

**1997 CARL G. HARTMAN AWARD
RALPH L. BRINSTER**



The Carl G. Hartman Award is sponsored by the R.W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey. Dr. Ralph L. Brinster is the recipient of the 1997 Carl G. Hartman Award.

Dr. Brinster's career is accentuated by spectacular, resourceful, and highly innovative contributions to basic and applied research and to veterinary medicine. After growing up in Cedar Grove, New Jersey, he attended Rutgers University School of Agriculture (1949–53), where he received a B.S. After graduation, he served in Korea as a lieutenant in the U.S. Air Force (1954–56). Following this, he earned a V.M.D. (1960), graduating first in his class at the School of Veterinary Medicine, University of Pennsylvania, and later obtained a Ph.D. in physiology (1964) from the Graduate School of Arts and Sciences. During this period, he held a fellowship from the American Veterinary Medical Association and later a Pennsylvania Plan Scholarship. Dr. Brinster's Ph.D. thesis is the fourth most cited from the University of Pennsylvania. While in graduate school, he undertook research at The Jackson Laboratory, Bar Harbor, Maine (1960), and at the Marine Biological Laboratory, Woods Hole, Massachusetts (1962). This extensive and excellent training is reflected in Dr. Brinster's stellar performance throughout his long productive career.

Continuing at the University of Pennsylvania, Dr. Brinster advanced from Instructor (1964–65) to Assistant Professor (1965–66), Associate Professor (1966–70) and Professor of Physiology (1970–) in the Department of Animal Biology, School of Veterinary Medicine. In 1975 he was appointed Richard King Mellon Professor of Reproductive Physiology, School of Veterinary Medicine and Graduate School of Arts and Sciences, a position he still holds. Dr. Brinster initiated the Veterinary Medical Scientist Training Program (1969–84) and served for 15 years as Program Director. It is still the only V.M.D./Ph.D. program funded by the National Institutes of

Health. For 27 years, he was also a member of Graduate Group Committees in Physiology, Molecular Biology, Genetics, and Comparative Medical Sciences. Clearly, Dr. Brinster has given a life time of enthusiastic energy to the University of Pennsylvania, serving it with industry, diligence, and devotion.

Dr. Brinster's remarkable, rigorous, and distinguished contributions have led to leaps in scientific progress, for which he has received many international lectureships, honors, and awards. Lectureships were invited by the Harvey Society (1984), Nobel Symposium (1991), and Juan March Foundation (1992). He received the New York Academy of Sciences Award in Biological and Medical Sciences (1983), Distinguished Service Award from the U.S.D.A. (1989), and Pioneer Award from the International Embryo Transfer Society (1992), and he was honored for his extensive research contributions at an International Symposium (1987) held at the W. Alton Jones Science Center, Lake Placid, New York.

Dr. Brinster is a Member of the Institute of Medicine, National Academy of Sciences (1986); Fellow of the American Academy of Arts and Sciences (1986); Member of the National Academy of Sciences (1987); Fellow of the American Association for the Advancement of Science (1989); Fellow of the American Academy of Microbiology (1992); and Doctor Honoris Causa in Medicine, University of the Basque Country (1994). Drs. Brinster and Richard D. Palmiter together were awarded the Charles-Léopold Mayer Prize (1994), the highest award given by the French Academy of Sciences. Recently, Drs. Brinster and Beatrice Mintz received the first March of Dimes Award (1996), and he was bestowed with the most prestigious Bower Award and Prize by The Franklin Institute (1997). These distinguished credits attest to Dr. Brinster's outstanding contributions in elucidating mechanisms regulating mammalian embryonic development and reproduction.

Dr. Brinster has published more than 320 scientific papers in leading, peer-reviewed journals with intellectual contributions from his Ph.D. students (4), postdoctoral fellows (25), and numerous colleagues. In particular, a long-term collaboration with Dr. Richard D. Palmiter, Professor, Department of Biochemistry, and Investigator, Howard Hughes Medical Institute,

University of Washington, Seattle, Washington, has been outstandingly productive, resulting in more than 137 joint publications on development of transgenic animals. More than 15 of Dr. Brinster's publications made the cover of *Cell*, *Nature*, *Science*, or other scientific journals.

The theme of Dr. Brinster's research has focused on the mechanisms regulating the proliferation and differentiation of the mammalian germ line and how these cells can be modified genetically. His approach to basic science has been enterprising and incredibly innovative, leading to major advances in our understanding of developmental and reproductive biology. For instance, he was responsible largely for elucidating the requirements for in vitro development of murine embryos. These studies led to the successful integration of cells into early embryos and to the subsequent introduction of transgenic methodologies and their application to study developmental processes per se. Remarkably, he also demonstrated the feasibility of freezing and transferring stem cells between males and hence enabling an efficient means of undertaking homologous recombination for correcting defective genes in any species. Dr. Brinster's contributions extend to four interdependent areas.

