FAMOUS ANIMAL EXPERIMENTS
A Historical Perspective

Dr. W. H. Stone, Distinguished Professor, BS, MS, PhD, DSc
Department of Hematology,
Hospital De La Santa Creu i Sant Pau
Barcelona, 08025, SPAIN

INTRODUCTION

I wish to make it clear at the outset that I am not presenting this paper as an argument for the use of animals in research. Indeed, we are all aware of the fact that experiments using animals are absolutely necessary for improving human welfare. The context in which I am presenting this paper is to remind us how proud we should be to have done experiments with animals. These experiments have changed the course of human history and have had momentous benefits for human health.

Most historians of biology agree that the modern use of animals for biomedical experiments began in 1600 when Dr. William Harvey described, for the first time, the circulation of blood in animals. In 1616, he gave the “Lumleian Lecture” to the Royal College of Physicians in London during which he recorded the first account of the circulation of blood.

In his famous Book, entitled, *EXERCITATIO ANATOMICA DE MOTU CORDIS ET SANGUINIS IN ANIMALIBUS,* Harvey described in detail observations he made on various animals, including hunted deer from the forests of the various Kings of England. Harvey’s research remained highly controversial until the great Italian biologist, Marcello Malpighi himself a highly respected professor of medicine and personal physician to Pope Innocent XII, using a refined microscope, proved the validity of Harvey’s observations.

Doubtless we are all aware that Malpighi was one of the first scientists to study various animal organs and cellular structures that still bear his name, such as the Malpighian corpuscle. Needless to say that since these famous experiments were performed, research using animals has played a major role in biology, medicine and veterinary science.

COMMENT: Clearly, it would take many pages to discuss all of the famous animal experiments. So, instead, I have chosen to present only a few experiments in a variety of animal species, in hopes that each person reading this paper will be familiar with one or more of these species. Also, many scientists may not be aware of the species of animal that was used in experiments that they probably know very well.

I hope I will be able to provide some of this information in what follows.

<u>RODENTS (*Rodentia)*</u>
MICE (*Mus musculus*)

Who would question that rodents, especially mice, have been the pre-eminent laboratory animal? In my judgement, the most famous experiment with mice (and possibly one of the most famous of all animal experiments) was reported in 1944 by Avery, McCleod and McCarty who worked at the Rockefeller Institute for Medical Research in New York City. This experiment unequivocally demonstrated that DNA was the genetic material. There is a fascinating history behind this seminal discovery, going as far back as 1871 when Freidrich Miescher first identified nucleic acids. He called them "nucleins" because he isolated them from salmon sperm which are largely nuclei. Then in 1928, the British scientist, Frederick Griffith, working with two strains of pneumococcus bacteria discovered a factor that was capable of transforming heat-killed type III bacteria into living virulent bacteria when the heat-killed bacteria were mixed with living type II non-virulent bacteria. Griffith correctly concluded that something which he called, the **Transforming Principle** was passed between the bacteria so that living type III virulent bacteria arose. It was the brilliant research with mice that identified this transforming principle as DNA. This discovery was a necessary antecedent for Watson and Crick's description of the detailed conformational structure of DNA in 1953.

Another landmark experiment using mice was the delineation of the genetic and immunological basis of transplantation by Little and Tyzzer . It is quite remarkable that as early as1914 these scientists were able to demonstrate that tumors transplanted from one strain of mice to mice of the same strain were accepted, whereas, tumors transplanted to a different strain were rejected. The details of their experiments are voluminous and we have no space to discuss them. But, for example, in one of their studies alone, they used no less than 692 mice. Incidentally, Dr. Little founded and was Director of the famous Jackson Laboratory in Bar Harbor, Maine—the leading world-wide supplier of mice strains housing over 3,000 unique strains.

We should note that these studies foreshadowed the description of the Major Histocompatability Complex (MHC) in mice that was the model for the same gene family in other species, including humans, and which has been the foundation of successful organ transplantation in humans.

These studies won a Nobel Prize in 1980 for George Snell of the Jackson Laboratory who was a major contributor to the delineation of the MHC of mice and also to the broader field of immunogenetics.

Before leaving our discussion of mice—"the King of Laboratory Animals"—we should quote from a recent article (June 30-July 7, 2003) in NEWSWEEK magazine entitled, <u>*Re-inventing the Mouse*</u>. *It (meaning the mouse) is easy to keep and quick to breed. Mice have played a key role in medical research for nearly a century. It is the smallest mammal that is physiologically and genetically similar to humans--sharing close to 99% of the genes thought to be relevant to health.*

<u>RATS</u> (*Rattus norvegicus*)

If I were a rat, I think I would be a little disappointed that my species has not received the recognition it deserves. In fact, rats have played a critical part in some of the most important discoveries. For example in the 1880's, August Weisman began cutting the tails of rats to see if any rats were born without a tail. Needless to say, after 20 generations rats continued to be born with normal tails. This experiment, along with others in rabbits (to be mentioned later) dealt a fatal blow to the Theory of Acquired Characters proposed by Lamarck which posited that characteristics acquired by an animal during its life could be inherited by its offspring.

