The Genesis and Validation of Animal Models

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Every one who deals with the phenomena of pathology soon comes to know that nature often speaks her secrets with a still, small voice out of a dense thicket of happenings. He who would hear and comprehend can have no pride of intellect, no fixed preconceptions; he can only listen intently and ask himself what he may have heard.[1]

THE DISCOVERY, recognition, and exploitation of an animal model to contribute ideally to human and animal welfare should accomplish the end of eliminating the disease in question. The descriptions of animal models of human disease published in the American Journal of Pathology and in the Bulletin of Comparative Pathology by the Registry for Comparative Pathology form an excellent nucleus for the scientist interested in the development of animal models and the utilization of those already described. The recent book by Andrews, Ward and Altman,[2] also is a fine source of information.

The lessons we can send from this conference about the development and use of future animal models must be drawn from observations of the examples set by our predecessors. Some of the frontiers that we face now include the development of animal models for such important human diseases as cystic fibrosis, hypertension, diabetes, and senile dementia.

An important area of animal model utilization that has not been properly exploited and will become more important in future years is that of the use of animals as monitors to detect carcinogens or other environmental toxins that might affect human beings. This is amply illustrated by the studies in falcons of soft-shelled eggs caused by DDT some years ago, and again during the tragic events surrounding PBB toxicosis in cattle in Michigan in 1973.

The story of immunologic diseases is one of the most fascinating in the utilization of animal models, because it illustrates the development of ideas about a group of diseases that kept pace with or attempted to keep pace with the development of techniques. It was not until 1967 that Lerner, Glassock, and Dixon [3] elaborated the role of antiglomerular basement

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membrane antibody in the pathogenesis of human glomerulonephritis. Dixon [4] demonstrated the potential for chronic low-grade disease to produce glomerulonephritis of antigen-antibody complex pathogenesis; then Notkins,[5] working with the lactic dehydrogenase virus in mice, showed that a virus could be the antigen. This was also shown to be the case in Aleutian mink disease by Henson et al in 1971.[6]

Knowledge about the animal models for immune complex disease has progressed rapidly. The specific antigen component is known in several of these, such as LDH, Aleutian mink disease, equine infectious anemia, and NZB mouse disease. The knowledge is much less complete about such human diseases as lupus erythematosus and indeed a majority of cases of human nephritis. When the knowledge comes about these human entities, it will be in large measure because of the progress made on the animal diseases.

These are examples in which elucidation of pathogenesis of the disorder in the animal model is ahead of understanding of human disease. This allows specific questions to be pursued in patients with the knowledge that there is a direction, a template, to follow.

A more difficult problem is posed by the human disease looking for an animal model. Such is the case with cystic fibrosis, an important human disease known to be inherited and one that has been studied exhaustively. As yet, we have not found the animal model of cystic fibrosis, unless the disease recently described in monkeys proves to be a valid model system.

There is an excellent model for subacute sclerosing panencephalitis in the form of distemper-associated demyelinating encephalomyelitis in old dogs or the so-called "old dog" encephalitis. Koestner discussed this as animal model number 68 from the Registry of Comparative Pathology. This is an apparent aberration of the replicative cycle of paramyxovirus of canine distemper and of measles in man. The problems and unsolved questions relate to why this virus starts to replicate and then does not complete that cycle and why at some later stage in life, perhaps from some outside stress, it begins to undergo changes that cause the subsequent demyelinating lesions seen in the disease. These dogs contain antibodies against myelin when they develop demyelinating encephalitis, and this may be one of the major clues to the pathogenesis of this disease.

When one ponders the replicative mechanisms of paramyxoviruses, questions arise about the genome of the virus, which, in the form of a nucleocapsid, as it approaches the plasma membrane of the cell sends messages that result in production of the lipoprotein outer coat of the virus by modification of the plasma membrane of the host cell. What does this do to the self-recognition mechanism? Does this play a role in the immunologic process? Can one generalize on this kind of process to other autoim-

mune diseases of suspected or unknown pathogenesis, such as multiple sclerosis, periarteritis, lupus erythematosus, or chronic glomerulonephritis?

Leprosy is an important infectious disease in many parts of the world and one about which little has been learned concerning its pathogenesis because of the great difficulty in handling the organism in the laboratory and the fact that no animal could be infected with the agent.

A group of alert researchers, headed by Storrs,[7] discovered that the nine-banded armadillo (*Dasypus novemcinctus*) is highly susceptible to infection with this organism. Animals injected either intradermally, subcutaneously, or intravenously with a suspension of *Mycobacterium leprae* develop a disseminated infection in from 15 to 34 months. This infection closely resembles that found in man. Furthermore, the individuals infected, as with human beings, have no cellular immunity against this organism. This provides for many possible experimental questions to be asked about pathogenesis. Also, large amounts of the infectious organism can now be collected for basic biologic studies of the bacterium, and lepromin can be prepared from the tissues of these animals for biologic tests. It is encouraging that this disease, in existence for so many years, has been made available for study only very recently by alert observers, thus emphasizing again the need for us to look carefully for the animal models that are available to us.

We have discussed several animal models of different types, including genetic, infectious, and immunologic diseases. Why are these specific diseases good animal models? In searching for animal models, what criteria do we apply to judge value? Must the animal model be an exact duplicate of a human condition, or can differences sometimes also be of value?

The models of coagulopathies are excellent examples in which the animal model system in many cases exactly duplicates the chemical and metabolic pathways of the human ailment.

