrapolating data between species. The use of chimpanzee CAMs also suffers from the *systemic disanalogy* argument. Since systems such as organs and tissues may differ in subtle and unknown ways, identical exposure to a given compound will often cause different reactions in different species. In other words, for a CAM to be predictive, there should be no causally relevant disanalogies between species. Considering our knowledge of evolutionary biology, this is arguably impossible without *total* knowledge of both the model (chimpanzee) and thing being modeled (human). Evolutionary biology suggests that a lack of disanalogy would mandate that chimpanzees and man were one species.

History has shown that sometimes chimpanzees react to substances as humans, and sometimes they do not. Only by comparing the results from each test can we determine whether the chimpanzee is sufficiently similar to humans to allow extrapolation. Meaning, we can only know if chimpanzees mimic humans *after* we study the human data. Chimpanzee studies give no new reliable information about humans and are not predictive; consequently using them as CAMs is disingenuous.

Chimpanzee experimenters will insist that chimpanzees, notwithstanding their lack of isomorphism and inability to be CAMs, are still necessary because without chimpanzees researchers could not evaluate the disease, drug, or procedure in an *intact system*. We agree that life processes are interdependent, that the liver influences the heart, which in turn influences the brain, which in turn influences the kidneys and so on. Thus, the response of an isolated heart cell to a medication does not confirm that the intact human heart will respond as predicted by the isolated heart cell. The liver may metabolize a drug to a new chemical that is toxic to the heart whereas the original chemical was not. We also concede that cell cultures, computer modelling, *in vitro* research etc., cannot replace the living intact system of a human being. But the question remains: "Does the intact chimpanzee model do better than the non-chimpanzee methods or better than chance alone?" The evidence, which we will review, suggests that it does not. While chimpanzee models may be *intact* they still suffer from the *systemic disanalogy* argument.

(For a more in depth review of how animals are used as models, please see LaFollette and Shanks, *Brute Science,* Routledge, 1996 and Shanks and Greek, *Animal Research in Light of Evolution,* Rodopi 2006.)

Evolution

There is a very good reason why chimpanzees make poor models for medical research: chimpanzees have evolved to be chimpanzees, not humans. Both species have immune systems but each species has evolved to combat different viruses in different ways. A virus lethal to humans e.g., HIV may be totally harmless to chimpanzees and an HIV vaccine that is effective in a chimpanzee may not be effective in humans. Researchers can infect chimpanzees with artifical versions of human diseases, but it does not follow that what they learn will help humans. It may even result in harm.

Researchers who use chimpanzees are operating under the paradigm that assumes humans and chimpanzees are more similar than different. Modern evolutionary biology reveals that the differences are far more important than the similarities. Differences between organisms occur at the cellular level, the same level where disease occurs. The paradigm of using chimpanzees to study human disease, anatomy, and physiology was plausible in the 19th century when we knew so little. On the gross level humans and chimpanzees were similar: chimpanzees had hearts, so did humans; chimpanzees had electrical activity in their brains, so did humans. But today we are studying the very level that defines the species as different – the molecular level. It is unreasonable to assume that at this level what we learn about one species will apply to another.

The promise of animal modeling vis-à-vis chimpanzees was based on two assumptions. First, that at the molecular level of the biological hierarchy of organization, humans and their animal CAMs were the same animal dressed up differently. There is not a shred of evidence to support this assumption at the level of molecular interactions. This is the level that is relevant to drug metabolizing activity – where biomedically significant effects of evolution are to be found. The second assumption was that the best animal CAMs for human biomedical phenomena were those that are closest to us from a phylogenetic standpoint. This means nonhuman primates in general and chimpanzees in particular. In such CAMs, similarities are common at levels in the hierarchy of organization above the molecular.

As humans and chimpanzees share a common ancestor, it is not surprising that we share certain characteristics; neither is it surprising that each species is unique. The question modern-day researchers must ask is, “Do the similarities outweigh the differences? Can we extrapolate the results of an experiment on one species to a different species?” There is evidence that we can. For instance, as we just said, chimpanzees and humans have hearts, lungs and immune systems and we share the same cell types and tissues. But there is also evidence to the contrary. JL Schardein wrote:

> It is the actual results of teratogenicity testing in primates which have been most disappointing in consideration of these animals' possible use as a predictive

> model. While some nine subhuman primates (all but the bush baby) have demonstrated the characteristic limb defects observed in humans when administered thalidomide, the results with 83 other agents with which primates have been tested are less than perfect. Of the 15 listed putative human teratogens tested in non-human primates, only eight were also teratogenic in one or more of the various species. The data with respect to 'suspect' or 'likely' teratogens in humans under certain circumstances were equally divergent. Three of the eight suspect teratogens were also not suspect in monkeys or did not induce some developmental toxicity.[7]

One way of speaking about the results of evolutionary biology is to categorize life forms into groups known as species. *Homo sapiens* will have unique characteristics but will also have characteristics shared with other species, such as *Drosophila melanogaster* or *Pan troglodytes*. With the advent of molecular biology, we have learned that each species will have some genes in common with other species. Only 1% of genes differ between chimpanzees and humans. One percent sounds like an obstacle easily overcome, but that statistic ignores differences in gene regulation and gene-gene interactions.

Genes can be divided into *structural* and *regulatory* genes. The structural genes are responsible for the similarities. They are responsible for building the proteins of which the body is made. The regulatory genes turn the structural genes on and off, controlling the development of the embryo and organism, as well as its physiology. Regulatory genes account for differences between species. Understanding regulatory genes is crucial for understanding how diseases and therapies vary among species. Regulatory genes force us to see not only the similarities between species but also the differences in *regulatory mechanisms*. By studying regulatory genes, we begin to understand why very small differences between the ways regulatory genes exert control over similar functional genes can be of enormous importance in the way a disease affects a species and the way a species responds to a drug.

The lineage leading to modern chimpanzees diverged from that of modern humans about 5 million years ago (about the same amount of time separating deer from giraffes). Thus, from an evolutionary standpoint, we expect there to be fewer differences between humans and chimpanzees than between humans and mice or humans and yeast. But since humans and our closest phylogenetic relatives are complex, organized, interactive systems, where small differences can be of great biomedical significance, it is far from clear what follows from this observation concerning the degree of phylogenetic closeness.

LaFollette and Shanks write in *Brute Science*:

> Since phylogenetically related species, say mammals, have all evolved from the same ancestral species, we would expect them to be, in some respects, biologically similar. Nonetheless, evolution also leads us to expect important biological differences between species; after all, the species have adapted to different ecological niches. However, Darwin's theory does not tell us how

> pervasive or significant those differences will be. This again brings the ontological problem of relevance to the fore. Will the similarities between species be pervasive and deep enough to justify extrapolation from animal test subjects to humans? Or will the biological differences be quantitatively or qualitatively substantial enough to make such extrapolations scientifically dubious?

While all plant and animal species share the same genetic material, it is the *composition*, or arrangement and regulation of this genetic material that makes all the difference. Lewis Wolpert, in *The Triumph of the Embryo*, explains:

> Compare one's body to that of a chimpanzee—there are many similarities. Look, for example, at its arms or legs, which have rather different proportion to our own, but are basically the same. If we look at the internal organs, there is not much to distinguish a chimpanzee's heart or liver from our own. Even if we examined the cells in these organs, we will again find that they are very similar to ours. Yet we are different, *very* different from chimpanzees. Perhaps you may wish to argue, the differences lie within the brain. Perhaps there are special brain cells which we possess that chimpanzees do not. This is not so. We possess no cell types that the chimpanzee does not, nor does the chimpanzee have any cells that we do not have. The difference between us and the chimpanzees lies in the spatial organization of the cells.[8]

One reason for the difference between species vis-à-vis the spatial organization of the cells lies within the genes. Wolpert continues,

> The face develops from a series of bulges in the head region and at early embryonic stages it is not easy to distinguish dog from cat, mouse from man. The differences in facial features are very dependent on just how much these bulges grow. One can begin to imagine how genes could control such changes in growth rates at different positional values. The key changes in the evolution of form are in those genes that control the developmental programme for the spatial disposition of cells. The difference between chimpanzees and humans lies much less in the changes in the particular cell types—muscle, cartilage, skin, and so on—than in their spatial organization. Direct confirmation of this comes from studies which compare the proteins of humans and apes. If we look at the genes that code for the average "housekeeping" proteins—proteins that function as enzymes or provide basic cell structure and movement—the similarity between chimpanzees and humans is greater than ninety-nine percent. The difference must reside not in the building blocks but in how they are arranged, and these are controlled by regulatory genes controlling pattern and growth.

King and Wilson write:

> Small differences in the timing of activation or in the level of activity of a single gene could in principle influence considerably the systems controlling embryonic development. The organismal differences between chimpanzees and humans would then result chiefly from genetic changes in a few regulatory systems, while amino acid substitutions in general would rarely be a key factor in major adaptive shifts.[9]

As LaFollette and Shanks explain, understanding the role of regulatory genes in evolution is:

> …crucial to a proper understanding of biological phenomena. First, they focus our attention not merely on structural similarities and differences between organisms but also on the similarities and differences in regulatory mechanisms. Second, they illustrate an important fact about complex, evolved animals systems: *very small differences between them can be of enormous biological significance. Profound differences between species need not indicate any large quantitative genetic differences between them. Instead, even very small differences, allowed to propagate in developmental time, can have dramatic morphological and physiological consequences.* (Emphasis added)[10]

Even a few examples of "dramatic morphological and physiological consequences" illustrate how the similarities in an organism's structure make it appear at first glance that we can use animal models, while the profound differences in molecular composition demonstrate why the model breaks down upon further examination. There is only a 91% similarity between the human and chimpanzee CD4 HIV-receptor, and single amino acid changes are known to ablate HIV-binding in these molecules. A single amino acid difference can be responsible for causing cystic fibrosis. That is how very small differences on the cellular and sub-cellular level lead to dramatic differences in the organism as a whole.

Similarly, the mouse and human genomes do not appear to be *qualitatively* very different. They both contain about 30,000 genes with mice having 300 humans lack and vice-versa. Humans and mice both have the genes that in mice result in a tail. The difference between the species lies in the regulation of the same genes.

Vis-à-vis the mouse and human genomes we would expect various genes to be regulated differently. This is illustrated in figure 1. This is a gene expression profile for the *CFTR* gene. The blue bars are the expression profile for humans while the red bars are for the mouse. Notice that the same tissues have very different expression profiles. For example, the gene is expressed much more in mouse ovary then human, about the same in the salivary glands, much more in mouse liver, thyroid and so forth. This is what makes one species a mouse and the other a human and is why different species react differently to the same stimuli such as a medication.

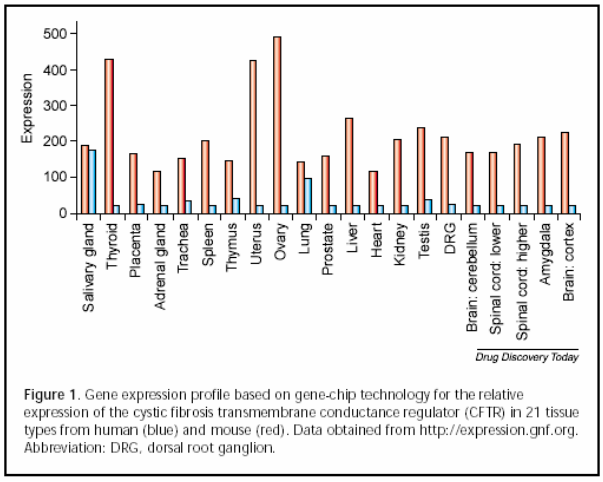

Figure 1. Gene expression profile based on gene-chip technology for the relative expression of the cystic fibrosis transmembrane conductance regulator (CFTR) in 21 tissue types from human (blue) and mouse (red). Data obtained from http://expression.gnf.org. Abbreviation: DRG, dorsal root ganglion.

Figure 1. (From *Drug Discovery Today*, Vol 8, No 6, 2003, pp 233-235.)

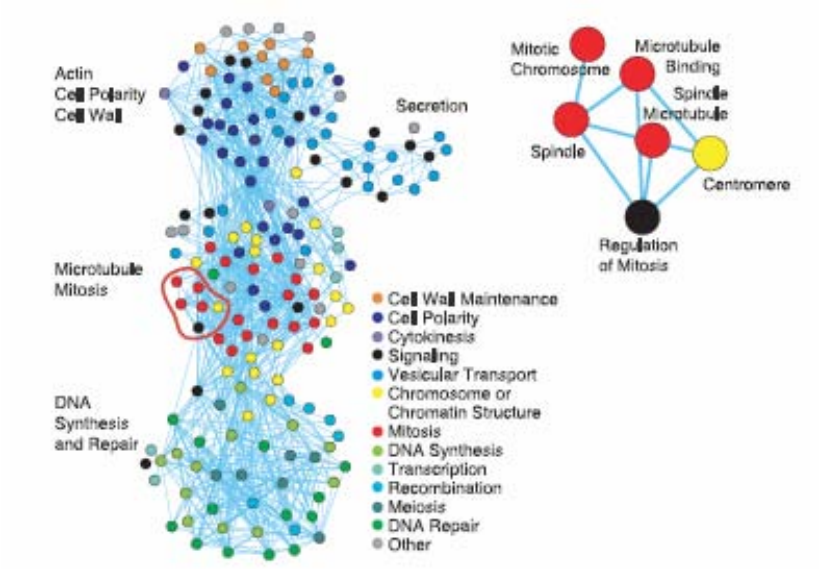

Figure 2. (From *Science* 2004;303:808-813)

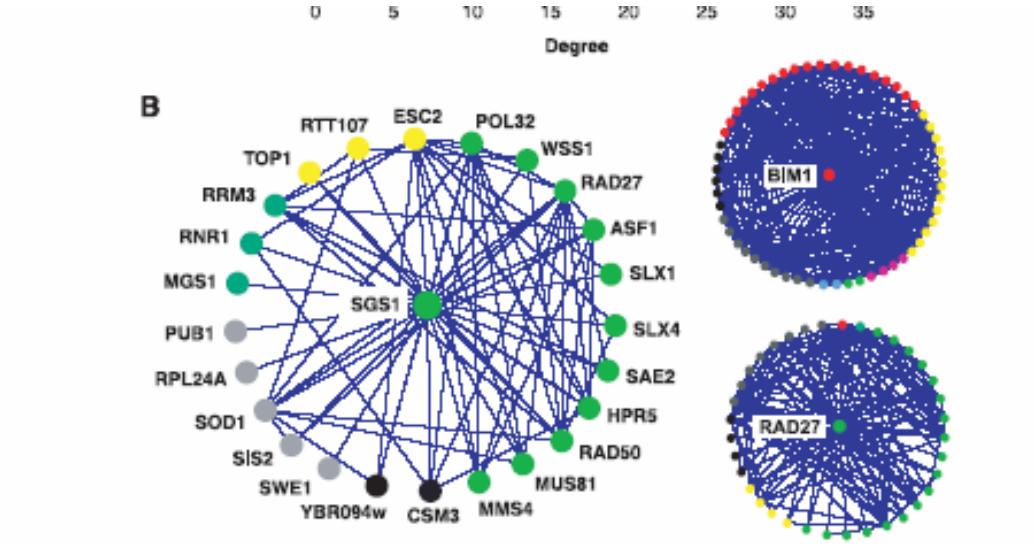

Figure 3. (From *Science* 2004;303:808-813.)