The American biologist and prolific science writer, Isaac Asimov, pointed out that Weisman could have cited the fact that many generations of circumcision by the Hebrews failed to produce a newborn boy without a foreskin, or that despite the century-old practice of docking the tails of lambs, lambs continue to be born with intact tails.

This and other brilliant research by Weisman led him to hypothesize that there was a difference between somatic and germinal cells and that only germinal changes (mutations) could be transmitted to the offspring. These ideas were elaborated in Weisman's classic book entitled, *On Heredity: Essays upon Heredity and Kindred Biological Problems*, published around 1890. Incidentally, to add to Weisman's distinction we should note that he was one of the co-discoverers of Mendel's Laws of Heredity.

GUINEA PIGS *(Cavia porcellas*)

Guinea pigs (GP) are often kept as pets because they are small, gentle to handle and have variously colored attractive fur. Humans who are used for experimentation or research **unknowingly** are often referred to as "guinea pigs"-- an unfortunate connotation for an animal that has made so many contributions to science. For example, Sewell Wright used GPs to develop his now universally accepted principles of population genetics. In a classic series of papers, beginning in 1915, he mathematically defined various systems of mating and developed a method to quantify inbreeding. Among his many contributions, he invented Path Diagrams and the Inbreeding Coefficient, "F", which are still used today.

The United States Department of Agriculture recently cited Wright's research with GPs as one of the 10 milestones of the past 100 years of research at the Beltsville, MD, Agricultural Research Center. After Wright retired at the age of 65 from the University of Chicago, the Genetics Department at the University of Wisconsin, Madison, in its wisdom invited him to join the faculty. This move culminated in the writing of 4-volumes of his brilliant studies, entitled: *Evolution and the Genetics of Populations.* Dr.Wright died in Madison at the age of 99**.**

RABBITS *(Oryctolagus cuniculus*)

Among the rodents, rabbits have made important contributions far beyond their use to produce antibodies and vaccines and as test animals in toxicity studies. One famous experiment using rabbits was performed around the turn of the 20^{th} century by Francis Galton, best known as the father of Eugenics. Galton wanted to test Darwin's Theory of Pangenesis, which postulated that the hereditary determiners were carried by so-called *gemmules* carried in the blood and transmitted during reproduction. Galton transfused blood from common lop-eared rabbits into the silver-gray breed of rabbits, and then mated the transfused silver grays. After observing that they did not produce hybrid rabbits, nor did any offspring possess any traits of the donor lop-eared breed, Galton correctly concluded that gemmules did not carry the hereditary determiners, as Weisman had shown in rats, the theory of inheritance of acquired characters was invalid.

Incidentally, the rabbit played an important role in the early studies of quantitative genetics by William Castle at Harvard, who was the pre-eminent mammalian geneticist of his time. Around 1910, Castle studied ear-lengths in rabbits and concluded that the inheritance was more complex than was suggested by a purely Mendelian Model of Inheritance.

Perhaps the most noteworthy experiments using rabbits were done by Pincus and Chiang (at the Worcester Institute in Massachusetts) in the 1950's. They developed the first practical oral contraceptive.

It is safe to say that the development of the birth-control pill has revolutionized sexual activity and social behavior throughout the world, especially in occidental societies. Clearly, the pill was a major factor in advancing women's independence.

CATS (*Felis catus*)

There is good evidence that cats were domesticated by the Egyptians around 3000 years ago. They soon became one of the most popular house pets in the world and now represent over 50 varieties or breeds. Humans valued cats not only for their beauty and independent personalities, but also because they were efficient hunters and could protect food from rodents. However, the domestic cat was not used as a research animal until the 20th century and even then, not very extensively.

Some of the most famous research using cats was done by Barr and Bertram in 1949. While they were microscopically examining cells from the nervous system of cats, they noticed a small dark-staining body on the periphery of the nucleus of female cells that was not present on male cells. They called this body a "nucleolar satellite", but it is now generally referred to as a *Barr body***.**

As expected, this discovery stimulated a tremendous amount of research in many different species all of which confirmed the original finding. Later it was discovered that the Barr body was, in fact, the inactivated sex chromatin of one of the female X chromosomes.

