Molecular geneticists looking for ways to model human disease and companies testing new drugs are creating an unprecedented demand for inbred rodents

The Rise of the Mouse, Biomedicine's Model Mammal

MOUSE ECONOMY

The mouse is taking over in the lab as a model of human genetics and physiology. *Science* examines three aspects of the boom: the growing sophistication of suppliers, new academic facilities, and the debate on patents.

▶ **SUPPLIERS**
ACADEMIC FACILITIES
PROPERTY CLAIMS

BAR HARBOR, MAINE— In a shiny new $23 million facility here at The Jackson Laboratory (TJL), mouse pups rate their own private elevator. The miniature "mousevator" whisks the thumb-sized newborns from ground-floor surgical suites up to germ-free nurseries, where whiskered foster parents and a bevy of human coddlers await. These animals—bred to mimic human diseases at costs approaching thousands of dollars each—are too valuable to be left to nature's mercies, notes geneticist Larry Mobraaten, a driving force behind the new Genetic Resources Building, dedicated to raising new and useful mutants. "They are extraordinarily valuable scientific resources," he says.

The mousevator is just one indication of the booming mouse economy. From lab benches to Wall Street, everyone from venture capitalists to cagemakers is scurrying to get in on the unprecedented international growth in the use and production of mice for scientific research. By some estimates, more than 25 million of the tiny mammals will be raised worldwide for studies this year, accounting for more than 90% of all mammals used in research. That's double the number used a decade ago, but still not enough to meet anticipated demand: Forecasters predict mouse use could grow by 10% to 20% annually over the next decade. "It's a feeding frenzy," says Ken Paigen, director of TJL, which ships nearly 2 million of its trademarked JAX mice to researchers each year.

Once a modest regional business, the mouse trade is now a global enterprise that is being transformed by scientists' growing ability to fine-tune the genetic variation of these model mammals. Major commercial breeders sold an estimated $200 million worth of rodents last year; now they are retooling to meet greater demand spurred by government and corporate spending on biomedical research. Universities are also aggressively building new animal facilities to lure top scientific talent, while lawyers on the "mouse bar" struggle to settle lingering controversies surrounding patented mice (see pp. 254 and 255). And government agencies are pouring cash into programs that will produce a new network of distribution centers and an avalanche of new strains—raising questions about which ones should be maintained as live breeding colonies and which ones frozen in vaults for future use. Meanwhile, researchers and breeders are keeping a nervous eye on animal rights activism, regulatory initiatives, and mouse diseases that could complicate these plans.

In the black. The versatile C57 Black 6 mouse is one of science's most popular and best documented strains, making it a perennial top seller. From $6.

Several factors have contributed to the boom, including the mouse's spectacular fecundity and relatively low maintenance costs. Some prolific pairs, for instance, can produce more than 250 descendants in just a year on little more than grain and water. But scientists like mice because they are physiologically and genetically similar to humans. Millions of mice are used to screen drugs and potentially dangerous compounds for safety, for instance. And most human genes appear to have a related mouse version, making it possible to gain insights into human diseases using gene-altered mouse

CREDIT: (TOP) TACONIC FARMS

A MOUSE CHRONOLOGY

1664 Robert Hooke observes the reactions of mice in experiments on air, the first recorded use of mice in scientific research.

1900 Retired schoolteacher Abbie Lathrop begins breeding "fancy" mice at her farm in Granby, Massachusetts. Initially sought as pets, the Granby mice become important in research.

THE JACKSON LABORATORY

1908 William Castle opens Harvard's Bussey Institution, where many early mouse geneticists get their start.

1909 Clarence Little begins to develop the first inbred strain, designated DBA for dilute, brown, and non-agouti.

THE JACKSON LABORATORY

1914–19 Lathrop sends mice that developed tumors to Leo Loeb at the University of Pennsylvania, who publishes pioneering papers on cancer.

THE JACKSON LABORATORY

1913–16 Halsey Bagg develops the BALB/c (Bagg albino) mouse for behavioral experiments.

models that suffer from similar ills but aren't subject to the same ethical concerns as human patients. Technologies that have made it easier than ever to tinker with the mouse's genome have only enhanced the rodent's value. For instance, the potential number of transgenic and "knockout" mice (which have one or more of their 80,000 genes disarmed) is mind-boggling, notes Donna Gulezian, product manager for transgenic models at Taconic Farms, a major mouse supplier in Germantown, New York. Mouse design, she says, is "limited only by the investigator's imagination."