Studies of hemorrhagic disorders probably represent some of the most productive animal model systems yet developed. From evaluating what we believe are criteria for good animal models in the light of a known productive animal model system, we may be able to agree on what those criteria should be.

What are the criteria of a good animal model system?

1. It should accurately reproduce the disease or lesion under study.

Nearly every bleeding disorder that has been reported in humans has been diagnosed in animals, and, for most of these disorders, a living animal is currently available that has that disease. The disease in the animals accurately reflects the disorder in people, and the sequelae of the disease in people also occur in the animals, eg, the joint lesions in the hemo-

philiacs. The mode of inheritance is the same in animals and humans.

This is not to say that differences cannot be exploited or that apparent differences might not mean that more work is needed on both the animal and human disorder. One good example is the work of Dr. Travis McGuire of Washington State University on combined immunodeficiency disease (CID) in the Arab foal. CID in the foal is inherited as an autosomal recessive trait. In the initial studies of CID in children the autosomal recessive form of CID showed a deficiency of adenosine deaminase (ADA), and intermediate levels of this enzyme were used to assess possible heterozygotes of the disorder. Since it would be nice to be able to identify heterozygotes specifically among a population of Arabian horses that is estimated to contain from 30% to 35% carriers, ADA activity was examined in affected and known carrier horses. It was found that ADA activities were normal in both affected and known heterzygote Arabs, so there was an apparent difference in the autosomal recessive form of the disease in the two species. It was later found that in the sex-linked form of CID in children ADA was normal and that in some autosomal recessive CID children ADA was normal. What was an apparent enzymatic difference in CID in the two species was thus resolved by further study.

2. It should be available to multiple investigators.

Approximately 70 investigators were involved in the study of the 16 hemorrhagic and thrombotic disorders in animals. It is obvious from the publications that many of these investigators studied the animals over long periods of time. Further, it is clear that separate investigators published jointly. Thus data and animals were made available across institutional lines. Published papers concerning crossbreeding studies of the disorders clearly show that the animals moved between groups of investigators and indicate that data are freely shared. Having the animals available to multiple investigators allows monitoring of the scientific validity of observations and stimulates further investigation.

3. It should be exportable.

It should be possible to ship good models around the country for most effective use. Brinkhous's hemophiliac dogs originated in Cornell, and the von Willebrand swine studied by Bowie and Fuster originated in Missouri. The effective use of the animals depended upon getting them to the appropriate location. This is not to say, for example, that finding a killer whale with the Chediak-Higashi syndrome in Victoria British Columbia is not an interesting observation, but it is clearly not the most effective model from a research standpoint.

4. If genetic, it should be in a polytocous species.

In genetic disorders, the numbers of animals produced is a limiting fac-

tor. Most of the animals involved in the hemorrhagic and thrombotic disorders, such as dogs, rats, and swine, reproduce relatively rapidly.

5. It should be large enough for multiple biopsies of samples.

The dogs with factor VII defect and the cattle with factor IX deficiencies provide the standard source of substrate for diagnosis and quality control in the identification of these disorders in people. This requires animals of sufficient size to draw repeated samples.

6. It should fit into available animal facilities of most laboratories.

The investment in animal facilities has accelerated over the past several years from the costs involved in making changes in animal housing and care standards, making this a highly desirable characteristic of a good model system. In most cases, the animals involved in hemorrhagic and thrombotic disorders can be handled by any good research laboratory in the country.

7. It should be easily handled by most investigators.

Sometimes investigators avoid using good animal model systems because they entail the use of animals with which they are unfamiliar. We, for example, based on experience, are perfectly happy working with a vicious little animal, the mink. We have also used cattle, horses, and numerous other animals in experimental protocols. But our training provides us with the background to handle such experimental animals. Some researchers who have different backgrounds are reluctant to utilize such animals. Therefore, the most useful model systems are found in those species with which investigators are most comfortable. Convenience, however, should not be the determining factor in selection of a model.

8. It should be available in multiple species.

To generate a research model system that is most adaptable, the disorder should be available in multiple species. For example, the platelet anomaly in the Chediak-Higashi syndrome was first described in cattle because large quantities of blood were available and the animals could be repeatedly sampled without bringing harm to them. On the other hand, the mouse is adequate for ultrastructural studies and is much more easily raised and handled. The increased susceptibility to infection was best demonstrated in the mouse and mink because the cost of doing the same study in cattle would clearly have been prohibitive.

The comparative studies of various species with hemorrhagic and thrombotic disorders have shown that the bleeding parameters found are associated with the disease in all species rather than being species specific.

9. It should survive long enough to be usable.

We are currently attempting to study a disorder in the Chihuahua in which there is an apparent paradox: an extremely low blood sugar level

along with the absence of islets of Langerhans. Three of these dogs have been seen, but they die apparently of hypoglycemic shock by five weeks of age, at which time they weigh 6 ounces. According to the owners there are few, if any, clinical signs prior to death. While the problem is an intriguing one, it is difficult to manipulate the system and thus it may well be an ineffective model.

The thrombotic and hemorrhagic animal models survive reasonably well with proper care, and many have been used over a number of years to yield the data necessary to characterize the disorders and serve as substitutes for humans in the development of significant diagnostic and therapeutic measures that are currently used on a worldwide basis.

The above are some of the most important criteria for good model systems, and a highly productive model system will fit these criteria closely. Other standards may also apply in certain situations, but this should at least be a start in the right direction.

Rene Dubos has more than once said that if we look carefully enough we will probably find an animal model for every human ailment. Have we looked or listened well enough? We must continue to be alert—to look and to listen—to detect, develop, and utilize these experiments in nature.

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