Figure 2 illustrates multiple gene interactions. Figure 3 is the topology of the genetic network of a three-gene neighborhood. Again note the multiple interactions. For our purposes this illustrates how difficult it would be to isolate all the functions of single gene and how by regulating the same genes differently evolution can produce two very different species.

PLoS Biology, in an editorial said this about mouse models of autoimmune diseases:

> These results fall in line with mounting evidence that background genes are not silent partners in gene-targeted disease models, but can themselves facilitate expression of the disease. This finding underscores the notion that genes are not solitary, static entities; their expression often depends on context. With genetically complex diseases, having the requisite combination of susceptibility genes does not always lead to disease.[11]

Finally we will briefly examine complexity and its implications in light of the above.

Complex Systems

Animals, human and nonhuman, are examples of complex systems. Complexity, as a concept in science, relates to structure and order that are found between the condition of total randomness or chaos, and total order. The significance of complexity within the context of this article, is that separate and independent routes of evolution can arrive at similar conclusions concerning function. The concept of nonlinearity exhibited by complex systems is also important. This nonlinearity is manifest in genetic networks and elsewhere.

The causes and effects of the events that a complex system experiences are not proportional to each other. The different parts of complex systems are linked to and affect one another in a synergistic manner. There is positive and negative feedback in a complex system. The level of complexity depends on the character of the system, its environment, and the nature of the interactions between the system and environment. Again, we are back to nonlinearity.

The world is made of many highly interconnected parts on many scales, the interactions of which result in a complex behavior that requires separate interpretations of each level. This realization forces us to appreciate the fact that new features emerge as one moves from one scale to another. So it follows that the science of complexity is about revealing the principles that govern the ways in which these new properties appear.

Complex systems are made up of a large number of interacting parts that affect one another. Complex systems also display a hierarchy of parts. Human society is composed of different populations of humans, which are composed of tissues or organs, which are made of cells, which are made of molecules, which are made of atoms, which are composed of elementary particles. Each level builds on the previous and complexity increases with each level. One cannot predict what happens at a level higher than the one that is being studied. Note that this does not contradict reductionism; when we speak of complex systems, we are by definition speaking of a system whose whole is greater than the sum of its parts.

Complexity also increases as size increases and as the number of different cells increases. The behavior at one level does not predict the behavior at another. For example: Water is simply two hydrogen atoms attached to one oxygen atom; it can be described exactly by the laws of physics. But there is nothing in those laws that predict what the compound will do when trillions of them combine. Liquidity, the name given to the properties of water, is emergent. The fact that water changes when cooled or heated has meaning only when water is present as billions of atoms, not as one molecule. Weather, such as the formation of hurricanes or tornadoes; life forming from DNA and proteins, and mind are also emergent phenomena. The universe is a hierarchy, where at each level of complexity new properties emerge (see figure 4). Psychology is not applied biology nor biology applied chemistry, nor chemistry applied physics. Hence, when we are studying complex organisms, like mice and humans, it should not come as a surprise that small differences, as we saw in gene profiles, will result in different emergent properties such as different species that respond differently to drugs and disease.

As an example of different species achieving the same end from different evolutionary pathways, consider the following: It is now generally recognized that chimpanzees, bonobos, and orangutans can recognize themselves in mirrors. It has recently been reported that bottlenose dolphins also pass this test. Most gorillas, monkeys, and other mammals, such as elephants, do not.

The research on dolphins is of particular interest since it suggests that self-recognition is not confined to the great apes and humans. The line leading to humans and chimpanzees

diverged from that leading to dolphins over 60 million years ago. Thus, although dolphins, chimpanzees, and humans show a high degree of encephalization and neocortical expansion, the brains of dolphins are very different from those of chimpanzees and humans, with respect to both cortical cytoarchitecture and organization. These findings imply that the emergence of self-recognition is not a by-product of factors specific to great apes and humans but instead may be attributable to more general characteristics such as high degree of encephalization and cognitive ability. Hypotheses about the evolution of self-recognition have, to date, focused on primate characteristics. These findings show that self-recognition may be based on a different neurological substrate in dolphins.

The authors of this dolphin study see self-recognition in dolphins as an example of *convergent cognitive evolution*— in other words the same cognitive capacity has evolved but was dependent on different neuroanatomical characteristics and evolutionary history. Further, it appears the linguistic abilities of chimpanzees are no better than those of dolphins. The evolutionary line that leads to dolphins diverged from that leading to humans at least 60 million years ago, resulting in at least 120 million years of independent evolution. Evolutionarily speaking, humans are no closer to dolphins than they are to rats. This is not an isolated example. To go even further apart in the family tree, some parrot species have linguistic abilities superior to some primates. (For more see Shanks. *Animals in Science.* ABC Clio 2002.)

For our purposes, this means that different structures can perform the same function, and that the same structures can perform different functions in different species. The similar function is the beguiling attraction of the animal model. We assume similar function means similar structures, which can be manipulated to produce similar results, just as Newton assumed in the 1600s.

However, combining this data with our previous discussion of gene expression profiles and gene networks, we can begin to see why studying one species will not necessarily yield information pertinent to another. Extrapolating results from one complex system to another is difficult because small differences between the systems can result in two very different species over evolutionary time.

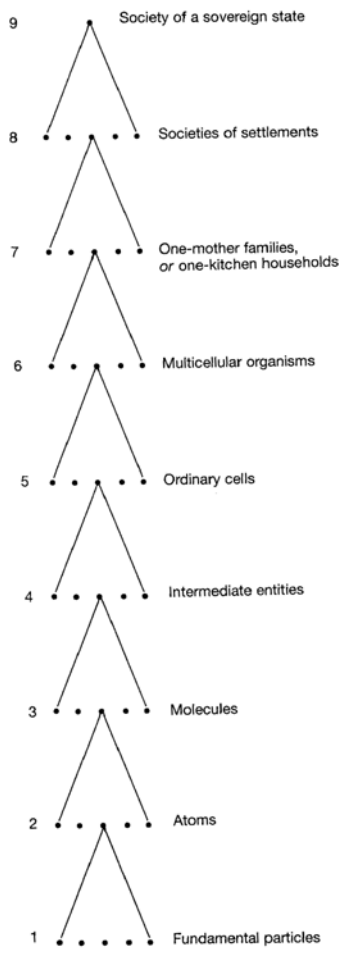

Figure 4

In the past, society learned to understand reality through simplification and analysis vis-à-vis reductionism. Some important simple systems are successful idealizations or primitive models of particular real situations — for example, a perfect sphere rolling down an absolutely smooth slope in a vacuum. This is the world of Newtonian mechanics, and it ignores a huge number of other, simultaneously acting factors. Although it might sometimes not matter that details such as the motions of the billions of atoms dancing inside the sphere's material are ignored, in other cases reductionism may lead to incorrect conclusions. In complex systems, we accept that processes that occur simultaneously on different scales or levels are important, and the intricate behavior of the whole system depends on its units in a non-trivial way. Here, the description of the entire system's behavior requires a qualitatively new theory, because the laws that describe its behavior are qualitatively different from those that govern its individual units. What we are witnessing in this context is a change of paradigm in our attempt to

understand our world. The laws of the whole cannot be deduced by digging deeper into the details.

Animal models fail as CAMs. This is expected and predictable following complexity theory. Complexity theory explores systems in which many independent agents are interacting with each other in many ways. Pertinent to this discussion, Nicolas and Prigogine have stated, "Complexity is somehow related to the various manifestations of life." We have the scientific understanding to travel to the moon and back, but are as yet unable to fully explain life on a cellular level. There is something fundamentally unique about the dynamics of living systems. Complex systems tend to give rise to new complex systems. A complex system is one in which numerous independent elements continuously interact and spontaneously organize and reorganize themselves into more and more elaborate structures over time, as evolution has reorganized genes and gene regulation. As with chaos, the behavior of self-organizing complex systems cannot be predicted by studying a different system, and complex systems do not observe the principle of additivity, their components cannot be divided up and studied in isolation. To repeat, the causes and effects of the events that a complex system experience are not proportional to each other. The different parts of complex systems are linked and affect one another in a synergistic manner. Consider for a moment humans and chimpanzees. Hochachka and Somero ask, "How much new or different genetic is required to make a new species? They comment:

> The problem (and in some senses the paradox) is that protein and gene sequences in the common chimpanzee and in the human are remarkably similar. In fact, human and chimpanzee proteins appear to be 99% identical at the amino acid level, and it is widely assumed that the same percentage similarity prevails at the DNA level. Yet no one would mistake the two species as one.[12]

Living systems such as chimpanzees, mice, and humans are obviously examples of complex systems. It should be equally obvious, therefore, why extrapolation between species will be problematic: small changes on the genetic level can lead to very large differences between species. Indeed, that is what evolution is all about.

Predicting human response based on an animal model is *not* an example of applying a "relatively simple set of well-established scientific principles" as we see with Newtonian physics. Living organisms are better examples of complexity theory than of Newtonian physics. Using the example of a model airplane, as so many defenders of the animal model do, is a good example of this. Studying a model airplane, especially a paper glider, will allow the observer to demonstrate the basics of flight. But if anyone seriously believes this method can be or is used to build or repair a 747, they are deluded. Just as animal models can be and were used to demonstrate very basic facts concerning anatomy and physiology so a model plane can be used to demonstrate basic physical laws concerning flight. But today, when we want to know why a 747 crashed we don't build paper airplanes and neither should we suggest to the public, who is paying for animal experiments, that a cure for AIDS, cancer or stroke will be derived from animal models.

Science, Models, Evolution, and Complexity in Real Life

So far the discussion has been somewhat abstract. The following examples illustrate in a more tangible way, the problems of complex systems and hence of extrapolation. Of ten medications withdrawn from the U.S. market between 1998 and 2001, eight were withdrawn because they were more likely to result in certain severe side effects in women than in men. Men and women are obviously similar in terms of evolutionary biology and gene regulation, but they responded very differently to these drugs.

A study in *Science*[13] revealed that one strain of mice could have a gene removed without obvious adverse effects, while another strain would die without the gene. Iressa was thought to be useless as an anticancer drug. Further analysis revealed it to be very effective for people with a specific mutation.

In many cases, identical twins do not suffer from the same severe diseases. Obviously, only a very small difference in gene expression between the two accounts for this.

This introduces the idea of "Personalized Medicine." Historically, medicine has been practiced based on statistics. If you suffered from high blood pressure and research revealed that 98% of people with high blood pressure responded to medication X, then you would want medication X. It may turn out that you were among the very small minority that needed medication Q but more likely than not, you needed X. If there was no way to determine if you were among the vast majority or minority, your best bet was to take X. Personalized medicine allows us to treat you like an individual not a statistic. This increases the likelihood of success. Considering the implications of personalized medicine, basing treatments on the response of a different species is like using phrenology to study mental illness or trephination to cure malaria.

Today, because of pharmacogenetics, and other advances, we are on the verge of being able to ascertain the best drug protocol for each individual. If the most appropriate drug protocol varies among individuals, what must it do between species? If men cannot predict the effects of a drug for women, and one strain of mouse cannot predict what will happen to another if a gene is removed, it follows that we are studying organisms at the level of organization or complexity that defines one species from another, and even defines one individual from another.

Evolution, complexity theory and evolutionary biology via molecular biology predict that animal testing should not be an effective means of conducting biomedical research and most importantly empirical data supports this. Physicians in clinical practice will frequently tell you that animal data is meaningless to them because it has no predictive ability.

Since those early days of Newtonian physics, important implications for the causal determinism theory in biological science have come to light as a result of Darwinian evolution, our increased understanding of DNA, genetics, genomics, evo-devo and complexity theory.

The *intact systems argument* has historically been the animal modelers' main argument: "We must test on animals because no experimental system be it *in vitro, in silico*, mathematical modeling, and so forth can predict what a drug will do to the *intact living human system*." Ironically, it is the fact that each intact living being is a different complex system that invalidates the use of animal models. Complex systems are more than the sum of their parts, and different complex systems respond differently to the same drug or disease.

The implicit claim in the intact systems argument, that humans and other animal species are the same biochemical animals just dressed up differently, is not true. Moreover, it is irrelevant to point to observed similarities in genetic makeup between species, since the differences are in the regulation of these conserved genes and the resultant interactions between them, not in the genes themselves. Remember the earlier example, it is as though all species have a common genetic keyboard on which different phenotypic concertos are being played—what matters is not similarity with respect to the keyboard but differences with respect to the order and timing of the pressing of the keys. Studying Mozart's keyboard could not have predicted Ray Charles. The piano itself does not predict the noise or music that emanates from it. Though genes are causal, they alone do not determine outcome.

In the 19th century, medical research was almost solely the domain of the experimental physiologist. But today, medical research, even the theory supporting it, is multidisciplinary. Physicists and mathematicians are involved vis-à-vis complex systems analysis. Evolutionary biologists, molecular biologists, mathematicians, computer scientists, physicians and others all play different but vital roles. It is incumbent upon all involved to understand the implications of the knowledge gained from other fields. Scientists should not continue to perform research based on unexamined assumptions from the 19th century.

We have now briefly examined the theoretical underpinning for what follows. The role of genes in complex systems will be illustrated in the next sections on neuroscience and xenobiotics.

Neuroscience

There are differences in the basic physiology and anatomy between humans and chimpanzees. Enard et al[14] compared the transcriptome in blood leukocytes, liver, and brain of humans, chimpanzees, orangutans, and macaques using microarrays, as well as protein expression patterns of humans and chimpanzees. They also studied three mouse species that are approximately as related to each other as are humans, chimpanzees, and orangutans. They identified species-specific gene expression patterns indicating that changes in protein and gene expression have been particularly pronounced in the human brain. They compared mRNA levels in brain and liver of humans, chimpanzees, and an orangutan. They examined approximately 12,000 human genes (see table and figures below):

Comparison	Analyzed spots	Differences	
		Qualitative	Quantitative
Human–chimpanzee	538	41 (7.6%)	169 (31.4%)
M. musculus–M. *spretus*	8767	668 (7.6%)	656 (7.5%)

Table 1. Brain protein pattern differences between humans and chimpanzees as analyzed by 2D gel electrophoresis. Differences between humans and chimpanzees were scored if confirmed in three individual human-chimpanzee pairs and were analyzed in the same way as in a larger mouse study comparing *M. musculus* and *M. spretus*. Qualitative differences represent changes in electrophoretic mobility of spots, which likely result from amino acid substitutions, whereas quantitative differences reflect changes in the amount of protein.