Indeed, the mouse's growing importance as a "fuzzy test tube" and its close kinship to humans has made it the only other mammal scheduled for complete genetic sequencing, a task that both the National Institutes of Health (NIH) and the private company Celera have targeted to complete within 5 years. The honor is one sign of the rodent's transformation from "lab urchin to scientific thoroughbred," says Bob Jacoby, director of the Animal Resources Center at Yale University in New Haven, Connecticut.

The mousketeers

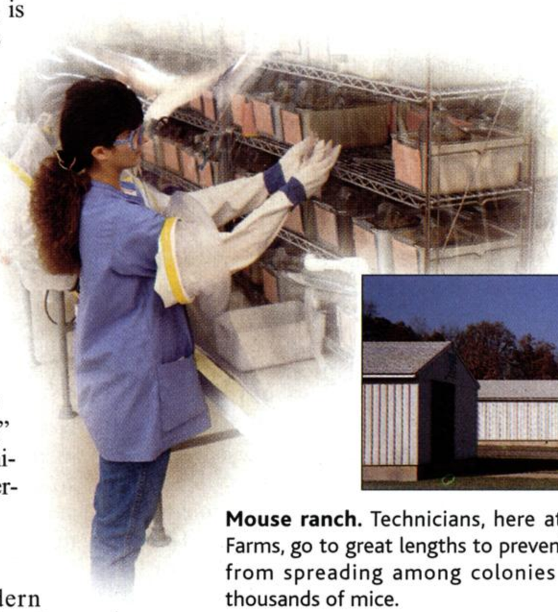
Like many businesses, the modern mouse economy is dominated by a few big names that coexist with some well-respected niche players and cottage industrialists. Globally, the commercial breeding heavyweight is Charles River Laboratories of Wilmington, Massachusetts, a 50-year-old concern with 49 facilities in 18 nations. Last year, it sold more than $140 million worth of mice, rats, and other research animals. (Company officials declined to detail how many mice they sold.) The other two U.S.-based, multinational industry leaders —number two breeder, Harlan Sprague Dawley of Indianapolis, Indiana, and Taconic—are smaller.

Analysts estimate that Harlan had $60 million in 1998 sales, while Taconic totaled $36 million last year. Also in the top pack is TJL, which rang up $29 million in mouse sales in 1999. But unlike its competitors, the lab—which one executive calls "the fourth mouseketeer"—is a taxpayer-funded nonprofit that plows profits back into research and warehousing thousands of mouse varieties that have little commercial value.

Charles River and Harlan are also major players in Europe, having purchased stakes in a number of homegrown providers. They are joined by Taconic ally M&B of Ry, Denmark—a significant supplier in Northern Europe and Germany—and government-sponsored mouse repositories, such as the European Mutant Mouse Archive in Italy. Charles River also has outposts in Japan, where it competes with CLEA Inc. of Tokyo and smaller suppliers.

On a regional scale, academic research labs also sustain a lively barter in specialized mice among scientists. Also serving a limited clientele, but charging big fees, are a few specialized high-end companies such as Lexicon Genetics of The Woodlands, Texas. For prices ranging from $18,000 to $65,000, depending on the company's share in any royalty income, Lexicon will spend 8 months creating four custom-tailored knockout mice for each customer.

The bulk of the world's lab mice, however, are bred by large academic or government labs for internal use or supplied by the high-volume breeders. And most of these suppliers are now reengineering themselves to keep pace with growing demand. Charles River, for instance, is completing a major financial reorganization after being sold last year by corporate parent Bausch & Lomb. The buyers—who paid $456 million—are Charles River's own longtime executives, backed by Global Health Care Partners, an investor group that includes the former CEOs of several major pharmaceutical companies.



Mouse ranch. Technicians, here at Taconic Farms, go to great lengths to prevent disease from spreading among colonies holding thousands of mice.

CREDITS: TACONIC FARMS

These executives are making what one industry insider calls a "gutsy gamble." Other breeders are impressed with the amount of debt that Charles River has taken on—nearly $350 million—given the risks of the live-animal trade, from mergers that can trim customer lists to diseases that can wipe out a close-packed breeding colony virtually overnight. Still, documents filed with the Securities and Exchange Commission in Washington, D.C., show that the company is the high-volume Wal-Mart of the mouse economy, with 62% of its $230 million in sales from animal models. Company executives are bullish that they can build on this position, noting that Charles River historically has "been able

1915 J. B. S. Haldane *et al.* publish the first genetic linkage study, establishing the linkage between two coat-color mutations.