The Barr body soon became a useful marker for cytogenetics, especially for sex determination. But, more importantly, it led to the Single-Active X Chromosome Theory which is now universally accepted, though still not thoroughly understood. In most mammals, one of the X chromosomes in somatic cells of females becomes condensed and partially deactivated during early development. Simply speaking, this is nature's way of equalizing the sex chromosome contribution of XY males and XX females. One of the most puzzling aspects of the Single Active X phenomenon is that deactivation is random--whether the maternal or the paternal chromosome is de-activated in a given cell is a matter of chance, yet all progeny of that cell have the same X chromosome de-activated.

It is worth mentioning that VandeBerg and colleagues in Australia made the amazing discovery that marsupials are exceptions to this random rule. In marsupials it is only the paternal X that is deactivated.

It is also worth mentioning that VandeBerg saw the need for a marsupial model to study this phenomenon and single-handedly, established the gray short-tailed opossum, *Monodelphis domestica*, as an important model organism for basic biological and biomedical research.

Those of us who have had cats as pets know that they are quite intelligent and exceedingly independent. Perhaps this is why Roger Sperry used cats for his pioneering split-brain experiments. Sperry was a psychobiologist who worked for 30 years at the California Institute of Biology (beginning in 1954) to sort out the relationship between the right and left hemispheres of the brain. He performed what is known as split-brain surgery—a procedure that effectively severs the *corpus callosum* which connects the two cerebral hemispheres and enables communication between the right and left sides of the brain. From these studies in cats, later in monkeys, and still later in humans, he was able to deduce that the left side of the brain was largely responsible for language ability and the right side of the brain for non-verbal processes. In 1981, Sperry received the Nobel Prize for his pioneering work that helped map the brain.

DOGS (*Canis familiaris*)

Dogs have also been used in what I have classified as "famous animal experiments". They were used to develop artificial insemination (AI) in1780 by the Italian physiologist, Spallanzani. Over 100 years later, the Russian scientist Ivanoff, perfected the technique in cattle which is used today in many millions of cattle and other animals throughout the world.

The Russian, Ivan Petrovich, also known as Pavlov, won a Nobel Prize in 1904 for his experiments with dogs that demonstrated conditioned response. He rang a bell and then fed the dog. He noted that the dog salivated, when it saw the food. He repeated this and then when he rang the bell without food the dog salivated automatically, i.e., had made a conditioned response (reflex).

Before leaving our discussion of the dog, we should mention that recently it has become one of the best models for transgenic therapy to treat hemophilia B (an X-linked recessive disease where Factor IX in the clotting cascade is deficient) which occurs naturally in certain breeds of dogs such as Dalmatians. The success of gene therapy in dogs has stimulated work in humans that is most encouraging.

Finally, dogs are being used increasingly in comparative genetics. For example, a gene mutation that causes narcolepsy (the uncontrollable tendency to sleep) in humans has recently been identified in dogs.

NOTE*: We should not forget that many famous experiments were done in farm animals. We shall mention only a few of these.*

CHICKENS (*Gallus domesticus)*

Without doubt, we must recognize the important role that chickens played in revealing how the immune system works. In 1956, Bruce Glick and associates published their famous paper (*THE BURSA OF FABRICIUS AND ANTIBODY PRODUCTION*) in Poultry Science (35: 244-246) describing the role of the bursa in immunity. Using chicks, they found that removing the bursa (a strange thymus-like organ at the tail end of the gastrointestinal tract similar to our appendix) soon after hatching inhibited normal immune development. Within a few years, Glick's findings were confirmed by Harold Wolfe at the University of Wisconsin and by Ben Papermaster at the University of Minnesota. About the same time, Robert Good and associates at the University of Minnesota reported the role of the mammalian thymus as the key organ in the development of transplantation immunity. Wisely, they used the chicken because it has both a thymus and a bursa -- so they could determine which organ functioned in which immune response. Their work culminated in the remarkable finding that the immune system consisted of two distinct lymphoid organs--the thymus responsible for cell mediated immunity and the acquisition of T lymphocytes, and the bursa or its equivalent (in mammals) responsible for humoral immunity and the acquisition of B lymphocytes.

CATTLE (*Bos taurus)*

A quantum leap in the understanding of the immune system came in 1945 from Ray Owen's finding (at the University of Wisconsin) that dizygotic twin cattle are often chimeric. That is--each twin possesses a mixture of its own blood and those of its co-twin throughout life. During embryological development, there is an anastomosis of the chorionic blood vessels between the twin embryos that allows for a reciprocal exchange of primordial hematopoietic tissue. Obviously, each twin must acquire the state of immunological tolerance against the foreign tissue of its co-twin or otherwise the tissue of the co-twin would be rejected (i.e., not tolerated). Also, reciprocal skin grafts between members of a chimeric twin pair are readily accepted. This provided conclusive evidence that tolerance had indeed been induced.

