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Letters

Evolution: Not Essential for Modern Medicine

This is a response to an Opinion article by Stephen Schiffer entitled *Evolution: The Founding Principle of Animal Models of Human Disease,* (Comp. Med., vol. 52, number 4, p. 305-306, 2002).

The main premise is that similarity proves common descent and this is somehow essential for modern medicine. However, this is equally well explained by a single common design as defined by the theory of intelligent design and being agnostic regarding the source of design (1). If everything were different, we might conclude there were many designers instead of one. There are differences in particular in how common structures develop to thwart evolutionary explanations. For example, the human hand and foot develop by different mechanisms than the frog foot (2, 3).

Regarding evolutionary medicine, it is vacuous to claim that we cannot treat a cough properly unless we realize that it is an evolutionary adaptation to expel particles from the trachea. In fact, it is possible to treat it equally well by regarding it as a designed mechanism to do just that.

Another example of evolutionary emptiness comes from mouse genome research where researchers were surprised to find that so-called junk DNA almost certainly has an important role, because 5% of the human and mouse genome is almost identical. Evolutionists call the almost identical sequences "highly conserved" because they interpret the similarities as arising from a common ancestor, but with natural selection eliminating any deviations in this 5% since precision is essential for it to function properly. One would wonder about a mechanism that relies on chance to provide this precision.

The cited figure of 98.7% similarity between ape and human DNA has now been discredited (4), but even if it were correct, it would still mean 1.3% difference. Since humans have 3 billion base pairs of information in the genome this amounts to 39 million base pairs of information (13 encyclopedia-sized books) that evolution has to generate by chance mutation and selection (5).

A growing number of biologists and scholars today are observing an apparent design in nature that may be a genuine organizing intelligence that is not the product of natural selection acting on random variations (6, 7).

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Comment on "Barrier Facilities for Transgenic Rodents in Academic Centers—A Two-Edged Sword"

In his Opinion article (Comp. Med., vol. 52, number 5, p. 397-402, 2002), Dr. Jon Gordon expressed some interesting views regarding barrier housing for genetically altered mice (1). We disagree with much of his reasoning; however, our purpose is not a point-by-point critique, but to re-emphasize some key issues. We say "re-emphasize" because these matters previously have been addressed by others, but we think it is important to remind ourselves now and then to continue trying to improve communication with our non-veterinarian investigator colleagues.

First, discussions of rodent health maintenance policies and procedures often are clouded by deficiencies in communication. For example, terms such as "barrier" and "conventional" commonly are used as if they had standard, universally accepted definitions, which they do not (2, 3). "Conventional" can mean use of open cages, unrestricted introduction and interchange of mice, uncontrolled traffic of people and equipment, and no health monitoring. This might seem acceptable to investigators unfamiliar with rodent infectious diseases, but one has only to recall how prevalent such diseases once were among laboratory rodents to predict the results if vendors and biomedical research institutions abandoned basic disease prevention measures. In today's research institutions, the risks from overt and opportunistic pathogens would be greatly amplified by the huge numbers of genetically engineered mice now in use, many of which have mutations with potential effects on disease resistance.

We agree that if barrier housing is used, the need should justify the cost and inconvenience, but Dr. Gordon doesn't describe barrier housing at his institution, nor does he address the needs of other investigators' research. "Barrier" commonly is used in the rigid and restrictive sense, but in the general sense means a combination of procedures and facilities features designed to control the microbiological status of the animals within, without specifying those procedures and features. Even basic preventive measures constitute a form of barrier, and, if accompanied by careful health monitoring, can be quite effective without exces-

sive cost or inconvenience. However, as Dr. Gordon notes, uncooperative and/or unknowledgeable investigators who don't follow protocols are a major reason for introduction of murine infectious agents. Thus, investigators who object to strict disease control measures should recognize that this in itself may be a major reason for imposition of increasingly rigid barrier protocols. In addition, considerable resources are required to develop and assure quality control of different performance-based protocols for various research needs; thus, institutions lacking such resources may instead implement a uniform "engineering" standard as the only available option. This may be perceived as imposing rigorous measures unnecessarily or without adequate justification, especially if a sound rationale is not clearly communicated. Investigators holding such views should consider whether efforts to control costs might be counter-productive if such efforts prevent an institution from developing the resources to design and implement flexible, cost-effective health maintenance programs.