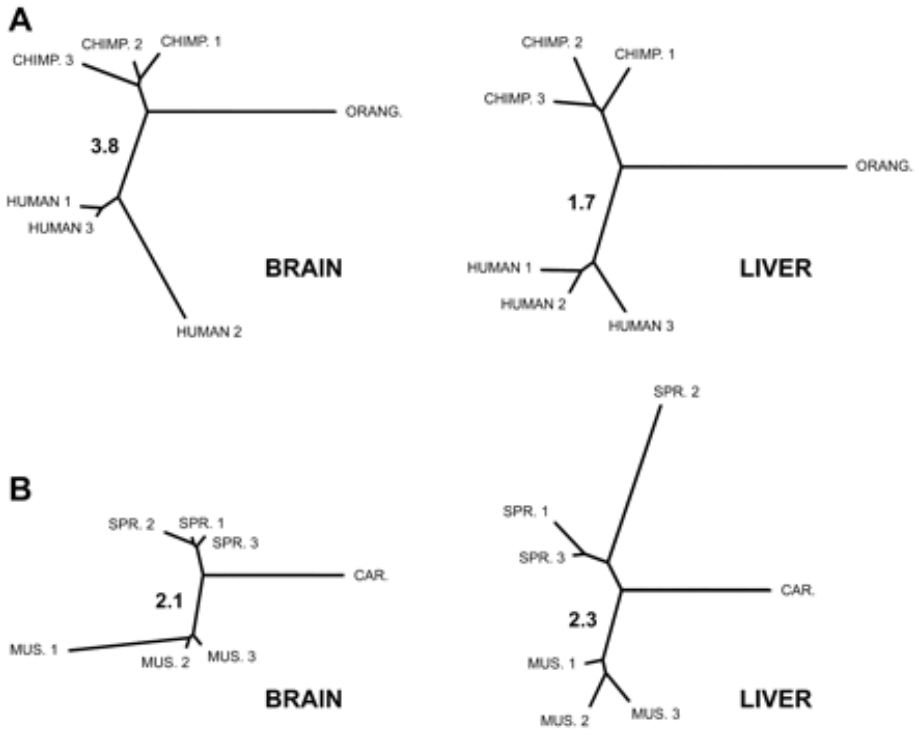

Figure 1. Distance trees representing the relative extent of expression changes in brain and liver among (**A**) three primate and (**B**) three mouse species: MUS., *M. musculus*; SPR., *M. spretus*; and CAR, *M. caroli* (6). Numbers refer to the ratio between the changes common to humans and chimpanzees, and *M. musculus* and *M. spretus*, respectively.

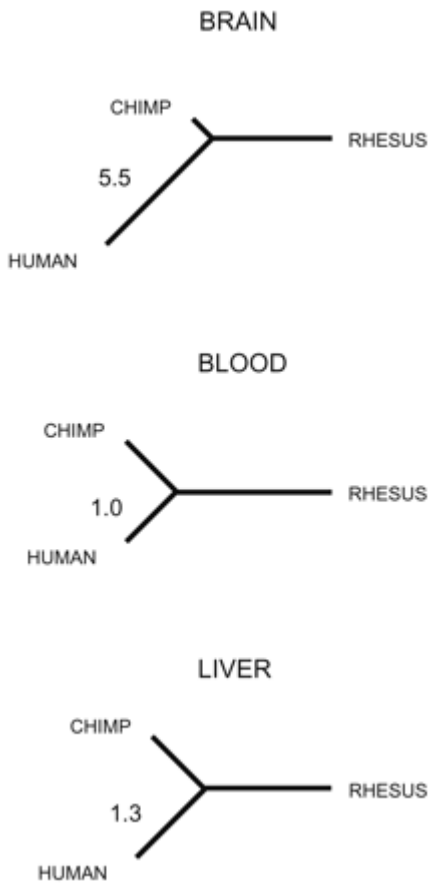

Figure 2. Distance trees representing the relative extent of expression changes among three primate species and three tissues as assayed by the cDNA arrays. Numbers refer to the ratio between the changes common to humans and chimpanzees.

Enard et al state:

> Our results show that that large numbers of quantitative changes in gene expression can be detected between closely related mammals. They furthermore suggest that such changes have been particularly pronounced during recent evolution of the human brain. The underlying reasons for such expression differences are likely to be manifold, for example, duplications and deletions of genes, promotor changes, changes in levels of transcription factors, and changes in cellular composition of tissues.

Models of human disease are generally developed in nonhuman primates since they are subjects with behaviors and anatomical characteristics similar to humans. But species differences play a role in the clinical expression as well as in the cellular specificity of disease. For example, striatal degeneration in humans is frequently associated with dyskinesia, whereas in nonhuman primates, striatal excitotoxic lesions alone are not sufficient to induce dyskinesia or chorea. Second, in addition to these species differences, the time course evolution of the nerve cell degeneration, which normally evolves over several years in neurodegenerative diseases in humans, is for practical reasons, being

replaced over a much shorter period of time in animal models. As researchers at the Salk Institute and the University of California wrote:

> What is known about the neuroanatomy of the human brain? Do we have a human cortical map corresponding to that for the macaque? And what does the human equivalent of the connectional map look like? The shameful answer is that we do not have such detailed maps because, for obvious reasons, most of the experimental methods used on the macaque brain cannot be used on humans ...For other cortical regions, such as the language areas, we cannot use the macaque brain even as a rough guide as it probably lacks comparable regions.[15]

Nonhuman primate brains and human brains *are* undeniably similar in structure, but that does not imply that structures in chimpanzee brains perform the same functions as similar structures in human brains. The chimpanzee brain is not a smaller, more primitive version of a human brain; it is a complex system with its own unique evolutionary history. There will be differential development of modules in humans and chimps that reflect that the two species have taken distinct evolutionary trajectories, adapting and solving different cognitive problems in the course of evolution. Structural similarities in the primate visual system, for example, tell us very little about the subjective character of visual experience in chimpanzees.

There are myriad other differences: The human type-1 hair keratin gene cluster contains a pseudogene ϕ*HaA.* This gene is functional in chimpanzees and gorillas. The reason the human gene is nonfunctional is that it has a nonsense mutation in exon 4 due to a SNP. Humans express the *Ha1* gene throughout the hair cortex, while chimpanzees and gorillas express *Ha1* in one half of the cortex and *HaA* in the other half.[16] There are differences in glycoproteins as well. Hacia writes:

> Many cell surface glycoproteins are modified with sialic acids such as *N*-glycolylneuraminic acid (Neu5Gc). African great apes and other nonhuman mammals have substantial amounts of Neu5Gc in most tissues except the brain. By contrast, humans do not have significant amounts of Neu5Gc in any tissue because of a frame-shift mutation in the human gene encoding CMP-sialic acid hydroxylase, which synthesizes CMP-NeuGc, a high-energy donor used in decorating glycoproteins with NeuGc. The biological significance of human Neu5Gc loss is unknown. It clearly has an effect on siglecs (Ig superfamily members that recognize sialic acids), which recognize Neu5Ac (N-acetylneuraminic acid) and Neu5Gc differentially. Unlike chimpanzees, most human macrophages express siglec-1 (sialoadhesin) on their surface. These macrophages are localized in different regions of human and chimpanzee spleens. Neu5Gc loss could cause differential susceptibility to pathogens that use Neu5Gc ligands to gain access into cells. It cannot be ruled out that Neu5GC loss alters glycoprotein function in human brains and potentially affects brain development.

There are also differences in disease susceptibility, manifestation, and response to treatment, nevertheless nonhuman primates are used as experimental models to study a wide range of human neurodegenerative diseases.

In 1936, the Portuguese neurologist Egas Moniz introduced a surgical operation, for which he won the Nobel Prize in 1949; prefrontal leukotomy or lobotomy. (Leukotomy is the surgical operation of interrupting the pathways of white nerve fibers within the brain. Lobotomy was the name given to a prefrontal leukotomy in which the nerve fibers connecting the frontal lobe with other parts of the brain were cut.) The operation consisted of incisions that destroyed connections between the prefrontal region and other parts of the brain. Lesioning the brain to treat mental illness was not new but it had never been widely adopted. Then, at a neurology conference in London in 1935, Jacobsen & Fulton presented data from operations on two chimpanzees which, after a leukotomy, managed to make mistakes without becoming aggressive, something which they had not managed to do before. Moniz took this chimpanzee data and applied it to humans. He operated on patients with affective disorders, i.e. various types of depression, obsessive-compulsive and hypochondriac states and so on. Suffice it to say, this operation is very rarely performed today as even though, as occurred in chimpanzees, it does make the human more docile, it also totally destroys their personality leaving them inactive and oblivious.

Marvanová et al. used human microarrays to profile genes from brains of human, macaque, and marmosets and combined this with available data from chimpanzee and orangutan to create a data set that revealed similarities and differences in expression of genes underlying Alzheimer's, Huntington's, and Parkinson's diseases.[17] They found that a large number of genes are expressed in human prefrontal cortex and that a significant percentage of these are also expressed in nonhuman primates. But they also found profound differences:

> Of the 12,386 probe sets analyzed on the Affymetrix U95A chip, 5460 (45%) genes in whole human brain were "present." This indicates that at least 45% of the genes covered on the human arrays are detected in adult human brain. In prefrontal cortical tissues the number of genes present were: human, 4794 (39%); chimpanzee, 4173 (34%); orangutan, 3501 (28%); macaque, 3376 (27%); and marmoset, 2960 (24%).
>
> …Approximately 20% of present human genes had a different expression profile (>2-fold change) in chimpanzees and >25% of genes in orangutan, macaque, and marmoset had a different expression profile. Distribution plots were generated to identify genes and compare expression of human to nonhuman primates. More than 80% of genes had a similar expression level (<2-fold change) in human compared with chimpanzee and > 60% of the other species studied. The percentage of genes present in prefrontal cortex and displaying a different expression level (>2-fold change) was chimpanzee, 18%; orangutan, 37%; macaque, 26%; marmoset, 33%. The number of genes more highly expressed (>2-fold change) in humans/more highly expressed in NHPs, and ratio were

chimpanzee (470/231 genes, 2.0), orangutan (910/249 genes, 3.7), macaque (528/280 genes, 1.9), and marmoset (539/311 genes, 1.7).

Genes involved in common neurodegenerative diseases AD, PD, and HD contained *qualitative and quantitative* differences in NHP PFCs. Some genes known to play a role in Alzheimer's disease were concordant in humans and NHPs (PS1, AD amyloid, amyloid precursor, CHRM3, tau, ubiquitin) but others were not (apolipoprotein E, NMDAR2C, TNF-α). The six genes related to dopaminergic system and thus possibly to Parkinson's disease (ubiquitin, gamma synuclein, dopamine 1 receptor, MAOB, MAOA, COMT) displayed good concordance in human, chimpanzee, and orangutan (except dopamine 1 receptor), but absent scores for dopamine 1 receptor and COMT were seen in macaque and for dopamine 1 receptor, MAOA, and COMT in marmoset. Huntington's-related gene HD was not detected in any species tested and HIP2 was detected only in PFC of profiled monkeys. Of the 12 genes related to basic mechanisms (growth factors and their receptors, transcription factors, cytokines, and apoptosis-related molecules), 9 were concordant and 3 were discordant, mostly in the case of marmoset PFCs, with the exception of Bcl-2 transcript, which was not detected in orangutan or macaque. Glutamate receptor 2 was up-regulated and four transcription-involved genes were down-regulated in all NHPs compared with humans. (Emphasis added)

…Many genes found in Alzheimer's pathology (amyloid precursor, CHRM3, tau, and ubiquitin) were also found in NHPs while those absent in the human PFC (presenilin 1, AD amyloid) were absent in NHPs. Several genes related to Huntington's disease pathology, such as the HD gene, were detected in human PFC but not in other NHP PFCs; Huntington protein 2 (HIP2) was not detected in human and ape PFCs but was in monkey PFCs. Moreover, COMT, an enzyme with a role in dopamine degradation, was present in humans and apes but was not detected in any of the monkey species tested… Four genes related to transcription were down-regulated by at least twofold in all NHP species analyzed compared with humans….

Considering the fact that a very small difference between two complex systems can lead to very divergent ways in how the complex systems act, these differences are not insignificant.

Marvanova et al[18] studied gene expression profiles of humans, chimpanzees and other nonhuman primates as they related to neurological diseases. Writing in *The FASEB Journal* in 2003, the authors concluded:

1. A large number of genes are expressed in human prefrontal cortex; a significant percentage of these are also expressed in nonhuman primates.
2. Approximately 20% of present human genes had a different expression profile (>2-fold change) in chimpanzees and >25% of genes in orangutan, macaque, and marmoset had a different expression profile.

3. Genes involved in common neurodegenerative diseases AD, PD, and HD contained qualitative and quantitative differences in NHP PFCs.

It is interesting to note that the authors concluded these NHPs would be valuable experimental models despite the above mentioned differences. It is errors like this, mistaking the concept of similar genes yielding similar gene functions that underlies much of biomedical research using chimpanzees. As mentioned earlier, it is not the genes themselves that are important but rather how they are expressed. Considering the differences in expression profiles, we should not be surprised that chimpanzees react differently to the environment and genes that in humans produce Alzheimer's, Parkinson's and so forth.

Now would be an appropriate time to bring up the use of chimpanzees in cognitive studies. There can be no denying the fact that if one wishes to learn about chimpanzee *thought* one needs to study chimpanzees. If one wishes to study the differences between human and chimpanzee brains one must study the brains of both humans and chimpanzees. Comparative anatomy and comparative medicine are legitimate fields of science, however they are not synonymous with biomedical research purporting to offer hope to people suffering from Alzheimer's, epilepsy, multiple sclerosis, Parkinson's, and other diseases of the brain and nervous system.

Furthermore, it is disingenuous to study depression or anxiety in chimpanzees, find they suffer from these conditions and suffer from them in the same situations humans would, and call this discovery important for humans suffering from mental illnesses. If one wishes to learn about human depression one must study humans. Just because chimpanzees suffer from illnesses that humans also suffer from does mean they suffer from these illnesses for the same reason or manifest the same pathophysiology or respond in the same way to the same treatments.

Chimpanzees can be used to study the neuroanatomy and neuropathology of chimpanzees but to imply this will yield knowledge beneficial to humans is disingenuous and contrary to current biology theory.

Xenobiotics

Chimpanzees in Research: Strategies for Their Ethical Care, Management and Use:

> Chimpanzees have been used as a final step in the evaluation of new therapeutic agents before their administration to humans. Evaluation of xenobiotics is generally brief and presents little or no potential hazard to the well-being of the chimpanzee. However, such studies can be essential in justifying the introduction of a xenobiotic to humans. A specific example is the development of novel inhibitors of the enzyme elastase, which is present at high concentrations in human neutrophils and has been implicated in tissue destruction associated with inflammatory diseases, such as those of the upper respiratory tract, including cystic fibrosis, bronchiectasis, and emphysema. The inhibitors are much less

> potent in lower species, only by using chimpanzees was it possible to validate their use in human trials (Mumford and others 1995), where its remarkable potency was confirmed.

Essentially there is no such thing as a *bad drug*. All chemistry, medical, and pharmacology students are taught that *the dose determines the poison*. In light of today's knowledge that should be amended to *the genetic and environmental factors influence at what dose a chemical becomes a poison*. A drug that kills one may cure another and the above in part explains the reasons for this. When one attempts to determine what a drug's efficacy or toxicity will be using other *humans* as test subjects the outcome is far from reliable and using a different species is exponentially more problematic.

Clozapine is an antianxiety, antipsychotic medication introduced in the 1970s. It was withdrawn secondary to causing agranulocytosis in several cases in Finland. Later it was allowed back on the market because it was so effective for some and the threat of the adverse reaction could be monitored.[19] Thalidomide is another example. It caused phocomelia in the children of pregnant women who took the drug; it is now used to treat multiple myeloma. All drugs can benefit some patients and kill others.