1919 Mouse genetics research begins in earnest at the Cold Spring Harbor Station for Experimental Evolution.

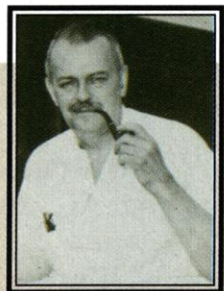
THE JACKSON LABORATORY

1921 L. C. Strong breeds a Bagg albino with an albino from Little's stock and starts the first of many tumor-prone strains, called the A strain, known for mammary and lung tumors.

1921 Using a pair of black mice from the Granby farm, Little develops the C57BL and C57BR strains.

THE JACKSON LABORATORY

1928 L. C. Dunn breeds Strain 129, which later proves to have a high incidence of testicular cancer; the strain is now valued as a source of embryonic stem cells for making knockout mice.



THE JACKSON LABORATORY

1929 Little starts The Jackson Laboratory in Bar Harbor, Maine, with help from Detroit industrialists who had previously recruited him to the University of Michigan.

Decoding a Mouse Name

129S7/SvEvBrd-$Hprt^{b-m2}$

For mice used in science, pedigree can be everything. So researchers have developed a precise naming code that tells users a bit about each mouse's history, source, and traits. Major breeders, for instance, begin names with their own acronym—Cr for Charles River, HSD for Harlan Sprague Dawley, Tac for Taconic, and J for The Jackson Laboratory—and then add strain information. Shown above is the name for one strain of the popular 129 mouse. It tells users that this is the #7 substrain with a steel-colored coat (S7), and that it has passed through labs run by researchers named Stevens (Sv), Evans (Ev), and Bradley (Brd). Finally, the name denotes a mutation on the "b" allele of the *Hprt* gene, with "m2" showing that it was the second mutation of that allele.

Names are getting longer as researchers demand more information on genotype and phenotype. "We live and die by the names," says Taconic's Sam Phelan, but "they are having a hard time keeping up." Soon, he says, names will be just the tip of an information iceberg, as researchers routinely turn to large electronic databases to get the complete skinny on their mouse model. **–D.M.**

to increase our prices at rates that are above the rate of inflation … by maintaining high quality." They predict "moderate but sustained growth in the research model business."

Harlan, Taconic, and TJL are also planning for multinational growth. TJL, for instance, is adding capacity. For the first time, it has also hired a business-savvy executive solely to manage and grow its production division, which generates nearly half of the funds the lab pumps into its research programs. New head mouse wrangler Warren Cook, a veteran of chemical and skiing businesses, says his top priority is improving the lab's ability to deliver mice in a timely manner, long a sore point with some researchers.

The problem is that while most suppliers offer 25 to 75 strains, TJL has about 2500—"by far the world's best selection," gushes one longtime customer. But diversity is also the lab's Achilles' heel, she says: "They can't keep every strain on hand, so you sometimes have to wait a long time for delivery." Cook jokes that buyers put up with delays because, "despite our lousy service, we still know mice better than anyone." But increasingly, says TJL's Phil Standel, the wait is unacceptable. Customers, he says, "see mice just the way you see a chemical kit you can order off the shelf in a few days."

Like TJL, Harlan and Taconic have added customer-service staff. Following an industrywide trend, they are also offering customized breeding services to clients who want to avoid the high cost of housing or raising their own mice, particularly the hard-to-maintain transgenic types. Taconic technicians, for instance, now care for more than 600 lines of "outsourced" mice belonging to other labs, along with about 75 of their own strains.

Contract breeding is attractive not only because it generates income. It also can give breeders an inside track on emerging models that may be worth adding to the product line. Indeed, to a greater degree than its competitors, Taconic is specializing in breeding the temperamental transgenics. It has created a "Transgenic Exchange" that helps researchers share their not-quite-ready-for-prime-time mice with other scientists. In exchange for Taconic's help in distributing and developing the model—a complicated breeding and characterizing process that can take years—the company positions itself to add the more popular contract-bred mice to its glossy catalog. Transgenics already account for nearly 10% of Taconic's revenue and are expected to be "a big part of our future," says part-owner and director of marketing Sam Phelan.