This finding led Macfarlane Burnet (in Australia) to hypothesize that a major function of the immune system was to distinguish self from non-self. A short time later, Medewar (in England) and colleagues induced tolerance to transplanted tumor tissue in mice and demonstrated that one could induce tolerance if the tolerogen was introduced before the immune system was fully developed. Burnet and Medewar shared the Nobel Prize (1960) for their seminal research.

SHEEP (*Ovis aries)*

Without doubt the most famous experiment in a farm animal within the past decade was the cloning of the sheep, "Dolly", in 1966 by Ian Wilmut of the Roslin Institute in Scotland. Cloning itself is not new, but what was new about cloning Dolly was that she was cloned from the cells of an adult animal. Previous successful cloning had been obtained only using embryonic cells. Dolly was derived from cells taken from the udder of a 6-year old ewe whose cells were induced to become quiescent in vitro and then fused with unfertilized eggs from which the nuclei had been removed. It must be emphasized that the technique is very inefficient. Of the 277 fused eggs, only 29 appear to have developed normally to the blastocyst stage. These 29 were transplanted into 13 surrogate ewes and only one gave birth to a viable lamb. Since Dolly was born, many different species have been cloned from adult cells including cattle, pigs, and even rhesus monkeys.

Cloning is certain to become an important process in farm animals, but its impact on humans is being hotly debated.

We can not leave the discussion of sheep without mentioning scrapie**,** which is a well-known degenerative, neurological disorder caused by a slow infectious agent called a "prion". Prions were discovered by Stanley Prusiner who proposed that a protein particle that somehow replicated with nucleic acid was responsible for scrapie. Although there were many who were initially skeptical about a self-replicating protein, Prusiner's hypothesis was confirmed and 15 years after his unconventional proposal (1997) he won the Nobel Prize.

RHESUS MONKEYS (*Macaca mulatta*)

We can not neglect the rhesus monkey when discussing famous animal experiments. We shall mention only a few such experiments but there have been several. For example, NASA's launched the rhesus monkey called, "Sam", into space in 1959 to test the effects of space flight on primates. Another example is the use of monkey kidney cells on which to abundantly culture viruses. This technique won a Noble Prize for Dr. John Enders at Harvard and later allowed Jonas Salk and Albert Sabin to produce and test a vaccine which has virtually eliminated polio.

Karl Landsteiner received the Nobel Prize in 1930 for his discovery of the human ABO Blood Groups which made blood transfusion safe for the first time. But, the prize did not deter Landsteiner from further research. In 1940, he and his student, Alexander Wiener divided humans into two groups, which they called Rh-positive and Rh-negative, after testing them with an antiserum produced in rabbits with the blood of rhesus monkeys. Later, he and another of his students, Philip Levine discovered that the Rh blood group was responsible for hemolytic disease of the newborn human if the mother was immunized transplacentally with Rh-positive blood of her fetus during gestation. This discovery has spared countless newborn babies from the irreparable brain damage caused by erythroblastosis fetalis also called hemolytic disease of the newborn.

Certainly, the exploration of social behavior in rhesus monkeys by Harry Harlow during the 1950-60's at the University of Wisconsin, Madison deserves to be classified as a famous experiment. Harlow separated newborn monkeys into two groups: one group was raised with artificial surrogate mothers (usually a blanket attached to a flat board) and the other group without one. When the monkeys were adults, those who had been raised with surrogate mothers (or actual mothers) were socially normal and capable of being attentive mothers themselves. In contrast, the babies raised without surrogate mothers were socially abnormal and incapable of normal motherhood. In effect, Harlow dramatically demonstrated that love and caring are essential ingredients of normal sexual and social development. Harlow won the National Medal of Science in 1967. He was the only primatologist to have received that honor.

CONCLUSIONS

Before closing, we would be remiss if we did not mention that Charles Darwin's revolutionary theory of evolution was based, in large part, on his keen observations of many different animals especially birds (finches) and other animals (turtles) that he studied on the Galapagos Islands. These studies culminated in his famous book, *The Origin of Species* in which Darwin presents his theory of evolution. Needless to say, the idea of evolution is essential to understanding biology.

CLOSING STATEMENT

Finally, one might think that with the advent of cell and molecular biology, animal experimentation will no longer be necessary. Quite the contrary, in the final analysis cells and molecules must be tested in living animals for such research to improve human health. Current thinking expresses the translation of laboratory research to the clinic as "*from bench to bedside"*. we dare say that this should be changed to read, "*from bench* ***to animals*** *to bedside*".

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