Toxicity is largely determined by mechanisms of drug metabolism. Many genes influence drug metabolism and as James P. Kehrer, PhD, of the Division of Pharmacology and Toxicology at the University of Texas at Austin stated: "Small differences in gene structure can make large differences in function."[20] Nonhuman primates are frequently used to study potential new medications. Theoretically, because they are closest to us in evolutionary terms they should be better models than rodents or rabbits. However, as we have seen, there are many differences between humans and nonhuman primates in drug absorption, distribution, metabolism, excretion, and toxicity (ADMET). For example, Actinomycin-D, one of the first of the chemotherapy drugs, kills monkeys[21] while drugs known to damage the human fetus are found to be safe in 70% of cases when tried on primates.[22] J. Caldwell stated,

> It has been obvious for some time that there is generally no evolutionary basis behind the particular-metabolizing ability of a particular species. Indeed, among rodents and primates, zoologically closely related species exhibit markedly different patterns of metabolism.[23]

> The reason nonhuman primates fail to respond to drugs as humans do is again because of speciation. When Ulrich et al[24] investigated the cellular expression of 9 cytochrome P450-isozymes (CYP1A1, CYP1A2, CYP2B6, CYP2C8, 9, 19, CYP2D1, CYP2E1, CYP3A1, CYP3A2, CYP3A4) and 3 glutathione S-transferase-isozymes (GST-*p* , GST-*a*, GST-*l*) in the pancreas of hamsters, mice, rats, rabbits, pigs, dogs and monkeys, and compared the results with the expression in the human pancreas, they found a wide variation in the distribution and cellular localization of the selected drug-metabolizing enzymes between the 8 species on one hand and the pancreas of humans on the other hand. (See tables

below.) An exclusive expression of enzymes in the islet cells was found in the hamster (CYP2E1), mouse (CYP1A1, CYP1A2, GST-*a*, GST-*l*), rat (CYP2C8,9,19), rabbit (CYP1A2, CYP2B6, GST-*p*), and pig (CYP1A1). Although no polymorphism was found in the pancreas of animals, in human tissue four enzymes were missing in about 50% of the cases.

TABLE 1.—The distribution of CYPs and GSTs in the ductal cells of the syrian hamster (SGH), nude mouse (NM), rat, rabbit, guinea pig, dog, monkey, and human.

CYP	SGH	NM	Rat	Rabbit	Pig	Dog	Monkey	Human
1A1	+	−	+	+	−	++	+	+
1A2	+	−	−	−	−	−	−	+
2B6	+	++	+	−	−	−	+	−/+[a]
2C8,9,19	+	++	+	−	−	−	+++	++[b]
2D1	+	+	+	++	+	+	+	++
2E1	−	+	+	+	−	−	−	+[c]
3A1	+	++	++	++	+	++	++	++
3A2	+	+	+	+	++	+	++	+
3A4	+	++	−	−	+	−	−	++
GST-π	++	+	+	−	+	++	+++	++
GST-α	+	−	++	−	+	++	++	++
GST-μ*	++	++	+	++	+	++	+++	++[*,d]

Staining intensities: −, none; +, weak; ++ moderate; +++ strong.
*Due to genetic polymorphism, GST-μ was expressed in 11/21 human specimens.
[a]No staining (9/21 samples), moderate staining (12/21).
[b]No staining (1/21), weak staining (13/21), moderate staining (7/21).
[c]No staining (3/21), weak staining (18/21).
[d]Weak staining (6/11), moderate staining (5/11).

TABLE 2.—The distribution of CYPs and GSTs in the acinar cells of the syrian hamster (SGH), nude mouse (NM), rat, rabbit, guinea pig, dog, monkey, and human.

CYP	SGH	NM	Rat	Rabbit	Pig	Dog	Monkey	Human
1A1	++	−	−	+	−	++	+	+
1A2	+	−	++	−	−	+++	+	+
2B6	+	++	−	−	+	++	++	−/+[a]
2C8,9,19	++	++	−	−	++	+	++	++[b]
2D1	++	+++	−	−	+	+	+	++[c]
2E1	−	+	+	+	−	−	−	−/+[d]
3A1	++	++	+	++	+	++	++	+
3A2	++	+	+	++	++	++	−	+
3A4	+	+	+	+	++	−	++	+
GST-π	−	−	−	−	−	−	−	−
GST-α	−	−	−	−	++	−	+	++[e]
GST-μ*	−	−	−	−	−	−	−	+*

Staining intensities: −, none; +, weak; ++ moderate; +++ strong.
*Due to genetic polymorphism, GST-μ was expressed in 10/21 human specimens.
[a]No staining (9/21 samples), weak staining (12/21).
[b]Weak staining (16/21), moderate staining (5/21).
[c]No staining (2/21), moderate staining (19/21).
[d]No staining (3/21), weak staining (18/21).
[e]Weak staining (7/21), moderate staining (12/21), strong staining (2/21).

TABLE 3.—The distribution of CYPs and GSTs in the islet cells of the syrian hamster (SGH), nude mouse (NM), rat, rabbit, guinea pig, dog, monkey, and human.

CYP	SGH	NM	Rat	Rabbit	Pig	Dog	Monkey	Human
1A1	++	+	++	+++[a]	+++	+	++	+/+++[b]
1A2	+++	+++[a]	+[a]	+++	−	−	−	+
2B6	+++[a]	+++	+++[a]	++[a]	+++[b]	−	+++[c]	−/+++[b,d]
2C8,9,19	+	+++	+++[a]	−	+++[b]	−	+	−/+++[b,e]
2D1	+	++	++	+	+	+	+	+++[f]
2E1	+++	++	+++	−	−	−	−	−/+[h]
3A1	+++[a]	++	+++	++	−	++[b]	+++	++[b]
3A2	+	+++[a]	+	+	+	+	+	++
3A4	+	++	+++	−	−	−	+	−/+++[k]
GST-π	+++	++[a]	+	+	−	++	++	−/+++[m]
GST-α	+++[a]	+	−	−	−	−	−	−/+[n]
GST-μ*	++	+	+	+++[a]	+[b]	−	++	++*

Staining intensities: −, none; +, weak; ++ moderate; +++ strong.
*Due to genetic polymorphism, GST-μ was expressed in 11/21 human specimens.
[a]Stronger staining in the periphery of the islet.
[b]Stronger staining of scattered cells in the islet.
[c]Stronger staining in the center of the islet.
[d]No staining (9/21), weak staining (12/21).
[e]No staining (2/21 samples), weak staining (16/21), strong staining (2/21).
[f]Moderate staining (8/21), strong staining (13/21).
[h]No staining (4/21), weak staining (17/21).
[k]Weak staining (12/21), moderate staining (4/21), strong staining (up to 25% of cells in 5/21).
[m]Strong staining of an average of 16% of the cells within the islets in all 21 samples.
[n]No staining (7/21 samples), moderate staining (up to 10% of cells in 14/21).

They state:

> The differences in the distribution of these drug-metabolizing enzymes in the pancreas between the species call for caution when extrapolating experimental results to humans….In humans, a genetic polymorphism has been reported for CYP1A1, CYP2C9, GST-P1 (gene 1 of GST*p*), GST-M1, GST-M3 (M1 andM3 represent 2 of the 5 GST*l* genes), and GST-T1 (gene 1 of two GST*h* genes). As shown in Tables 1–3, seven of the enzymes showed differences in their expression in the human specimens. The differences could be related to several factors, including exposure to different substrates, nutrition and ethnic differences (e.g., more Asians than Caucasians have inactive alleles of CYP 2C19)…. In contrast to humans, no interindividual differences existed between animals of the same strain.
>
> Despite some similarities in the expression of the CYP enzymes between the species extreme care is needed when extrapolating the test results gathered from these animals to humans. Among the very closely related proteins there may be considerable catalytic differences. Even between the rodents, like rat and mouse, there is little comparison in the metabolic pathways for activation and detoxication of xenobiotics including carcinogens.
>
> Moreover, it appears that the metabolic capacity of the same tissue from different species varies considerably, as does the localization of the enzymes in different cells of the same tissue in the same species.

Another example is indinavir. Nonhuman primates are supposedly the best models for drug toxicity and metabolism because of the homology of their drug-metabolizing enzymes. It is known that the nucleotide and amino acid sequences of P450 isoforms (CYP2D and 3A) in cynomolgus monkey and marmoset (*Callithrix jacchus*) have > 90% identities to the human P450s. Chiba *et al.* studied the metabolism of indinavir, an HIV protease inhibitor, using liver microsomes from humans, cynomolgus monkeys (*Macaca fascicularis*), rhesus monkeys (*Macaca mulatta*), and chimpanzees (*Pan troglodytes*). (See table below.) They found that in vitro metabolism of indinavir varied markedly between species:

> The overall rate of indinavir metabolism varied > 4-fold among primates (84 pmol/min/mg protein in cynomolgus monkey versus 20.4 pmol/min/mg protein in human) and followed the rank order: cynomolgus monkey > rhesus monkey > chimpanzee > human. The cis- (indan) hydroxylated metabolite of indinavir was formed only in cynomolgus and rhesus monkey livers, whereas trans-(indan)hydroxylation and N-dealkylation were observed as the major metabolites in all primates tested. Inhibition studies with P450selective inhibitors (ketoconazole, quinine, quinidine) and monoclonal antibodies (against CYP2D6 or CYP3A4) indicated that a cytochromeP450 isoform of the CYP2D subfamily is

involved in the formation of the unique cis-(indan) hydroxylated metabolite in monkey, whereas all other oxidative metabolites, including the tracts-(indan)hydroxylated metabolite, are formed by CYP3A isoform(s)… Although the homology of the nucleotide and the deduced amino acid sequences of a CYP2D isoform in cynomolgus monkey (CYP2D17) were reported to be 96 and 94% respectively to human CYP2D6, there is little known about the substrate specificity of monkey CYP2D isoforms. It has been demonstrated that cloned CYP2D isoforms (CYP2D1, 2D2, 2D3, 2D4), all belonging to the rat CYP2D subfamily, had distinct substrate specificities toward bufuralol, debrisoquin and lidocaine (Wan et al. 1997). Furthermore, Smith et al. (1998) demonstrated that a single amino acid substitution (F483I) had conferred on CYP2D6 the ability to metabolize testosterone to the novel product, 15ahydroxytestosterone without losing its catalytic activity for bufuralol metabolism. Therefore, it is not surprising that a minor difference in the critical structure of monkey CYP2D isoform(s) could result in the acquisition of a metabolic capability to form a stereoselective metabolite distinct from the other primates….

The species differences in regio- and stereo-selective metabolism catalyzed by P450s have been studied for many compounds including digitoxin, mephenytoin, phenanthrene, progesterone, testosterone, tolbutamide and warfarin. It has been suggested that primates are better surrogates for human than rodent or dog in qualitatively predicting human *drug* metabolism. However, the present data strongly indicate that the metabolism of indinavir in monkey is qualitatively different from that in human, whereas the indinavir metabolism in chimpanzee is similar to that in human….

The present results suggest that chimpanzee might be a good animal model in predicting drug metabolism in human. However, it was reported that the stereoselectivity of ABT-418 metabolism catalyzed by flavin-containing monooxygenase (FMO) in chimpanzee was different from rat, rabbit, dog and human. Therefore, the species differences and similarity in drug metabolism appears to depend on the enzyme system(s) involved in the metabolism of interest….

The present study, however, re-emphasizes the fact that qualitative differences in metabolic profiles can still exist among primates. Thus, caution must be taken when extrapolating drug metabolism and pharmacokinetic data from monkey to human.[25]

Table 1. Hepatic microsomal metabolism of indinavir in primates[*],

Species[h]		Metabolite formation rate (pmol/min/mg)						
		M2+M5`	M3	M4a	M4b	M6	M7	Total
Rhesus monkey	Mean	5.26	15.8	12.3	3.90	19.6	17.6	74.5
	SD	1.71	2.9	1.7	0.79	3.6	1.0	11.0
Cynomolgus monkey	Mean	9.19	12.7	13.6	3.25	29.2	16.0	84.0
	SD	1.99	2.7	3.6	0.59	6.8	3.6	17.5
Chimpanzee	Mean	5.62	2.94	4.83	0.80	12.5	nd'	26.7
	SD	1.53	0.89	0.92	0.10	2.5		2.6
Human	HL-1	7.82	7.09	5.94	2.5	16.0	nd	39.4
	HL-2	1.28	1.13	1.19	0.727	2.11	nd	6.44
	HL-3	1.06	0.763	0.744	0.202	1.61	nd	4.38
	HL-4	3.98	2.89	3.67	2.27	5.69	nd	18.5
	HL-5	2.56	0.959	1.46	0.478	3.65	nd	9.11
	HL-6	8.58	7.60	7.27	4.82	16.1	nd	44.4
	Mean	4.21	3.41	3.38	1.83	7.53		20.4
	SD	3.27	3.15	2.73	1.75	6.75		17.4

[a]Metabolite formation rates were measured at 10 (monkey) or 20 (chimpanzee and human) min after the onset of incubation in the presence of NADPH and 4 mg/ml microsomal protein. Indinavir concentration was 10 pM. Data are the mean of results from three individual microsomal preparations except human *(n* = 6).
[b]All experimental animals were male.
[c]Formation rates for secondary metabolites (M2 and M5) were summed.
d Data were taken from Lin *et al.* (1996) for the comparison.
[e] ND, Not detectable.

In light of the knowledge we have obtained about interspecies differences, vis-à-vis the Human Genome Project, evolutionary biology, and studies like the above, it should come as no surprise that trans-species extrapolation is unreliable. Even intra-species extrapolation is troublesome. By examining the records of 786 patients and then another 1,093 women and 1,355 men, scientists found that women treated with 5-FU-based chemotherapy for colorectal cancer, had more severe stomatitis and leukopenia compared with men.[26] Caucasians and African-Americans have a similar prevalence of early age-related macular degeneration. However, the progression to the late form of this disease, which is characterized by proliferation of new vessels in the pigmented layer of the eye (known as the choroid), is very rare for African-Americans.[27] Similarly, infantile hemangiomas of the skin are commonly seen in Caucasians but are rare in African-Americans.[28]

Currently, an estimated 4,000 to 6,000 Americans die each year while awaiting a bone marrow match. Only about 60 percent of white Americans now find a suitable donor, and the rates for minorities range from just 20 percent to 50 percent. Physicians have more difficulty finding a kidney or bone marrow match for Blacks than Whites because Blacks have more antigen combinations on their cell surface and some of the antigens are very rare in non-Black populations. There are other important differences between races, individuals, and sexes. Black women have a 50% higher incidence of breast cancer prior to age 35 than Whites. They also have a greater probability of developing aggressive tumors and have the highest incidence of pre-menopausal cancer.

An article published in the *New England Journal of Medicine* in May 2001 revealed that Blacks did not respond as well to medications known as ACE-inhibitors, medications

routinely used to treat heart failure. One theory as to why this is the case is that Blacks have less nitric oxide, a chemical important in how ACE-inhibitors work. This theory led to the development of a medication named BiDil, a heart drug that increases the amount of nitric oxide. It appears to work very well in Blacks, when given to Whites it worked no better than a placebo, as would be expected if Whites already had adequate amounts of nitric oxide.[29]

As we have previously mentioned, among ten medications withdrawn from the U.S. market between 1998 and 2001, eight had more severe side effects in women than in men. The ten drugs were Pondimin, which led to valvular heart disease; Redux, which also led to valvular heart disease; Rezulin, which led to liver failure; Lotronex, which led to ischemic colitis; Seldane, which led to a life-threatening heart condition known as Torsades de Pointes (TdP); Posicor, which lowered heart rate and caused drug interactions; Hismanal, also caused TdP; Propulsid, also caused TdP; Raxar, also caused TdP; and Duract which led to liver failure. All but Raxar and Duract were more toxic to women.[30] Similarly, a study in *Science* revealed that one strain of mice could have a gene removed without obvious adverse effects while a similar strain of mice would die without the gene.[31]

If men cannot predict the effects of a drug for women and one strain of mice cannot predict what will happen to another if a gene is removed, is it not likely that medicine has reached the level of organization that distinguishes one species from another and even individuals from each other?

Infectious Diseases

Chimpanzees were useful in infectious disease research of the past. But were they models of human disease or merely incubators and living bioassays? And if they were merely incubators, was the role they played essential?