But few researchers should count on a cushy retirement as a mouse tycoon, industry officials caution. "We get calls all the time from researchers who think they've created the next big mouse," says Taconic's Gulezian. "But the reality is very few models have broad enough applications to be commercially attractive." At TJL, for instance, "most of our strains are money-losers," but "it serves our public purpose to maintain them," notes Paigen.

There are exceptions. Although details are shielded by proprietary agreements, researchers who invented now-widespread patented techniques for engineering mice or who hold stock in biotech companies with rights to unusually useful strains have done very well. Earlier this year, for instance, a mouse engineered to grow human tissues proved so valuable that it scuttled a planned \$350 million buy-out of a California biotech company. The promise of the Xeno mouse, owned by Abgenix Co. of Alameda, so excited investors that the company's stock value shot from \$130 million to \$370 million in just a few weeks—making the buy-out offer pale in comparison.

But the big mouse breeders can't count on controlling such patented mice; instead they rely on their brand name to market common strains available from many vendors. TJL, for instance, bans direct sales to its competitors, in order to "maintain the strength and integrity of our brand," says Cook: "Our Black 6 is different from Charles River's Black 6." And TJL's Carol Linder adds that studies have shown that mice from different vendors have developed significant genetic differences over time, though they may share the same name. "I'd never recommend switching suppliers midway through an experiment, even if you think you are ordering the same mouse," she says.

1939 International Committee on Standardized Nomenclature for Mice begins, bringing order to the naming of mice and their genes.

1937 Peter Gorer shows in mouse studies at The Jackson Lab that transplant rejection is primarily governed by what he calls the H2 genetic locus, later described as the major histocompatibility complex, a key component of immunity.

1947 Britain launches the Medical Research Council (MRC) Radiobiology Unit—now known as the MRC Mammalian Genetics Unit and the U.K. Mouse Genome Centre—in Harwell, U.K., using radiation to carry out large-scale mutagenesis experiments. Harwell becomes Europe's hotbed of mouse genetics.


THE JACKSON LABORATORY

Researchers at Oak Ridge National Laboratory in the United States also do radiation studies. The mutant mice lead to major advances in mouse genetics.

A fire destroys most of The Jackson Lab and its mice. Researchers rally to rebuild stocks.

Down on the ranch

The toughest part of mouse ranching, however, may not be differentiating your product but keeping it healthy. "Raising mice can be a nightmare," says Phelan. "Most researchers are blown away when they see what it takes to run a production facility." Companies spend millions, for instance, to prevent human caretakers from infecting their wards with disease. At Taconic and elsewhere, masked and gowned workers are required to shower and don sterile jumpsuits before entering "barrier facilities"—mouse barns with sophisticated ventilation and watering systems. Some of the immunocompromised transgenic and mutant mice are particularly vulnerable and must be housed in germ-free plastic bubbles. (To introduce the "good" microbes mice need to digest food, caretakers often add a single pellet of mouse feces to their drinking water.) Other strains can't stand bright light, need cages mounted on vibration-damping shock absorbers, or stop reproducing or die if their food or ventilation isn't just right. Abigail Smith, an animal-care specialist who recently left Loyola University in Chicago, Illinois, for TJL, recalls that one strain would "start seizuring if you just clapped your hands."

Despite the precautions, almost every producer has had to destroy vast numbers of animals to halt epidemics. Indeed, disease is such a grave threat to sales that major producers are quick to investigate and address any suggestion that their animals are contributing to an outbreak. As rumors spread last year that TJL and Taconic mice appeared to be testing positive for a feared mouse hepatitis virus, for instance, both companies took aggressive steps to clear their names. After detailed testing—the results of which were posted on their Web sites—Taconic and TJL researchers concluded that the "outbreak" was either a rash of false-positive results or a hepatitis strain spread by mice from some other source.

Mouse suppliers obsess over animal health in part because studies have shown that mice carrying pathogens can produce flawed research results. However, suppliers—and researchers—are also becoming sensitive about the high price tags on some mice. "When a mouse cost a buck and it got sick, no problem: You'd get another one," notes Yale's Jacoby. But with transgenic mice routinely costing $175 each, and some rare pairs worth up to $30,000, "providing health care is becoming an increasingly attractive option," he says. Mouse doctors—and pathologists, for essential postmortems—are in short supply, however. As a result, the NIH is calling for training a new generation of specialists who can keep animals healthy and help researchers understand the sometimes subtle genetic and environmental factors that influence an animal's behavior and physiology.