First, Dr. Brinster's success in developing transgenesis, a process of genetic manipulation, depended on his introduction of key in vitro techniques for the mass manipulation and culture of mouse oocytes, zygotes, and early cleavage-stage embryos. These procedures, involving the use of simple metabolic substrates (pyruvate, lactate), were developed as part of his Ph.D. thesis under the tutelage of Dr. John D. Biggers and were reported in 1963 in a publication that remains among the top 300 most highly cited in the life sciences. The defined medium, and subsequent variations, formed the backbone for research on in vitro embryonic development for the next 33 years. The overwhelming influence of this body of work on the rapid expansion of mammalian embryo research cannot be overestimated. These discoveries enabled scientists to pursue research on oocyte maturation, fertilization, embryogenesis, nuclear transplantation, and gene injection; to manipulate eggs from other mammals, including the human; and to generate identical twins in domestic species. The latter, in turn, facilitated exciting

genetic research in livestock production and management.

Second, Dr. Brinster's interests in the mechanisms regulating embryonic development evolved in innovative and intriguing ways. His subsequent experiments led to the successful introduction and integration of asynchronous embryonic cells and embryonal carcinoma cells (EC cells) into an embryo's blastocoelic cavity. The blastocysts were colonized with cells collected from older embryos, even bone marrow stem cells and EC cells. Dr. Brinster demonstrated that foreign cells would integrate into blastocysts to form chimeras, and later it was shown that these cells were transmitted through the germ line, to contribute to the genetic lineage of offspring. These data were verified quickly by other scientists, and EC cells later were proven to be the direct antecedents of embryonic stem cells (ES cells). Thus, Dr. Brinster showed that cells of a different origin and genetic constitution could contribute to a common embryonic lineage. Germ line transmission of EC and ES cells made it possible to manipulate the mammalian genome by introducing targeted mutations through homologous recombination with endogenous genes.

Third, during the early 1980s, Dr. Brinster demonstrated that globin mRNA injected into oocytes led to the translation of the appropriate protein. These studies evolved to injecting the 5S RNA gene into the zygotic pronucleus and subsequently showing expression of the gene product. Then, Dr. Brinster initiated very productive, long-term collaborations with Dr. Palmiter, a well-known molecular biologist. By using Dr. Palmiter's construct of the mouse metallothionein promoter fused to the herpes simplex virus thymidine kinase gene, Dr. Brinster obtained integration of the construct into the oocyte genome, and, more importantly, expression of the gene in adult organs under the control of the inducible promoter. In collaboration with Dr. Ursula Storb, Dr. Brinster demonstrated that foreign genes were expressed in a tissue-specific manner, further unveiling the enormous potential of transgenesis. This research provided the foundation for the subsequent rapid outgrowth and exploitation of insertional and targeted transgenesis. These innovative experiments greatly enriched our understanding of the genetic events regulating normal and abnormal embryonic

and fetal development in surprising and fascinating ways.

Subsequently, Drs. Brinster and Palmiter showed that the rat growth hormone gene could be integrated and expressed in mice. The resulting high levels of circulating rat growth hormone dramatically changed the phenotype of the transgenic mice by stimulating them to grow twice as large as normal. The giant mice instilled major excitement in the scientific and public communities, markedly enhancing attention on the transgenic mouse system. Thereafter, in a series of dazzling collaborative endeavors, Drs. Brinster and Palmiter delineated regulatory elements in promoter regions of genes, examined effects of ectopically expressing gene products in tissue-specific patterns, showed haploid spermatids acted functionally as diploid cells, demonstrated that introduction of oncogenes led to cancer, and showed that microinjection of xenogeneic hepatocytes restored liver development. Further, they extended the transgenic system to domestic livestock, thereby demonstrating the potential to enhance growth, modify resistance to disease, and produce milk containing human proteins of medical importance, such as blood clotting factors for hemophiliacs and growth hormone. The impact of transgenesis is emphasized by the huge number of research groups and corporations that have and will continue to utilize the technology to study important fields of embryonic and adult physiology.

Fourth, Dr. Brinster recently made another breakthrough of immense importance, continuing the theme of stem cell manipulation, in this case, that of the male germ line. Totipotent spermatogonial stem cells collected from fertile male mice were transferred into the testes of recipients experimentally depleted of endogenous germ cells. The stem cells, carrying the marker 3-galactosidase transgene, colonized the recipient testes and re-established spermatogenesis to levels that resulted in fertility and gave rise to offspring of the donor haplotype. Further, Dr. Brinster froze, thawed, and transferred mouse germ cells into recipient testes, where the germ cells successfully reinitiated spermatogenesis, thus demonstrating the potential of storing the cells for long periods. Lastly, after transferring rat stem cells into mouse testes, he noted that the foreign cells differentiated to form morphologically normal rat sperm. These experiments are highly

innovative and fascinating. When methods become available for propagating totipotent spermatogonial stem cells, it will be feasible to restore fertility in individuals subfertile or sterile due to stem cell defects. Further, it will be possible to clone and modify the stem cells genetically by homologous recombination, prior to transplanting them into recipients to undergo spermatogenesis. This pioneer technology will provide a direct route to the modification and/or restoration of selected genes. A new era dawns in the genetic manipulation of totipotent germ cells by homologous recombination, selection, and transplantation to recipient animals.

Amidst these pioneering discoveries, Dr. Brinster remains extremely humble, quick to shower credit on those who have contributed toward the research, and exceedingly grateful for the continued support of friends and colleagues.