Again from *Chimpanzees in Research: Strategies for Their Ethical Care, Management and Use:*

> Over the last 20 years, chimpanzees have been used as experimental models of humans in several research fields, including infectious disease, reproduction, language, and behavior. The contributions with the greatest effect on human health have come from infectious-disease research that focused on the development of vaccines and new classes of therapeutic agents. Instances in which the use of chimpanzees was considered either critical or a prerequisite to introducing an agent into humans include development and safety testing of vaccines for hepatitis B virus (HBV) and identification of the hepatitis C virus (HCV) both of which had enormous benefit to humankind; and development of novel inhibitors of neutrophil elastase.

> Experimental infection of chimpanzees as animal models in biomedical research has involved such diverse microorganisms as mycoplasma species, the filarial nematode Onchocerca volvulus, numerous viruses, and unconventional agents associated with subacute degenerative diseases of the central nervous system (such as spongiform encephalopathies, including kuru and Creutzfeldt-Jakob disease). Major contributions to human health have resulted from the use of chimpanzees in studies to control transmission of and disease induced by the hepatitis viruses, respiratory syncytial virus, and human immunodeficiency virus (HIV).

Let examine the use of chimpanzees in research involving the infectious diseases hepatitis and HIV/AIDS.

Hepatitis A and B

Again from *Chimpanzees in Research: Strategies for Their Ethical Care, Management and Use:*

> Early research on HBV was hindered by the inability to propagate it in tissue culture. Because chimpanzees are the only nonhuman primates susceptible to infection with HBV, they were critical to the development of a vaccine by providing a source of virus and viral antigens and by making it possible to evaluate the safety and the effectiveness of candidate vaccines. The benefits of HBV vaccination to humanity can be characterized as not only controlling an important disease but also presenting a potential approach to controlling the transmission of disease from mother to child, thereby eliminating a major problem for mankind, particularly in Asia, but also in the Unites States. Even though hepatitis B is relatively rare in the United States, the major vaccine-recommending bodies, including the American Academy of Pediatrics and the Immunization Practices Advisory Committee, now recommend universal hepatitis B vaccination of newborns. This is important because about 75% of newborns who acquire it from their mothers become chronic carriers, which provides the potential for lifelong transmission of the disease, lifelong carriage of the virus in an active replicating form, and an increase by a factor of 200 in the relative risk of developing hepatocellular carcinoma, compared with a noninfected person. The latter possibility makes this the first vaccine against a form of cancer. That enormous long-term benefit to humanity represents the harvest that we will continue to reap from the *research on hepatitis B that was carried out in chimpanzees*. (Emphasis added.)
>
> Although the exact number of chimpanzees used in the successful development of a vaccine against HBV is not known, institutions reporting past exposures of chimpanzees to specific agents indicate that 195 animals that they now house, including those also exposed to HCV and HIV, participated in hepatitis virus research (Table 2.1). That number substantially underestimates the total used, because of normal attrition and the fact that many chimpanzees housed at New

> York University's Laboratory of Experimental Medicine and Surgery in Primates (LEMSIP) were not counted but are known to have been used in HBV studies.

Note the above says *research on hepatitis B that was carried out in chimpanzees.* This is a common fallacy in the pro-animal experimentation propaganda. The sentence makes it sound like chimpanzees were used as models for HBV, as CAMs, when in fact they were used as incubators and bioassays.

What is the real story of hepatitis?

Hepatitis is inflammation of the liver, most often due to an acute viral infection, of which there are several varieties. The initial symptoms are the same for all varieties – fever followed by weakness, loss of appetite, achy muscles and digestive problems. The upper abdomen can be very sensitive and jaundice occurs. Liver failure caused by infectious hepatitis is still one of the leading non-alcohol related reasons patients require liver transplants.[32]

Hepatitis A is very similar to the poliovirus, in that it stems from contaminated food, although some victims contract it from injections with unsterilized hypodermics. In most underdeveloped parts of the world, children are exposed to the virus and develop a lifelong immunity. It was during WWII that research with volunteers yielded the knowledge that it is transmitted via the fecal-oral route. Diagnosis is based on a clinical exam and laboratory analysis looking for the virus. The disease could not be propagated except in humans until 1972 when it was found in marmosets. Of all mammals tested, some nonhuman primates are the only other species that can contract it. The virus was isolated from human tissue in 1973. Hepatitis A vaccine is made from virus grown in tissue culture and processed similarly to the polio vaccine. Scientists use *in vitro* methods to test the quality of hepatitis A and hepatitis B vaccines.

Hepatitis B was differentiated from hepatitis A in 1967, based in part on human studies on children in a home for the mentally retarded. Krugman found that the children who were infected actually had two different diseases. Also in the 1960s, Baruch Blumberg isolated an antigen in the blood of Australian aborigines. He thought initially that he had found another blood antigen. But when his assistant came down with the “antigen,” he realized he had found an infectious agent. Hepatitis B, usually transmitted via contaminated needles and syringes or through sexual contact, continues to be a significant cause of sickness and mortality in many countries. Continued infection with hepatitis B can lead to cancer.

Researchers have managed to infect animals as disparate as woodchucks and ducks with hepatitis B, and have developed transgenic mice that can be infected. But the mice remain tolerant to the antigens.[33] The animal of choice for research has been the chimpanzee. However, there are major differences between hepatitis B infection in humans and in chimpanzees. Chimpanzees are essentially asymptomatic when infected. Humans are not. The virus continues to reproduce as long as it is in their body. This is not true in humans. The liver is not affected in the same way in chimpanzees as it is in humans. Liver

enzymes, which are measured to assess the progression of the disease, respond differently in humans and chimpanzees. Therefore, comparing the human disease with that of the chimpanzee is meaningless.[34, 35, 36]

Epidemiology and *in vitro* technology have identified several other forms of hepatitis – C, D, E, and GB virus-C/hepatitis G (a distant cousin of hepatitis C) are also problematic though less prevalent. Similar technology also allows blood testing for the disease.[37] State-of-the-art instrumentation allows scientists to observe hepatitis receptors on liver cells (hepatocytes). The clearer picture of the way humans are infected may lead to a technology for interfering with virus binding.[38] However, there is still no cure for any hepatitis infections.

Compare and contrast this version of history and the one from *Chimpanzees in Research: Strategies for Their Ethical Care, Management and Use* with the following publication by the National Academy of Science:[39] [Our comments are in brackets. The gray highlighting has also been added.]

> Debilitating and deadly, hepatitis has plagued humankind since the beginning of recorded history. But the course of this disease was irrevocably changed with the accidental convergence of a medical researcher curious about why some people are especially prone to various ailments, another medical researcher wondering why people often become sick after receiving blood transfusions, and the blood of an Australian aborigine. [Serendipity.]
>
> That convergence led to a discovery that in less than a decade spurred a blood-screening campaign that dramatically reduced the incidence of hepatitis spread by blood transfusions—hepatitis B. The discovery also led to a highly effective hepatitis vaccine that not only introduced a novel way of protecting people from infectious diseases but also is the first effective vaccine against liver cancer. Yet the scientists whose work revolutionized the study of hepatitis did not even have the disease in mind when they embarked on their investigations. As often happens in science and medicine, the landmark discovery grew not out of "targeted research" but from studies aimed at answering more fundamental questions about nature. The following article, adapted in part from an account by researcher Baruch Blumberg, who shared the 1976 Nobel Prize for physiology or medicine, explores the trail of research that led to the discovery of many of the viruses that cause hepatitis and to blood screening for and revolutionary vaccines against some of them. It provides a dramatic example of how science works and how basic research can lead to practical results that were virtually unimaginable when the research was done.
>
> …Although hepatitis has been known for centuries, before World War II doctors did not know that it was caused by a virus. It was assumed to be contagious because epidemics of hepatitis often occurred in crowded, unsanitary conditions, but how it was passed from person to person was a mystery.

Headway into solving the mystery was made in the 1940s by a British doctor, F. O. MacCallum, who specialized in liver disease. He was concerned not so much with hepatitis as with the extremely deadly yellow fever transmitted by mosquitoes, which was killing soldiers in Africa and South America. Charged with the production of a yellow fever vaccine, MacCallum was perplexed as to why a sizable proportion of soldiers who received the yellow fever vaccine developed hepatitis a few months later. The yellow fever vaccine contained human serum, and MacCallum was aware of other hepatitis cases reported in the medical literature that followed inoculation with vaccines containing human serum. He also knew of cases that followed the reuse of unsterilized syringes and needles in the treatment of diabetes or venereal disease, instruments that could contain particles of blood. MacCallum came to suspect that a virus carried in human blood could cause hepatitis. [Clinical observation.]

A series of observations of volunteers by MacCallum and others during and shortly after the war strengthened that hypothesis and made it clear that hepatitis can also be spread by other means than through blood. MacCallum coined the terms hepatitis A for the form of the disease that is spread primarily through food and water contaminated with minute quantities of fecal material and hepatitis B for the form that is transmitted mainly by exposure to contaminated blood.

During the next decade and a half, researchers at many laboratories tried in vain to isolate the infectious agents that cause the two types of hepatitis. Scientists suspected that the culprit organisms were viruses because they were small enough to pass through some of the smallest-pore filters used in experiments, but the scientists were unable to grow them in order to identify and study them. By the mid-1960s, hepatitis research had reached a discouraging deadlock. Then a remarkable advance in knowledge of the causes of hepatitis was made by someone who was not working on the disease at the time. Baruch Blumberg, a medical researcher specializing in internal medicine and biochemistry, was interested in a more basic question—why were some people prone to particular diseases?

As a medical student in the early 1950s, Blumberg had conducted research in Surinam on elephantiasis, a parasitic disease common in the tropics. His investigations showed that some of the ethnic populations in the town in which he worked were more susceptible to elephantiasis than others, even though everyone was apparently exposed to the same conditions. A few years later he began to suspect that differences in susceptibility stemmed from variations in the genetic makeup of different ethnic populations, but the tools of modern molecular biology that now allow scientists to link disease susceptibility to variations in genes had not yet been invented. At the time, researchers trying to detect genetic differences that might be tied to disease susceptibility looked for inherited differences in specific blood proteins.

These differences, called polymorphisms, were in some cases assumed to be maintained over generations because they gave those who carried them a survival advantage, such as resistance to a disease. Researchers had already discovered a number of polymorphisms in blood proteins—for example, the different blood proteins that determine type A, O, or B blood—but this field was a vast and relatively unexplored terrain that promised to unlock the secrets of disease susceptibility. In the late 1950s, Blumberg embarked on research aimed at finding new polymorphisms in blood proteins. To that end he began collecting blood samples from populations all over the world.

In the late 1950s, as part of his basic research into inherited variations in blood proteins, Baruch Blumberg began collecting blood samples from populations all over the world. Several years later, his efforts resulted in the discovery of the hepatitis B surface antigen (HBsAg), initially identified in the blood serum of an Australian aborigine. [Clinical research.]

In the early 1960s, Blumberg was at the National Institutes of Health (NIH), where he collaborated with biochemist Anthony Allison on a way to detect novel blood proteins quickly and easily. The scientists reasoned that patients who received multiple blood transfusions had probably encountered blood proteins sufficiently different from their own to prompt their bodies to generate an immune reaction, or antibodies, against the foreign proteins, or antigens. They used a technique known as agar gel diffusion, which relies on the immune system's ability to spot minor differences in proteins and to produce an antigen-antibody interaction in response to a novel blood protein…. [Clinical and *in vitro* research.]

Meanwhile, reactions to someone else's blood were also of interest to blood specialist Harvey Alter at the NIH Blood Bank. Alter wanted to find out why some patients developed fever, chills, or rashes after blood transfusions. He thought they might be suffering from immune reactions to foreign proteins (antigens) in donated blood. When Alter learned that Blumberg was looking for immune reactions in the blood of patients who had received many transfusions, he went to see him, and they decided to collaborate.

Blumberg and Alter used agar gel diffusion to test sera from patients who had received multiple transfusions (for example, hemophilia and leukemia patients) against panels of serum in Blumberg's international collection from people of widely varied origins. In 1963, after months of experiments, the researchers discovered that serum from a New York hemophilia patient reacted with serum from a person residing in the opposite corner of the world—an Australian aborigine. [Clinical and *in vitro* research.]

This finding was not unusual in itself; up to that point, the transfused patients' blood in these experiments had reacted with high frequency to other sera, indicating that the patients had been exposed to many common antigens through

transfusions. As a result, though, it had not been possible to draw any definitive conclusions as to which antigen or antigens were causing the reaction—until now.

It turned out that in the particular experiment with the Australian aborigine's serum, only one of 24 hemophilia patients' sera reacted with it. The significance of this was exciting, for it implied that a single and rare antigen was causing the reaction. So what was the antigen? Since it occurred only rarely, it was unlikely to be an antigen caused by genetic variation in human blood. Instead, it was more likely to be from an infectious source. Intrigued by this question, although still not working on hepatitis B directly, Blumberg and Alter tested the serum of the hemophiliac in question against thousands of serum samples. They found that samples from only one in 1,000 healthy nonhemophiliac American blood donors reacted with the hemophiliac's serum, whereas samples from one in 10 of the leukemia patients reacted. [Clinical and in vitro research.]

Whatever antigen in the Australian aborigine's blood had caused the reaction in Blumberg and Alter's tests was also found often in the blood of leukemia patients. Moreover, the antigen was rarely found in normal patients' blood but frequently in hemophiliacs and leukemia patients. The researchers labeled the mysterious protein Australian antigen (Aa) in reference to the homeland of the aborigine whose blood led to its discovery. They hypothesized that an unknown antigen in the Australian aborigine's blood was reacting with antibodies in the blood of certain hemophilia and leukemia patients.

Blumberg thought he might have detected an inherited blood-protein polymorphism that affected people's susceptibility to leukemia, but he knew that other possibilities (including an infectious agent like a virus) might explain the link between Aa and leukemia. To clarify that link, he began searching for Aa in the blood of children with Down syndrome, who run a particularly high risk of developing leukemia. Almost one-third of these children had Aa.

Blumberg then tested Down's patients of various ages who were housed in various settings. Newborn patients tested negative for Aa, but the bigger the institution in which the patient resided, the more likely that he or she tested positive. This suggested that Aa might be linked to an infection of some sort. Usually, the children who tested negative for Aa remained negative when retested and those who tested positive remained positive, as expected for a blood protein polymorphism. But in 1966, Blumberg, W. Thomas London, and Alton Sutnick discovered that a 12-year-old boy with Down syndrome who had no trace of Aa in his serum when he was first tested showed presence of the antigen in his blood a few months later. Significantly, this boy not only displayed Aa by the agar gel diffusion test but he also had hepatitis. The coincidence suggested that, rather than being associated with an inherited blood-protein polymorphism, Aa was linked to hepatitis. [Clinical and *in vitro* research.]

Immediately researchers began exploring this hypothesis. In testing patients with and without hepatitis, they found that those with hepatitis tested positive for Aa more often than those without the disease. The hypothesis was dramatically bolstered when Blumberg's laboratory technician began to feel ill. Aware of the link between Aa and hepatitis, she tested her own serum for the presence of Aa—and found it positive. She later developed hepatitis and became one of the first people whose viral hepatitis was diagnosed with the Aa test. [Clinical and *in vitro* research.]