Flesh tone. Hairless nude mouse strains carry impaired immune systems, allowing researchers to implant and study human tumors. From $25 to $75.

CREDIT: (TOP) TACONIC FARMS

Disease concerns have also prompted renewed calls recently for international testing standards; researchers want to know that the mice they get are clean. Several decades ago, animal-care experts thought they had solved that problem by introducing "specific pathogen free" (SPF) standards. The push, which prompted mouse users to take greater care in testing and accepting new mice into their colonies, helped produce a dramatic leap in health quality. Before SPF, "your average mouse was basically a sewer—it had every microbe known to mousedom," recalls Smith, adding, "Things are much better now." Today, however, the SPF label is used so routinely and enforcement is so lax that it has become virtually meaningless, some animal-care experts say. Health enforcers have not kept up with the proliferation of new and newly detectable mouse diseases, says Smith, who calls SPF "a garbage term unless they specifically tell you what pathogens they've tested for."

To restore SPF's good name, some countries, such as the United Kingdom, have adopted new policies that prohibit laboratories from accepting animals that haven't been certified as free of a "hot list" of pathogens. So far, however, major producers in the United States, Japan, and elsewhere in Europe have resisted the regulations, arguing that they are unnecessary in a market that already places a premium on health. Says Taconic animal-care chief James Geistfeld: "Our approach is to test the heck out of the animals and then publish the results."

Quiet resistance to greater health regulation also comes from researchers impatient to begin experiments with newly acquired animals. Some scientists sneak untested animals around quarantine restrictions, mouse health experts claim. The results can be disastrous. Loyola, for instance, had to shut down its colonies earlier this year after a pathogen was introduced by what officials believe were smuggled-in animals, disrupting dozens of experiments. It can take a year or more to complete the expensive—about $5000 per strain—process of rederiving stocks by implanting embryos in disease-free foster mothers. But Smith believes that, as scientists become more aware of the risks of working with untested animals, "they'll respond appropriately"—perhaps by turning in rogue colleagues.

Companies not only need a clean bill of health, but they are increasingly pressured to certify that their mice are—genetically

THE JACKSON LABORATORY

Late 1940s George Snell develops congenic strains of mice—identical but for a small chromosomal segment—by breeding for differences only at the H2 locus. This opens new areas of immunological research and earns Snell a Nobel Prize.

1949 The informal *Mouse News Letter* begins its 40 years of publication under that name. At its peak, some 60 labs contribute to it.

1950 Obese mouse is discovered at The Jackson Lab. The first animal model for obesity, the mouse later proves to have a key mutation in the *leptin* gene.

THE JACKSON LABORATORY

1954 Leroy Stevens develops an ovary transplant procedure that enables mutant strains to be propagated even if the mutation causes the animal to die before it can reproduce.

1958 Margaret Green at The Jackson Lab starts a card-file database of mouse linkages and loci, which forms the foundation of the Mouse Genome Database. Eventually, the National Institutes of Health (NIH) begins supporting the database.

speaking—the real thing. Many researchers have horror stories about mice that turned out to be genetically different from the advertised strain. Even these problems, however, can be an opportunity to mouse providers: For a fee, they will do the sophisticated genetic testing necessary to cull imposters.

Hickory, dickory, stock

As mouse strains proliferate, one of the biggest challenges facing every retailer is figuring out how to keep live mice "on the shelf" awaiting a buyer. "One of the hardest things to control is fluctuating demand," says Taconic's Gulezian. The problem is especially acute at TJL, which as a federally funded mouse repository has a mandate to keep as many potentially useful strains on hand as possible. Deciding what to keep is becoming more difficult: Scientists will create more strains this year than used to be developed in an entire decade. And the problem will only get worse, as special mouse initiatives in Europe and the United States ramp up.

The U.S. initiative—championed by former NIH director Harold Varmus—includes millions of dollars to create potentially thousands of new mutants and transgenic mice. Last December, for instance, the National Cancer Institute funded 19 groups at 30 institutions to "accelerate the tempo at which mouse models of cancer are developed." And NIH officials have tapped TJL, Taconic, and Harlan to help operate a new network of regional distribution centers that will help house and characterize new mutants created by exposing mouse sperm to ethylnitrosourea, a powerful mutagenic chemical. A related effort by the European Community hopes to pump up stocks at the European Mutant Mouse Archive. Although such centers will help ease the housing shortage, selection panels will still face some tough choices. "We'll have to do some crystal balling about what will be in demand years from now," says TJL's Mobraaten, whose facility can accept about 90 new mutants a year.