Dr. Gordon refers to the lack of definitions for such inherently imprecise terms as "clean animal" and "clean conventional." The problem of definitions readily is solved by not using such terms, and using "specific pathogen-free" (SPF) instead, as its definition is clear from the list of agents for which the animals in question are tested and found to be free ("SPF" also can be misused by being assumed to mean more than it really does, as if it were akin to "defined flora" or "gnotobiotic," but, as "SPF" actually defines only a limited number of excluded agents, the microflora of SPF mice is little more defined than that of conventional mice). However, a universally accepted set of agents to be excluded from SPF mice is unlikely ever to exist, in large part because it is impossible to classify all murine infectious agents simply as either pathogenic or nonpathogenic. Dr. Gordon notes that there is disagreement as to whether certain agents should be considered pathogens, and cites mouse hepatitis virus (MHV) and *Helicobacter* species as examples. Most agents, including these, are not simply either pathogenic or nonpathogenic. Rather, pathogenicity is more often a variable property dependent upon host, environmental, and microbial factors. Thus, it is pointless to classify as pathogenic or nonpathogenic agents that alter biological responses without causing detectable injury to the host, or that cause mild or subclinical disease, or that cause disease in susceptible hosts but not resistant ones. Most infectious agents of mice fall into one or another of these categories. MHV strains vary widely in pathogenicity, and manifestations of infection are highly influenced by host genotype, age, concurrent infections, and various experimental manipulations. Thus, infection can be subclinical or cause overt disease, depending on the interactions among these variables. Furthermore, effects are not necessarily transient, and the liver is not the only organ affected in the case of polytropic MHV strains in susceptible mice. Similarly, the majority of known murine *Helicobacter* species are not pathogenic for immunocompetent mice, but chronic active hepatitis and induction of hepatocellular neoplasms by *Helicobacter hepaticus* in mice of certain strains has had major adverse consequences for toxicologic bioassays, and new *Helicobacter* species continue to be discovered that are pathogenic only under certain circumstances (4-8).

Discussions of these matters also can be hindered by confusing absence of evidence with evidence of absence. Dr. Gordon asserts that "conventional animal housing does no obvious harm to animals" and takes the position that widespread use of conventional housing would not entail significant risks in other than a few unusual circumstances. However, because most common murine infectious agents don't generally cause evident morbidity and mortality in immunocompetent mice doesn't warrant the assumption that wider use of conventional housing would not entail a significant risk of morbidity and mortality from overt or opportunistic pathogens, that subclinical infections or variations in normal microflora don't affect biological responses, or that only immunodeficient mice are affected (2, 3, 9-11). As another example, Dr. Gordon states that he is "not aware of a single research publication that has had to be withdrawn because the animals were not of VAF status." We'd have been surprised if he were, because few editors or reviewers ever consider such issues, aside from those of a very few laboratory animal-related journals. The actual number of publications in which microbiological status was at least a potential complicating factor cannot be known, of course, but that doesn't justify the assumption that none exist. Related to this is the idea that SPF status decreases the value of mice as research tools because it is "unnatural." Of course it is unnatural; however, laboratory mice themselves are unnatural, having been bred from several subspecies. Inbred laboratory mouse strains and genetically engineered mice are even more unnatural. This doesn't negate the usefulness of mice as scientific tools, a major reason for which is their amenability to genetic and microbiological manipulation. In regard to microbiological and other variables that can affect mutant phenotypes, and the advisability of knowledgeable and thorough phenotypic characterization of mutants to evaluate the effects of such variables, suffice it for us to refer to recent communications in *Comparative Medicine* (9, 12).

The above issues notwithstanding, the major problem in our view is that far too little is known about most murine infectious agents in terms of potential adverse effects, transmissibility, prevalence, and effectiveness of detection and prevention methods. In the absence of such information, assessing risks and developing optimal control measures is educated guesswork at best. Better standards for diagnostic tests are also badly needed. As it stands, users have no way to evaluate the sensitivity, specificity, and quality control of the tests offered by various laboratories, even though it is widely recognized that sending split samples to different laboratories can produce discordant results. There also is a serious need to develop automated molecular methods for direct detection of specific agents for cost effective screening of large numbers of mice housed in filtered cages. Thus, a far better use of the funds Dr. Gordon proposes for regional barrier facilities would be to support research to provide a solid scientific basis for designing effective and efficient SPF programs. Unfortunately, the interest of NIH in supporting such research seems to be at an all-time low. We suggest that Dr. Gordon and others having similar views should press NIH to make available the necessary funds, and, if necessary, direct their use by the National Center for Research Resources.

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Author's Response

I am grateful to Dr. Shoeb and Dr. Davis for the effort they made to provide a response to my article. Exchanges such as these can only improve animal management. I do not feel compelled to argue the question of how to define "clean" animals. Clearly, every barrier needs to develop such a definition before proceeding to develop maintenance protocols. Of course, the "cleaner" the animal is required to be, the more difficult it becomes to maintain that state of cleanliness.

The comment that too little is known about the impact of infectious agents on animal experiments to expect papers to have been withdrawn because of problems with animal pathogens is interesting, and I entirely agree that we need to know more about the physiological manifestations of infection with common rodent pathogens. However, if we balance the absence of negative information on the affect of pathogens against the documented finding of transgene phenotypes that do not appear when animals are clean, I believe the most rational approach is to produce transgenic animals and evaluate these animals under conventional conditions, where transgene phenotypes are less likely to be missed. It is important to recall in this regard the several examples I cited of trangene-related phenotypes that do not appear in an artificially clean setting. This strategy not only takes potentially undisciplined personnel out of the barrier, it also facilitates barrier maintenance by eliminating complex equipment and elaborate experimental manipulations from the facility. Lines of animals can always be rederived to whatever level of cleanliness is required for specific experiments after the production and initial evaluation period. In addition, while it is true that the absence of evidence is not the evidence of absence, we should not simply assume that barrier housing is superior because it avoids imagined rather than documented problems.

I also agree, of course, that barrier housing should be justified. If the decision to perform experiments within a barrier is made judiciously, barrier use can be more efficient, more cost-effective, and more adaptable to progress in both the scientific and technological arenas.

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