Hearing of Blumberg's findings, virologist Alfred Prince of the New York Blood Center started an experiment in the mid-1960s that would eventually confirm the link between Aa and hepatitis. Knowing that at least one in 10 patients who received multiple blood transfusions would come down with hepatitis, Prince wanted to determine whether Aa appeared in the blood during the incubation period of the disease, before any symptoms of illness, as would occur if Aa were part of the virus that caused the hepatitis. Prince began taking blood samples from certain patients at the New York Blood Center at regular intervals and storing them in a freezer. Finally, in 1968, he heard that a patient whose blood he had collected had developed clear symptoms of hepatitis. When he tested the man's blood samples, he found no evidence of Aa in the early batches but clear evidence of it in blood taken a few weeks before onset of the illness. Such seemingly direct evidence strongly suggested that Aa was indeed involved in the development of hepatitis B. [Clinical and *in vitro* research.]

At about the same time, University of Tokyo's Kazuo Okochi showed that blood that tested positive for Aa was much more likely to transmit hepatitis to transfused patients than blood that tested negative. Alberto Vierrucci, of the University of Sienna, Italy, independently confirmed Prince's and Okochi's reports in the same year, 1968. Further strengthening the link between Aa and hepatitis were discoveries made with an electron microscope in 1970 by D. S. Dane and colleagues at Middlesex Hospital in London and K. E. Anderson and colleagues in New York of what looked like virus particles in the sera of people who tested positive for Aa. They also found particles in the liver cells of patients with hepatitis. By the end of 1970, mounting evidence led nearly everyone in the field to the same conclusion: Aa was part of the virus that causes hepatitis B. (At this point nomenclature for Aa was changed to HAA, or hepatitis- associated antigen; it is now officially called HBsAg, for hepatitis B surface antigen.) The leukemia and hemophilia patients whose blood showed a high incidence of HBsAg all had needed frequent transfusions and therefore were more likely to have received blood contaminated with hepatitis B virus. [Human-based research vis-à-vis clinical and *in vitro* research and technology-based research.]

The HBsAg-hepatitis B discovery had stunning clinical implications. In the United States in the 1960s, a large percentage of donated blood was obtained from paid donors, who were more likely than the general population to have hepatitis B. As a consequence, the incidence of posttransfusion hepatitis was high; in some

studies the disease developed in half the patients who received large numbers of transfusions for extensive surgical treatments. The medical community recognized that it could dramatically reduce posttransfusion hepatitis if it could screen HBsAg-contaminated blood by an appropriate test.

But the gel diffusion technique that Blumberg and Alter used to detect HBsAg in blood was not sufficiently sensitive for accurate blood screening. Fortunately, the curiosity of two researchers at the Bronx Veterans Administration Medical Center as to what happens to insulin in the blood of diabetics had led in the early 1950s to a revolutionary technique for detecting and measuring tiny amounts of serum proteins and antibodies. Rosalyn Yalow and Solomon Berson had been perplexed as to how it was that diabetics produce insulin, a hormone produced by the pancreas, even though diabetes is characterized by symptoms that indicate a lack of insulin. To determine what happens to insulin in diabetics once it enters the bloodstream, they prepared a radioactive form of the hormone that could be easily detected.

However, while studying the blood of diabetics who had received injections of radioactive insulin, the researchers discovered that the insulin was binding to antibodies generated by the patients' immune systems. That discovery led Yalow and Berson to devise a technique called radioimmunoassay, which can trace minute quantities of a substance as it binds to an antibody or other protein. Not only was it simpler than gel diffusion techniques, the radioimmunoassay was also a thousand times more sensitive. For her development of the radioimmunoassay, Yalow shared the 1977 Nobel Prize for physiology or medicine. Several commercial companies and academic researchers adapted the radioimmunoassay to produce kits for the accurate detection of HBsAg in blood. In the United States, laws were passed in 1972 requiring that donated blood be tested for hepatitis B virus (HBV). As a result, all blood banks tested every sample of blood, and posttransfusion hepatitis due to hepatitis B became a rarity. Screening of donated blood for HBV has produced an estimated savings in medical treatments of some half-billion dollars a year in the United States alone. [Technology and human-based research led to radioimmunoassays.]

The benefits of the HBsAg/hepatitis B discovery soon extended beyond protecting people who received blood transfusions from hepatitis B to the broader arena of protecting all people from the disease. In the late 1960s, Blumberg, working at the Fox Chase Cancer Center (FCCC) with immunologist and virologist Barbara Werner, electron microscopist Manfred Bayer, and molecular biologist Lawrence Loeb, described further the small particles isolated from HBsAg-positive blood and visualized with the electron microscope. Some particles were whole viruses; others were shown to contain no nucleic acid—the gene or genes responsible for causing infection and disease.

Several experiments showed that the particles could induce protective immunity. In 1971, infectious disease expert Saul Krugman, of New York University,

published a paper on the accidental discovery that injections of hepatitis B-contaminated blood that had been heated to kill viruses gave some protection against hepatitis B. Although the nucleic-acid-free particles Blumberg isolated could not cause disease, several findings suggested they could be used to stimulate immunity against the infectious virus. Okochi and colleagues found that patients who had received transfusions and whose blood contained antibodies to HBsAg were less likely to develop posttransfusion hepatitis than were patients without the antibody.

Intrigued by the notion that HBsAg provokes an immune response that protects people from hepatitis B, Blumberg and Irving Millman, working at FCCC, proposed that a vaccine could be made from HbsAg particles obtained from the blood of hepatitis B carriers. This was an unusual approach to developing a vaccine. Before 1969 all vaccines were made in one of three ways. In one method they were prepared from whole viruses or bacteria that had been killed to prevent infection. In another they were made from weakened strains of pathogenic organisms that caused mild or no symptoms when injected as a vaccine yet protected recipients from more severe wild strains.

Vaccines had also been made from whole viruses that, while not causing disease themselves, were closely related to viruses that did. But no vaccines had been made from human blood using only parts, or "subunits," of human virus. FCCC filed a patent for a method involving this concept in 1969.

Maurice Hilleman and colleagues at the Merck Institute for Therapeutic Research recognized the importance of the possibility of developing a vaccine from particles, or subunits, of the virus. In 1971, Merck, where scientists were independently working along related lines, took a license from FCCC and, after many years of extensive research and testing, developed a subunit hepatitis B vaccine made from HBsAg purified from blood. In 1980, Wolf Szmuness, of the New York Blood Center, and colleagues at Merck showed that the vaccine provided more than 90 percent protection against hepatitis B and had no adverse side effects. In 1981, the serum-derived subunit vaccine was made available for general use. [Human- and technology-based research]

In an independent line of basic research in animals, a group of scientists led by Howard Bachrach at the U.S. Department of Agriculture reported in 1981 the first effective protein vaccine for use in animals or humans. His work resulted in the first viral protein vaccine, against foot-and-mouth disease. [Immaterial to the HBV vaccine development.]

Production of the hepatitis B subunit vaccine in large quantities was hampered by the need for the blood of hepatitis B carriers and the realization that such blood could be contaminated with other viruses. Building on an interest in this problem, William Rutter and colleagues at the University of California-San Francisco in 1977 obtained material containing the virus from Merck. They proposed to

develop a hepatitis B vaccine by preparing HBsAg particles using recombinant technology. This new process would both ensure no contamination from other sources and allow production of large quantities of the vaccine. The concept of producing a vaccine in this way was totally new. [Technology-based research.]

After cloning the hepatitis B virus and obtaining the genetic sequence of HBsAg, Rutter and colleagues explored a variety of different biological systems in which to produce the particles using recombinant techniques. They were unsuccessful using bacteria. Then, in 1980 and 1981, Rutter collaborated with Benjamin Hall and colleagues, of the University of Washington, who had developed a model system using yeast cells. Rutter and Hall successfully produced pure HBsAg particles from genetically altered yeast cells. Rutter and colleagues then founded Chiron Corporation, in part to develop the HBsAg vaccine through a contractual relationship with Merck and also to develop other medical therapies using recombinant techniques. At Merck, Hilleman used the recombinant yeast-derived HBsAg, rather than blood plasma-derived antigen, to make an improved version of a hepatitis B vaccine.

This recombinant vaccine was the first of its kind for use in humans and was licensed by the U.S. Food and Drug Administration for general use in 1986, after nine years of research. [Technology-based and *in vitro* research.]

Further studies have revealed that hepatitis B can be passed from person to person not only through blood but also through sexual contact or from a carrier mother to her newborn child. An important study in Taiwan by Palmer Beasley and colleagues in 1975 showed that nearly two-thirds of infants born to HbsAg-positive women became HBsAg carriers themselves. [Human-based clinical research.]

The hepatitis B vaccine protects people from all forms of transmission. Because infants or children infected with hepatitis B virus have an extremely high risk of becoming lifelong carriers of the disease, universal childhood vaccination for hepatitis B has now been adopted by more than 85 countries, including the United States…..

Encouraged by successful pinpointing of the hepatitis B virus, many researchers pursued research aimed at learning more about the hepatitis A virus as well as other suspected hepatitis viruses. In 1973, Stephen M. Feinstone and colleagues at NIH used an electron microscope to visualize viral particles in the stools of infected individuals. At about the same time, Hilleman and colleagues at Merck defined and characterized the human hepatitis A virus that Feinstone had purified from the infected livers of marmosets, a type of monkey. By 1996, Hilleman and his colleagues had made an attenuated hepatitis A vaccine (that is, a vaccine made from a virus that is modified in such a way that it cannot cause disease) that was licensed for general use. Another hepatitis A vaccine was developed by

SmithKline Beecham Laboratories. [Monkeys used a reservoirs or incubators, not CAMs.]

In 1978, Italian gastroenterologist Mario Rizzetto and molecular virologist John Gerin, of Georgetown University, discovered the delta, or hepatitis D, virus. This rare virus depends on hepatitis B to survive and in combination with hepatitis B causes a much more severe form of the disease. In 1983, Mikhail Balayan of the Institute of Poliomyelitis and Viral Encephalitides in Moscow discovered hepatitis E virus. Like hepatitis A, hepatitis E is spread by contaminated food and water and is usually found during localized epidemics. Despite blood screening for hepatitis B, some patients still came down with posttransfusion hepatitis due to what was termed "non A-non B" hepatitis. Scientists suspected that yet another virus or viruses could be transmitted via blood and turned their attention to developing strategies first to isolate non A-non B hepatitis and then a test to identify it in blood. [Human-based and *in vitro* research.]

After reaching those milestones, they hoped to someday work toward developing a recombinant vaccine. But the non A-non B hepatitis agent proved especially elusive. In 1983, Chiron Corporation began supporting a large research program to solve the puzzle, involving a collaboration between Daniel Bradley at the Centers for Disease Control and Prevention and Michael Houghton, George Kuo, and Que Lim Choo and colleagues at Chiron. Bradley, who had been studying chimpanzees infected with human serum containing non A-non B hepatitis agent or agents, provided contaminated chimpanzee sera to Chiron. In 1989, Michael Houghton and colleagues ushered in a new era for the discovery of infectious agents when they used molecular biological techniques to clone hepatitis C, the agent responsible for 80 to 90 percent of non A-non B hepatitis. This was a scientific tour de force because the unknown agent, unlike the other hepatitis viruses identified up to that point, had not been visualized, grown in culture, or immunologically defined. Following the introduction of sensitive and effective blood tests for the detection of hepatitis C in 1990, the risk of transfusion-related hepatitis is now in the range of one in 100,000 units transfused. [Chimpanzees as incubators or reservoirs not CAMs. The real advance here was the in vitro and technology-based research that allowed the identification and cloning.]

As the above article from the NAS proves, chimpanzees were used as incubators or reservoirs, not as models for the disease. It is disingenuous to sell research on chimpanzee as CAMs based on this instance of their use as reservoirs. Perhaps if more time and money had been spent HBV could have been grown in tissue culture, just as eventually poliovirus was. But it is immaterial to the point: The discovery of the various types of hepatitis and development of hepatitis vaccines was not incumbent upon the use of chimpanzees as *models*.

The following is from *Lab Animal*:[40]

In vivo models of HBV based on cell culture generally involve primary hepatocytes or cell lines derived from hepatocytes. However, infection of these cells with HBV has produced poor viral replication and low viral yields. Therefore, although these cell culture systems demonstrate infectivity by the virus and may be useful for some drug studies, they are inefficient models for studying the viral life cycle. Although HBV can be generated from integrated HBV genome into host cell chromosomes, in this model the mode of viral replication is different from that in natural infection.

Among the *in vivo* models, chimpanzees are natural hosts for HBV. Chimpanzees develop acute hepatitis after HBV infection and mount immune responses, but they do not develop chronic liver disease. Limited availability, endangered status, and expense further limit the routine use of these animals….

The restricted host range of the hepatitis viruses has hampered the development of suitable animal models. Table 1 provides a partial list of existing models.

Currently, one of the few animal models for HBV infection is the chimpanzee. In the wake of the discovery of the Australia antigen and the establishment of its relationship to HBsAg, as well as the discovery that Africans had a very high rate of positivity to HBsAg, a marker of chronic HBV infection, there was a search for the presence of HBV infection among chimpanzees caught in the wild in Africa.

Researchers soon demonstrated that ~3–6% of the wild-caught chimpanzees were positive for HBsAg, and ~50% of the older animals were positive for the antibody to HBsAg, a marker for resolved HBV infection. The thinking was that these HBV infections resulted from the practice of injecting human serum in wild-caught animals to improve their survival during transit and confinement; however, various groups have recently demonstrated the presence of a unique chimpanzee HBV strain that was verified by sequencing of the entire genome. Chimpanzees inoculated with HBsAg+ chimpanzee plasma developed characteristic hepatitis, HBs antigenemia, and eventual anti-HBs seroconversion.

Since these seminal studies, virologists have characterized the biological properties of HBV in chimpanzees. Thomssen *et al*. demonstrated that the 42-nm Dane particles were the infectious virion of HBV. When chimpanzees were immunized with HBsAg, all developed high titers of antibodies to HBsAg. Soon researchers were using chimpanzees to evaluate the safety and immunogenicity of candidate HBV vaccines that had been prepared from the plasma of infected human chronic carriers. These studies also proved that these plasma vaccines were actually free from infectious viruses. Because there were no *in vitro* assays for detection of infectious HBV, the chimpanzees were the only means of ensuring that batches of plasma-derived HBV vaccine did not contain live HBV.

Later, chimpanzees also served in the evaluation of the inactivation of HBV and HCV for the manufacture of virus-free plasma derivatives, such as coagulation

> Factors VIII and IX preparations. The use of chimpanzees in testing for the development of new immunization strategies such as DNA vaccines continues. [Chimpanzees as bioassays not CAMs.]
>
> Chimpanzees have contributed immensely in the development of HBV vaccines, evaluation of the safety of blood products, and the discovery of HCV. However, their limited availability, expense, endangered status, and the lack of chronic liver disease precludes the study of pathogenesis of cirrhosis and HCC. It is also not practical to use chimpanzees for the preclinical evaluation of novel drugs and therapies of viral hepatitis….
>
> Thus, although no single cell culture system or animal model is ideal for studying all features of HBV hepatitis, researchers are developing imaginative and novel animal models that are designed to investigate specific aspects of pathobiology, prevention, and therapy of HBV.

As the above shows, chimpanzees were again not used to model the disease (CAMs) but merely as indicators of the presence or absence of antibodies. This is an example of chimpanzees being used as bioreactors. Chimpanzees and other animals can be so used, but the way chimpanzee-based research is sold to the U.S. taxpayer is as a model of human disease capable of providing cures for human disease. Again, to use this instance of chimpanzee use as bioassays or bioreactors to support their use as CAMs that may result in cures for Alzheimer's and cancer is disingenuous at best.