In the long run, however, it will be impossible for mouse researchers to build their way out of the space shortage, observers say. "You can't throw bricks, mortar, plastic, and stainless steel at the problem forever," says Yale's Jacoby. Like others, he is hoping that new storage technologies—from freezing embryos or eggs to sperm and chunks of ovary—will eventually reduce the need to maintain live colonies. With that in mind, Mobraaten can equip TJL's new building with up to 18 cryogenic freezers—up from an existing four. But he notes that, so far, only the relatively expensive embryo-freezing process has proven effective with mice, while newer sperm- and ovary-freezing techniques remain hit or miss. Few labs, for example, have been able to routinely repeat the success that Ryuzo Yanagimachi of the University of Hawaii, Honolulu, has had in reconstituting strains from frozen germ cells. TJL staff "have been trying for a year and can't produce a mouse," says Mobraaten. To overcome the obstacles, NIH is funding a special mouse reproduction initiative that Mobraaten says "is looking promising."

A gene short. Knockout mice like this popular Taconic model can help reveal gene function. From $100 to $15,000.

CREDIT: (TOP) TACONIC FARMS

But even freezing sperm may have limitations. "It seems to work very well for strains that have a mixed background," says Mobraaten, but inbred strains don't do well. "If you have a valuable [inbred] strain, I wouldn't rely on it."

Ironically, Mobraaten notes, the freeze-storage plans now viewed as a form of salvation once were criticized as extravagant. "The complaint early on was that we were going to create a mouse museum" of unused strains, he says. Today, however, TJL—which stores about 1000 strains as embryos—is "recovering to the tune of 150 strains a year."

The mouse redefined

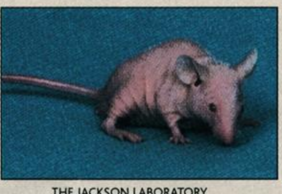
While mouse experts are confident that they can leap technical hurdles, some worry that future animal rights issues may be more difficult to surmount. Traditionally, mice have slipped "under the animal rights activists radar screen—they just don't have the sympathy factor generated by a dog or chimp," says one industry executive. But that is changing. In Europe, groups are pushing the Council of Europe to more stringently regulate mouse use. And in the United States, breeders are keeping a close eye on a bid by animal rights groups to have the mouse redefined as a "regulated animal" under the U.S. Animal Welfare Act (AWA) (*Science*, 5 February 1999, p. 767), which currently exempts mice, rats, and birds from caging and inspection requirements.

If the effort is successful—and preliminary signs are that it will be—mouse breeders and researchers may have to submit to new caging rules that could reduce colony densities. TJL's Cook, for one, worries that such rules could increase researchers' costs "by 20% or more." But Charles River shrugs off the threat that increased AWA regulation could pose to its business, noting that competitors would all have to play by the same price-raising rules. And Taconic's Phelan is philosophical about regulatory changes, viewing them as one of many winds buffeting the mouse economy. "This is a very rapidly changing and maturing business," says Phelan. "We're doing things now we wouldn't have dreamt possible a few years ago. We just have to get used to the fact that when it comes to mice, we're dealing with a whole new world."

–DAVID MALAKOFF

1961 Harwell's Mary Lyon proposes X-chromosome inactivation, in which one chromosome in an X-chromosome pair shuts down to maintain the right balance of gene activity.

1962 The nude mouse, lacking hair, is discovered in Ruchill Hospital, Glasgow, U.K. Several years later, scientists realize that its lack of a thymus means it produces no T cells. It becomes an important tool for immunological studies.



THE JACKSON LABORATORY

c. 1970 Richard Gardner of Cambridge, U.K., performs surgery on mouse embryos, opening the way to embryo transfer, embryonic stem cell research, and transgenic mouse technology.

1971 Donald Bailey develops the first recombinant inbred strains of mice by crossing two inbred strains. The resulting inbreds prove useful for genetic mapping and gene hunting.

1972 U.K. researcher David Whittingham shows that frozen mouse embryos can survive thawing, making it possible to preserve strains without continuous breeding.