Summary

In summary: Scientists could not find the hepatitis A virus except in humans until 1972 when it was discovered in marmosets. Research on the marmosets has not yielded results of clinical significance. Hepatitis B was differentiated from hepatitis A based on clinical studies. The animal model for HBV was and is the chimpanzee, which is essentially asymptomatic when infected; humans aren't. Chimpanzees continue to produce the virus as long as it is in their body; humans don't. The liver, which is the organ primarily affected, is not affected in chimpanzees as it is in humans. Liver enzymes, which are measured to assess the progression of the disease, respond differently in humans and chimpanzees. Therefore, comparing the human disease with that of the chimpanzee is impossible. The first hepatitis B vaccine was made from blood of infected humans and is now made from bacterial culture.

It is also suspect to claim that without chimpanzees there would be no HBV vaccine. Just because chimpanzees were used in manufacturing the vaccine does not mean that there would be no vaccine today had they not been used. Clearly, history unfolded as it did and the use of chimpanzees was part of that history. But when we consider where we are today we must consider everything:

1. There is a HBV vaccine that came about in part due to the use of chimpanzees as bioreactors not CAMs.

2. There is no cure for HBV. Guha et al: "Despite the existence of a preventative vaccine, HBV represents a substantial threat to public health, suggesting the need for research to develop new treatments to combat the disease."[41] Akbar et al: "Despite the presence of an effective prophylactic vaccine since 1982, more than 350 million people of the world are now chronically infected with hepatitis B virus (HBV). In one scenario, a considerable number of chronic HBV carriers would eventually develop serious complications like liver cirrhosis and hepatocellular carcinoma. In another, chronic HBV carriers would be permanent sources of HBV infection and transmit HBV to uninfected healthy individuals. Taken together, chronic HBV infection represents a major global public health problem, especially in the developing nations of the Asia and Africa, where most of the chronic HBV-carriers reside. Unfortunately, there is no good curative therapy approach for these patients. The prospect of treatment of chronic HBV infection by antiviral agents like type-1 interferons and lamivudine is not satisfactory due to their low efficacy, considerable side effects and high costs."[42]

3. There is still no adequate cell culture system for HBV. Guha et al: "A major obstacle to the research on the development of drug and gene-based therapies for HBV infections has been the lack of an efficient cell culture system or a readily available small-animal model, permissive for viral infection and replication. Lack of a robust in vitro cell culture system has seriously hampered the progress of HBV research…For reasons that are not clear, infection of primary hepatocytes and established cell lines with hepatitis viruses has produced poor viral replication and low viral yields and has suffered from poor reproducibility although the addition of polyethylene glycol to primary hepatocyte cultures maintained in the presence of 2% dimethylsulfoxide markedly increases the infection of HBV. In vitro cell culture models can at best demonstrate infectivity by the virus but are not suitable to study viral life cycle because of very low levels of viral replication. They could still prove useful for drug studies."

If chimpanzees had not been used the HBV vaccine would not have been developed as it was. It does not follow that it would not have been developed at all. It might have been developed later, or even in the same time frame, using tissue cultures. If money spent on chimpanzee based research had been allocated to the development of tissue culture, the knowledge gained might have led to a cure as well as a vaccine. After all, necessity is the mother of invention. When one plays the *what if* game, as the vested interest groups often do to frighten the public and lawmakers into accepting animal-based research on human disease, one must be careful in drawing conclusions.

Insulin is an example of this concept. After insulin was purified, it was harvested from pigs and cows. Researchers essentially thought the problem of diabetes was solved. However, nonhuman insulin was problematic for diabetics. Many of these problems are no longer an issue now that human insulin is manufactured via *in vitro* methods. For

nearly fifty years, very little effort was made to develop human insulin. Why bother, if it could be collected at slaughter? When the inadequacies of cow and pig insulin became apparent, research was directed toward synthesizing human insulin.

Hepatitis C

Chimpanzees in Research: Strategies for Their Ethical Care, Management and Use:

> Infection of chimpanzees with the hepatitis A, C, and delta viruses also provided important models for gaining an understanding of disease. HCV virus is a bloodborne pathogen that can establish a chronic infection and lead to cirrhosis or hepatocellular carcinoma. It is rapidly evolving, and already 1-2% of people in the United States are infected (Purcell 1994). Using molecular biological techniques and plasma samples from a chimpanzee chronically infected with HCV, previously called non-A, non-B (NANB) hepatitis virus, Choo and others (1989, 1990) successfully identified the causative agent of the infection. That would not have been possible without the clearly documented titration and transmission studies that were performed in chimpanzees. A successful vaccine for hepatitis C remains elusive because of the extensive genetic diversity of the virus. Chimpanzees continue to be important in the search for a solution to this problem (Lemon and Thomas 1997).

Are chimpanzees useful in HCV research? They can be infected with HCV, as they can with other viruses that infect humans. Their liver enzymes respond to HCV in a manner similar to humans. Researchers can harvest the virus from their blood as they can from humans blood. Are those similarities sufficient for developing treatments or a vaccine?

Hepatitis C infection is estimated to have infected 1- 3% of the population of the planet, or 170-200 million people worldwide. It is the most common cause of chronic liver disease in many countries. In the United States 40% of chronic liver disease is related to HCV. HCV infection can lead to cirrhosis, liver failure, and hepatocellular carcinoma. Between 1990 and 1992 routine antibody testing by enzyme-linked immunoassay (EIA) became available. Recombinant immunoblot assay (RIBA) is also available as are viral RNA detection tests for HCV. Treatment with interferon in combination with ribavirin, is ineffective in the majority of cases hence the need for a vaccine.

HCV is a single-stranded RNA virus and was cloned in 1989 at which time it was found to be the cause of 80% to 90% of cases of non-A, non-B hepatitis. Like HIV, numerous genotypes exist and the virus can mutate rapidly, this means, again like HIV, that developing a vaccine will be difficult.

Studying HCV has been difficult, in part, because of the lack of a reliable tissue culture for testing neutralizing antibodies or for passage and expanding of the virus. Historically, the invention of such a tissue culture system has been crucial to the development of other vaccines, such as polio.

Using chimpanzees has presented numerous problems, as once again they respond to HCV differently than humans. Mother-infant transmission has been reported in humans but not chimpanzees.[43] Chronic infection occurs approximately 75% of the time in humans but only 30-50% of the time in chimpanzees.[44] [45] Humans progress to liver fibrosis and cirrhosis while chimpanzees do not. Environmental differences, such as alcohol use may account for some differences. Humans suffer from hepatocellular carcinoma as a result of HCV. Hepatocellular carcinoma after HCV infection is very rare in chimpanzees.[46]

Robert E. Lanford and Catherine Bigger stated:

> Evaluation of the NANBH and HCV-exposed chimpanzees in the colony at the Southwest Foundation for Biomedical Research revealed that chimpanzees have a high viral clearance rate. Viral clearance was documented in 61% of the animals with confirmed infections. This obviously represents a high rate of clearance compared to previous estimates from human cohorts. Increased viral clearance in chimpanzees can be explained if chimpanzees experience a different clinical course than humans, or if the full clinical spectrum of HCV infections in humans has not been observed because human cohorts are biased for persistent infection.[47]

All this presents a problem when extrapolating results from chimpanzees, as there are obviously differences between the species.

Martin Lechmann, Ph.D. and T. Jake Liang, M.D., of the Liver Diseases Section, NIDDK, National Institutes of Health, Bethesda, Maryland wrote:

> Furthermore, the course of HCV infection in chimpanzee may not necessarily represent that in humans. Earlier experiments in chimpanzees in which challenge of apparently recovered chimpanzees with a homologous or heterologous strain of HCV resulted in reinfection suggest an absence of protective immunity from natural infection. In addition, HCV manages to persist in chronically infected persons despite the presence of broad antibody and T-cell responses. The viral and host factors that lead to persistence are not fully understood and remain to be elucidated in the future. [48]

Nonhuman primates (NHPs) also differ in their response to HCV vaccines. Martin Lechmann, Ph.D. and T. Jake Liang, M.D., of the Liver Diseases Section, NIDDK, National Institutes of Health, Bethesda, Maryland wrote:

> Mice and macaques immunized with a plasmid in which the E2 protein was targeted to the cell surface by replacing the C-terminus with a transmembrane domain showed an antibody response against E2 that occurred earlier and had higher titers than chimpanzees immunized with a plasmid expressing the full-length E2. Based on these results, two chimpanzees were immunized three times

> with this construct. Only one chimpanzee developed anti-E2 antibodies, and preliminary data from challenge studies showed no protection against viral challenge. [49]

Even if the disease affected human and chimpanzee in an identical fashion, interspecies variation would still be troublesome in terms of making a vaccine. Vaccines, drugs, and diseases have manifest marked differences between chimpanzees and humans.

There are options other than chimpanzees. Hugo R. Rosen, M.D. and Paul Martin, M.D. wrote:

> Moreover, human liver transplantation represents the only available model system to study HCV, as a suitable animal model (the endangered chimpanzee model has significant limitations) and tissue culture systems for its propagation do not exist. Despite significant advances in our understanding of the epidemiology and molecular biology of HCV, the mechanisms responsible for hepatocellular injury in chronic HCV infection remain poorly understood. [50]

To date, the breakthroughs in HCV have been mainly *in vitro* research-based. Laboratory tests to identify the presence of the virus facilitate diagnosis and provide a screen of blood slated for transfusion; the test to quantify HCV in the blood; the ability to inactivate or kill the virus in blood products like plasma, factors VIII and IX were all *in vitro* accomplishments. The sequencing of the virus, which also was primarily an *in vitro* accomplishment, did use virus-containing blood from a chimpanzee.

Human-based research led us to the discovery of HCV and revealed that HCV can take very different courses. In some, it progresses rapidly and causes death in less than a decade while in others, it mild and nonprogressive. To date, no test exists that can predict which course it will take in any given person. Epidemiologic research into factors affecting prognosis deserves more funding. Human-based research will eventually lead to a cure. Liver biopsies from humans revealed changes characteristic of viral hepatitis. Clinical observation revealed the association of infection with exposure to blood products and distinctive patterns of ALT elevation. Studying humans is difficult but yields the most useful data. Studying HCV in humans is difficult in part, because HCV infection is usually asymptomatic and presents without jaundice. Many do not know they are infected and hence physicians cannot follow and study them to see how their body responds early in the course of the disease. This is not impossible however. Studying illicit drug users and HCV positive individuals who have undergone liver transplant, provide opportunities hitherto underutilized.

Since 1989, we *have* learned things about HCV and about HCV in chimpanzees. But that begs the question, “Is any of what we have learned relevant to humans?” HCV affects more people today than it did in 1989. There is no vaccine and there is no cure. Just as we grew poliovirus in NHPs in the first half of the twentieth century because we could not grow it in culture, so we have maintained a supply of HCV by infecting chimpanzees.

But it was not until Ender grew the poliovirus in culture that the vaccine was possible. It was not until we were able to study the poliovirus *in culture* that we were able to discern the similarities and differences in how the virus affected humans and NHPs. Only in retrospect could we say what had been important in the chimpanzee-based research. Knowing something in *retrospect* is not useful in *developing* a vaccine.

Development needs knowledge that can be counted on. Retrospective analysis is interesting, indeed comparative medicine on the whole is interesting, but what is needed in order to ease human suffering is data that we know is applicable to humans. We have no such knowledge based on chimpanzee studies. Unfortunately history provides many examples where data from a nonhuman primate species led to human deaths.

Researchers thought they had knowledge applicable to humans based on studying HIV in chimpanzees and the French blood bank crisis was the result; thousands died. Based on chimpanzee and monkey models of HIV, scientists thought the virus entered the white blood cell via one receptor only; it requires two in humans. Based on studies of chimpanzees, researchers thought HIV reproduced slowly in humans; it doesn't.

Note the following quote regarding how researchers studied the poliovirus:

> The experimental path he [Draper] had elected to follow...only led him further and further away from the human disease and deeper into the woods. He had convinced himself that the virus was strictly a neurotropic one that entered the body via the nasal route and preceded directly to the central nervous system…He steadfastly held out against the alimentary tract as the portal of entry. Remarkably enough he was resistant to the idea that polioviruses are actually a family composed of several types with different antigenicity. But more than that he held out doggedly against methods of clinical investigation which included clinical virology - approaches that eventually made possible the unraveling of the whole story…Draper had gone completely over to Flexner's views on the nasal portal of entry. The clock had been set back about twenty five years in poliovirus research." Based on the concept, that polio gained entrance to the human body through the nose as it does in non human primates, researchers thought that they could block the route of transmission. They applied zinc sulfate and picric acid alum to the noses of children. The children lost their sense of smell permanently but experienced no protection from polio.

More than once polio vaccines thought effective based on research with NHPs proved harmful to children. In 1934, Dr. Maurice Brodie ground up the spinal cords of monkeys infected with polio and made a vaccine. He tested the vaccine on monkeys and found it effective. When given to children it actually caused polio. Some children died while others were paralyzed. Dr. John Kolmer repeated this mistake when he made a polio vaccine. He tested it on monkeys then gave it to children who then went on to develop polio, die or were left paralyzed.[51]

The inventor of the polio vaccine, Dr. Sabin stated, under oath before the U.S. Congress, that the polio vaccine was long delayed because of inaccurate results from nonhuman primates: "...the work on prevention (of polio) was long delayed by the erroneous conception of the nature of the human disease based on misleading experimental models of the disease in monkeys."[52]

Another concern about using chimpanzees in HCV research pertains to zoonoses. Researchers have taken blood samples from humans with HCV, injected them into chimpanzees, and cultured the viruses back from the chimpanzees. But are they the same virus? Viruses mutate, especially RNA viruses. By infecting chimpanzees we run the very real risk of the virus mutating into a form that more virulent and/or more contagious than the current versions. Infecting different species with human viruses places the public's health at grave risk.

Successful HCV research will require the ability to culture the virus, just as polio did. Note what Dr. Robert Gallo said about culturing viruses *in vitro*, "The development of *in vitro* systems greatly facilitated progress in this field, enabling scientists to make better quantitative estimates of the amount of virus in a sample, to determine the target cells of a particular virus, and to see whether the virus produced a cytopathic effect… Although techniques for tissue culture were a development of the 1930s, the techniques have been continually refined up to the present. A big advance came in the 1950s when John Enders grew the poliovirus in cell culture. The 1950s and 1960s became one of the greatest periods of medical virology because of these cell culture advances."[53] Notably, in 2005, two groundbreaking papers demonstrated the successful, robust and efficient propagation of HCV in tissue culture, which the authors believe will greatly aid the search for improved antivirals and vaccines.[54]

HIV/AIDS

Chimpanzees in Research: Strategies for Their Ethical Care, Management and Use:

> As with HBV and HCV, the only animal species initially tested that could be infected with AIDS-patient material, or with the virus itself after it was isolated, was the chimpanzee (Francis and others 1984; Gajdusek and others 1985). This primate species remains the only one (except humans) that can be persistently infected with multiple HIV-1 strains by both intravenous and mucosal routes. That chimpanzees can be infected with HIV strains representing different subtypes is critical because of the unprecedented genetic diversity of strains circulating worldwide (WHO Network for HIV Isolation and Characterization 1994). That diversity (and data obtained in studies with chimpanzees) indicates that a vaccine based on only one HIV subtype will have limited protective value, so it will be necessary to test different combinations of antigens to identify the ones that together induce the broadest cross-reactivity. This is even more important in light of the high costs associated with the current successful advances in the treatment of AIDS; these costs will limit their use not only in the United States, but especially in poorer nations. Thus, the formulation of clinically

> effective, inexpensive vaccines is likely to be the best long-term solution to this global problem.
>
> Although only one of about 200 chimpanzees infected with HIV-1 has so far succumbed to an AIDS-like disease, several animals that have been infected for a long time exhibit decreased CD4:CD8 lymphocyte ratios. Virus isolated from the chimpanzee that died of AIDS elicited a rapid decline in $CD4^+$ cells in all of three chimpanzees experimentally infected with this HIV variant (Fultz unpublished data; Novembre and others 1997). Thorough evaluation of immune responses and virus-host interactions in these infected animals, compared with chimpanzees infected with other, less pathogenic isolates, might provide new insights into HIV pathogenesis. In addition, chimpanzees have been and will continue to be important in studies to develop HIV vaccines and to evaluate their immunogenicity and protective efficacy against infection.
>
> Although HIV infection of chimpanzees has not been an ideal model of disease, at least 198 chimpanzees have been used to date in HIV-related studies; this number reflects only HIV-1-infected animals now held at various institutions, excluding animals exposed at LEMSIP of which the committee has no knowledge.

Probably no animal species has been more studied for the cause and effects of a disease than chimpanzees (and recently monkeys) have been for HIV/AIDS. But what have been the results? Claude Reiss, an eminent French biologist with 40 years of research experience, including many years spent at the prestigious French National Centre for Scientific Research (CNRS), stated:

> We recall that at the beginning of the 1980s, the observation that HIV was innocuous to great apes convinced experts that the virus was of negligible harm to man. The green light had thus been given in France for the distribution of contaminated blood samples, whose consequences we know. The true cause of the contaminated blood scandal is the animal model. The emergence of other scandals, maybe even more dramatic, is to be feared if the animal model continues to be used as a basis for gauging health risks.

This is supported by Pierre Tambourin, then head of the life science department of CNRS (National Center for Scientific Research, the largest research organization in Europe, Tambourin indirectly supervised over 2500 researchers and 4000 engineers and technicians, all civil servants) when he testified before the board of Ministers of Parliament on July 9, 1996: "What are the chances of developing a prion disease following ingestion of contaminated meat? Nobody knows, but we must not repeat the error we did in 1983-1985 with AIDS, when we referred to animal models to dramatically underestimate the risk to which humans are exposed." As MPs asked later for more precise wordings, he admitted that he alluded to negative chimpanzee experiments which convinced experts that transfusion of contaminated blood is devoid of risk.[55]

Once again, speciation results in profound differences in response to disease. Chimpanzees and bonobos appear to be less susceptible to AIDS, malaria, hepatitis B virus, Alzheimer's disease, and cancer.[56] Some of the most well known differences between the human and chimpanzee genomes are those in the genes encoding HLA types; there appear to be no shared alleles between human and chimpanzee.[57]

HIV is a very simple virus; it has only nine genes. Unfortunately, it lacks the usual repair mechanisms and as a result, mutations are common. In ten years HIV can undergo the human equivalent of one million human-years worth of mutations. That is a mutation rate of about 1% per year. (Consider that the 1% difference between humans and chimpanzees took about 5 million years.) There are six subclasses of HIV, which differ by 30 percent in their genes, another significant difference when looking at disease sequelae. In the United States, the main variety of HIV is Type B, while in countries like Thailand, 90 percent of the HIV is Type E. One difference between the two strains is that Type E infects cells of the vaginal walls much more readily than Type B.

Three enzymes are vital for HIV's interaction with humans: HIV protease, reverse transcriptase and HIV integrase. HIV attacks human cells in at least four steps. First, the virus attaches itself to receptors located on the surface of the host cell, such as a CD4 cell and injects its RNA into the cell, where an enzyme converts it to DNA. Second, the DNA penetrates the cell's nucleus and co-opts the machinery normally used to replicate DNA to reproduce itself. Third, to make copies of itself, HIV uses the protease enzyme to slice up the proteins into shorter strips, suitable for making new viruses. Finally, thousands of HIV containing capsules are released through the cell membrane, flooding the body with a new generation of the virus.

Many breakthroughs seen in nonhuman primates in vaccine development have not transferred to humans.[58] AIDS research in NHPs has been unsuccessful in both monkeys and chimpanzees. Why is this the case? With few exceptions, HIV-1 infection of chimpanzees is universally mild with no notable decline in CD4+ T-cell levels, immunosuppression or other signs of an AIDS-like illness. Rather, HIV-1 infection of chimpanzees results in detectable plasma HIV that decreases within 2–3 months of infection and becomes low to undetectable within a few years. The ability to detect or culture HIV after this initial time period is variable.

The virus used to infect the chimpanzee named Jerom (the only chimpanzee to come down with an AIDS-like illness) in Yerkes Primate Center was different from the type that usually infects humans. After Jerom was infected his blood was transfused into other chimpanzees that then dropped their CD4 counts, but in contrast to Jerom they did not exhibit signs of illness. When HIV infects humans for the first time it binds to the CCR-5 receptor, then it develops a preference for the CXCR-4 receptor. The virus used to infect Jerom relied on the CXCR-4 receptor from the outset.[59] These differences are significant.

Louis R. Sibal, Director of the NIH Office of Laboratory Animal Research, and Kurt J. Samson wrote in *ILAR Journal*:

> Although progressive infection with HIV-1 can occur in some chimpanzees, chronically infected animals usually maintain normal numbers of CD4+ T-lymphocytes and do not become immunodeficient. The one exception stems from a report that a chimpanzee [Jerom] infected with three different isolates of type-1-HIV over a period of 10 years revealed a persistent decline in CD4+ T-lymphocytes that progressed to AIDS or an AIDS-like disease. Blood from this animal that was transfused into an uninfected chimpanzee induced a rapid depletion of CD4+ T-lymphocytes but did not cause clinical disease. Without disease as an endpoint, researchers can measure only the infection-blocking effect of candidate vaccines.[60]

Although Samson and Sibal strongly support the use of chimpanzees for AIDS research, they also state, "However, because AIDS is a complicated disease involving many molecular events in several different cell types, a vaccine that works in NHPs may not work in humans."[61]

The reasons HIV does not infect chimpanzees as it does humans are myriad and again, the result of speciation. In chimpanzees, HIV does not reproduce well. Chimpanzees have higher baseline levels of T8 cells, a greater proliferative response, and a lower ratio of T4/T8 cells. Since T4 cells are selectively attacked by HIV, this difference is not insignificant. Unlike humans, chimpanzees do not drop their T4 counts to zero with infection. They do go down, but not as dramatically. B-lymphocytes produce more antibodies in chimpanzees and they produce them earlier, thus stopping disease spread. Humans drop their antibody count prior to systemic illness; chimpanzees do not. Chimpanzees have HIV only in their blood cells, while humans also have the virus in plasma. Chimpanzees exhibit only a flu-like illness in response to being infected with the virus, while humans go on to full-blown AIDS. Humans develop opportunistic infections and cancers associated with HIV, which chimpanzees do not. Chimps do not reveal classic changes in the central nervous system that humans do. They do not have virus particles in saliva or cerebral spinal fluid.[62] Margaret I. Johnston states about HIV in chimpanzees:

> With few exceptions, HIV-1 infection of chimpanzees is universally mild with no notable decline in CD4+ T-cell levels, immunosuppression or other signs of an AIDS-like illness. Rather, HIV-1 infection of chimpanzees results in detectable plasma HIV that decreases within 2–3 months of infection and becomes low to undetectable within a few years. The ability to detect or culture HIV after this initial time period is variable.[63]

Because of differences such as these, the *Handbook of Laboratory Animal Science*, in 1994 called primate models of AIDS "unsuccessful."[64]

The vaccine made by VaxGen, AIDSVAX showed promise when given to chimpanzees[65] but failed when tested on 3,330 humans, mostly men. An equal percentage of those receiving the vaccine contracted HIV compared to the controls.[66]

There has been progress on HIV and AIDS. The following is a brief summary of our progress. Much of this data was taken from several review articles that appeared in the July 2003 issue of *Nature Medicine*.

Human-based discoveries and developments:

1981. AIDS was first noticed when homosexual men experienced an increased incidence of rare diseases, notably Kaposi sarcoma and opportunistic infections such as *Pneumocystis carinii* pneumonia, as well as cases of unexplained, persistent lymphadenopathy. Physicians quickly discovered that these individuals had a common immunological deficit resulting from a significant decrease of circulating $CD4^+$ T cells.

1982. Clinical and epidemiological investigations had provided persuasive evidence that the disease was caused by an infectious agent, probably a virus, transmitted by sexual routes and in blood derivatives. Initial attempts to establish a link between the epidemiological and clinical features of this disease and a known virus failed. The French working group became convinced that the cause was probably an as yet unidentified virus.

As with many emerging infectious diseases, the initial and most powerful tool to illuminate the etiology of the disease was classic epidemiology. Initial observations suggested that the disease might have a retroviral etiology. Two retroviruses, human T-lymphotrophic virus (HTLV)-I and HTLV-II, which had been recently recognized at that time, were the only viruses known to preferentially infect $CD4^+$ T cells. The transmission pattern of HTLV was similar to that seen among individuals with AIDS; in addition, HTLV-I and related retroviruses were known to cause varying degrees of immune deficiency in humans and animals. Thus, the search for a new retrovirus was undertaken in earnest.

1983. The HIV was discovered and a blood test was developed to identify infected patients.

1987. The first effective drug against HIV was the reverse transcriptase inhibitor (NRTI) zidovudine, or AZT. It was identified when large numbers of compounds that had been produced for other purposes were screened for possible efficacy against the new virus. (AZT was originally developed as an anticancer drug but did not prove effective in that capacity.)

1991. More NRTIs available.

1994. Zidovudine prescribed for mothers-to-be to prevent mother-to-baby transmission.

1994. Ineffectiveness of monotherapy noted.

1995. Drugs are developed to target specific vulnerable points in the virus replication cycle, providing a cogent example of the importance of the basic research endeavors in viral biology and the translational approaches in drug development. The prototype of this approach was the expression, purification, and crystallization of the HIV protease enzyme to facilitate the tailored design of protease inhibitors—a class of antiretroviral drug that was first approved by the U.S. Food and Drug Administration (FDA) in 1995.

1995. HAART (Highly Active Anti-Retroviral Therapy)

The structure of HIV was identified. The virus was found to have nine genes.
Light was shed on the pathogenesis of AIDS such as CD4 depletion.

1996. First NNRTI (nevirapine) developed.

1996. A test to estimate viral load in widespread use.

2000. Lymph tissue was identified as the chief target of HIV. Other tissues were identified as reservoirs thus making it hard to eradicate HIV from body. The treatment of HIV-1 is complicated by the existence of tissue compartments and cellular reservoirs. Much of the virus in the central nervous system and in semen evolves independently of virus found in blood cells. Latently infected, resting $CD4^+$ T lymphocytes can survive for many years, and these lymphocytes can archive many quasispecies of virus that can re-emerge and propagate after the withdrawal of HAART. Macrophage populations can also express virus in HIV-1-infected individuals on virally suppressive HAART. Moreover, HAART does not inhibit all viral replication; low levels of viral replication occur 'cryptically' below the limits of clinical plasma viral load detection.

Resistance testing of HIV isolates has become part of standard HIV-1 care. There are phenotypic and genotypic assays available to help predict which drugs are likely to have activity against HIV-1 and which agents are likely to fail because of resistance. Phenotypic assays measure drug susceptibility directly. Genotypic assays identify mutations in HIV-1 that are known to confer phenotypic changes. Genotypic testing, which is more widely used than phenotypic testing, is an example of one of the earliest applications of gene sequencing in clinical medicine.

2003. The newest class of drug, fusion inhibitors, represents another example of successful targeted drug development led by basic science discovery. These compounds block the fusion of the viral envelope to the cell membrane, and became available with the FDA approval of enfuvirtide (Fuzeon). New and improved drugs in all three classes (reverse transcriptase inhibitors, protease inhibitors, and fusion and entry inhibitors) are being actively pursued along with drugs against alternative targets such as the viral integrase. Currently, there are 20 FDA-approved drugs or combinations of drugs for HIV.

Summary

One argument that is frequently used to justify the current bias against using chimpanzees is the high cost of maintaining them in labs. We find this specious as chimpanzees eat fruit, and have been kept in cages hanging off the floor so that their waste will fall out and can be hosed away. The cost must be low compared with the cost of say a CT machine, a researcher assistant's salary, other lab equipment, and so forth. It is in fact incredibly low compared to making a genetically modified mouse. If chimpanzees fulfilled the requirements for CAMs we would have no need of genetically modified mice. The fact that we have so many varieties of genetically modified mice speaks volumes about the utility of chimpanzees. Further, the reason chimpanzees make such poor models also rules out the use of genetically modified animals: complex systems react to change in a nonlinear fashion. Simply changing one or two genes will not result in a complex system that can be used as a CAM.

If chimpanzees actually were productive models, who can imagine that we wouldn't have chimpanzee labs at every institution? Would we really forego a productive method of investigation?

If any animal is going to be a reliable model for humans vis-à-vis drug testing and disease, it would have to be the chimpanzee. As we have seen, the chimpanzee differs from humans in gene regulation and expression and hence reacts very differently than humans to disease conditions and drugs.

Using chimpanzees to model humans is an archaic paradigm that began in the 2nd century A.D. many years before Darwin's theory of evolution and before the discovery of DNA. When scientists lacked knowledge about the fundamentals, it appeared that humans and chimpanzees had more in common than not. And we did in fact learn things about humans from studying chimps. Chimpanzees have hearts and other organs, suffer from infectious diseases, and think with their brains, and so forth, as do humans. But modern-day biomedical research is not looking for answers that can be found in chimpanzees. Very small differences between species, on the genetic level, the level we cannot see, lead to lethal errors in the practice of medicine. And not just differences between species but even between men and women of the same species or between siblings.

We now understand that if a woman and her twin sister are diagnosed with the same type of breast cancer on the same day, because of the fact that one gene was turned on in sister A and not sister B, they may receive very different chemotherapies. Even though the twin sister has far more genes in common than either has with a chimpanzee, even the one cannot predict the correct medication for the other.

Drug testing is another example. As we mentioned earlier, among ten medications withdrawn from the U.S. market between 1998 and 2001, eight had more severe side effects in women than in men. Men could not even predict what a drug would do in

women. How could we possibly believe chimpanzees would have predicted the correct response?

We are living in the beginning of the age of *personalized medicine*; an age when your genetic profile will be known to you and your physician. You will be able to predict which diseases you are subject to and take measures to avoid them, and the medications most appropriate for your genetic makeup will be prescribed. If we are to expand and refine our current gene-based treatments, it is time to hone the focus of our medical research. Avenues of distraction, fostered by studying entirely different species, are best avoided.

Ethically conducted human-based research such as occurs using human tissues, stem cells, autopsies, clinical research and epidemiology, and advances in technology such as that which led to artifical neural networks, balloon angioplasty, mammography, hip and knee replacements, and functional MRI and PET scanners, and advances in the basic sciences such as physics, chemistry, molecular biology and math will lead to the cures and treatments we are most in need of today.

Chimpanzees are no longer viable for use:
1) as models for the study of human disease; as CAMs; or
2) as models of humans for testing drugs.

Society funds research on chimpanzees because it believes that doing so will lead to cures for diseases like AIDS, Alzheimer's, cancer, diabetes, heart disease, multiple sclerosis, Parkinson's, stroke, and so forth. It is time to put our funds toward the future and leave antiquated animal models behind.

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